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ORIGINAL PAPER

Diuretic efficacy and phytochemical profiling of the 80% methanol extract of *Sida schimperiana* Hochst. ex A. Rich

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ABSTRACT

Introduction and aim. Diuretics are used for edema, heart failure, and hypertension, but may cause side effects and resistance. *Sida schimperiana* is used for urinary retention in Ethiopian folk medicine, although its diuretic effects are unverified. This study aims to evaluate the diuretic potential and phytochemical content of the 80% methanol extract of *S. schimperiana*.

Material and methods. Male rats were administered distilled water (2 mL/100 g), furosemide (10 mg/kg), or 80% methanol extract (100, 200, or 400 mg/kg). Diuretic effects were assessed by monitoring urination onset, urine volume, electrolyte levels, and pH. Phytochemical analysis was performed to explore underlying diuretic mechanisms.

Results. The 80% methanol extract demonstrated significant diuretic and natriuretic effects in a dose- and time-dependent manner, surpassing the negative control. At doses of 200 and 400 mg/kg, diuresis ($p<0.001$) and sodium and chloride excretion ($p<0.001$) were notably increased at all time points. The treated group also showed significant changes in urine pH. Phytochemical analysis identified alkaloids, flavonoids, polyphenols, and tannins, with high concentrations of flavonoids (187.3 mg quercetin equivalent/g) and polyphenols (128.4 mg quercetin equivalent/g).

Conclusion. This study affirmed the methanolic extract's diuretic potency, comparable to furosemide, emphasizing its prospective therapeutic value.

Keywords. diuretic activity, in vivo, phytochemicals, rats, *Sida schimperiana*

Introduction

Diuretics are drugs commonly prescribed for managing conditions such as edema, heart failure, and hypertension, they facilitate the excretion of sodium and water, thus mitigating excess extracellular fluid accumulation.[1-4]

Despite the significant therapeutic benefits offered by conventional agents, their use is frequently accompanied by a wide range of adverse effects, including electrolyte disturbances, ototoxicity, and renal dysfunction.[5] Among these, the most prominent issues are fluid and electrolyte balance disruptions, particularly hypokale-

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mia and volume depletion, which can severely compromise normal physiological functions. Such imbalances can give rise to serious complications, including hyperglycemia, cardiac arrhythmias, hyperlipidemia, and other potentially life-threatening conditions that may exacerbate underlying health problems.[5,6]

The most prominent are fluid and electrolyte balance perturbations, specifically hypokalemia and volume depletion, which can critically undermine physiological processes. These imbalances may lead to severe complications, such as hyperglycemia, cardiac arrhythmias, hyperlipidemia, and other potentially life-threatening conditions.[6] Additionally, diuretic therapy may induce metabolic disturbances, including acidosis or alkalosis, and escalate the overall cost of treatment.[7]

These limitations have prompted an increasing interest in natural products as alternative or complementary sources of diuretic agents, particularly those with fewer side effects and additional health benefits. Owing to the ease of access and affordability of herbal remedies, many people rely on them instead of modern medications, which are often expensive and difficult to obtain.[8]

Among these, *Sida schimperiana* Hochst. ex A. Rich. *(S. schimperiana)*, also known as "Chifrig" in Amharic in the genus Sida, belongs to the family Malvaceae and is commonly used as a diuretic for the treatment of urinary retention.[9-11] It is a perennial subshrub with woody, erect, or procumbent stems that can grow up to a height of about 90 centimeters and grows primarily in the seasonally dry tropical biome.[12,13]

Geographically, *S. schimperiana* is native to eastern Africa, specifically found in Eritrea, Ethiopia, Kenya, Tanzania, and Uganda. Additionally, it has been introduced to India.[14]

In Ethiopian traditional medicine, the leaves and stems of *S. schimperiana* are used orally for the treatment of gonorrhea, and urinary retention.[9] The roots treat abdominal pain and evil eye disease.[10] *S. schimperiana* has several pharmacologically proven activities. Its root extract has antifungal,[15] antibacterial, and immunomodulatory activities.[16] The crude root extract's phytochemical analysis has revealed a diverse array of bioactive compounds, including polyphenols, flavonoids, coumarins, and phytosteroids.[15]

Aim

The aim was to evaluate the diuretic potential and phytochemical contents of the 80% methanol extract of *S. schimperiana*.

Material and methods

Drugs, chemicals, and reagents

Distilled water and normal saline were obtained from EPHARM (Ethiopia). Acetic anhydride, atropine, chloroform, Folin-Ciocalteu reagent, gallic acid, glacial acetic acid, magnesium ribbon, methanol, phosphate buffer solution, quercetin, and tannic acid were purchased from Sigma-Aldrich (Germany). Aluminum chloride, ammonium hydroxide, bismuth nitrate, bromocresol green, dimethyl sulfoxide, ferric chloride, hydrochloric acid, iodine, isopropyl alcohol, lead acetate, mercuric chloride, potassium hydroxide, potassium iodide, sodium carbonate, sodium hydroxide, sodium nitrate, and sulfuric acid were purchased from Fisher Scientific (UK). Furosemide was obtained from Denk Pharma GmbH and Co. KG (Germany) and sodium pentobarbitone was purchased from Taj Pharmaceuticals Ltd. (India). All drugs, chemicals, and reagents used were of analytical grade.

