Original Research Article

Evaluation of Diuretic activity of Fractional extracts of *Ajuga remota* Benth (Lamiaceae) in Albino mice

ABSTRACT

Crude extracts of *Ajuga remota* B., an erect rhizomatous pubescent herb belonging to Lamiaceae family, which is recognized for its pharmacological and pharmaceutical properties. The present study was evaluated to know the in vivo Diuretic activity of fractional extracts of *Ajuga remota* Benth (Lamiaceae) in albino mice. The dried aqueous crude extracts were subjected to soxhlet extraction by *n*-butanol, methanol and water solvents. The mice were randomly divided into eleven groups with 8 mice in each. All fractions were administered orally at doses of 250, 500 and 1000 mg/kg to adult male mice, and the positive and negative controls were treated with furosemide (10 mg/kg, p.o) and the vehicle distilled water (2 ml/100 gm of body weight) respectively. The diuretic effect of the extracts was evaluated by measuring urine volume, urinary electrolytes and urinary pH. The result indicates that aqueous and methanolic fractions at 1000 mg/kg dose produced significant ($p<0.001$) increase in urine output and electrolyte excretion ($p<0.001$) when compared to control. Additionally, potassium sparing activity (*27%*, $p<0.05$) and high natriuretic index (2.7-3.03) were produced by the *n*-butanol fraction relatively even if it showed minimal effect on urine output. Therefore, from the present study it may be concluded that the constituents present in methanolic and aqueous fraction are responsible for diuretic activity. This finding together with previous results on the aqueous crude extracts provides a quantitative basis for developing a new diuretic medicine from *A. remota* plant.

Keywords: Ajuga remota, Fractionation, Furosemide, Diuresis, Natriuresis, Saluresis

1. INTRODUCTION

1.1. History of diuretics

The term '*diuretic'* is derived from the Greek '*diouretikos'* meaning to promote urine. Although infusion of saline or ingestion of water would qualify as being diuretic, the term diuretic usually represents drug that can reduce the extracellular fluid volume by increasing urinary solute or water excretion [1]. The diuretic is different from the term *aquaretic* that has been applied to drugs that increase excretion of solute free water [2]. It is also signifies an increase in urine volume distinguishing it to the term '*natriuresis'*, term for an increase in renal sodium excretion [3,4].

In our present study, we have extensively reviewed the literature on biological properties of *Ajuga remota*, and research findings available on the leaves extract on phytochemical and biochemical profile is scanty. Hence, we are presenting our findings on in vivo studies on the phytochemical and biochemical profile of the *Ajuga remota* for the development of a natural product for application.

1.2. *Ajuga remota*Benth (Lamiaceae)

Ajuga remota B. is an erect rhizomatous pubescent herb found growing in east Africa. It belongs to family Lamiaceae. The herb is not eaten by animals, birds or insects probably due to the very bitter taste of almost all of its parts [5]. In Ethiopia its vernacular names include *Harmaguusaa* (Oromiffa), *Akorarchign* (Amharic). The aerial part of *A. remota* is employed against diarrhea, as antifungal, antihypertensive and a remedy against diabetes by traditional healers. Additionally, the plant was found to be analgesic, remedy for fever, antimalaria and antimycobacteria [6-11].

2. MATERIAL AND METHODS

2.1. Plant materials collection

The leaves of *A. remota* were collected from a place called Sebeta, Western to Addis Ababa, Ethiopia. The plant was identified as *A. remota* at the National Herbarium, Addis Ababa University and given a voucher specimen number of U001.

2.2. Extraction procedures

The leaves of *A. remota* were sliced to smaller pieces and dried at room temperature in the shade for about two weeks. Then, the dried and sliced pieces of the leaves were powdered finely and extracted by using water as crude extracting solvent [12].

2.3. Crude extraction

600 gm of the dried powder of leaves of *A. remota* was weighed and boiled at 100^{o}C in proportional amount of tap water for 30 minutes. Then, the decoction was cooled to room temperature for 15 minutes in the same manner as it is prepared traditionally. Subsequently, the cooled decoction was centrifuged at 120 rotation per minute for five minutes, filtered and frozen in refrigerator overnight and then freeze dried in a lyophilizer (Operan, Korea) at -40 ^{o}C to obtain freeze dried aqueous extract. The dried crude extract was collected and weighed. The dried plant extract was used for further fractionation processes.

