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Diuretic activity evaluation and chemical composition analysis of *Hedyotis scandens* extract from Mizoram, India, in rat models

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A R T I C L E I N F O

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A B S T R A C T

Ethnopharmacological relevance: Diuretics play a crucial role in addressing various medical conditions such as hypertension and edema. Across numerous communities, plants have served as diuretic agents, leveraging their abundant phytochemical composition. In certain instances, plant-based remedies have gained preference over synthetic drugs due to their affordability and ready availability. *Hedyotis scandens* Roxb., commonly recognized as Laikingtuibur/Kelhnamtur in Mizoram, belongs to the Rubiaceae family. This plant has been harnessed worldwide within diverse societies as a medicinal resource to combat a spectrum of ailments. Notably, in Mizoram, the leaves are employed in creating a decoction with diuretic properties. The ethnopharmacological exploration of plant diuretics not only preserves cultural traditions but also contribute to the potential discovery of novel therapeutic agents.

Aim of the study: Our study endeavours to explore the traditional employment of this plant as a diuretic in Mizoram. Furthermore, we seek to elucidate the plant's chemical composition through the utilization of GC-MS analysis.

Materials and methods: In this investigation, we conducted plant extraction using methanol and distilled water as solvents within a soxhlet apparatus. Prior to commencing the main experiment, we conducted an acute toxicity test to ensure the safety of the plant extract. For the assessment of diuretic activity, we adopted the methodology outlined by Lipschitz et al. (1943). All in vivo experiments were conducted in strict accordance with the guidelines set forth by the OECD. Based on the outcomes of the acute toxicity evaluation, we opted for three dosage levels: a high dose (1000 mg/kg), a medium dose (500 mg/kg), and a low dose (250 mg/kg). Furosemide, recognized as a loop diuretic, was employed as the standard reference, while the control group received distilled water.

Results: Our investigation unveiled the presence of several uncharacterized bioactive compounds within the plant. Of particular interest, the GC-MS analysis identified a specific compound named 'phytol,' which has previously been associated with diuretic properties. Notably, the acute toxicity assessment demonstrated the plant extract's safety even at a high dose of 5000 mg/kg, as no toxic effects were observed. The diuretic evaluation of the *H. scandens* extract exhibited a dose-dependent increase in diuresis, with the methanolic extract yielding notably superior outcomes compared to the aqueous extract. Moreover, the treated animals displayed an elevated output of electrolytes and an enhanced glomerular filtration rate in comparison to the control group. Notably, the histological examination of the kidneys from the treated animals depicted a normal structural configuration, devoid of any cellular-level modifications attributed to the plant extract across all tested doses.

Conclusion: The Hedyotis scandens extract demonstrated a pronounced diuretic effect in contrast to the control group. As such, our study substantiates the traditional employment of this plant as a diuretic within the Mizoram region.

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Abbreviations

HSME	*Hedyotis scandens* methanol extract
HSAE	*Hedyotis scandens* aqueous extract
BSTFA	O-Bis (trimethylsilyl) trifluoroacetamide
TMCS	Trimethylchlorosilane
GC-MS	Gas chromatography mass spectrometry
NaCl	Sodium chloride
Na^+	Sodium
K^+	Potassium
Cl^-	Chloride
ALT	Alanine transaminase
SGOT	Serum Glutamic Oxaloacetic Transaminase
SGPT	Serum Glutamic Pyruvic Transaminase
BUN	Blood Urea Nitrogen
DPX	Dibutyl phthalate Polystyrene Xylene
OECD	Organisation for Economic Cooperation and Development
HSAE + F	*Hedyotis scandens* aqueous extract + Furosemide
HSME + F	*Hedyotis scandens* methanol extract + Furosemide

1. Introduction

Diuretics play a pivotal role in enhancing urine production and facilitating the excretion of water and electrolytes from the body, making them indispensable for managing a wide spectrum of medical conditions such as hypertension, congestive heart failure, kidney disorders, and certain edematous states (Arumugham VB, 2023). These diuretics are generally categorized into four distinct classes based on their modes of action: thiazide diuretics, loop diuretics, carbonic anhydrase inhibitors, and potassium-sparing diuretics (Kehrenberg and Bachmann, 2022). Nevertheless, the utilization of these pharmacological agents is coupled with potential adverse effects, underscoring the rising inclination toward exploring natural alternatives (Gupta and Neyses, 2005; Martín et al., 1991). Plants, enriched with a plethora of secondary metabolites, have long served as diuretic agents, indicating substantial potential in treating ailments and offering medicinal benefits (Abdala et al., 2012; Craig, 1999; Perez Gutierrez and Baez, 2009). In numerous studies, plant-based remedies have exhibited heightened effectiveness and preferability in comparison to synthetic counterparts (Rastogi et al., 2016). The burgeoning interest in utilizing plants as potential sources of diuretic properties is substantiated by a wealth of studies that spotlight their efficacy and advocate for their adoption as natural alternatives to conventional diuretic drugs (Dearing et al., 2001).

Within this context, the spotlight turns to *Hedyotis scandens* Roxb., a member of the Rubiaceae family, characterized by its creeping growth pattern and elongated stems, frequently found in disturbed environments. The plant has attracted attention for its extensive therapeutic use, spanning the treatment of bone fractures, respiratory ailments, kidney stones, ocular complications, and sprains (Panmei et al., 2019). Known as "Laikingtuibur" or "Kelhnamtur" among the inhabitants of Mizoram, it boasts a rich traditional history in mitigating kidney and urinary disorders (Lalfakzuala, 2007; Rai and Lalramnghinglova, 2010). Notably, the Jorhat ethnic community also harnesses the plant's potential, employing a leaf decoction to address kidney and urinary issues (Sengupta, 2017). These traditional applications hint at the presence of distinctive compounds responsible for engendering these therapeutic effects. The plant's antioxidant and antimicrobial attributes have already been established through scientific investigation, unravelling noteworthy secondary metabolites, including novel phenolic glycosides (Vanlalruati Ngamlai et al., 2022; Wang et al., 2013). However, despite the paucity of scientific validation concerning its diuretic potential, insights gleaned from indigenous practices and the wisdom of traditional healers in Mizoram offer compelling evidence that the leaves of *H. scandens*, when prepared as a decoction, hold diuretic properties. As such, the root of our study rests on the fundamental objective of evaluating the diuretic impact of the crude extract derived from *H. scandens*. In this context, our study postulates a hypothesis that the traditional utilization of *H. scandens* in promoting diuresis aligns with its potential bioactive compounds, which may contribute to enhanced urinary output. We anticipate that our investigation will not only validate the traditional claims but also shed light on the underlying mechanisms that facilitate the diuretic effect, potentially leading to the identification of novel therapeutic agents.