Plant material collection and extraction

In September 2023, the stems of *S. schimperiana* were collected from the region surrounding Debre Markos. A taxonomist authenticated the botanical specimen and a voucher specimen (BGM04) was duly archived. Freshly collected stems were meticulously rinsed with tap water and subsequently air-dried in a shaded environment at ambient temperature. After that, the desiccated stems were ground into coarse powder. A 150-gram of the coarse powder was subjected to maceration in 1.5 liters of 80% methanol within a conical flask, and the mixture was agitated at 120 rpm for 72 hours at ambient temperature. The resultant extract underwent a sequential filtration process, initially utilizing muslin cloth, followed by filtration through Whatman® grade 1 filter paper. The residual marc was then subjected to two subsequent rounds of re-extraction, employing fresh solvent in identical volumes to those used in the initial extraction, as outlined previously. The fluid extract was concentrated using a rotary evaporator (Buchi, Rotavapor R-210/215, Switzerland) under reduced pressure at 40°C, and then dried in a lyophilizer (OPR-FDU-5012, Korea). The dried extract was 12.4% (w/w). It was subsequently stored in a deep freezer at -20°C until use.

Experimental animals

Sprague-Dawley rats (males for the main study and females for the acute toxicity study), aged 6–10 weeks and weighing 280–320 g, were used in the experiment. The rats were housed in polypropylene cages (6 rats per cage) under standard environmental conditions (25±2°C, 55±5% humidity, and a 12 h/12 h light/dark cycle). They had free access to tap water and laboratory pellets and were acclimatized to the laboratory conditions for one week before the experiment. Each rat was placed in a metabolic cage (Tecniplast, Italy) 24 hours before the experiment.

Acute oral toxicity test

An acute oral toxicity test for the 80% methanol extract of *S. schimperiana* was conducted following the guide-

lines of the Organization for Economic Co-operation and Development.[16] Rats were fasted overnight before and 4 hours after administration of the extract. Initially, a sighting study was performed to determine the starting dose, with one rat receiving 2000 mg/kg 80% methanol extract as a single dose via oral gavage. As no death was observed within a day, an additional four rats were administered the same dose. The rats were monitored continuously for 4 hours at 30-minute intervals and subsequently observed daily for 14 days to assess the general signs and symptoms of toxicity (diarrhea, weight loss, tremor, lethargy, and paralysis), food and water intake, and mortality. Three dose levels were selected for further testing: a middle dose, which was one-tenth of the maximum dose used in the acute toxicity study; a low dose, which was half of the middle dose; and a high dose, which was twice the middle dose.[17]

Experimental design

Rats were randomly assigned to five groups (two control and three test groups), comprising six animals per group. Negative controls were treated with the vehicle used for reconstitution (2 mL/100 g distilled water), positive controls were treated with the standard drug furosemide 10 mg/kg (F10), and the three test groups were treated with 100, 200, and 400 mg/kg doses of the 80% methanol extract of *S. schimperiana* as SSME100, SSME200, and SSME400, respectively. Each group received the assigned treatment via oral gavage. Doses were determined using data from an acute oral toxicity study. At the end of the experiment, all rats were euthanized by an intraperitoneal injection of sodium pentobarbital at a dose of 150 mg/kg.

Determination of diuretic activity

The diuretic activity was determined using the method described by Hailu and Engidawork.[18] Rats were fasted overnight with free access to water, and pretreated with normal saline at an oral dose of 15 mL/kg to impose uniform water and salt load. Rats were divided into groups and treated as described in the Experimental Design section. Immediately after the assigned treatment, the animals were placed in metabolic cages (a rat in a cage). Urine was collected and measured at the time of first urination, and 1, 2, 3, 4, and 5 h after dosing, and stored at –20°C for electrolyte analysis. No food or water was available at the time of the urine collection.

The following parameters were recorded for each rat: first urinary latency (time to the first urination); urine volume; urine concentration of Na^+, K^+, and Cl^-; and urine pH. To compare the effects of three different doses of the 80% methanol extract with each other and with the controls (positive and negative), parameters such as percent urinary excretion, diuretic action, and diuretic activity were also calculated using the following formulas:

$$Percent\ \ urinary\ \ excretion = \frac{Total\ \ urinary\ \ output}{Total\ \ liquid\ \ administered} \times 100,$$

$$diuretic\ \ action = \frac{Mean\ \ urinary\ \ excretion\ \ of\ \ treatment\ \ groups}{Mean\ \ urinary\ \ excretion\ \ of\ \ control\ \ group},$$

$$and\ \ diuretic\ \ activity = \frac{Diuretic\ \ action\ \ of\ \ test\ \ sample}{Diuretic\ \ action\ \ of\ \ standard\ \ drug},$$ [18]

Urinary electrolyte level determination

The 80% methanol extract of *S. schimperiana* and rat urine were analyzed for the levels of three electrolytes (Na^+, K^+, and Cl^-). The Na^+ and K^+ concentrations were determined using a high-precision flame photometer (FP8400 Flame Photometer, A. KRUSS OPTRONIC GmbH, Germany), and the Cl^- was determined using an ion-selective electrode analyzer (AVL 9181 Electrolyte Analyzer, Roche, Germany). Electrolyte ratios, including test/control, Na^+/K^+, and $Cl^-/[K^+ + Na^+]$, were calculated to assess saluretic, natriuretic, and carbonic anhydrase inhibitory activities, respectively. The urine pH was measured using a pH meter.[19]

Qualitative phytochemical screening

Qualitative phytochemical analysis of the 80% methanol extract of *S. schimperiana* was conducted to identify bioactive secondary metabolites with potential medicinal properties. The investigation targeted compounds such as alkaloids, cardiac glycosides, flavonoids, polyphenols, saponins, steroids, tannins, and terpenoids, employing established methods for detection.[20-24]

Alkaloid detection test

About 10 mg of the 80% methanol extract was dissolved in 2 mL of 5% hydrochloric acid and subsequently filtered. The resulting filtrate was divided into separate test tubes, with each aliquot treated with a few drops of one of the following reagents: Bouchardat, Dragendorff, Mayer, or Wagner. The formation of distinct precipitates; brown (Bouchardat), red-orange (Dragendorff), yellowish-white (Mayer), and red-brown (Wagner), provides conclusive evidence for the presence of alkaloids.