2.4. Fractionation

100 gm of aqueous crude extract of *A. remota* was weighed by analytical balance and submitted to extraction in a soxhlet extractor in two divided places each weighing 50 gm. This was subjected to fractionation by two additional solvents by increasing polarity order (*n*-butanol and absolute methanol), and the remaining residue being aqueous fraction. Accordingly, 50 gm of the crude extract weighed and added into thimble. Then, it was subjected to extraction by using about 250 ml of *n*-butanol in soxhlet apparatus until the color of the solvent dropping from the thimble was clear enough to judge the whole soluble components were extracted. The residues remaining in the thimble was then dried and further extracted by 250 ml of absolute methanol sequentially, and the final residue being the aqueous fraction. The same procedure was repeated to gain the required amounts of the extract required. The *n*-butanolic and methanolic fractions were concentrated in a Rota vapor (BUCHI Rotavapour R-200, Switzerland) at 40^{0}C. The resulting dry extract was weighed and calculated for percentage yield which was 5.6%, 25.5% and 68.9% (w/w) for *n*-butanol, methanol and aqueous fractions respectively. The dried fractional extracts were reconstituted with distilled water (DW) and administered to experimental animals orally.

2.5. Phytochemical screening

Phytochemical investigations were carried out for all the fractional extracts as per the standard methods [13,14].

2.6. Grouping and dosing of animals

Animals were randomly assigned into eleven groups each consisting of 8 mice for diuretic test of each fraction. Three treatment groups are found in each fraction for test. Dose selection was made based on the acute toxicity test performed prior to the commencement of the experiment and the previous study undergone on the plant crude extract. Accordingly, each treatment group was treated with different doses of 250, 500 and 1000 mg/kg of the aqueous (Fr.Aq), the methanolic (Fr.MeOH) and *n*-butanolic (Fr.BuOH) fractions. The positive and negative control groups were treated with standard drug, furosemide (FRSD) (10 mg/kg, p.o.) and DW (2 ml/100 gm of body weight) respectively.

2.7. Diuretic activity

Diuretic activity was determined following methods used [15] with slight modification. Male Swiss mice were divided into eleven groups of eight animals each, in laboratory cages.Each male mouse was placed in an individual metabolic cage 24 h prior to commencement of the experiment for adaptationandthen fasted overnight for 18 hours with free access to water before testing. To impose a uniform water and salt load, the mice were pretreated with physiological saline (0.9% NaCl) at an oral dose of 0.15 ml/10 g body weight. Then, each group was treated orally according to their estimated dose by using oral gavage with appropriate agents and immediately the mice were individually placed in a metabolic cage. Urine volume was measured for a total of 5 hours every 1 hour after administration with the respective extracts and the controls. The total urine collected over five hours were then filtered and finally stored at -20 ^{0}C for further electrolyte analyses for each fraction and controls [16].

The parameters like urinary excretion, diuretic action and diuretic activity were calculated in order to compare the effects of the extracts to both the negative control and standard drug on urine excretion using the following formula.

Urinary Excretion = $\frac{\text{Total urinary output}}{\text{Total liquid administered}}$ x 100% (Formula-a)

Diuretic Action = $\frac{\text{Urinary excretion of treatment groups}}{\text{Urinary excretion of control group}}$ (Formula-b)

Diuretic Activity = $\frac{\text{Diuretic action of test drug}}{\text{Diuretic action of standard drug}}$ (Formula-c)

The urinary excretion independent of the animal weight was calculated as total urinary output divided by total liquid administered (Formula -a). Diuretic Action each dose was the estimated by using the ratio of urinary excretion in treatment group of a given dose to urinary excretion in the control group (Formula -b). By using diuretic action obtained for all groups, the diuretic activities of each dose of the extracts were computed to compare them with standard drug in the test group. (Formula - c) [17].

2.8. Urinalysis procedures

The cationic electrolytes in the urine (Na^+ and K^+), and an ionic Cl^- were determined by using flame photometry and Ion Selective Electrode (ISE) analysis (AVL 9180 Electrolyte Analyzer, Roche, USA) respectively to evaluate the saluretic activity of the extracts. A calibration was performed automatically for equipment prior to analysis with different levels of standards. The electrolytes were measured at laboratory of St Paul specialized General Hospital. The pH was directly determined on fresh urine samples using a pH-meter at laboratory Pharmaceutical Analysis of pharmacy school at Addis Ababa University.

2.9. Statistical analysis

Data are expressed as mean ± S.E.M (standard error of mean) of eight mice for the test. Statistical analysis of the data were performed with ANOVA (one-way analysis of variance) followed by Tukey's multiple comparison test. Significant differences were set at pvalues lower than 0.05.

