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ORIGINAL RESEARCH

Evaluation of Diuretic Activity of Aqueous and Hydro Methanolic Crude Extracts and Solvent Fraction of the Hydromethanolic Flower Extract of *Erica Arborea L. (Ericaceae)* in Swiss Albino Mice

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Purpose: To evaluate the diuretic effects of aqueous (AQ) and hydromethanolic crude extract (HM) the as well as the solvent fractions of the HM extract from Erica arborea flowers in mice.

Methods: Mice were administered AQ and HM crude extracts, along with solvent fractions of HM extracts of *E. arborea* flowers, including HXF (n-hexane fraction), EAF (ethyl acetate fraction), and AQF (aqueous fraction), at doses ranging from 100 to 400 mg/kg orally. The effects of these extracts and solvent fractions on urine and salt excretion over 5 hours were compared to the effects of the solvent used for reconstitution and a standard drug (furosemide 10 mg/kg), as well as to each other.

Results: The HM crude extract at a lower dose (100 mg/kg) significantly increased urine volume and salt excretion starting from the 3rd h compared to the AQ crude extract. Similar effects were observed for EAF. Notably, the HM extract and its EAF at 400 mg/kg showed comparable urine and salt excretion profiles to the standard drug.

Conclusion: This study demonstrated that HM extract and EAF promote better diuresis, likely due to their saluretic properties. Furthermore, it confirms the diuretic activity of Erica arborea flowers.

Keywords: diuresis, natriuresis, urine electrolyte, urine pH, mice

Introduction

Cardiovascular diseases are the leading cause of death globally, taking an estimated 17.9 million lives each year, which accounts for one third of global mortality.[1] Hypertension is the most important risk factor, and heart failure is estimated to account for 80% of the cardiovascular disease burden.[2] Congestion, which is linked to pressure or fluid overload is a central in the pathogenesis of these diseases states of condition.[3] Diuretics are among the most commonly prescribed medications in Reno cardiovascular disorders.

They inhibit electrolyte reabsorption from the lumen of the nephron, thereby increasing osmolality and inducing a negative fluid balance.[4] In the human body, numerous compounds display diuretic effects. However, conventionally, diuretics are classified into five categories and taught as such. These categories include carbonic anhydrase inhibitors (CAIs), loop diuretics, osmotic diuretics, potassium-sparing diuretics, and thiazides.[5]

Despite the fact that the currently available diuretics have an overall favourable benefit/risk ratio, the issue of fluid electrolyte imbalance, reduced efficacy,[6] and the issue of diuretic resistance poses a significant clinical

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hurdle often associated with an unfavorable prognosis.[7] Although newer drugs like vaptans and Vasopressin receptor (V2R) antagonist antagonists have demonstrated efficacy in extensive trials, they lack significant effects on long-term morbidity and mortality.[8] As a result, pharmaceutical researchers have been working to create new medications with an improved pharmacological profile.[9] Natural diuretics sourced from plants are extensively employed, owing to their minimal toxicity. They are deemed a secure and economically viable option when compared to synthetic formulations.[10] This attribute positions them as an excellent choice for the development of diuretic drugs derived from plants, offering advantages such as affordability, improved pharmacological properties, and enhanced safety profiles.[11] *Erica arborea* (Ericaceae) is among the medicinal plants, whose flowers traditionally believed to possess diuretic properties. Nevertheless, there has not been in vivo study validating its ethno medicinal use. Hence, the rationale behind this investigation is to conduct a scientific evaluation of the diuretic impact of the flower extracts. This aims to establish a foundation for subsequent researches aimed at identifying the lead active compounds for drug development from natural sources.

Erica arborea L is a flowering plant, indigenous to the Mediterranean region, as well as to the Canary Islands, the Ethiopian Highlands, the mountains of central Africa between Uganda and the Democratic Republic of the Congo, and Cameroon.[12] It belongs to family of Ericaceae, which is a large metropolitan family represented by 124 genera and about 4100 species.[13] This plant thrives in moist and rainy agroclimatic zones, typically at altitudes of 2500–3300 meters, and is often found on dry, rocky ground with thin soil.[14] Ethno botanical studies from different parts of the world reported its flowers use for diuretic purpose.

An ethnobotanical study conducted in Italy reported the oral consumption of decoctions and infusions of Erica arborea flowers as diuretic tea.[15] In North Africa, it is common to orally consume three cups of flower decoction before as a treatment of renal lithiasis.[16] In Turkish folkloric medicine, the decoction and infusion of the flowering tips are utilized as a diuretic and urinary antiseptic.[17,18] A field investigation into the medicinal plants found in the flora of the Canary Islands, documented the decoction of flowers as a remedy for urinary antiseptic, anti-inflammatory, and hypotensive purposes. Additionally, the branches and leaves of this plant are mashed and applied externally for the treatment of insect bites.[19] Algerian traditional healers utilized the flower as a hypo tensor agent, for treatment of kidney disease and prostate.[20] E. arborea is recognized as possessing astringent properties, with its aerial parts being attributed to a diverse array of traditional applications including antiulcer, antimicrobial, anti-edema, antidiarrheal, and wound-healing agents.[21] In Ethiopian traditional healer communities, the decoction of the shoot is used for treatment of giardiasis,[22] and the powder of dried leaves is mixed with butter and rubbed to the wounds.[23] Previous ethno pharmacological investigations conducted on the plant have demonstrated its antioxidant,[24] analgesic,[25] and anti-inflammatory[26] effects.