2. Materials and method

2.1. Plant material collection and extract preparation

Hedyotis scandens (*H. scandens*) was procured from the premises of Mizoram University (coordinates: 23.7365° N, 92.6642° E) during the months of June and July. The botanical identity of the plant was authenticated and confirmed by the Natural History Museum, Mizoram University (Accession no- NHMM-P/000158). The leaves were meticulously washed, shade-dried, and subsequently powdered for sequential extraction using a Soxhlet apparatus. Distilled water and methanol were employed as solvents for extraction, with each extraction lasting 72 h. The resultant extracts were concentrated using a rotary evaporator and stored at 4 °C for future use. The accurate nomenclature of *Hedyotis scandens* Roxb was cross-verified and validated at, 'World Flora Online' http://www.worldfloraonline.org/taxon/wfo-0000980836.

2.2. Gas chromatography-mass spectrometry (GC-MS) analysis

Phytocompounds present in HSME and HSAE were detected using the GCMS-QP2010 Ultra, with column oven temperature of 120 °C, injection temperature 200 °C and column flow rate of 1.21 ml/min. The ion source was maintained at 220 °C. Roughly 500 μg of each *H. scandens* methanol extract (HSME) and *H. scandens* aqueous extract (HSAE) at a concentration of 1 mg/ml was mixed in a separating funnel and agitated with a mixture of water and ethyl acetate in a 1:4 ratio. The upper phase was collected, concentrated to 1 ml using a rotary evaporator, and treated with 50 μl of N, O-Bis (trimethylsilyl) trifluoroacetamide and trimethylchlorosilane (BSTFA + TMCS), followed by the addition of 10 μl of pyridine. The mixture was heated at 60 °C for 30 min. After this, the samples were transferred to GC vials, dried using nitrogen gas, and subsequently dissolved in methanol prior to GC-MS analysis. Compounds detected were identified by comparing with known compounds found in the National Institute of Standards and Technology (NIST) library.

2.3. Experimental animals

Adult Wistar rats, weighing between 180 and 185 g, were procured from authorized suppliers. These rats were individually housed in propylene cages under controlled conditions (temperature: 22±2 °C, 12-h light/dark cycle) with unrestricted access to food and water. The experimental protocols were conducted in alignment with the Mizoram University Institutional Animal Ethical Committee's guidelines (Approval No. MZU/IAEC/2021–22/13).

2.4. Acute toxicity study

The acute toxicity study was conducted following the Organization of Economic Cooperation and Development (OECD) 423 recommendation. Rats were divided into four groups (n = 6) and administered HSME and HSAE orally at doses of 1000, 3000, and 5000 mg/kg. Distilled water was administered to the control group. Animals were observed for

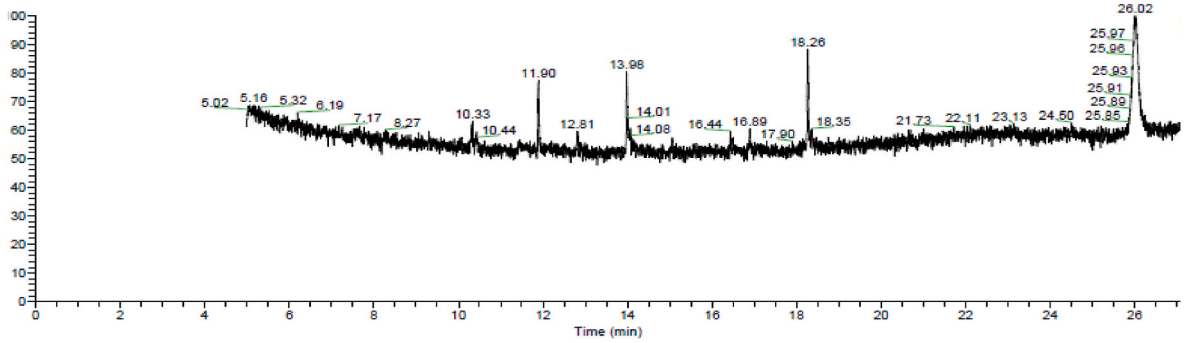

Fig. 1. GC-MS chromatogram of HSME.

acute toxicity signs within the first 4 h and then once daily for 14 days. On the 14th day, rats were sacrificed, and organs and serum were collected.

2.5. Diuretic activity assessment

The diuretic activity assessment followed a modified version of the method established by Lipschitz (1943). Each treatment group comprised six Wistar rats. Prior to the experiment, a fasting period of 18 h was observed. Rats received an oral load of 2.5 mL of 0.9% NaCl/100 g of body weight to ensure a consistent water and salt load. The control group received normal water in addition to the saline solution. Furosemide was used as a positive control at a dose of 10 mg/kg. Urine volume was recorded after 5and 24 h post-treatment. After the experiment, animals were euthanized, and serum and organs were collected.

2.6. Urinary pH and urine electrolytes

Urinary pH was measured using a calibrated digital pH meter. Urinary concentrations of Na+, K+, and Cl-were determined using Ion Selective Electrode analysis (9180 Electrolyte analyzer, Roche). Ratios of electrolytes (Na+/K+ and Cl-/Na+ + K+) were calculated to estimate natriuretic and carbonic anhydrase inhibitory activity (Somova et al., 2003).

2.7. Biochemical assay

Serum samples were collected for biochemical assays including ALT, SGOT, SGPT, bilirubin, total bilirubin, urea, blood urea nitrogen, and creatinine using Coral Clinical Systems kits.

2.8. Glomerular filtration rate

Glomerular filtration rate was estimated via creatinine clearance based on serum and urine creatinine levels (Bazzano et al., 2015; Pellicer-Valero et al., 2022).

2.9. Histology of the kidney

Histological investigations were conducted following established procedures as detailed by Bancroft and Gamble (2008). Briefly, the kidneys from the treated rats were extracted and subsequently immersed in Bouin's solution for a 24-h duration. Following this, the tissues underwent dehydration through a sequence of graded ethanol solutions (70%, 90%, and 100%) and were then clarified using xylene prior to embedding in paraffin wax. Tissue blocks were created and sliced into sections with a thickness of 7 μm using a Leica rotary microtome (model RM2125 RTS). To ensure proper adhesion, slides were coated with poly-L-lysine, and the tissue sections were gently spread using a water bath maintained at 43 °C. Deparaffinization was accomplished by treating the slides with xylene, succeeded by rehydration via a gradual series of ethanol concentrations for 10 min at each level. Staining of the slides was performed using haematoxylin and eosin, with subsequent dehydration utilizing diverse alcohol concentrations. The final steps included treating the slides with xylene to facilitate clarity, followed by mounting using DPX. Ultimately, scrutiny and imaging of the slides were undertaken utilizing a Nikon E200 microscope.