Anthraquinone glycoside detection test

Borntrager's assay: To 3 mL of the 80% methanol extract, 3 mL of chloroform was added, and the chloroform layer was meticulously separated. Subsequently, a 5% potassium hydroxide solution was introduced to the mixture. The emergence of a red coloration serves as a definitive indication of the presence of anthraquinone glycosides.

Ammonium hydroxide assay: A solution comprising 10 mg of the 80% methanol extract was meticulously dissolved in 10 mL of isopropyl alcohol. To this resultant mixture, a single drop of concentrated ammonium hydroxide was introduced. Following a two-minute incubation peri-

od, the emergence of a pink-red hue serves as an indicative marker for the presence of anthraquinone glycosides.

Cardiac glycoside detection test (Keller-Kiliani assay): An aliquot of 0.25 g of the 80% methanol extract was dissolved in distilled water. To 5 mL of the resulting 80% methanol extract solution, 2 mL of glacial acetic acid, fortified with a single drop of ferric chloride solution, was carefully added. The mixture was then underplayed with 1 mL of concentrated sulfuric acid. The development of a distinct brown ring at the interface of the two layers serves as a definitive indication of the presence of cardiac glycosides.

Flavonoid detection test

Alkaline reagent assay: A volume of 1 mL of the 80% methanol extract was subjected to treatment with a few drops of a 20% sodium hydroxide solution. The development of a vivid yellow coloration, which subsequently decolorizes upon the introduction of hydrochloric acid, serves as a qualitative indicator of the presence of flavonoids.

Shinoda assay: To 5 mL of the 80% methanol extract, fragments of magnesium ribbon were introduced, followed by the addition of concentrated hydrochloric acid. The emergence of a red-to-pink hue after a brief interval signifies the presence of flavonoids.

Polyphenol detection test

Lead acetate assay: A few drops of a 10% lead acetate solution were introduced into the 80% methanol extract. The formation of a white precipitate serves as a definitive indication of the presence of phenolic compounds.

Ferric chloride assay: To 2 mL of the filtered 80% methanol extract, four drops of a 10% ferric chloride solution were added. The manifestation of a blue-green coloration signifies the presence of phenolic compounds.

Saponin detection test (Frothing assay)

To 0.25 g of the 80% methanol extract, 20 mL of distilled water was added, followed by vigorous agitation for 15 minutes. The development of a stable and persistent froth is indicative of the presence of saponins.

Steroid detection test (Libermann-Buchard assay)

A volume of 1 mL of the 80% methanol extract was combined with 10 mL of chloroform and subsequently filtered. The filtrate was treated with a few drops of acetic anhydride, subjected to boiling, and then allowed to cool. Concentrated sulfuric acid was cautiously added to the mixture. The emergence of a distinct brown ring at the phase interface unequivocally confirms the presence of steroids.

Tannin detection test (Ferric chloride assay)

A quantity of 10 mg of the 80% methanol extract was dissolved in 2 mL of distilled water and subsequently filtered. To the resultant filtrate, a few drops of a 10% ferric chloride solution were introduced. The appearance of a blue or green coloration serves as a definitive indicator of the presence of tannins.

Terpenoid detection test (Salkowski assay)

To 10 mg of the 80% methanol extract, 2 mL of chloroform was introduced. To this mixture, 3 mL of concentrated sulfuric acid was cautiously added. The development of a reddish-brown coloration at the interface serves as a definitive indicator of the presence of terpenoids.

Quantitative estimation of phytochemical constituents

Phytochemicals detected through qualitative screening such as alkaloids, flavonoids, polyphenols, and tannins were subsequently quantified using meticulously validated standard methods outlined below.

Total alkaloid content determination

The total alkaloid concentration in the 80% methanol extract of *S. schimperiana* was quantified using a spectrophotometric method, relying on the interaction between alkaloids and bromocresol green (BCG).[25] One milligram of the plant extract was dissolved in dimethyl sulfoxide (DMSO). To this solution, 1 mL of 2N hydrochloric acid was added, and the resulting mixture was filtered. The pH of the filtrate was adjusted to neutral using 0.1 N sodium hydroxide. The solution was then transferred into a separatory funnel, and 5 mL of BCG solution, along with 5 mL of phosphate buffer (pH 4.7), was introduced. The mixture was vigorously shaken with chloroform in volumes of 1, 2, 3, and 4 mL, then diluted to a total volume of 10 mL with additional chloroform. Similarly, standard solutions of atropine (12.5, 25, 50, 100, 200, and 400 µg/mL) were prepared by combining accurately measured atropine aliquots with 5 mL of BCG solution and 5 mL of phosphate buffer (pH 4.7), followed by shaking with chloroform in volumes of 1, 2, 3, and 4 mL. These solutions were collected in a 10 mL volumetric flask and diluted with chloroform to a final volume of 10 mL. The absorbance of both the atropine standards and the test solutions was measured against a reagent blank at 470 nm using a UV-visible spectrophotometer (SHIMADZU UV 1800, Kyoto, Japan). To ensure accuracy, all procedures were conducted on the same day and repeated in triplicate. The total alkaloid content was expressed as milligrams of atropine equivalents per gram of dry plant extract (AE/g).