Materials and Methods

Chemicals and Reagents

Absolute methanol (Folium Pharmaceuticals, Ethiopia), ethyl acetate (Sisco Research Laboratories, Pvt. Ltd., India), and n-hexane (Loba Chemie Pvt. Ltd. India), normal saline (Fresenius Kabi AG, Germany), furosemide (Salutas Pharma GmBH, Germany), or Tween 80 (Care Laboratories and Medical Supplies, India). All substances used were of analytical grade.

Plant Material

The flowers of E. arborea (Ericaceae) were collected from Choke Mountain, Amhara region, East Gojam Zone, northeast Ethiopia, in April 2022 (Figure 1). The plant specimen was authenticated by Dr. Getinet Masresha (PhD) and a voucher specimen (NL1/2022) was deposited at the institutional herbarium of the College of Natural and Computational Sciences, University of Gondar, Ethiopia.

Figure 1 Photographs of *Erica arborea*. L at its habitat.

Experimental Animals

Swiss albino mice of both sexes were used to evaluate diuretic activity, while only female mice were utilized for the acute toxicity test. These mice weighed between 25–35 g and were sourced from the Department of Pharmacology, School of Pharmacy, University of Gondar. They were kept under standard laboratory conditions at 25 ± 2 °C with a 12/12-hour light-dark cycle and had ad libitum access to standard dry pellet diet and water. Prior to experimentation, the mice were individually housed and allowed to acclimate for one week. All experiments were conducted following the guidelines for experiments involving animals.[27] The protocol was approved by the Ethics Review Committee of the Department of Pharmacology with reference number (SOP 4 /59/2014).

Plant Extraction

The flowers of E. arborea were rigorously washed with tap water, dried in the shade, and extracted.

Aqueous Crude Extraction (AQ)

Four hundred grams of the dried and powdered E. arborea flowers (400 g) were soaked in hot distilled water (1:10 w/v) at 40 °C for 15 min and subsequently cooled for 30 min to simulate traditional usage. The obtained infusion was filtered through a muslin cloth, followed by Whatman filter paper. The filtrate was then frozen at −40 °C and lyophilized until dried. The final yield obtained was 22.35 g (5.59%).

Hydromethanolic Crude Extraction (80% Methanol) (HM)

One and a half kilograms of plant powder were divided into five 300-gram portions and added to five Erlenmeyer flasks, each containing 1.5 liters of hydro-methanol. The mixture was macerated at room temperature for three days, with occasional shaking, and each Erlenmeyer flask was covered with aluminum foil. The solvent-to-sample ratio was maintained at 5:1. After three days, the extract was filtered through Whatman filter paper using pressurized suction filtration. The marc was then re-macerated twice with 80% methanol for three days each time, totaling nine days, to maximize the yield. The solvent was removed from the extract using a rotary evaporator set at 40°C to eliminate methanol. Subsequently, the extracts were frozen at −40°C and lyophilized until dry. A final yield of 220 g (14.6%) was obtained and stored in a refrigerator until reconstituted for use.

Hydromethanolic Solvent Fraction

Seventy grams (70 g) of the methanolic extract underwent further fractionation through successive extractions using solvents of increasing polarity, including n-hexane, ethyl acetate, and water, utilizing a separatory funnel. This process was repeated until complete extraction was achieved. Following extraction with each solvent, the solvent was evaporated by drying in an oven at 40°C to obtain the respective fractions. Ultimately, a total yield of 9 g HXF (12.8%), 15.13 g EAF (21.6%), and 32.11 g AQF (45.87%) was obtained and stored in a refrigerator until reconstituted for use. The aqueous crude extract (AQ), hydromethanolic crude extract (HM), and the aqueous fraction (AQF) were reconstituted with distilled water (DW), while the n-hexane fraction (HXF) and ethyl acetate fraction (EAF) were reconstituted with 2% (v/v) Tween 80 in water (2% TW80).

Acute Toxicity Study

The acute oral toxicity test was conducted following the guidelines outlined in the Organization for Economic Co-operation and Development (OECD) guideline number 425, as per the 2008 edition.[28] Ten non-pregnant female mice (five per extract) underwent a fasting period prior to the test. Following fasting, two mice (one for AQ and one for HM) were received the limit dose of 2 g/kg. They were then meticulously observed for 4 hours at 30-minute intervals to detect any behavioral changes or signs of death. Since no signs of death were observed within 24 hours, an additional eight mice (four for each extract) received the same dosage and were continuously monitored every 24 hours for 14 consecutive days.

Grouping and Dosing of Animals

A total of 120 Swiss albino mice were utilized in this study. For the evaluation of diuretic activity of the crude extracts, the animals were randomly divided into eight groups, each comprising six mice. Group 1 served as the negative control and received distilled water (1 mL/100 g body weight of mice), while group 2 acted as the positive control and received the standard drug furosemide (10 mg/kg). Experimental groups (groups 3–5) received AQ extract at varying doses (100, 200, and 400 mg/kg), and groups 6–8 received HM extract at equivalent doses as those of AQ extract.

To assess the diuretic activity of HM solvent fractions, the animals were allocated into 12 groups. Groups 1 and 2 served as negative controls and received 2% Tween 80 in water (2% TW80) and distilled water (DW), respectively. Group 3, the positive control, received 10 mg/kg of standard drug furosemide. Experimental groups (groups 4–6) received the n-hexane fraction at different doses (100, 200, and 400 mg/kg), while groups 7–9 received the ethyl acetate fraction, and groups 10–12 received the aqueous fraction, all at doses equivalent to those of the n-hexane fraction.