3. Results

3.1. Acute toxicity and GC-MS analysis of H. scandens

Over the entire 14-days observation period, aligning with OECD guideline 423, all six animals subjected to the acute toxicity study exhibited sustained survival without any evident neurological or behavioural deviations. This comprehensive outcome substantiates that this extract lies outside the ambit of the 'Global Harmonization System' classification (Jadhav et al., 2010). Given the absence of discernible toxicity at the administered doses, the necessity to ascertain the precise LD50 value was considered unwarranted. A total of 42 peaks were identified in the GC-MS chromatogram of both HSME and HSAE extracts (Figs. 1 and 2). Through meticulous analysis encompassing retention time, peak area (%), height (%), and fragmentation patterns observed in mass spectrometry, these peaks were aligned with known compounds

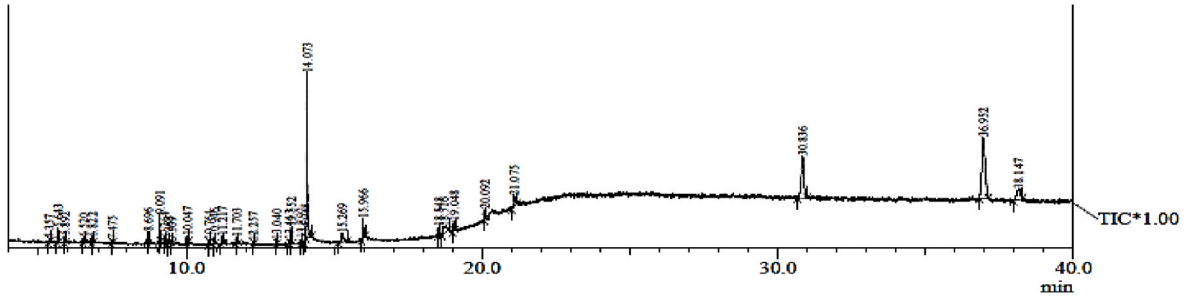

Fig. 2. GC-MS chromatogram of HSAE.

Table 1
Phytochemical constituents identified from HSME using Gas Chromatography-Mass Spectrometry.

Peak	RT	Area	Name	Activity	Reference
1	10.33	9724679	4-Trifluoroacetoxytetradecane	Antimicrobial	(Sarada et al., n.d.)
2	10.43		1,6:3,4- Dianhydro-2-deoxy-a-d-lyxo-hexopyranose	Antimicrobial	Sushma B K and Raveesha H R (2020)
3	11.90	17546587	Phenol, 2,5-bis(1,1-dimethylethyl)	Anticancer, antimicrobial, anti-inflammatory, antibacterial, antioxidant, antifungal	(Kuppuswamy1 et al., 2013; Rajeswari,2011; Ramand Rao, 2015; Pereira et al., 2009)
4	12.01	576560	E−14-Hexadecenal	Antibacterial	(Ram and Rao, n.d.)
5	13.98	20031683	Dodecyl acrylate	No activity reported	
6	14.06		1-Ethyldodecyl acrylate	No activity reported	
7	16.89	10474501	Pthalic acid, butyl nonyl ester	No activity reported	
8	18.26	25262966	Phytol	Diuretic, to treat rheumatoid arthritis antioxidant, anti-inflammatory, anticancer	(Ogunlesi et al., 2009; Rajeswari et al., 2013; Malipeddi and Das, 2014; Thangapandian, n.d.; Nagalakshmi et al., 2015)
9	18.35	6748762	Cyclopentaneundecanoic acid, methyl ester	Antimicrobial	Maha Lakshmi et al. (2018)
10	26	4017022	Pregnan-18-oicacid	No activity reported	
11	26.23	71237434	3 [(Trimethylsilyl)oxy]androstane-11,17-dione 17-(O-benzyloxime)	Antimicrobial, anti-inflammatory	Susheela et al. (2018)

Table 2
Phytochemical constituents identified from HSAE using Gas Chromatography-Mass Spectrometry.

Peak#	R. Time	Area	Name	Activity	Reference
1	5.357	12383	3,4- Diaminobenzonitrile	No activity reported	
2	5.643	58414	L-Proline, N-(3-fluorobenzoyl)-, isohexyl ester	No activity reported	
3	5.892	28242	Docosane	Antibacterial, lytotoxic, antioxidant	Godwin et al. (2015)
4	6.822	23245	Ethaneperoxoic acid, 1-cyano-1-[2-(2-phenyl-1,3-dioxolan-2-yl)ethyl]pentyl ester	Antimicrobial, insectiside	Janakiraman et al. (2012)
5	7.475	10459	9,10,11-Trioxabicyclo [6.2.1] undecane	Anti-infective, antimyopathies, antineoplastic,antirickettsial	Ali et al. (2022)
6	8.696	30808	Decane, 1-iodo-	No activity reported	
7	9.091	81553	Phenol,2,4-Bis (1,1- dimethylethyl)-	Anticancer, antimicrobial, antiinflammatory, antibacterial, antioxidant, antifungal, antimalarial	Mathivanan and Suseem (2015)
8	9.264	25658	Decane, 1 -iodo-	No activity reported	
9	9.388	12141	C13-E2	No activity reported	
10	9.509	6697	3-Hexanone, 2,2-dimethyl-	No activity reported	
11	10.047	27120	Ethyl 1,2,3,4,5,6,7,8- octahydro-8-oxo-1 Naphthalenecarboxylate ethyl	No activity reported	
12	10.764	11270	3- Methoxy-2-undecanone	No activity reported	
13	10.946	23735	Cheloviolin	No activity reported	
14	11.217	53987	1- iodotetradecane	No activity reported	
15	11.703	26440	3,5-Dimethyl-4-octanone	No activity reported	
16	12.257	7636	Docos-13-Enoic acid	No activity reported	
17	13.040	16295	Phthalic acid, 4-bromophenyl heptyl ester	Potential antioxidant	Karolina et al. (2019)
18	13.552	55982	7,9-Di-tert-butyl-1-oxaspiro(4,5)deca-6,9-diene-2,8-dione	Antioxidant	Merlin, N. et al.: Pharmacogn. Res., 1, 152 (2009); Conforti, F. et al.: Food Chem., 116, 898 (2009), Riquet, A. et al.: Food Chem., 199, 59 (2016);
19	14.018	21873	Ethyl-6- beta-D- Ribofuranosylimidaz0	No activity reported	
20	14.073	758922	n-Hexadecanoic acid	Prostaglandin inhibitor, antioxidant	Mohy El-Din and El-Ahwany (2016a)
21	15.269	86812	1-Octadecene	Used for nano crystal synthesis	Dhaene et al. (2019)
23	15.966	119891	Octadecanoic acid	antibacterial	Mohy El-Din and El-Ahwany (2016b)
24	18.548	35885	1H- Indole-3-Ethanamine	No activity reported	
25	18.716	103182	1H-Indene, 1-Hexadecyl-2,3-Dihydro-	No activity reported	
26	19.048	49995	1,2-Benzenedicarboxylic acid	Cytotoxic activity	Krishnan et al. (2014)
27	20.092	31033	5-ethoxy-5-oxonorvaline	No activity reported	
28	21.075	97121	13-Docosenamide, (Z)-	Human and plant metabolite	National Center for Biotechnology Information (2023). PubChem Compound Summary for CID 5365369, 13-Docosenamide. Retrieved May 8, 2023 from https://pubchem.ncbi.nlm.nih.gov/compound/13-Docosenamide
29	30.836	530481	Phenol, 2,4-bis(1,1-dimethylethyl)-, phosphite (3:1)	Selective antioxidant and antimicrobial activity	Malhotra (2008)
30	36.952	973257	Tris(2,4-di-tert-butylphenyl) phosphate	antioxidant	Wolf and Kaul (2000)
31	38.147	180266	Benzenepropanoic acid, 3,5-bis(1,1-dimethylethyl)-4-hydrox	Antifungal, antioxidant	Akpuaka et al. (2013)

enlisted in the National Institute of Standards and Technology (NIST) library. HSME accounted for the presence of 11 distinct compounds, while HSAE revealed 31 such compounds. The outcomes of the GC-MS evaluation for HSME and HSAE validated the existence of several pivotal bioactive components, outlined in Tables 1 and 2. Notably, some of these compounds have garnered prior recognition for their diuretic