Total flavonoid content determination

The total flavonoid content in the 80% methanol extract of *S. schimperiana* was assessed using the aluminum chloride colorimetric method.[26] A series of quercetin standards in methanol were prepared at con-

centrations of 12.5, 50, 100, 200, and 400 µg/mL. To each standard, 250 µL of the solution was combined with 125 µL of water and 75 µL of sodium nitrate solution. The mixtures were kept in the dark at 25°C for 6 minutes. Afterward, 150 µL of aluminum chloride was added, and the samples were left in the dark for 5 hours. Subsequently, 500 µL of sodium hydroxide and 275 µL of water were added to each solution. The absorbance was recorded at 510 nm using a UV-visible spectrophotometer, and a standard curve correlating concentration to absorbance was constructed. The test sample was treated in the same manner and analyzed. All experiments were performed in triplicate. The total flavonoid content was expressed as milligrams of quercetin equivalents per gram of dry plant extract (QE/g).

Total phenolic content determination

The total phenolic content of the 80% methanolic extract of *S. schimperiana* was quantified using the Folin-Ciocalteu reagent assay.[27,28] Serial dilutions of methanolic gallic acid solutions at concentrations of 12.5, 25, 50, 100, 200, and 400 µg/mL were prepared, and 50 µL of each solution was combined with 100 µL of 10% Folin-Ciocalteu reagent and 100 µL of 7% sodium carbonate. The reaction mixture was incubated at ambient temperature for 30 minutes, after which the absorbance was measured at 765 nm using a UV-visible spectrophotometer, with a reagent blank serving as the reference. A calibration curve was generated by plotting absorbance values against gallic acid concentrations. The test sample was prepared and analyzed following the same procedure. All measurements were conducted in triplicate on the same day. The total phenolic content was reported as milligrams of gallic acid equivalents per gram of dry plant extract (mg GAE/g).

Total tannin content determination

The total tannin content of the 80% methanolic extract of *S. schimperiana* was determined using the method described by Chandran and Indira.[29] To 0.1 mL of the sample extract, 0.5 mL of Folin-Ciocalteu reagent, and 1 mL of 35% sodium carbonate solution were added. The resulting mixture was diluted to a final volume of 10 mL with distilled water, thoroughly mixed, and allowed to stand at room temperature for 30 minutes. In parallel, a series of tannic acid reference standards at concentrations of 12.5, 25, 50, 100, 200, and 400 µg/mL were prepared and processed similarly. The absorbance of both the test samples and the standard solutions was recorded at 700 nm using a UV-visible spectrophotometer, with a reagent blank serving as the baseline reference. All analyses were performed in triplicate on the same day. The total tannin content was expressed as milligrams of tannic acid equivalents per gram of dry plant extract (mg TAE/g).

Statistical analysis

The study findings were expressed as mean±standard error of the mean (S.E.M). Statistical analysis was conducted using one-way analysis of variance (ANOVA), followed by Tukey's post-hoc multiple comparison test to discern specific group differences using SPSS version 25 (IBM, Armonk, NY, USA). Dose-dependent effects were examined using linear regression analysis. Statistical significance was determined using a threshold p-value of less than 0.05.

Ethical consideration

Animal handling was conducted following the guidelines set forth by the National Institutes of Health for the Care and Use of Laboratory Animals, as well as with the standards for international animal care and welfare.[30,31] The study received formal approval from the Research Ethics Committee of the Department of Pharmacy, College of Medicine and Health Sciences at Debre Markos University, as documented in the ethical approval letter with reference number 1921/23.

Results

Acute oral toxicity test

The acute oral toxicity test of the 80% methanol extract of *S. schimperiana* demonstrated that it did not induce gross behavioral changes or mortality either within 24 hours or over the course of 14 days. Consequently, the median lethal oral dose of the 80% methanol extract was estimated to exceed 2000 mg/kg in rats.

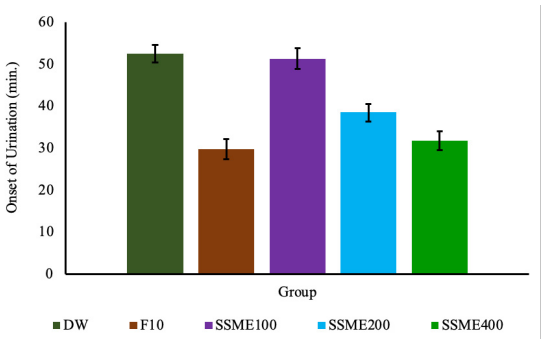

Fig. 1. Effect of 80% methanol extract on the onset of urination in rats (DW – distilled water; F10 – furosemide 10 mg/kg; SSME100 – *S. schimperiana* methanol extract 100 mg/kg; SSME200 – *S. schimperiana* methanol extract 200 mg/kg; SSME400 – *S. schimperiana* methanol extract 400 mg/kg)

Determination of diuretic activity

Effect on the onset of urination

The effect of the 80% methanol extract of *S. schimperiana* on the onset of urination is shown in Figure 1. Rats in the treatment group that received 100 mg/kg of the extract did not exhibit a significant difference in the onset of urination compared to the negative con-

trol. However, rats treated with 200 mg/kg and 400 mg/kg of the extract showed a significantly faster onset of urination, with times of 38.51±1.94 and 31.68±2.26 minutes, respectively ($p<0.001$), compared to the negative control (52.46±2.02 minutes).

Effect on urine volume

The effect of the 80% methanol extract of *S. schimperiana* on urine volume in rats is presented in Table 1. The extract-induced diuresis appeared to be dose- and time-dependent ($r^2=0.901$; $p<0.001$). Although rats treated with SSME100 showed a significant increase in urine output ($p<0.01$) after the fifth hour, no detectable change in urine volume was observed during the first four hours post-dosing, compared to the negative control. In contrast, rats treated with SSME200 and SSME400 demonstrated a significant increase in urine volume at all time points, with maximum diuresis of 111% ($p<0.001$) and 124% ($p<0.001$), respectively. Additionally, the diuretic effects of SSME200 and SSME400 were comparable to those of F10. Among the three doses, SSME200 and SSME400 produced significantly greater diuresis than SSME100 at all time points. Specifically, SSME200 and SSME400 exhibited urinary excretion rates of 103% and 110%, respectively, and diuretic actions of 2.11 and 2.24 (Table 1).