3. RESULTS AND DISCUSSION

The different parameters analyzed for the different fraction extracts of *A. remota* in the treated groups as well as the FRSD and control groups are included in figure 1 which shows the urinary pH for all groups over five hours of urine collection, and tables 1–4.Table 1-3 lists the urinary volume results (ml/5 h) for fractions and table 4 the electrolyte (Na+, K^+, and Cl^-) content ($mmolL^{-1}5\ h^{-1}$) in the urine of all groups.

3.1. Phytoconstituents of fractional extracts

The extracts of the leaves of *A. remota* by all fractions were preliminarily tested for phytochemical contents in order to predict the chemicals/compounds which are probably responsible for the diuretic and electrolyte excretory effects of each fraction. The test explored that both Fr.Aq and Fr.MeOH were found to be positive for phenolic compounds, saponins, and tannins. The Fr.MeOH was positive for cardiac glycoside and steroids in addition to the above secondary metabolites. However, terpenoids were found only in the Fr.Aq. On the other hand, Fr.BuOH was positive for the test of saponins, phenolic compounds and tannins. Generally, phenolic compounds and tannins were found in all fractions used in this study. None of the fractions were positive for the presence of anthraquinone and alkaloids.

3.2. Acute toxicity study

The mice were observed for 15 days to see if fractional extracts of the plant had acute toxicity in mice. The LD_{50} of fractional extract of *A. remota* is estimated to be above 5000 mg/kg orally as they did not provoke any visible signs of toxicity. This had been evidenced by absence of tremor, loss of weight, lethargy, paralysis, stress or adverse behaviors; no sign of diarrhea and none of the treated mice were dead.

3.3. Urinary pH

The urinary pH was measured and the different treatment groups of the aqueous, methanolic and butanolic fractions have shown different results (figure 1).

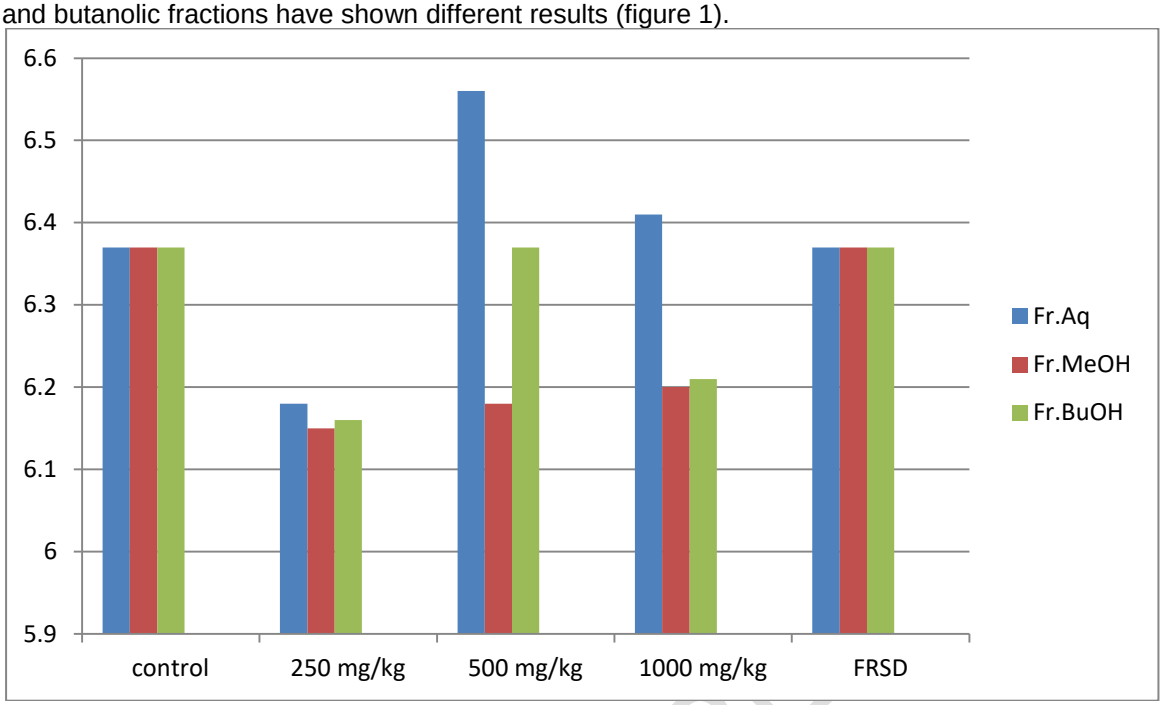

Figure 1: Effects of fractional extracts on urinary pH of mice. *Control*: negative control; *FRSD*: 10 mg/kg of furosemide; *Fr.Aq*: aqueous fraction; *Fr.MeOH*: methanolic fraction; *Fr.BuOH*: *n*-butanolic fraction; *250 mg/kg*: 250 mg/kg dose; *500 mg/kg*: 500 mg/kg dose; *1000 mg/kg*: 1000 mg/kg dose.