Table 3
Effect of HSME and HSAE on urinary pH.

	5 h	24 h		5 h	24 h
Control	7.08 ± 0.5		Control	7.08 ± 0.5	
Furosemide, 10 mg/kg	7.46 ± 0.08		Furosemide, 10 mg/kg	7.46 ± 0.08	
HSME, 250 mg/kg	7.14 ± 0.4	7.25 ± 0.5	HSAE, 250 mg/kg	7.04 ± 0.08	7.1 ± 0.5
HSME, 500 mg/kg	7.23 ± 0.2		HSAE, 500 mg/kg	7.15 ± 0.05	7.23 ± 0.5
HSME, 1000 mg/kg	7.36 ± 0.6		HSAE, 1000 mg/kg	7.28 ± 0.07	7.36 ± 0.5
HSME 250 mg/kg + F, 10 mg/kg	7.1 ± 1		HSAE 250 mg/kg + F, 10 mg/kg	7.2 ± 0.3	
HSME 500 mg/kg + F, 10 mg/kg	7.4 ± 0.05		HSAE 500 mg/kg + F, 10 mg/kg	7.31 ± 0.5	
HSME 1000 mg/kg + F, 10 mg/kg	7.53 ± 0.09		HSAE 1000 mg/kg + F, 10 mg/kg	7.42 ± 0.9	

Data are expressed as mean ± SEM, $n = 6$.

Table 4
The effects of *Hedyotis scandens* methanol extract (HSME) and its combinations with furosemide (HSME + F) on urine volume at two time points: 5 h (5hr) and 24 h (24hr).

Group	Urine volume (ml)		Diuretic action		Diuretic activity	
	5 h	24 h	5 h	24 h	5 h	24 h
Control	2.2 ± 0.44				0.15	
Furosemide, 10 mg/kg	15 ± 0.23*		7.1		1	
HSME, 250 mg/kg	5.7 ± 0.12	5.2 ± 0.31	2.6	2.4	0.380	0.34
HSME, 500 mg/kg	6.4 ± 0.22		3		0.2	
HSME, 1000 mg/kg	7.3 ± 0.21		3.32		0.48	
HSME 250 mg/kg + F, 10 mg/kg	6.5 ± 0.2		2.9		0.43	
HSME 500 mg/kg + F, 10 mg/kg	7.180 ± 0.18		3.26		0.47	
HSME 1000 mg/kg + F, 10 mg/kg	14 ± 0.71**		6.36		0.9	

The data presented in the table represent the mean values along with the standard error of the mean (SEM) for a sample size of 6. Statistical analysis was conducted using a one-way analysis of variance (ANOVA), followed by the Tukey's test for multiple comparisons. Significance levels are indicated as follows: *p < 0.05: Indicates statistical significance at the 0.05 level, suggesting that the compared groups are significantly different from the control group. **p < 0.001: Indicates high statistical significance at the 0.001 level, suggesting that the compared groups are significantly different from the control group with a higher level of confidence.

attributes, underscoring the latent diuretic properties intrinsic to *Hedyotis scandens*.

3.2. Effect of H. scandens on urinary pH

The impact of *Hedyotis scandens* on urinary pH was investigated, revealing noteworthy alterations in the pH levels of treated rats compared to controls (Table 3). The baseline urinary pH of the control rats was measured at 7.08 ± 0.5 The rats treated with furosemide at a dose of 10 mg/kg exhibited a higher urinary pH of 7.46 ± 0.08 at both 5 h and 24 h. The rats treated with different doses of HSME and HSAE showed varying effects on urinary pH. For instance, when treated with HSME at 250 mg/kg, the urinary pH was 7.14 ± 0.4 at 5 h and increased to 7.25 ± 0.5 at 24 h. Similarly, when treated with HSAE at 250 mg/kg,

Table 5
The effects of various treatments on urine volume, diuretic action, and diuretic activity at both 5-h (5 h) and 24-h (24 h) time points.

Group	Urine volume(ml)		Diuretic action		Diuretic activity	
	5 h	24 h	5 h	24 h	5 h	24 h
Control	2.2 ± 0.44				0.15	
Furosemide, 10 mg/kg	15 ± 0.23*		1			
HSAE, 250 mg/kg	2.7 ± 0.46	5.45 ± 0.15	1.23	2.5	0.18	0.36
HSAE, 500 mg/kg	4.32 ± 1.4	6.5 ± 0.15	1.97	2.9	0.28	0.43
HSAE, 1000 mg/kg	5.63 ± 1.1	7.5 ± 0.18	2.56	3.4	0.37	0.5
HSAE 250 mg/kg + F, 10 mg/kg	5.7 ± 0.95		2.5		0.38	
HSAE 500 mg/kg + F, 10 mg/kg	6.1 ± 1.01		3		0.41	
HSAE 1000 mg/kg + F, 10 mg/kg	8.9 ± i1.35		4.04		0.59	

The data presented in the table represent the mean values along with the standard error of the mean (SEM) for a sample size of 6. Statistical analysis was conducted using a one-way analysis of variance (ANOVA), followed by the Tukey's test for multiple comparisons. Significance levels are indicated as follows: *p < 0.05: Indicates statistical significance at the 0.05 level, suggesting that the compared groups are significantly different from the control group. **p < 0.001: Indicates high statistical significance at the 0.001 level, suggesting that the compared groups are significantly different from the control group with a higher level of confidence.

the urinary pH was 7.04 ± 0.08 at 5 h and slightly elevated to 7.1 ± 0.5 at 24 h. Similar patterns are observed for other doses of HSME and HSAE as well. When combining HSME or HSAE with furosemide at 10 mg/kg, there's a shift in urinary pH. For instance, HSME at 1000 mg/kg combined with furosemide resulted in a urinary pH of 7.53 ± 0.09 at 5 h. Overall, Table 3 demonstrates how different treatments, including *Hedyotis scandens* extracts and furosemide, influence urinary pH levels over time, suggesting potential effects on the renal system. These observations highlight the potential of *H. scandens* extracts, both aqueous and methanol-based, to influence urinary pH, suggesting their potential utility in managing urinary system dynamics.