Effect on cumulative urine output

The cumulative urine output of rats administered the 80% methanol extract of *S. schimperiana* is illustrated in Figure 2. SSME100 showed no statistically significant change in cumulative urine output during the first 4 hours; however, it induced a notable increase ($p<0.01$) by the end of the fifth hour compared to the negative control. Both SSME200 and SSME400 demonstrated urine outputs comparable to that of F10. Furthermore, SSME400 resulted in a cumulative urine output that exceeded that of the standard at both the third and fourth hours (Fig. 2).

Table 1. Effect of 80% methanol extract of *S. schimperiana* on urine volume in rats*

Group	Volume of urine (mL)					% Urinary excretion	Diuretic action	Diuretic activity
	1 h	2 h	3 h	4 h	5 h			
DW	1.62 ±0.18	2.40 ±0.21	2.75 ±0.08	3.04 ±0.11	3.22 ±0.20	48	1.00	
F10	3.62 ±0.30 a###	4.72 ±0.23 a###	5.73 ±0.40 a###	6.82 ±0.29 a###	7.27 ±0.36 a###	112	2.26	1.00
SSME100	1.89 ±0.17 b###,c##,d###	2.46 ±0.19 b###,c###,d###	3.18 ±0.22 b###,c###,d###	3.46 ±0.20 b###,c###,d###	3.95 ±0.09 a##,b###, c###,d###	63	1.23	0.54
SSME200	2.86 ±0.26 a##	3.87 ±0.23 a###	5.32 ±0.29 a###	6.64 ±0.25 a###	6.80 ±0.10 a###	103	2.11	0.93
SSME400	3.08 ±0.24 a###	4.08 ±0.27 a###	6.14 ±0.42 a###	6.96 ±0.34 a###	7.21 ±0.28 a###	110	2.24	0.99

* each value represents the mean±S.E.M, n=6, [a] – against negative control (DW), [b] – against standard (F10), [c] – against 200 mg/kg, [d] – against 400 mg/kg, [#] – $p<0.05$, [##] – $p<0.01$, [###] – $p<0.001$, DW – distilled water, F10 – furosemide 10 mg/kg, SSME100 – *S. schimperiana* methanol extract 100 mg/kg, SSME200 – *S. schimperiana* methanol extract 200 mg/kg, SSME400 – *S. schimperiana* methanol extract 400 mg/kg

Effect on urinary electrolyte excretion

The electrolyte contents (Na^+, K^+, and Cl^-) in the collected urine samples were quantified and are presented in Table 2. SSME100 did not significantly increase the excretion of any of the three electrolytes compared to the negative

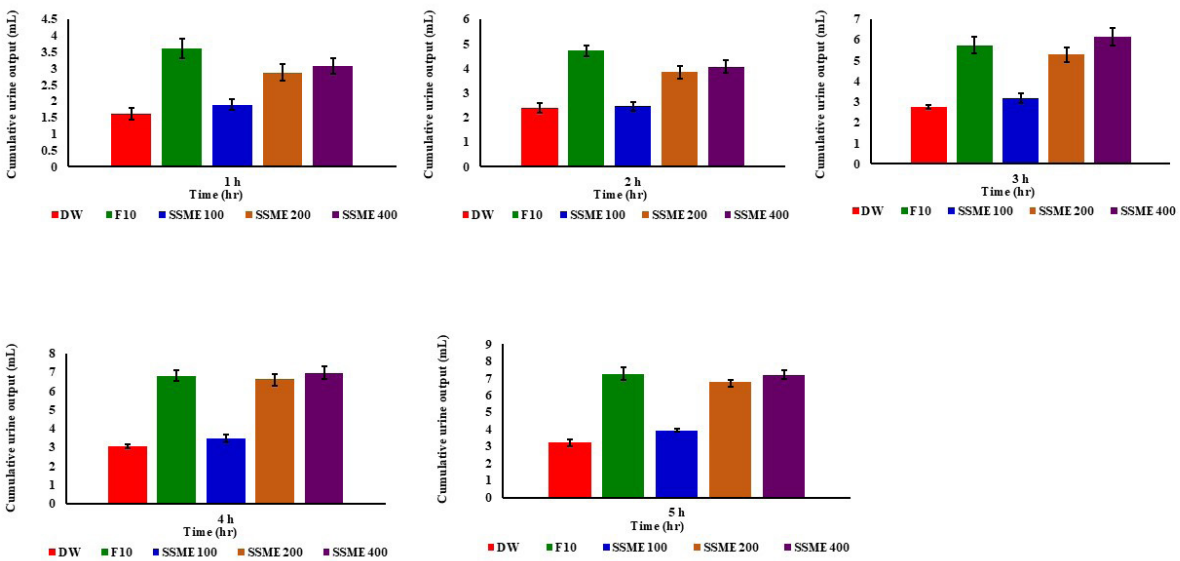

Fig. 2. Effect of 80% methanol extract on cumulative urine output in rats (DW – distilled water; F10 – furosemide 10 mg/kg; SSME100 – *S. schimperiana* methanol extract 100 mg/kg; SSME200 – *S. schimperiana* methanol extract 200 mg/kg; SSME400 – *S. schimperiana* methanol extract 400 mg/kg)

control. In contrast, SSME200 and SSME400 significantly enhanced the excretion of Na^+ and Cl^- ($p<0.001$), as well as K^+ ($p<0.01$), when compared to the negative control. Specifically, SSME200 increased Na^+ and Cl^- excretion by 131% and 44%, respectively, while SSME400 increased Na^+ and Cl^- excretion by 184% and 52%, respectively, relative to the negative control (Table 2). No significant differences were observed in Na^+ and Cl^- excretion between F10 and the two doses of the 80% methanol extract. However, F10 significantly outperformed both SSME200 and SSME400 in K^+ excretion, with a 209.2% increase ($p<0.001$) compared to the negative control, which was significantly greater than the K^+ excretion induced by SSME200 (63%, $p<0.01$) and SSME400 (85%, $p<0.01$).