Dose selection was guided by the results of an acute oral toxicity study conducted according to OECD guidelines, with 10% of the limit test dose chosen as the medium dose. The standard dose was determined based on previous studies and a pilot study.[29,30] All were administered via oral gavage.

Determination Diuretic Activity

Diuretic activity was determined using the methods described in previous studies.[29,31]

All animals were fasted overnight with free access to water. The effects of different doses (100,200,400 mg/kg po) of AQ, HM crude extract, and HM solvent fractions on the increase in urine volume were investigated. The administration of each test substance was preceded by fluid overload of 15 mL/kg NS. The mice were individually placed in metabolic

cages. Urine was then collected and measured, and pH was determined at 1, 2, 3, 4, and 5 h. Urine samples collected at 5 h were transferred to Falcon tubes and used for urine electrolyte analysis. The parameters measured for each mice were total urine volume, urine electrolyte concentration of sodium (Na^+), potassium (K^+), chloride (Cl^-) ions, and urine pH. Finally, the diuretic effects of crude extracts and solvent fractions were compared to those of controls and the standard using the following parameters.

$$\text{Urinary Excretion} = \frac{\text{Total urinary output}}{\text{Total liquid administered}} \times 100\% \quad (1)$$

$$\text{Diuretic Action} = \frac{\text{Urinary excretion of treatment groups}}{\text{Urinary excretion of control group}} \quad (2)$$

$$\text{Diuretic Activity} = \frac{\text{Diuretic action of test drug}}{\text{Diuretic action of standard drug}} \quad (3)$$

Analytical Procedures

Na^+, K^+ and Cl^- levels in both the collected urine and plant extracts were determined using an ion-selective electrode (ISE) analyser at the University of Gondar Comprehensive Specialized Hospital. The effects of the extracts on the Sal uretic index for the measured ions, natriuretic activity, and carbonic anhydrase inhibition (CAI) activity were determined using the following parameters.

$$\text{Saluretic index} = \frac{\text{Urine } Na^+, k^+Cl^- \text{test group}}{\text{Urine}Na^+, k^+, Cl^- \text{control group}} \quad (4)$$

$$\text{Natriuretic index} = \frac{\text{Urinary } Na^+ \text{level in same test group}}{\text{Urinary } k^+ \text{level in same test group}} \quad (5)$$

$$\text{CAI index} = \frac{\text{Urinary } Cl^- \text{level in same teste group}}{\text{urinary}Na^+ + k^+, \text{level in the same test group}} \quad (6)$$

Phytochemical Screening

Phytochemical screening was conducted on both the extracts and HM solvent fractions to explore the potential presence of various secondary metabolite classes, following the method outlined by Shaikh and Patil.[32]

Statistical Analysis

Data are presented as mean ± SEM. Statistical analyses were performed using SPSS, version 24 software. Significance was assessed using one-way analysis of variance (ANOVA), followed by Tukey's post-hoc test for multiple comparisons. Statistical significance was set at $p < 0.05$.

Results

Acute Toxicity Study

Both AQ and HM crude extracts were safe at a dose of 2 g/kg body weight. All mice survived for 14 days.

Phytochemical Test

The AQ and HM crude extracts of E. arborea flowers as well as the HM solvent fractions were screened for the possible presence or absence of phyto-constituents. Terpenoids and tannins were absent in AQ extract and AQF. Alkaloids, flavonoids and phenols were present in all extracts and HM solvent fractions (Table 1).

Table 1 Phytochemical Screenings of Aqueous, Hydromethanolic Extract & Solvent Fraction of Flower of E.arborea

	AQ	HM	HXF	EAF	AQF
Alkaloids	+	+	+	+	+
Glycosides	+	+	–	–	+
Flavonoids	+	+++	+++	++	+
Phenols	++	+++	++	+++	++
Tannins	–	+++	++	+++	–
Steroids	–	–	–	–	–
Terpenoids	–	+++	++	+++	–
Saponins	+++	++	+	+	+++

Notes: -: absent, +: mild presence, ++: moderate presence, +++: high presence.
Abbreviations: AQ, Aqueous extract; HM, Hydro methanol extract; HXF, Hexane fraction; EAF, Ethyl acetate fraction; AQF, Aqueous fraction.

Effect on Urine Volume

The hydromethanolic flower extract of E. arborea resulted in better diuresis than the aqueous extract did. HM100 significantly increased urine output ($P < 0.001$) start from 3rd h to 5th h. However, no apparent difference was observed for AQ100 at all 5 h compared with the negative control. Although the standard drug had better diuresis, the diuretic indices of HM400 and SF were nearly similar (3.3 vs 3.4) and not statistically different in the first 3 h. (Table 2).

Table 2 Effect of AQ & HM Flower Extract of Erica Arborea on Urine Volume in Mice