3.3. Effect of H. scandens on urine volume and urine electrolyte

The cumulative urine volume over 5 and 24 h was computed across all experimental groups. Detailed information regarding urine volume, diuretic activity, and diuretic action can be found in Tables 4–8. Both *Hedyotis scandens* methanol extract (HSME) and *Hedyotis scandens* aqueous extract (HSAE) exhibited a dose-dependent escalation in urine output in comparison to the control group. Notably, HSME at a dose of 1000 mg/kg demonstrated the highest diuresis at 5 h, whereas HSAE at the same dosage exhibited peak activity after 24 h. Upon co-administration with furosemide (HSME + F and HSAE + F), increased diuretic activity was observed when compared to the extracts administered alone. However, furosemide displayed the highest urine output and reached its peak activity within 5 h.

Comparing the extracts, both HSME and HSME + F showcased higher urine output than their HSAE counterparts across all administered dosages. Additionally, HSME and HSME + F exhibited significantly greater urine output compared to the control group, even at the lowest dose of 250 mg/kg. In terms of diuretic activity, furosemide exhibited the highest value of 1, followed by HSME 1000 mg/kg + F with a value of 0.9. HSME 500 mg/kg + F demonstrated a diuretic activity of 0.58, while both HSME 250 mg/kg + F and HSAE 500 mg/kg displayed an identical diuretic activity of 0.43. The control group exhibited the lowest

Table 6
The impact of HSME and HSME + F on urinary electrolyte concentrations at the 5-h mark.

Group	Na^+(mmol/L)	K^+(mmol/L)	Cl^-(mmol/L)	Saluretic index			Na^+/K^+	$Cl^-/Na^+ + K^+$
				Na+	K+	Cl-		
Control	46.5 ± 3	34.3 ± 2	77.5 ± 1.4	1	1	1	1.355	0.95
Furosemide, 10 mg/kg	115 ± 1.8**	49.3 ± 0.8*	170.5 ± 1*	2.5	1.43	2.2	2.3	1.03
HSME, 250 mg/kg	78.16 ± 2**	41 ± 2.8	135.6 ± 4.8**	1.68	1.19	1.74	1.9	1.13
HSME, 500 mg/kg	90.83 ± 2.57**	49.6 ± 2.3	162 ± 3.2**	1.95	1.44	2.09	1.83	1.15
HSME, 1000 mg/kg	91.6 ± 2.23**	50.5 ± 2.5	163 ± 7.6**	1.9	1.47	2.1	1.81	1.14
HSME 250 mg/kg + F, 10 mg/kg	76.3 ± 4.8**	42.5 ± 5.1	141.83 ± 6.4**	1.64	1.23	1.83	1.7	1.19
HSME 500 mg/kg + F, 10 mg/kg	95.1 ± 3.2**	46.3 ± 2.4	152.8 ± 1**	2.04	1.34	1.97	2.1	1.1
HSME 1000 mg/kg + F, 10 mg/kg	109 ± 2.3**	48.6 ± 2.9	158.6 ± 3.3**	2.34	1.41	2.04	2.2	1

The data presented in the table represent the mean values along with the standard error of the mean (SEM) for a sample size of 6. Statistical analysis was conducted using a one-way analysis of variance (ANOVA), followed by the Tukey's test for multiple comparisons. Significance levels are indicated as follows: *$p < 0.05$: Indicates statistical significance at the 0.05 level, suggesting that the compared groups are significantly different from the control group. **$p < 0.001$: Indicates high statistical significance at the 0.001 level, suggesting that the compared groups are significantly different from the control group with a higher level of confidence.

Table 7
The influence of HSAE and HSAE + F on urinary electrolyte concentrations at the 5-h interval. The parameters under study include Sodium (Na+), Potassium (K+), Chloride (Cl-), Saluretic index, Na+/K+ ratio, and Cl—/Na++ K+ ratio.

	Na^+(mmol/L)	K^+ (mmol/L)	Cl^- (mmol/L)	Saluretic index			Na+/K+	Cl^-/Na++ K+
				Na+	K+	Cl^-		
Control	46.5 ± 3	34.3 ± 2	77.5 ± 1.4	1	1	1	1.355	0.95
Furosemide, 10 mg/kg	115 ± 1.8**	49.3 ± 0.8*	170.5 ± 1*	2.5	1.43	2.2	2.3	1.03
HSAE, 250 mg/kg	40.6 ± 2.5	35.8 ± 1.6*	76 ± 2.4*	0.87	1.04	1.01	1.13	0.9
HSAE, 500 mg/kg	60.6 ± 2.7*	41.6 ± 3.7	83 ± 2.9**	1.3	1.21	1.1	1.45	0.81
HSAE, 1000 mg/kg	62.3 ± 2.26*	42.5 ± 2.28	78 ± 2.5**	1.33	1.24	1	1.46	0.74
HSAE 250 mg/kg + F, 10 mg/kg	68.1 ± 2.5*	46.8 ± 2.5*	118.5 ± 2.2**	1.46	1.36	1.52	1.45	1.03
HSAE 500 mg/kg + F, 10 mg/kg	72.3 ± 4.6**	48.1 ± 5.2*	127.5 ± 3**	1.55	1.4	1.64	1.5	1.05
HSAE 1000 mg/kg + F, 10 mg/kg	75.6 ± 4.4**	50 ± 1.5*	138.16 ± 7.8**	1.62	1.45	1.78	1.51	1.1

The data presented in the table represent the mean values along with the standard error of the mean (SEM) for a sample size of 6. Statistical analysis was conducted using a one-way analysis of variance (ANOVA), followed by the Tukey's test for multiple comparisons. Significance levels are indicated as follows: *$p < 0.05$: Indicates statistical significance at the 0.05 level, suggesting that the compared groups are significantly different from the control group. **$p < 0.001$: Indicates high statistical significance at the 0.001 level, suggesting that the compared groups are significantly different from the control group with a higher level of confidence.

Table 8
The influence of HSAE and HSAE + F on urinary electrolyte concentrations at the 5-h interval. The parameters under study include Sodium (Na+), Potassium (K+), Chloride (Cl-), Saluretic index, Na+/K+ ratio, and Cl—/Na++ K+ ratio.