As it can be seen from figure 1, the maximal pH (6.56) was produced by 500 mg/kg of the aqueous fraction which was a bit greater than both the negative and the positive controls (6.37). Compared to other groups, the Fr.MeOH showed the least pH values at all doses. But, the pH differences of the extract at different doses are insignificant in respect of the dose of the extract administered. For instance, except in case of Fr.MeOH500 (6.18), the other two fractions showed higher pH at 500 mg/kg than the maximal dose which indicated that the urinary pH was not dose dependent.

3.4. Diuretic activity of the fractional extracts: Effect on urine volume

The doses tested were 250 mg/kg, 500 mg/kg, and 1000 mg/kg for all fractions. The results showed that the reference drug FRSD induced excretion values for water of 155% and that all the different fractions of *Ajuga remota* tested in the present study also produced an increase in the urinary volume excretion, but in different amount as compared to control.

3.4.1. Aqueous fraction

The Fr.Aq of extract of *A.remota* leaves produced diuresis which appeared to be dose-dependent (Table 1). The Fr.Aq250 did not produce significant increase in urine volume throughout the experiment to compare to control. At 500 mg/kg dose, the effect of fraction on urine output started to be seen from the first hour of urine collection (52%, $p<0.05$) even though a maximum diuresis was produced at the fifth hour (66.7%, $p<0.01$). (Table 1).

The comparison to the standard drug showed that Fr.Aq produced diuretic activity of 0.87 at the maximal 1000 mg/kg dose which has no significant difference. However, Fr.Aq500 and Fr.Aq250 showed diuretic activity of only 0.65 and 0.43 respectively comparatively. Furthermore, Fr.Aq at the 1000 mg/kg dose has shown increased urine output over 500 mg/kg dose tested which was significant throughout the experiment ($p<0.001$)(Table 1).

Table 1: Effect of aqueous, methanolic and *n*-butanolic fractions of the leaves of *A. remota* extracts on urine volume in mice.

Groups	Volume of urine (ml)					Diuretic action	Diuretic activity
	1h	2h	3h	4h	5h		
Control	0.5 ± 0.05	0.51 ± 0.04	0.62± 0.02	0.68 ± 0.03	0.69 ± 0.03	1.0	
FRSD	1.24 ± 0.05^{a2}	1.44 ± 0.09 ^{a3}	1.60±0.07 ^{a3}	1.675 ± 0.07 ^{a3}	1.765 ± 0.03 ^{a3}	2.55	1.0
Fr.Aq250	0.31 ± 0.04 ^{b3,c3,d3}	0.34 ± 0.06 ^{b3,c3,d3}	0.55 ± 0.05 ^{b3,c2,,d3}	0.61± 0.04 ^{b2,c2,d3}	0.76 ± 0.03 ^{b2,c2,d3}	1.10	0.43
Fr.Aq500	0.76 ± 0.04 ^{a1,b3,c3}	0.84 ± 0.08 ^{a1,b3,c2}	0.975 ± 0.07 ^{a2,b3,c2}	0.975± 0.07 ^{a1,b3,c2}	1.15 ±0.04 ^{a2,b3,c3}	1.67	0.65
Fr.Aq1000	1.03 ± 0.03 ^{a2,b2}	1.16 ± 0.05 ^{a2,b1}	1.33 ± 0.062 ^{a3}	1.36 ± 0.08 ^{a3}	1.53 ± 0.065 ^{a3}	2.21	0.87
Fr.MeOH250	0.36±0.042^{b3,e2}	0.6± 0.073^{b3,e3}	0.62±0.067^{b3,e3}	0.69±0.08^{b3,e3}	0.73±0.092^{b3,e3,f3}	1.05	0.41
Fr.MeOH500	0.51±0.05^{b3}	0.64±0.04^{b3}	0.8±0.04^{b3}	0.925±0.05 ^{b3}	1.25±.05^{a2,b1,e1}	1.81	0.71
Fr.MeOH1000	0.6±0.04^{b3}	0.81±0.08^{a2,b3}	1.31±0.06 ^{a2,b1}	1.37±0.07 ^{a2,b1}	1.75±0.03 ^{a3}	2.53	0.99
Fr.BuOH250	0.37± 0.02^{b3,g2}	0.39 ±0.03^{b3,g2,h2}	0.63 ±0.04^{b3,g1,h1}	0.73±0.06^{b3,g2,h2}	0.84±0.10^{b3,g2,h1}	1.21	0.47
Fr.BuOH500	0.44±0.03^{b3}	0.7±0.05^{b3}	0.85±.04^{b3}	1.07±0.6^{b3}	1.11±0.05 ^{a2,b3}	1.61	0.63
Fr.BuOH1000	0.58±0.04^{b3}	0.7±.03^{b3}	0.87±0.09^{b3}	1.12±0.05 ^{a3,b3}	1.19±0.05 ^{a2,b3}	1.72	0.67