Group	Volume of Urine (mL)					Diuretic Action	Diuretic activity
	1h	2h	3 h	4 h	5 h		
NC	0.13±0.02	0.27±0.03	0.33±0.02	0.40±0.03	0.50±0.03	–	–
SF10	1.17±0.08	1.65±0.11	1.90±0.15	2.12±0.16	2.37±0.16	3.4	1
AQ100	0.13±0.02^{b***}	0.25±0.02^{b***}	0.43±0.03^{b***}	0.53±0.02^{b***}	0.62±0.02^{b***}	1.6	0.48
AQ200	0.15±0.03^{b***}	0.28±0.03^{b***}	0.45±0.02^{b***}	0.83±0.02^{a***b***}	0.95±0.03^{a***b***c*}	1.8	0.54
AQ400	0.27±0.03^{b***}	0.58±0.03^{a***b***}	0.95±0.02^{a***b***c***d*}	1.13±0.02^{a***b***c***d*}	1.35±0.03^{a***b***c***d**}	2.1	0.62
HM100	0.17±0.02	0.35±0.03	0.83±0.02 ^{a***c**}	0.93±0.02^{a***c**}	1.12±0.02^{a***c***}	1.7	0.5
HM200	0.35±0.02^{a**b***}	0.68±0.04^{a***b***c***d**}	1.13±0.02^{a***b***c***d***f*}	1.33±0.02^{a***b***c***d***f**}	1.53±0.03^{a***b***c***d***f**}	2.5	0.72
HM400	0.55±0.04^{a***c**}	1.00±0.05^{a***d***f***}	1.43±0.02^{a***e***f***g*}	1.73±0.02^{a***b**}	2.03±0.03^{a***b**e***}	3.3	0.97

Notes: Each value represents mean ± SEM; (n=6), Analysis was performed by one-way ANOVA. [a]Against NC; [b]Against SF; [c]Against AQ100; [d]Against AQ200; [e]Against AQ400; [f]Against HM100; [g]Against HM 200; *p<0.05; **p<0.01; ***p<0.001. Numbers following AQ and HM indicate dose/kg.
Abbreviations: NC, Negative Control group receiving vehicle; AQ, Aqueous crude extract receiving groups; SF, Standard Furosemide 10 mg/kg receiving group; HM, Hydro methanol crude extract receiving group.

Regarding the HM solvent fraction, The Ethyl acetate fraction produced prominent diuresis followed by the n-hexane and the least urine output observed for the aqueous fraction. EAF100 and HXF100 to produce statistically significant diuresis at the end of 5th h ($p < 0.001$) compared with the negative control. However, AQF100 did not show any significant differences throughout the five hours. At higher doses, EAF400 ($p < 0.001$) and HXF400 ($p < 0.05$) significantly increased the urine output starting from the very 1st h. In contrast, AQF400 significantly increased urine output ($p < 0.01$) lately from 2nd h as all groups compared with the negative control. SF had better diuresis against HX400 and AQF400 but was unable to produce statistically significant diuresis compared to EAF400. (Table 3).

Table 3 Effect of HM Solvent Fractions of Flower of Erica Arborea on Urine Volume in Mice

Group	Volume of Urine (mL)					Diuretic action	Diuretic activity
	1h	2h	3 h	4 h	5 h		
2%TW80	0.25±0.04	0.48±0.05	0.65±0.06	0.77±0.04	0.95±0.02	–	–
SF	0.92±0.15	1.67±0.17	2.08±0.08	2.58±0.08	3.00±0.01	3.06	1
HXF100	0.37±0.4[b]***	0.68±0.05[b]***	0.95±0.06[a]***[b]***	1.07±0.04[a]***[b]***	1.23±0.02[a]***[b]***	1.10	0.4
HXF200	0.48± 0.05[b]**	0.88±0.05[a]**[b]***	1.25±0.06[a]***[b]***c**	1.47±0.04[a]***[b]***c***	1.73±0.02[a]***[b]***c***	1.59	0.52
HXF400	0.58 ± 0.05[a]*[b]*	1.08±0.05[a]***[b]***c**	1.55±0.06[a]***[b]***c***d**	1.87±0.04[a]***[b]***c***d***	2.23±0.02[a]***[b]***c***d***	2.20	0.72
EAF100	0.55± 0.02[b]**	1.05±0.02[b]***	1.45±0.02[a]***[b]***	1.75±0.02[a]***[b]***	1.95±0.02[a]***[b]***	2.05	0.67
EAF200	0.65 ±0.02[a]**[b]*	1.25±0.02[a]***[b]***	1.75±0.02[a]***[b]**c*	2.05±0.02[a]***[b]***c**	2.45±0.02[a]***[b]***c***	2.43	0.79
EAF400	0.75± 0.02[a]***	1.45±0.02[a]***c**	2.05±0.02[a]***c***d**	2.35±0.02[a]***c***d*	2.95±0.02[a]***c***d***	2.55	0.83
DW	0.33 ±0.07	0.47±0.003	0.68±0.05	0.87±0.06	1.03±0.04	–	–
AQF100	0.45±0.06[b]***	0.57±0.03[b]***	0.78±0.05[b]***	0.97±0.06[b]***	1.13±0.04[b]***	1.19	0.38
AQF200	0.55 ±0.06[b]***	0.77±0.03[a]*[b]***	1.08±0.05[a]***[b]***	1.27±0.06[a]***[b]***c**	1.53±0.04[a]***[b]***c***	1.6	0.52
AQF400	0.55±0.06[b]***	0.89±0.03[a]**[b]***c**	1.38±0.05[a]***[b]***c***d**	1.65±0.06[a]***[b]***c***d***	2.03±0.04[a]***[b]***c***d***	2.07	0.68

Notes: Results are expressed as mean ± SEM; (n=6), [a]Against negative control group receiving the vehicle (DW for AQF or 2%TW80 for other fractions) [b]Against SF, [c]Against 100mg/kg [d]Against 200 mg/kg (in each fraction types). *p<0.05; **p<0.01; ***p<0.001. Numbers following abbreviations indicate dose/kg.
Abbreviations: 2%TW80, 2% Tween 80 in water; DW, Distilled water; SF, Standard Furosemide 10 mg/kg receiving group; HXF, n-Hexane fraction; EAF, Ethyl acetate fraction; AQF, Aqueous Fraction.