Group	Na+(mmol/L)	K+ (mmol/L)	Cl- (mmol/L)	Saluretic index			Na^+/K^+	$Cl^-/Na^+ + K^+$
				Na+	K+	Cl-		
Control	46.5 ± 3	34.3 ± 2	77.5 ± 1.4	1	1	1	1.355	0.95
Furosemide, 10 mg/kg	115 ± 1.8**	49.3 ± 0.8*	170.5 ± 1*	2.5	1.43	2.2	2.3	1.03
HSAE, 250 mg/kg	51.8 ± 1.6	30.1 ± 2.1	87 ± 1.3	1.1	0.87	1.12	1.72	1.06
HSAE, 500 mg/kg	61.8 ± 3.7*	58 ± 5.6	117.8 ± 1**	1.3	1.6	1.52	1.06	0.98
HSAE, 1000 mg/kg	67.5 ± 2.8*	34 ± 2.5	123.1 ± 2.1**	1.4	0.99	1.58	1.58	1.21
HSME, 250 mg/kg	75.05 ± 2*	43 ± 1	130.5 ± 2**	1.6	0.87	1.6	1.7	1.105

The data presented in the table represent the mean values along with the standard error of the mean (SEM) for a sample size of 6. Statistical analysis was conducted using a one-way analysis of variance (ANOVA), followed by the Tukey's test for multiple comparisons. Significance levels are indicated as follows: *$p < 0.05$: Indicates statistical significance at the 0.05 level, suggesting that the compared groups are significantly different from the control group. **$p < 0.001$: Indicates high statistical significance at the 0.001 level, suggesting that the compared groups are significantly different from the control group with a higher level of confidence.

diuretic activity. The urinary electrolyte content is outlined in Tables 6–8. Furosemide once again showcased the highest urinary electrolyte content, with the control group exhibiting the lowest. Intriguingly, HSME + F at 1000 mg/kg exhibited a sodium (Na) output of 109 ± 2.3, closely resembling the Na output of furosemide at 115 ± 1.8. Additionally, HSME + F at 500 mg/kg displayed a Na output of 95.1 ± 3.2, somewhat comparable to HSME at 1000 mg/kg (91.6 ± 2.23). This experiment revealed that when combined with furosemide, both HSME and HSAE exhibited the highest Na output.

Notably, HSME at dosages of 500 and 1000 mg/kg demonstrated a higher potassium (K) output of 49.6 ± 2.3 and 50.5 ± 2.5, respectively, compared to furosemide, which had a K output of 49.3 ± 0.8. HSME 500 mg/kg exhibited slightly higher chloride (Cl) output (162 ± 3.2) than HSME 1000 mg/kg + F (158.6 ± 3.3). HSAE 1000 mg/kg + F, 10 mg/kg displayed a high Na output of 75.6 ± 4.4. HSAE 500 mg/kg + F exhibited a K output similar to furosemide at 48.1 ± 5.2, while the K output of HSAE 1000 mg/kg + F was higher than furosemide at 50 ± 1.5, akin to the K output of HSME at 500 mg/kg. Cl output of HSAE alone did not significantly differ from the control group. However, when combined with furosemide, the Cl output significantly exceeded that of the control group.

Table 9 presents the result of biochemical analysis of serum from the treated animals. Biochemical analysis of serum indicated that the administered extracts, across various doses, did not lead to significant increases in tested parameters when compared to the control group. Nevertheless, doses of HSME 250 mg/kg + F and HSAE 250 mg/kg + F, 10 mg/kg, exhibited a minor increase in urea concentration within the normal range. Furthermore, HSAE 500 mg/kg + F, 10 mg/kg, resulted in

Table 9
Results of biochemical analyses conducted on serum samples, revealing the impact of different treatments on various parameters. The parameters assessed include Serum Glutamic Oxaloacetic Transaminase (SGOT/U/L), Serum Glutamic Pyruvic Transaminase (SGPT/U/L), Urea (mg/dl), Blood Urea Nitrogen (BUN, mg/dl), and Creatinine (g/L).

Group	SGOT (U/L)	SGPT (U/L)	UREA (mg/dl)	BUN (mg/dl)	CREATININE (g/L)
Control	44 ± 0.7	28.5 ± 2	36.6 ± 1.5	17.8 ± 1.5	0.85 ± 0.4
Furosemide, 10 mg/kg	28.5 ± 0.4	10* ± 0.9	13.34 ± 1	11.1 ± 0.5	0.6 ± 0.2
HSME, 250 mg/kg	32.61 ± 1	7.8** ± 2.6	22.8 ± 1	11.5 ± 1	0.66 ± 0.37
HSME, 500 mg/kg	32.6 ± 1.5	6.1** ± 0.8	25.7 ± 2	12.1 ± 0.15	0.37 ± 0.4
HSME, 1000 mg/kg	27.4 ± 0.9	8.6** ± 0.2	11.09 ± 0.6	6 ± 0.7	0.762 ± 0.1
HSME 250 mg/kg + F, 10 mg/kg	33.9 ± 1.2	7.1** ± 2.3	38.6 ± 3	14 ± 0.29	0.261 ± 0.52
HSME 500 mg/kg + F, 10 mg/kg	34.4 ± 2	10* ± 2.8	29.7 ± 1.5	16.83* ± 0.8	0.5 ± 0.1
HSME 1000 mg/kg + F, 10 mg/kg	31.65 ± 2.3	14** ± 0.7	6.7 ± 1.2	3.22 ± 0.28	0.45** ± 0.2
HSAE, 250 mg/kg	24.8* ± 1.6	25.02 ± 1	25 ± 0.14	3.2 ± 0.7	0.7 ± 1
HSAE, 500 mg/kg	29.1 ± 0.8	25.96 ± 2.4	12.1 ± 0.11	4 ± 1	0.5 ± 1
HSAE, 1000 mg/kg	29.6 ± 2	8.1** ± 0.9	11.13 ± 1.8	4.55 ± 0.3	0.4 ± 0.5
HSAE 250 mg/kg + F, 10 mg/kg	24.7* ± 0.5	13.5* ± 0.7	36 ± 1.5	5.43 ± 0.29	0.28* ± 1.9
HSAE 500 mg/kg + F, 10 mg/kg	29.2 ± 1.8	14* ± 6	12.6 ± 1.3	5.91 ± 0.6	0.86 ± 0.9
HSAE 1000 mg/kg + F, 10 mg/kg	19.06** ± 2	6.6** ± 2	23 ± 1.2	12.7 ± 0.13	0.52 ± 1.2

The data presented in the table represent the mean values along with the standard error of the mean (SEM) for a sample size of 6. Statistical analysis was conducted using a one-way analysis of variance (ANOVA), followed by the Tukey's test for multiple comparisons. Significance levels are indicated as follows: *p < 0.05: Indicates statistical significance at the 0.05 level, suggesting that the compared groups are significantly different from the control group. **p < 0.001: Indicates high statistical significance at the 0.001 level, suggesting that the compared groups are significantly different from the control group with a higher level of confidence.

a slight elevation in creatinine levels, though still within the normal range.

In conclusion, the findings suggest that HSME and HSAE exhibit dose-dependent diuretic effects, with HSME demonstrating notable diuretic action and diuretic activity across various dosages. The combined administration of extracts with furosemide enhances diuretic activity. Moreover, HSME and HSME + F showcased greater urinary output than HSAE and HSAE + F. The experiment also highlighted the distinctive effects of these extracts on urinary electrolyte content and indicated no significant adverse impact on serum biochemical parameters, reinforcing their potential as diuretic agents within safe levels of administration.