The data are expressed as mean ±S.E.M, n=8

^{a}: against control; ^{b}: against standard;^{c}: against Fr.Aq1000; ^{d}: against Fr.Aq500; ^{e}: against Fr.MeOH1000; ^{f}: against Fr.MeOH500; ^{g}: against Fr.BuOH1000, ^{h}: against Fr.BuOH500. The significant differences in each group vs. the controls were as follows*: ^{1}: p<0.05, ^{2}: p<0.01, ^{3}: p<0.001.*FRSD: 10 mg/kg dose of furosemide; Fr.MeOH250: 250 mg/kg dose of methanolic fraction; Fr.MeOH500: 500 mg/kg dose of methanolic fraction; Fr.MeOH1000: 1000 mg/kg dose methanolic fraction.;Fr.Aq250: 250mg/kg dose of aqueous fraction; Fr.Aq500: 500mg/kg dose of aqueous fraction; Fr.Aq1000: 1000mg/kg dose of aqueous fraction; Fr.BuOH250: 250mg/kg dose of n-butanol fraction at; Fr.BuOH500: 500mg/kg dose of n- butanol fraction; Fr.BuOH1000: 1000mg/kg dose of n- butanol fraction.

3.4.2. Methanolic fraction

Compared to control, Fr.MeOH250 has also no difference on the urine volume throughout the study period like that of Fr.Aq250. But, at 500 mg/ kg dose, Fr.MeOH produced significant increase at fifth hour (81.2%, *p< 0.01*) relative to the control. At a dose of 1000 mg/kg, significant increase in effect of this fraction began to be seen from the second hour of urine collection (58%, *p<0.05*) and it were persistent through the fifth hour (153.6%, *p<0.001*).None of Fr.MeOH doses tested has shown significant diuresis at the first hour of urine collection (Table 1).

Compared to furosemide, which showed the diuretic action of 2.55, the Fr.MeOH produced diuretic action of 1.05, 1.81, and 2.53 at 250, 500, and 1000 mg/kg doses respectively. The diuretic activity calculated was 0.41, 0.71, and 0.99 for the increasing order of the doses (Table 1). Hence, the maximum dose tested of the Fr.MeOH produced the activity which was equivalent to that of FRSD. The Fr.MeOH also has dose dependent effect as that of Fr.Aq. Moreover, this fraction produced better diuresis when compared to both the Fr.Aq and Fr.BuOH (Table 1).

3.4.3. Butanolic fraction

The animals treated with the Fr.BuOH of *A.remota*leaves extract also showed insignificant increase on urine volume over five hours of the study at 250 mg/kg dose compared to the control. Even both 500 mg/kg and 1000 mg/kg tested doses did not show significant increase of urine volume the first four hours of the study period. However, Fr.BuOH500 started to increase significant urine output at fourth hour (57.7%, *p<0.05*) and fifth hour (61%, *p<0.01)* at 500 mg/kg dose. Similarly, the maximal dose also has produced significant increase in urine output at fourth (64.7%, *p<0.01*) and fifth (72.4%, *p<0.01*) hour. These results indicated the diuretic action of 1.21, 1.61, and 1.72 for 250, 500, and 1000 mg/kg doses consecutively.

Compared to furosemide, albeit Fr.BuOH has produced the diuretic activity of 0.47, 0.63, and 0.67 for increasing order the doses tested, the effect was a kind of modest (Table 1). Therefore, even the maximal dose has not proved acceptable effect on urine volume difference.