Table 2. Effect of 80% methanol extract of *S. schimperiana* on urinary electrolyte excretion in rats*

Group	Urinary electrolyte concentration (mmol/L)			Saluretic index			Na^+/K^+	Na^+/Cl^-	$Cl^-/(Na^+ + K^+)$
	Na^+	K^+	Cl^-	Na^+	K^+	Cl^-			
DW	53.81 ± 3.74	38.56 ± 2.89	76.87 ± 4.08	1.00	1.00	1.00	1.39	0.70	0.83
F10	156.34 ± 4.38 $^{a\#\#}$	119.21 ± 4.03 $^{a\#\#}$	138.07 ± 5.42 $^{a\#\#}$	2.91	3.09	1.79	1.31	1.13	0.50
SSME100	68.88 ± 2.78 $^{b\#\#\#,c\#\#\#,d\#\#\#}$	41.64 ± 2.08 $^{b\#\#\#,c\#\#\#,d\#\#\#}$	89.94 ± 3.16 $^{b\#\#\#,d\#\#\#}$	1.28	1.08	1.17	1.65	0.77	0.81
SSME200	124.30 ± 4.93 $^{a\#\#\#}$	62.85 ± 3.75 $^{a\#\#,b\#\#}$	110.69 ± 4.31 $^{a\#\#\#}$	2.31	1.63	1.44	1.98	1.12	0.59
SSME400	152.68 ± 3.66 $^{a\#\#\#}$	71.35 ± 3.24 $^{a\#\#,b\#\#}$	116.49 ± 5.86 $^{a\#\#\#}$	2.84	1.85	1.52	2.14	1.31	0.52

* each value represents the mean±S.E.M, n=6, ^{a} – against negative control (DW), ^{b} – against standard (F10), ^{c} – against 200 mg/kg, ^{d} – against 400 mg/kg, $^\#$ – $p<0.05$, $^{\#\#}$ – $p<0.01$, $^{\#\#\#}$ – $p<0.001$, DW – distilled water, F10 – furosemide 10 mg/kg, SSME100 – *S. schimperiana* methanol extract 100 mg/kg, SSME200 – *S. schimperiana* methanol extract 200 mg/kg, SSME400 – *S. schimperiana* methanol extract 400 mg/kg

The indices for saluretic, natriuretic (aldosterone secretory), thiazide secretory, and carbonic anhydrase inhibitory activities of the 80% methanol extract of *S. schimperiana* at three different doses are summarized in Table 2. SSME400 exhibited saluretic indices of 2.84 for Na^+, 1.85 for K^+, and 1.52 for Cl^-, while F10's indices were 2.91, 3.09, and 1.79 for these three ions, respectively. The natriuretic indices (Na^+/K^+) for SSME100 (1.65), SSME200 (1.98), and SSME400 (2.14) were all higher than that of the standard drug (1.31). The carbonic anhydrase inhibitory activity indices, represented by the $[Cl^-/(Na^+ + K^+)]$ ratios, were 0.52 for SSME200 and 0.59 for SSME400 (Table 2).

Electrolyte content of the 80% methanol extract

To preclude potential interference, the 80% methanol extract of *S. schimperiana* was rigorously analyzed for the presence of three specific electrolytes: Na^+, K^+, and Cl^-. The results revealed that none of these ions were detectable across any of the concentrations tested.

Effect on urine pH

The pH of the urine samples was measured, yielding varying results (Fig. 3). Both SSME200 and SSME400 generated relatively alkaline urine, with pH values of 8.54±0.32 and 8.82±0.37, respectively. F10 also resulted in slightly basic urine, with a pH of 8.37±0.21 (Figure 3).

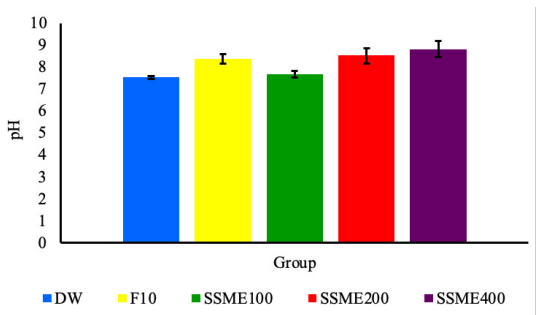

Fig. 3. Effect of 80% methanol extract on urine pH of rats (DW – distilled water; F10 – furosemide 10 mg/kg; SSME100 – *S. schimperiana* methanol extract 100 mg/kg; SSME200 – *S. schimperiana* methanol extract 200 mg/kg; SSME400 – *S. schimperiana* methanol extract 400 mg/kg)

Table 3. Qualitative phytochemical screening of the 80% methanol extract of *S. schimperiana*