3.4. Effect on histopathology and glomerular filtration rate

Histological photomicrographs of kidney from both treated and control animals are presented in Fig. 5. The histopathological analysis of kidney tissue indicated a consistent preservation of normal histoarchitecture across all treatment groups, even in the group administered the highest dosage. The absence of any discernible signs of toxicity in the

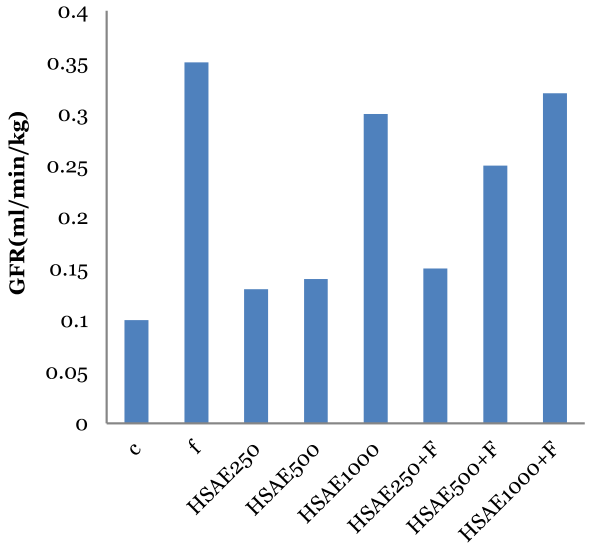

Fig. 3. Effect of HSAE on glomerular filtration rate (ml/min).

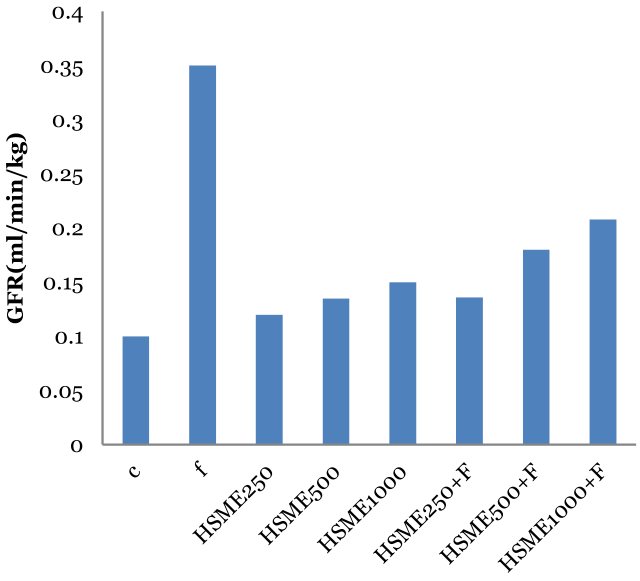

Fig. 4. Effect of HSME on glomerular filtration rate(ml/min).

treated groups, when compared to the control group, underlined the safety profile of the administered substances. To measure renal function, the Glomerular Filtration Rate (GFR) was quantified through the calculation of creatinine clearance. A comparison of the GFR of the treated animals at different doses with the control and standard group is shown in Figs. 3 and 4. The findings revealed notable shifts in GFR dynamics. Both HSME and HSAE demonstrated a remarkable increase in GFR in comparison to the control group, suggesting their potential positive influence on renal filtration processes. Remarkably, furosemide, a well-known diuretic, exhibited the highest GFR value within the tested groups, signifying its robust impact on renal filtration. Following furosemide, HSAE administered at a dosage of 1000 mg/kg emerged as the second-highest GFR enhancer, indicating its potential in promoting efficient kidney function. These findings collectively underscore the potential renal benefits of HSME and HSAE, further solidifying their