Effect on Urine Electrolyte Excretion

Na^+ and Cl^- excretion of AQ100 were significantly lower than HM100 ($p < 0.001$) as compared to each other. HM400 resulted similar Na^+, Cl^- excretion (Na^+, 106.96%: Cl^- 78.35%) as SF (Na^+, 111.05%: Cl^-: 74.06%, $p < 0.001$). The k^+ loss for HM400 (110.02%, $p < 0.001$) was lower than both SF (129.1%, $p < 0.001$) and AQ400 (152.45%, $p < 0.001$) as, compared to negative control. All the extracts produced satisfactory natriuresis (index >1) and CAI activity (index<0.8). (Table 4).

Table 4 Effect of AQ & HM Flower Extract of Erica Arborea on Urine Electrolyte in Mice

Group	Urinary Electrolyte Excretion (mmol/L)			Saluretic Index			Na^+/K^+	$Cl^-/Na^+ + K^+$
	Na^+	K^+	Cl^-	Na^+	K^+	Cl^-		
NC	48.98±0.32	37.83±0.48	69.90±0.86	-	-	-	1.29	0.8
SF10	103.37±0.55	86.67±0.84	121.67±0.71	2.11	2.29	1.74	1.19	0.64
AQ100	54.00±0.32 [a]***[b]***[f]***	49.00±0.58[a]***[b]***	72.32±1.17[b]***[f]***	1.1	1.29	1.03	1.10	0.7
AQ200	68.67±0.84 [a]***[b]***c***[g]***	65.50±0.89[a]***[b]***[g]***	81.83±0.80[a]***[b]***[g]***	1.4	1.73	1.17	1.05	0.61
AQ400	96.83±0.60[a]***[b]***c***d***[h]*	95.50±0.43[a]***[b]***d***[h]***	114.00±1.46[a]***[b]***c***d***[h]***	1.98	2.52	1.63	1.01	0.59
HM100	75.83±0.60[a]***[b]***[g]***[h]***	48.27±0.37[a]***[b]***	88.83±0.87[a]***[b]***	1.55	1.28	1.27	1.57	0.72
HM200	83.50±0.76[a]***[b]***	53.50±0.76[a]***[b]***[f]***	96.43±0.67[a]***[b]***[f]***	1.70	1.41	1.53	1.56	0.70
HM400	101.37±0.83[a]***	79.45±0.78[a]***[b]***[f]***[g]***	124.67±1.17[a]***[f]***[g]***	2.07	2.10	1.78	1.28	0.69

Notes: Each value represents mean ± SEM; (n=6), Analysis was performed by one-way ANOVA followed by Tukey's test; [a]Against NC; [b]Against SF; [c]Against AQ100; [d]Against AQ200; [f]Against HM100; [g]Against HM 200; [h]Against HM400 *p<0.05; **p<0.01; ***p<0.001. Numbers following abbreviations indicate dose/kg. Saluretic index = Na^+, K^+, Cl^- of the test group / Na^+, K^+, Cl^- of the control group; natriuretic index (the ratio of Na^+ to K^+)= Na^+/K^+ and CAI index (the ratio of cl^- to sum of Na^+andk^+) =$Cl^-/Na^+ + K^+$ in the same group.
Abbreviations: NC, Negative Control group receiving vehicle; SF, Standard Furosemide 10 mg/kg receiving group; AQ, Aqueous crude extract receiving groups; HM, Hydro methanol crude extract receiving group.

Regarding the HM solvent fractions, EAF100 significantly increased urinary excretion of Na^+ (38.33%, $p < 0.001$), K^+ (17.10%, $p < 0.001$) and Cl^- (15.16%, $p < 0.001$) as compared to the negative control but not for HXF100 & AQ100. When HXF400 and AQF400 compared to negative control, HXF 400 showed better performance as it increased Na^+ (54.33%, $p < 0.001$), Cl^- (36.38%, $p < 0.001$) whereas AQF 400 increased Na^+ (43.66%, $p < 0.001$), Cl^- (34.42%, $p < 0.001$) excretion. SF resulted Na^+ (111.3%, $p < 0.001$) and Cl^- (74.06%, $p < 0.001$). Whereas, EAF 400 resulted Na^+ (104.4%, $p < 0.001$) and Cl^- (80.69%, $p < 0.001$) excretion. Although SF elicited the maximum increase in Na^+ excretion, it was comparable to that of EAF400. Regarding the k^+ loss, the maximal K^+ loss was observed with AQF400 (155.09%, $p < 0.001$), followed by SF (128.08%, $p < 0.001$), EAF 400 (86.79%, $p < 0.001$), and HXF400 (65.53%, $p < 0.001$) as all compared to negative control. (Table 5).

Table 5 Effect of HM Solvent Fractions of Flower of Erica Arborea on Urinary Electrolyte in Mice