3.4.4. Effect of fractional extracts on electrolytes excretion

Furosemide which is known by its potential saluretic and diuretic effects has increased the excretion of Na^+, K^+ and Cl^- in this study. It was observed that effect of FRSD on sodium excretion increased by 119.3% (*p<0.001*) compared to the control. The data showed a remarkable parallelism among electrolytes excretion and urine output by FRSD. Among fractions, the Fr.BuOH produced a significant and comparable increase in the excretion of Na^+ at all doses to FRSD (between 70.8% and 118.6%, *p<0.001*).

The effect of aqueous fraction on urinary Na^+ concentration was also measured at all dose tested. At 250 mg/kg dose, even if its diuretic effect was not significant, Na^+ excretion was increased significantly (52.2%, *p<0.05*) compared to control. Similarly, increased Na^+ excretion were also observed with both Fr.Aq500 (50.5%, *p<0.05*) and Fr.Aq1000 (68.5%, *p<0.01*).

The methanolic fraction showed a dose dependent effect on Na^+ excretion in parallel to urine output. At the first two doses, 250 mg/kg and 500 mg/kg, Na^+ excretion was increased by 26.4% and 41.2% (*p<0.05*) respectively compared to control. However, the highest Na^+ excretion of Fr.MeOH was produced at 1000 mg/kg dose (76.6%, *p<0.01*) (Table 2).

Table 2: Effect of fractional extracts of *A. remota*leaves on urinary electrolytes excretion in mice.

Groups	Urinary electrolyte concentration (mmol/L)			Saluretic Index*			Na^+/K^+	$Cl^-/ Na^+ + K^+$
	Na^+	K^+	Cl^-	Na^+	K^+	Cl^-		
Control	59.50±1.37	49.73 ± 1.14	63.1±2.04				1.2	0.57
FRSD	130.5 ±1.22^{a3}	87.87 ± 4.54 ^{a2}	138.75±1.16^{a3}	2.19	1.77	2.19	1.48	0.64
Fr.Aq250	90.58 ±1.88^{a3,b3}	83.51 ± 1.15^{a2}	111.27±1.50 ^{a3,b1}	1.52	1.68	1.76	1.08	0.64
Fr.Aq500	83.52 ±1.24^{a3,b3}	45.08± 0.66 ^{b3}	85.75±1.06 ^{a1,b3}	1.40	0.90	1.36	1.85	0.67
Fr.Aq1000	100.28 ± 0.76 ^{a3,b2}	54.18 ± 0.75^{b3}	93.65±0.74 ^{a2,b2}	1.68	1.09	1.48	1.85	0.60
Fr.MeOH250	75.25 ±0.97 ^{a3,b3}	48.42±0.84 ^{b3}	87.02±0.83 ^{a1,b2}	1.26	0.97	1.38	1.65	0.70
Fr.MeOH500	84.03±0.68^{a3,b3}	51.1±0.61^{b3}	95.41±2.1 ^{a2,b2}	1.41	1.03	1.51	1.64	0.70
Fr.MeOH1000	105.10±0.81^{a3,b2}	69.3±0.83^{a2,b3}	107.98±1.61 ^{a3,b2}	1.77	1.39	1.71	1.52	0.62
Fr.BuOH250	130.11±2.81^{a3}	48.08±1.81^{b3}	128.06±2.42 ^{a3}	2.18	0.96	2.03	2.70	0.72
Fr.BuOH500	101.62±3.13 ^{a3,b2}	40.21±1.88^{b3}	95.91±2.51 ^{a2,b2}	1.70	0.81	1.52	2.53	0.67
Fr.BuOH1000	110.12±1 .88 ^{a3,b2}	36.31±2.12^{a1,b3}	105.98±2.02 ^{a3,b2}	1.85	0.73	1.68	3.03	0.72

Data are expressed as mean ±S.E.M, n=8

*Saluretic Index = urinary electrolytes concentration ($mmolL^{-1}$) of control group / urinary electrolyte concentration ($mmolL^{-1}$) of treated groups.

***a** : against control; **b** : against standard.*The significant differences in each group vs. the controls were as follows*: **1**: P<0.05, **2**: P<0.01, **3**: P<0.001*.FRSD*: 10 mg/kg dose of furosemide; Fr.MeOH250: 250 mg/kg dose of methanolic fraction; Fr.MeOH500: 500 mg/kg dose of methanolic fraction; Fr.MeOH1000: 1000 mg/kg dose methanolic fraction.;Fr.Aq250: 250mg/kg dose of aqueous fraction; Fr.Aq500: 500mg/kg dose of aqueous fraction; Fr.Aq1000: 1000mg/kg dose of aqueous fraction; Fr.BuOH250: 250mg/kg dose of n- butanol fraction at; Fr.BuOH500: 500mg/kg dose of n- butanol fraction; Fr.BuOH1000: 1000mg/kg dose of n- butanol fraction.