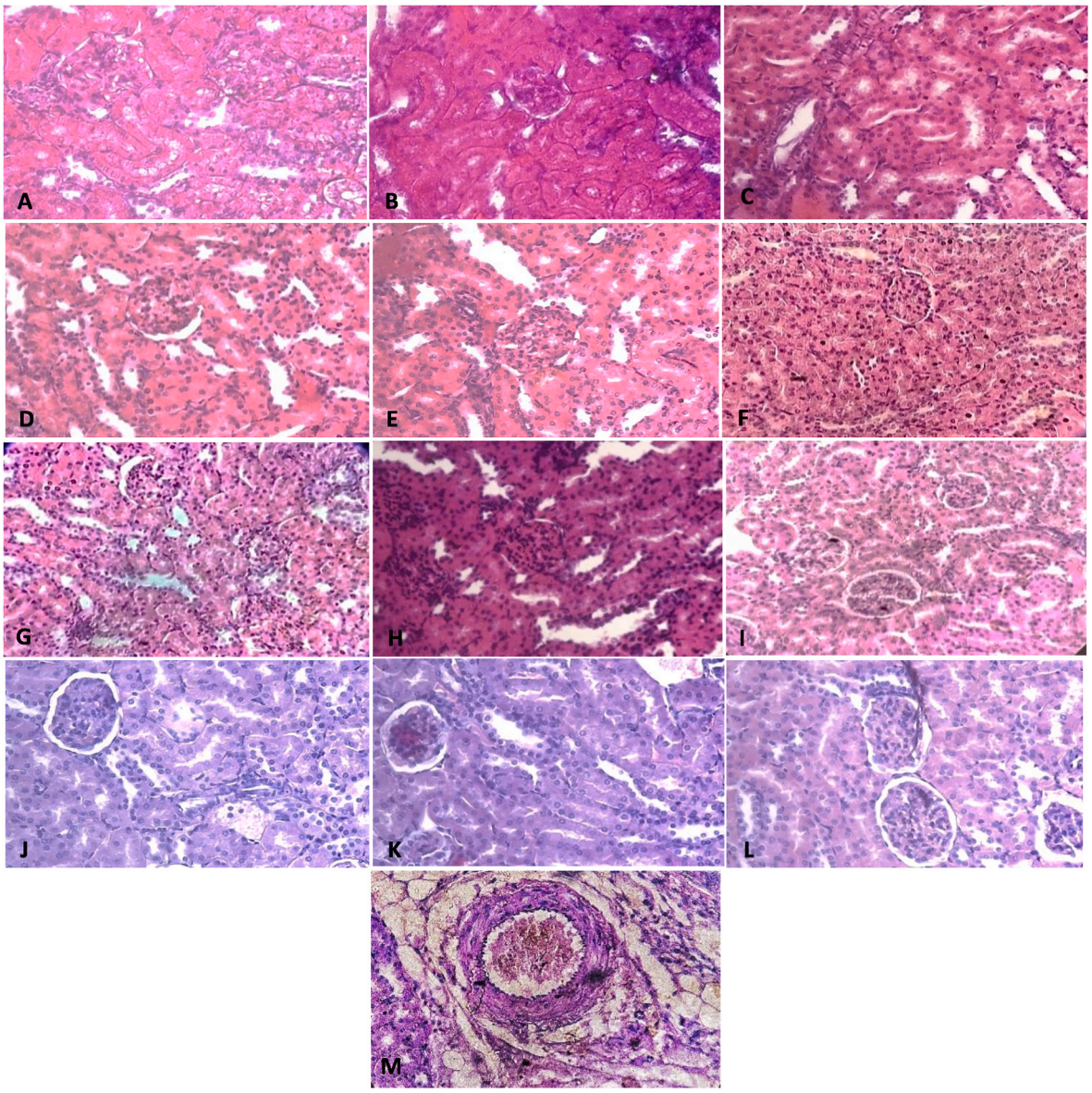

Fig. 5. Photomicrographs depicting the histology of kidney tissues across various groups, including both the control and experimental groups treated with HSAE and HSME. In (A), the control group showcases the normal histological architecture of the kidney. Moving on to the experimental groups, (B) through (G) correspond to the HSME and HSAE treated groups at doses of 250 mg/kg, 500 mg/kg, and 1000 mg/kg, respectively. Similarly, (H) through (M) correspond to the HSME + F and HSAE + F treated groups at the same dosage levels. No observable alterations are evident in the histological features of the kidney tissues in these experimental groups, reaffirming the preservation of normal kidney structure throughout the study.

promising role in promoting renal health.

4. Discussion

Diuretics play a pivotal role in the management of fluid volume and composition, particularly in conditions like hypertension. This study delved into the diuretic properties of Hedyotis scandens, and the results align with its traditional application as a diuretic in Mizoram. These findings are consistent with studies exploring diuretic effects in other plant species like Herniaria glabra L, Retama raetam (Forssk.) Webb & Berthel., and Ficus glumosa Delile (Hakim et al., 2021; Maghrani et al., 2005; Ntchapda et al., 2016), further emphasizing the potential of plant-based diuretics.

Importantly, the diuretic action of HSME and HSAE was pronounced, with HSME demonstrating a rapid surge in urine output within 5 h, while HSAE exhibited its peak activity within 24 h. This temporal distinction aligns with traditional knowledge of diuretic plants, where the onset and duration of diuretic effects are often modulated by the plant's constituents and mechanisms of action. To better understand the extent of diuretic activity, the diuretic action value was employed as a metric. Values above 1.50 indicate a robust diuretic effect, while those between 1.00 and 1.50 suggest moderate activity. Diuretic actions in the range of 0.72–1.00 imply mild effects, and values below 0.72 denote no diuretic action (Asif et al., 2014). In the current study, both HSME and HSAE consistently exhibited diuretic action values surpassing 1.50, indicating potent diuretic effects. Notably, HSAE250 mg/kg at 5 h demonstrated a diuretic action falling within the moderate range, providing insight into the nuanced dynamics of diuretic activities.

When HSME and HSAE were combined with furosemide, an established diuretic agent, synergistic effects were observed. This combination led to heightened diuresis, elevated diuretic action, and enhanced diuretic activity compared to the administration of extracts alone. This intriguing interaction underscores the potential of *H. scandens* extracts to complement established diuretic therapies, potentially offering more

effective management of fluid balance in clinical contexts. Saluretic activity, characterized by the excretion of sodium and chloride ions, exhibited interesting patterns. HSME, particularly at doses of 500 mg/kg and 1000 mg/kg, along with HSME500+F and HME1000+F, induced significant increases in sodium and chloride excretion. Similarly, HSAE displayed dose-dependent increments in sodium and chloride excretion. Although the saluretic effect of HSAE was comparatively milder than HSME, it still attested to the plant's diuretic potential.

Remarkably, the diuretic influence of *H. scandens* extracts was observed to be of the saluretic type, distinct from the aquaretic effect seen in many phytodiuretics. This unique mode of action could offer advantages in addressing specific clinical scenarios that demand alterations in electrolyte balance and sodium excretion. Furthermore, the extract's impact on both water and electrolyte outputs paralleled that of furosemide, renowned for its saluretic and diuretic properties (Ratnasooriya et al., 2004). The noteworthy increase in the sodium-to-potassium ion excretion ratio underscored the extract's potential to facilitate sodium elimination, a valuable trait in avoiding hyperkalemia, a common electrolyte imbalance associated with conventional diuretics (Horisberger and Giebisch, 1987; Sarafidis et al., 2010).

Significantly, the investigation into various biochemical parameters yielded encouraging results. Changes in the biochemical ratios can result in the disruption of normal working of the organs.(Afolayan and Yakubu, 2009). Oral administration of the plant extracts up to 5000 mg/kg demonstrated no adverse effects on liver and kidney function in the studied rats. This suggests that *H. scandens* extract holds promise as a safe and well-tolerated option for diuretic therapy. Notably, the presence of saponins in *H. scandens*, as identified in previous studies (Amuthan et al., 2012; Martín-Herrera et al., 2007), could potentially contribute to its diuretic effects. Moreover, the plant's antioxidant properties, supported by the GC-MS analysis, resonate with findings in other diuretic plants, implying a plausible link between antioxidant content and diuretic effects across diverse plant species (Ntchapda et al., 2016). However, in-depth exploration is essential to unravel the intricacies of this relationship.

The presence of bioactive compounds, such as phytol, as revealed by GC-MS analysis, provides further support for the diuretic potential of *H. scandens*. Bioactive compounds present in the plant could contribute synergistically to the observed diuretic effects, warranting continued research to unravel their exact mechanisms of action. Collectively, the findings support the multifaceted potential of *H. scandens* as a valuable diuretic agent, inviting further exploration into its therapeutic applications and mechanisms of action.

5. Conclusion

In conclusion, this study highlights the diuretic potential of *Hedyotis scandens* in line with its traditional use for diuretic purposes in Mizoram. HSME exhibited rapid diuresis within 5 h, while HSAE displayed peak activity at 24 h. Both extracts demonstrated strong diuretic effects, except for HSAE 250 mg/kg at 5 h, which showed moderate action. Combining the extracts with furosemide amplified their diuretic impact, offering potential synergy in fluid management. Saluretic activity was notable, particularly in HSME at 500 mg/kg and 1000 mg/kg, and HSME500+F and HME1000+F. Biochemical analyses affirmed the extracts' safety for oral consumption up to 5000 mg/kg. The presence of saponins and antioxidants in the extracts supports their diuretic effects. This study underscores *Hedyotis scandens* as a promising natural diuretic candidate, urging further exploration of its mechanisms and clinical applications for fluid balance management.

CRediT authorship contribution statement

Elizabeth Vanlalruati Ngamlai: designed the experiment, wrote the manuscript. **R.B. Pradhan:** assisted with the experiment. **P.C. Lalbiaknii:** assisted with the experiment. **Vanlalhruaii Ralte:** supervised the work. **F. Lalnunmawia:** supervised the work, The final manuscript was read and approved by all of the authors. **P.C. Vanlalhluna:** helped with plant collection. **S.K. Mehta:** provided lab equipment, chemicals and edited manuscript.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

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