Group	Urinary Electrolyte Excretion (mmol/L)			Saluretic Index			Na^+/K^+	$Cl/Na^+ + K^+$
	Na^+	K^+	Cl^-	Na^+	K^+	Cl^-		
2%TW80	48.92±0.33	38.00±0.43	69.90±0.86	-	-	-	1.29	0.8
SF	103.37±0.55^{a***}	86.67±0.84 ^{a***}	121.67±0.71^{a***}	2.11	2.28	1.74	1.19	0.64
HXF100	51.83±0.79	41.17±1.01	71.50±0.56	1.06	1.08	1.02	1.24	0.76
HXF200	59.00±1.15^{a***b***}	54.00±0.86^{a***b***}	82.18±0.74^{a***b***}	1.20	1.42	1.18	1.09	0.72
HXF400	75.50±0.56^{a***b***c***}	62.90±0.79^{a***b***c***d***}	95.33±0.61^{a***b***c***d***}	1.54	1.66	1.36	1.20	0.69
EAF100	67.67±0.49^{a***b***}	44.50±1.89^{a*b***}	80.50±0.43^{a***b***}	1.38	1.15	1.15	1.15	0.61
EAF200	91.00±0.58^{a***b***c*}	61.83±0.48^{a***b***c***}	82.83±0.60^{a***}	1.86	1.63	1.18	1.47	0.54
EAF400	100.00±0.97^{a***b*c***}	70.98±0.56^{a***b***c***d***}	126.30±0.32^{a***b**c***d***}	2.04	1.84	1.81	1.40	0.73
DW	46.17±0.48	37.83±0.48	70.23±0.58	-	-	-	1.29	0.83
AQF100	48.98±0.32^{b***}	39.50±0.43^{b***}	72.20±0.31^{b***}	1.03	1.04	1.03	1.21	0.81
AQF200	49.00±1.03^{b***}	66.50±0.67^{a***b***}	74.20±0.31^{b***}	1.06	1.75	1.05	0.73	0.64
AQF400	66.33±0.33^{a***b***c***}	96.50±1.12 ^{a***b***c***}	94.33±0.21^{a***b***}	1.38	2.44	1.42	0.68	0.57

Notes: Results are expressed as mean ± SEM; (n=6), [a]Against negative control group receiving the vehicle (DW for AQF or 2%TW80 for other fractions) [b]Against SF, [c]Against 100mg/kg [d]Against 200 mg/kg (in each fraction types). *p<0.05; **p<0.01; ***p<0.001 Saluretic index = Na^+, K^+, Cl^- of the test group / Na^+, K^+, Cl^- of the control group; natriuretic index (the ratio of Na^+ to K^+) = Na^+/K^+ and CAI index (the ratio of cl^- to sum of Na^+andk^+) =$Cl^-/Na^+ + K^+$ in the same group. Numbers following abbreviations indicate dose/kg.

Abbreviations: 2% TW80, 2% Tween 80 in water; DW, distilled water; SF, Standard Furosemide 10 mg/kg receiving group; HXF, n-Hexane fraction; EAF, Ethyl acetate fraction; AQF, Aqueous fraction.

With respect to the saluretic index, EAF400 elicited saluretic indices of the measured ions (Na^+, Cl^-) closer to the SF. All doses of EAF and HXF elicited satisfactory natriuresis (index > 1), whereas AQF did not. (Table 5).

CAI decreased as the dose increased. EAF 200 showed the highest CAI activity (0.54). In contrast, AQF100 showed the lowest value (0.81). SF and EAF400 exhibited intermediate CAI activity (0.64 and 0.73). (Table 5).

Effect on pH

There was no significant difference in pH between higher doses of AQ and HM extracts (400 mg/kg) and SF. They had an alkaline pH (Figure 2). Likewise, the HM solvent fractions at their middle and high doses produce significantly alkaline urine pH (p<0.001) compared to the negative controls. The standard drug also produced an alkaline pH (Figure 3).

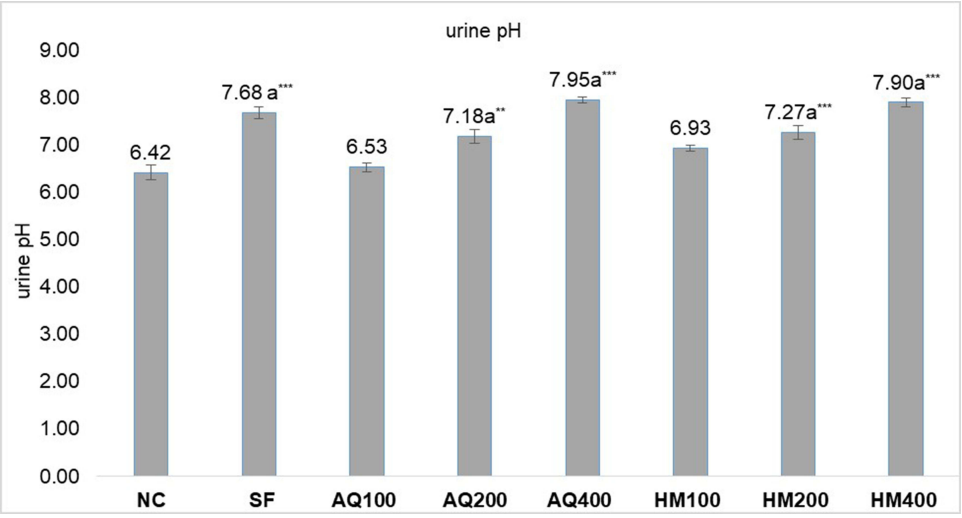

Figure 2 Effect of AQ and HM flower extract of *Erica arborea* on urine pH in mice.

Notes: Each value represent the mean ± SEM, n = 6, [a]against NC;**p<0.01; ***p<0.001. Numbers following AQ and HM indicate dose/kg.

Abbreviations: NC, Negative Control group receiving vehicle; AQ, Aqueous crude extract receiving group HM, Hydro methanol crude extract receiving group.