The data for potassium excretion for Fr.Aq showed that only the Fr.Aq250 produced significant (68%, *p<0.01*) increase which was comparable effect to a standard drug, FRSD (76.7%, *p<0.01*). This can also be noted from its considerably low Na^+/K^+ index (1.08) that augments the absence diuretic effect at this dose for the fraction. The other two doses of Fr.Aq showed inconsequential effects on K^+ excretion. In case of Fr.Aq1000, there was insignificant increase of K^+ loss (9%) compared to control. However, Fr.Aq500 saved potassium loss (9.3%) compared to the control though it has insignificant difference to Fr.Aq1000. The increase of K^+ excretion in case of Fr.MeOH was only seen at 1000 mg/kg dose which produced 39.3% (*p< 0.05*) loss. The Fr.MeOH250 and Fr.MeOH500 has not modified the K^+ excretion when compared to control. From all fractions, the Fr.BuOH has better potassium sparing effect which was dose dependent. For instance, Fr.BuOH500 and Fr.BuOH1000 has reduced potassium loss by 19.1% and 27% (*p<0.05*) respectively (Table 2).

The natriuretic index (Na^+/K^+) of different doses within the fraction indicated that Fr.Aq500 (1.85) and Fr.Aq1000 (1.85) had produced a significant natriuresis as compared with Fr.Aq250 (1.08). All doses of Fr.MeOH had natriuretic index which are more or less equal (1.65, 1.64 and 1.52). The only fraction that showed greater than two natriuretic indexes was Fr.BuOH at all tested doses (between 2.5 and 3) (Table 2).

In the case of Cl^-, the effect fractional extracts were in parallel with Na^+ excretion. For instances, the Fr.Aq produced an increased chloride excretion which was between 48.6% (*p<0.05*) and 76.3% (*p<0.01*) for the three doses of the fraction (Table 2). Fr.MeOH extract enhanced the loss of Cl^- by 38.6%, 51.2% and 71.1% directly in relation to the increasing dose as compared to the control. Moreover, the greater chloride excretion was seen with Fr.BuOH which produced between 53.7% (*p<0.05*) and 104% (*p<0.001*) compared to control. And also it was comparable effect on chloride excretion to that of the standard FRSD (120%, *p< 0.001*).

The measure of $Cl^-/Na^+ + K^+$ ratio is important indicator of the CAI activity of the diuretics. As described in table 2, it is observed that all doses of the fractions studied showed the $Cl^-/Na^+ + K^+$ ratio (0.6-0.72) values of almost related which are comparable to the standard drug (0.62). However, the values are greater than that of the control (0.57).

To compile, the ionic excretion obtained for all the tested fractions showed that the less polar solvent, *n*-butanol fraction, increased the sodium and chloride excretion comparative to the other two relatively polar solvents. Moreover, aqueous and methanolic fractions have comparable effect on the urine Na^+ and Cl^- concentration. Regarding to K^+ excretion, however, the Fr.BuOH has saved significant amount of K^+ and the two other fractions, especially Fr.MeOH1000 and Fr.Aq250, have shown high level of kaliuresis compared to control (Table 2).

The aim of the present study was to confirm the diuretic activity previously observed with crude aqueous and hydro-alcoholic extract of *A. remota* in albino mice [11]. The leaves of *A. remota* have been used for many medicinal purpose based on ethnobotanical information in Sebeta area of Western Addis Ababa. According to this information, *A. remota* was widely used in hypertension phytotherapy and control in Ethiopia [7]. Primarily the aqueous crude extract was prepared as a decoction to simulate traditional use of the plant. Then the freeze dried crude extract was subjected to fractionation in soxhlet apparatus in different solvent of increasing polarity. The solvents used include *n*-butanol, methanol and the residue as aqueous extract. The choice of the solvent at first was chloroform but, there were no extract that went into this solvent. Therefore, it was changed from chloroform to *n*-butanol for less polar constituents.

All the three fractions were assayed for diuretic activity at 250, 500 and 1000 mg/kg of body weight. In addition to attempting to determine dose effect results with each fraction, in this phase of the study it was intended to determine which of the fractions was most active, assuming that the most active fraction(s) would contain the chemical compounds or a higher concentration of active compound(s) responsible for the diuretic activity.