Phytochemical	Test method	Observation	Result
Alkaloids	Bouchardat	brown precipitate was formed	the presence of alkaloids confirmed
	Dragendorff	a red-orange precipitate was formed	the presence of alkaloids confirmed
	Mayer	a yellowish-white precipitate was formed	the presence of alkaloids confirmed
	Wagner	a red-brown precipitate was formed	the presence of alkaloids confirmed
Anthraquinone Glycosides	Borntrager's test	the red color did not occur	absence of anthraquinone glycosides
	Ammonium hydroxide test	the pink-red color was not formed	absence of anthraquinone glycosides
Cardiac Glycosides	Keller-Kiliani test	no formation of brown-ring between layers	absence of cardiac glycosides
Flavonoids	Alkaline reagent test	yellow fluorescence was present	presence of flavonoids
	Shinoda test	the red-pink color was formed	presence of flavonoids
Polyphenols	Lead acetate test	a bulky white precipitate was formed	presence of polyphenols
	Ferric chloride test	the blue-green color was formed	presence of polyphenols
Saponins	Frothing test	a stable persistent froth was not formed	absence of saponins
Steroids	Libermann-Buchard test	no formation of a brown ring at the interface	absence of steroids
Tannins	Ferric chloride test	the blue-purple or green precipitate was formed	presence of tannins
Terpenoids	Salkowski test	a reddish-brown precipitate was not formed	absence of terpenoids

Qualitative phytochemical screening

The preliminary phytochemical analysis revealed the presence of alkaloids, flavonoids, polyphenols, and tannins in the 80% methanolic extract of *S. schimperiana* (Table 3).

Quantitative estimation of phytochemical constituents

The analysis of phytochemical constituents demonstrated a marked increase in the total concentrations of flavonoids and polyphenols, with values quantified at 187.3 mg of GAE/g and 128.4 mg of QE/g, respectively (Table 4).

Table 4. Quantitative phytochemical content estimation of the 80% methanol extract of *S. schimperiana**

Parameter	Content (mg/g dry weight of plant extract)
Total alkaloid content (mg AE/g dry weight of plant extract)	61.8
Total flavonoid content (mg QE/g dry weight of plant extract)	187.3
Total polyphenol content (mg GAE/g dry weight of plant extract)	128.4
Total tannin content (mg TAE/g dry weight of plant extract)	22.5

* AE – atropine equivalent, QE – quercetin equivalent, GAE – gallic acid equivalent, TAE – tannic acid equivalent

Discussion

An ethnopharmacological survey conducted in Northwestern Ethiopia highlighted the traditional use of *S. schimperiana* stem powder for treating urinary retention.[9] This study investigates the plant's diuretic potential and analyzes the phytochemicals found in the 80% methanol extract. The results reveal that the 80% methanol extract of *S. schimperiana* (SSME) enhanced diuresis in a dose- and time-dependent manner (r^2=0.901, p<0.001). While the 100 mg/kg dose (SSME100) did not induce significant diuresis, both the 200 mg/kg (SSME200) and 400 mg/kg (SSME400) doses substantially increased urine volume at all time points, with maximum diuresis of 111% (p<0.001) and 124% (p<0.001), respectively. These findings suggest that 100 mg/kg is below the minimum effective dose, while 200 mg/kg and 400 mg/kg are sufficient to induce a diuretic effect. Notably, the diuretic action of SSME400 was comparable to the standard, with ratios of 2.24, 0.99, 2.26, and 1.00, respectively.

The onset of urinary latency was significantly delayed in rats administered SSME100 (51.29±2.51 min, p<0.001). In contrast, both SSME200 and SSME400 caused only a modest delay in the onset of diuresis, with latencies of 38.51±1.94 min and 31.68±2.26 min, respectively (p<0.001), compared to F10, which exhibited a latency of 29.72±2.37 min (Fig. 1). The rapid onset of diuresis observed with the higher doses is likely attributable to their action on the ion co-transport mechanism, similar to the mode of action of loop diuretics.[32]

Treatment with SSME100 did not significantly affect the excretion of any of the three electrolytes compared to the negative control. However, SSME200 and SSME400 treatments notably increased the excretion of Na^+, Cl^- (p<0.001), and K^+ (p<0.01) relative to the negative control. Specifically, Na^+ and Cl^- excretions were elevated by 131% and 44% with SSME200, and 184% and 52% with SSME400, respectively. No significant difference in Na^+ and Cl^- excretion was observed between F10 and the two doses of the 80% methanol extract. In contrast, F10 induced a 209.2% increase in K^+ excretion (p<0.001) compared to the negative control, a result that was significantly higher than the K^+ loss observed with SSME200 (63%, p<0.01) and SSME400 (85%, p<0.01).

The extract significantly enhanced the excretion of three urinary electrolytes (Na^+, K^+, and Cl^-) in a dose-dependent manner. The lowest dose did not significantly affect the excretion of any of these electrolytes, while the intermediate and highest doses notably increased Na^+ and Cl^- excretion compared to the standard. However, K^+ excretion was lower than that observed with the standard drug. The natriuretic and carbonic anhydrase inhibitory activities can be quantified via the aldosterone secretory index, employing the Na^+/K^+ ratio to measure natriuresis and the $[Cl^-/(Na^+ + K^+)]$ ratio to evaluate carbonic anhydrase inhibition.[19,33]

The natriuretic indices of the extract were found to be higher than those of the standard across all measures: 1.65 for SSME100, 1.98 for SSME200, and 2.14 for SSME400, compared to 1.31 for F10. Similarly, at the highest dose, the extract exhibited carbonic anhydrase activity comparable to that of F10, as determined by the $Cl^-/[Na^+ + K^+]$ ratio. Ratios between 1.0 and 0.8 indicate no inhibition of carbonic anhydrase, while a reduction in this ratio suggests enzyme-inhibitory activity.[19,33] As shown in Table 2, the values of 0.52 for SSME200 and 0.59 for SSME400 indicate significant carbonic anhydrase inhibition, which is further supported by the observed increase in urinary pH (Fig. 3); a characteristic effect of carbonic anhydrase inhibitors.[5]