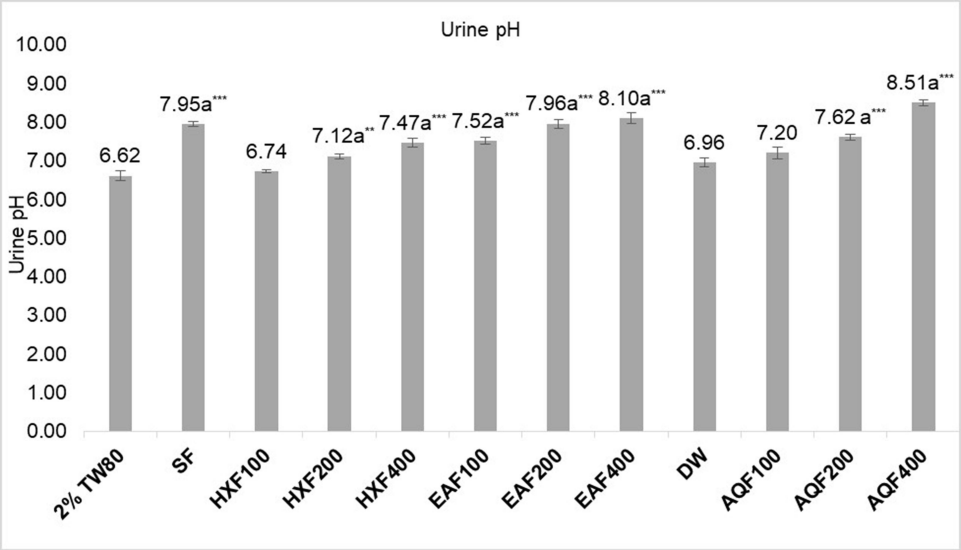

Figure 3 Effect of HM solvent fractions of flower of *Erica arborea* on urine pH in mice.
Notes: Each value represent the mean ± SEM, n = 6, [a]Against negative control group receiving the vehicle (DW for AQF or 2%TW80 for others) **p<0.01; ***p<0.001. Numbers following HXF, EAF, and AQF indicate dose/kg.
Abbreviations: 2%TW80, negative control group receiving 2% Tween 80 in water; DW, distilled water; HXF, n-Hexane fraction; EAF, Ethyl acetate fraction; AQF: Aqueous fraction.

Electrolyte Content of the Extracts and Solvent Fractions

Na^+ and Cl^- were not detectable at any dose in the crude extract or solvent fraction. However, k^+ content was detected in both the crude extracts and aqueous solvent fractions. AQ100 (14.5), AQ200 (27.93), and AQ400 (62) mmol/l were detected. The hydromethanolic crude extracts resulted in lower values of HM100 (7.51), HM200 (14.41), and HM400 (32) mmol/l. For the aqueous fractions, AQF100 (10.85), AQF200 (20.72), and AQF400 (26) mmol/l were detected (Figure 4).

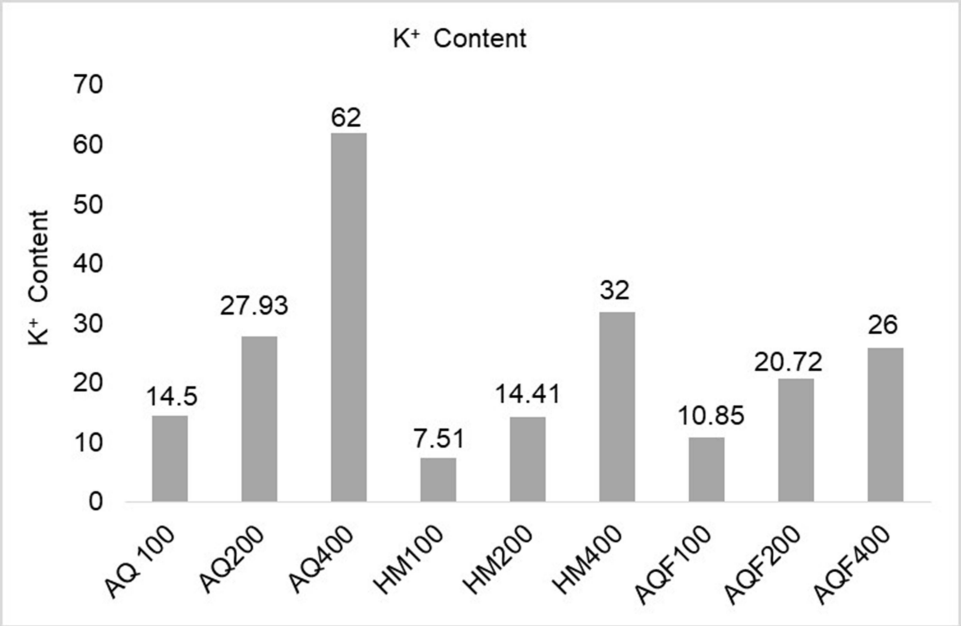

Figure 4 Electrolyte content of flower of Erica arborea (mmol/l).
Notes: K^+ represent potassium content in mmol/l. Numbers following AQ, HM, and AQF indicate dose/kg.
Abbreviations: AQ, Aqueous crude extract; HM, Hydromethanolic crude extract; AQF, Aqueous fraction.