The mice in all groups were administered normal saline orally before diuretic activity test was done. This is to simulate edema as it would be highly important to demonstrate effectiveness in the presence of electrolyte and water, and diuretics are employed clinically in the treatment of edema [17]. Oral route is chosen to meet the way used by people in traditional medicine. Additionally, FRSD, a loop diuretic known for its diuretic and saluretic effect, was selected as the reference drug, since it is used clinically as a diuretic in edematous states (CHF, hepatic cirrhosis and nephritic syndrome) and hypertension [18].

The present study shows that all fractional extracts (aqueous, methanol and *n*-butanol) of *A. remota* produced an increase in urine volume over five hours as compared to the control group. Among fractions, Fr.Aq and Fr.MeOH produced significantly increased urine output comparable to the standard drug at 1000 mg/kg dose.

Even if their saluretic effects were equivalent to the medium and maximal doses within the fraction, the minimal doses applied in the present study (250 mg/kg) of all fractions did not produce significant effect in urine output compared to control. This could be due to that the saluretic effect of *A. remota* can be obtained even low dose without producing aquaresis indicating the lack of enough concentration of active components which are responsible for diuretic effect at these lower doses. In case of medium dose (500 mg/kg), there was an increase of urine volume (60-80%, $p< 0.05$) over five hours of the study period relative to control. On the other hands, there is no significant difference among the fractions at this dose even if the effect of the Fr.MeOH500 is greatest (80%) relative to Fr.BuOH500 (61%) and Fr.Aq500 (67%). However, this increase on urine output was not still sufficient.

To differentiate the diuretic effect among the fractions, increasing the dose of the extract to be tested was done. However, at1000 mg/kg, the increase in urine volume produced by both the Fr.Aq and Fr.MeOH was significantly greater than the medium dose (500 mg/kg). That means, the diuretic activity of Fr.Aq500 (0.65) is lower than that of Fr.Aq1000 (0.87) and that of Fr.MeOH produced a diuretic activity of 0.71 and 0.99 at 500 and 1000mg/kg respectively (Table 1). But, in case of Fr.BuOH, further increase in dose above 500 mg/kg did not show significant difference in urine output. This may be due to the saturation of the receptors for active ingredient found in the Fr.BuOH responsible for diuretic effect. Additionally, this could also reflect that the mechanism of action involved by the Fr.BuOH is different from the other two fractions (aqueous and methanolic). These results can help suggest that the ingredient(s) of the plant material responsible for the diuretic effect in could probably be more polar and hence less partitioned in to the less polar *n*-butanol solvent. This suggestion was supported by different findings [19,15,20,21,22,23,24].

In relation to the onset of action, the standard drug was significant starting from the first hour of the study compared to the control group. The aqueous fraction from the extract tested, showed similar onset of action to the reference drug. For instance, Fr.Aq500 showed

significant difference (*p<0.05*) to the control at the first hour of urine collection even if the diuretic activity at this dose is of little type; and that of Fr.Aq1000 dose of *A. remota* extract is sufficiently rapid and has a fairly long duration of action as it produced its significant effect from the first hour *(p<0.01)* to the fifth hour *(p< 0.001)* (table 1). This result is similar to the diuretic effect of the extract of *Ericamultiflora* and that of *Spilanthesacmella* flowers in rats producing activity like clinically used loop diuretic [25,26]. However, in case of Fr.MeOH, none of the doses administered has shown significant urine output difference at the first hour of urine collection compared to the control group. Even the Fr.MeOH1000 effect starts to be seen from second hour which yet not that much significant. On the third hour of urine collection, this dose produced comparable effect to the Fr.Aq1000. This may indicate slow onset of action of the active ingredients extracted by this semi-polar solvent, and forecasting the other difference between Fr.MeOH and Fr.Aq. The possibilities in the delayed diuretic activity methanolic fraction may be related to the gastrointestinal absorption characteristics of the active principle(s) or due to biotransformation to its active metabolite or in vivo stimulation of endogenous diuretic compound [26,27].

4. CONCLUSION

In general, three fractions of aqueous crude extract were tested, and methanolic and aqueous fractions were found to be the most active as diuretic. On basis of electrolyte excretion, Fr.BuOH is found to be the best K^+ sparing fraction among all tested groups. This finding supports the suggestion that the plant *A. remota* leaves extract holds more than one mechanism of actions; loop diuresis and potassium sparing mechanisms being some of them. This finding together with previous results on the aqueous crude extracts provides a quantitative basis for developing a new diuretic medicine from *A. remota* plant.

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