The plant extract exhibited a potassium-sparing effect while promoting the excretion of sodium and chloride ions, presenting a notable advantage over conventional pharmacological agents. This is especially significant given that a primary adverse effect associated with loop and thiazide diuretics is hypokalemia, a condition that often requires oral potassium supplementation or the use of potassium-sparing diuretics to mitigate potassium loss in urine.[5,34,35]

Collectively, these results suggest that the potent natriuretic, potassium-sparing, and carbonic anhydrase-inhibitory effects of the extract may be attributed to its secondary metabolites, as has been observed in several other plants.[18,36,37] While the precise bioactive constituents responsible for the diuretic and natriuretic properties of *S. schimperiana* remain unidentified, qual-

itative phytochemical assays have identified the presence of alkaloids, flavonoids, polyphenols, and tannins (Table 3). These phytoconstituents may exert their effects either individually or synergistically, contributing to the observed physiological outcomes.

Existing literature supports the notion that these phytochemicals possess diuretic and natriuretic properties through various biochemical mechanisms. For example, alkaloids inhibit carbonic anhydrase activity, promote renal perfusion through vasodilation, and may increase sodium excretion while conserving calcium.[38-40]

Flavonoids induce diuresis by enhancing renal perfusion, inhibiting carbonic anhydrase, blocking the sodium-potassium-chloride cotransporter, and modulating Na^+/K^+-ATPase activity. They also inhibit angiotensin-converting enzyme (ACE) and act as A1 adenosine receptor antagonists, promoting sodium excretion and diuresis through reduced sodium reabsorption and afferent arteriole dilation.[41-44]

Similarly, polyphenols promote diuresis by modulating ion channels and pumps involved in sodium and chloride reabsorption, inhibiting carbonic anhydrase, and regulating ACE and Na^+/K^+-ATPase activity.[43-46]

Flavonoids exhibit dual roles, providing both diuretic and potassium-sparing effects. The minimal impact of the plant extract on potassium excretion supports its potassium-sparing potential. Meanwhile, tannins contribute to hypotensive effects by promoting the excretion of water and electrolytes.[6] Overall, empirical evidence suggests that *S. schimperiana* exhibits diuretic activity through multiple mechanisms, which can be attributed to its diverse phytochemical profile.

Above all, this study presents the first comprehensive, dose-dependent assessment of the diuretic activity of 80% methanol extract from *S. schimperiana*, revealing its efficacy to be comparable to that of the standard diuretic, furosemide, at higher doses. Furthermore, the study provides a systematic exploration of the plant's phytochemical composition, establishing a connection between these compounds and the observed pharmacological effects. This suggests that *S. schimperiana* operates through multiple mechanisms of action. Notably, the findings underscore the potential of *S. schimperiana* as a natural diuretic, particularly for the management of fluid retention disorders. Its potassium-sparing properties offer promise as a safer alternative, with the potential for synergistic effects when combined with conventional diuretics.

Study limitations

Although the study offers compelling evidence for the diuretic potential and phytochemical composition of 80% methanol extract of *S. schimperiana*, it is not without limitations. First, the specific bioactive compounds responsible for the observed effects remain unidentified. While preliminary phytochemical analysis indicates the presence of several bioactive classes, advanced techniques such as high-performance liquid chromatography-mass spectrometry or nuclear magnetic resonance spectroscopy are necessary to isolate and characterize these compounds. Second, as a preclinical study, the findings are based solely on a rat model, which, despite its relevance, may not fully replicate human physiology. To confirm the extract's safety, efficacy, and therapeutic potential in humans, further isolation and characterization of bioactive compounds, along with clinical trials, are indispensable. It remains difficult to extrapolate findings from animal models to humans in the absence of such studies.

Moreover, the long-term effects of *S. schimperiana* methanolic extract were not investigated. Prolonged administration may lead to cumulative toxicity or adverse outcomes, particularly electrolyte imbalances. The study also lacked pharmacokinetic profiling, including absorption, distribution, metabolism, and excretion, which are essential for determining bioavailability and the therapeutic window. Finally, potential interactions between the extract and conventional diuretics or other medications were not evaluated, potentially limiting its clinical utility.

Conclusion

The 80% methanolic extract of *S. schimperiana* exhibits significant dose-dependent diuretic activity with confirmed safety at tested doses. Its efficacy, comparable to furosemide, is linked to its rich phytochemical profile. Further studies are needed to isolate bioactive compounds, clarify mechanisms, and validate clinical efficacy and safety.

Declarations

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This study did not receive any funding.

Author contributions

Conceptualization, B.G.M. and T.A.T.; Methodology, B.G.M.; Software, B.G.M.; Validation, D.A.G., A.D.T., G.N.A., G.T.B., and Y.Y.L.; Formal Analysis, B.G.M.; Investigation, B.G.M. and A.A.; Resources, Y.W.E.; Data Curation, M.T.K.; Writing – Original Draft Preparation, B.G.M.; Writing – Review & Editing, B.G.M.; Visualization, D.A.G.; Supervision, B.G.M.; Project Administration, B.G.M.; Funding Acquisition.

Conflicts of interest

The authors have no conflicts of interest to declare.

Data availability

All relevant data and materials are available from the corresponding author upon reasonable request.

Ethics approval

The study received formal approval from the Research Ethics Committee of the Department of Pharmacy, College of Medicine and Health Sciences at Debre Markos University, as documented in the ethical approval letter with reference number 1921/23.

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