Discussion

Diuretic activity was deemed good if it exceeded 1.50, moderate if it ranged from 1.00 to 1.50, little if it fell within the 0.72 to 1.00 range, and nil if it was less than 0.72.[33] HM200 (0.72), HM400 (0.97), EAF 200 (0.79), EAF400 (0.83), and HXF400 (0.72) exhibited mild diuretic activity. In contrast, both AQ extract and AQF at all doses showed no diuretic activity (Tables 2 and 3). Better diuresis resulting from HM extract and its most active EAF suggests that the active constituents responsible for diuretic activity might be more abundant in the less polar extract. This is consistent with studies accentuating that less polar components are responsible for diuretic activity, as seen in plants such as *Rumex abyssinicus* Jacq,[34] *Clerodendrum myricoides* Hochst[35] and *Avicennia officinalis*.L.[36] In contrast to other studies emphasizing that more polar fractions are responsible for diuretic activity, as seen *in Withania somnifera* L.[37]

The genuine enhancement of urine volume in conjunction with the increment of electrolyte/salt excretion effect, as observed in this study, supports the concept that the diuretic effect of the plant is of the saluretic type in contrast to the aquaretic-type aspect of most phytodiuretic agents.[38]

Natriuretic activity or Na^+/K^+ ratio can predict the nature of the diuretic mechanism. If Na^+/K^+ ratios > 1, 2, and 10 indicated satisfactory natriuresis, favourable natriuresis, and favourable K^+-sparing activity, respectively.[39] In accordance with this criterion, none of the extracts or solvent fractions exhibited potassium-sparing activity (Tables 4 and 5). This finding is consistent with studies on the diuretic activity of hydroalcoholic leaf extract of *Moringa Oleifera*,[40] and the ethanolic seed extract of *Nigella sativa*,[39] as these studies demonstrated satisfactory natriuresis but with lower potassium loss compared to the standard. In contrast, a study on the diuretic activity of leaves *of Medicago Sativa* L[41] and *Ajuga remota* B[29] showed low potassium excretion only at high doses of the extract.

The ratio of Cl^- / (Na^+ + K^+) falls within the range of 1.0 to 0.8, indicating a potential weak to strong carbonic anhydrase inhibitor (CAI) effect.[42] In this study, both extracts and solvent fractions exhibited a decrease in CAI indices with increasing doses (Tables 4 and 5). The incidental rise in urinary pH (Figures 2 and 3) suggests that CAI might be a mechanism of action of the plant. Although the CAI effect of EAF400 was lower compared to EAF200 (Figure 3), the maximum diuresis induced by EAF400 persisted, implying the presence of another mechanism of action at higher doses beyond CAI. This observation, where there's a modest CAI effect but a significant increase in urine volume and electrolyte excretions at high doses, aligns with findings from a study on the diuretic activity of Hydro-Ethanolic leaves Extract of *Moringa Stenopetala*.[43]

The osmotic mode of action owing to the salt overloading effect of the plant can be prevented by the HM extract. In contrast, potassium detected in the aqueous extract and at a high dose in the aqueous fraction (Figure 4) might exert an effect aligned with the intrinsic components.[37]

The most prognostic mechanism for HM extract and its most active EAF could be the inhibition of tubular reabsorption of water and electrolytes. Thus, they may act similarly to loop diuretics. Loop diuretics increase urinary flow rate and urinary excretion of Na^+, K^+ and Cl^- by inhibiting Na^+-K^+-$2Cl^-$ symporter in the thick ascending limb (TAL), stimulating production of renal prostaglandins and by inhibiting carbonic anhydrase enzyme in the proximal convoluted tubule (PCT).[44] Moreover, HM400 & EAF400 resulted similar onset of diuresis, similar Na^+ and Cl^- excretion profile, similar pH to that of SF. However, k^+ excretion for HM400&EAF400 were lower than that for SF, suggesting that the mechanism of diuresis may not be exactly similar to that of loop diuretics.

Preliminary tests revealed the presence of tannins and terpenoids in the HM crude extract and its EAF and HXF fractions but were absent in the AQ crude extract and AQ fraction (Table 1). Terpenoids have been claimed to have diuretic effects owing to their interference with the Na^+-K^+- $2Cl^-$ co-transport carrier in the luminal membrane of the TAL of the loop of Henle.[45] Tannins promotes urinary excretion of water and electrolytes.[46] Implying that terpenoids and tannins could be the most active components responsible for the prominent diuretic effect of HM extract and it's most active EAF. Alkaloids, flavonoids, and phenols, recognized for their CAI activity,[47] were found in both crude extracts and solvent fractions (Table 1). This implies that they may be accountable for the observed CAI effect and the simultaneous increase in urine pH. Saponins inhibit furosemide-sensitive Na^+-ATPase expressed in the basolateral membrane of PCT

in the kidney.[31] So that the saponins present in AQ extract and AQF (Table 1) might be responsible for preserving the least diuretic effect.

Conclusion

This study supports the traditional claim that E.arborea is a diuretic medicinal plant used for the treatment of renal lithiasis. The findings suggest that better extraction through hydro-methanol (using the cold maceration technique) than the traditional method via aqueous extraction (hot decoction technique) and fractionation using ethyl acetate were found to be more effective compared to other solvents.

Data Sharing Statement

The datasets used and/or analysed during the current study are available from the corresponding author upon reasonable request.

Ethics Approval

The study commenced after ethical clearance was secured from the Department of Pharmacology, College of Medicine and Health Sciences, University of Gondar (protocol number SOP 4 /59/2014. Experiments were carried out, and the data were compiled in compliance with the National Institutes of Health Guidelines for the Care and Use of Laboratory Animals, National Research Council (2012).[27]

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Author Contributions

All authors made distinguishable contributions to the conception of the study, design of the experiments, and acquisition, analysis, and interpretation of the data. They drafted and edited the grammatical corrections of the manuscript, agreed to submit it to the current journal, gave final approval to the version to be published, and agreed to be accountable for all aspects of the work.

Disclosure

The authors report no conflicts of interest in this work.

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