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Diuretic activity of *Euphorbia cotinifolia* extracts in adult Wistar albino rats: A herb used locally in the management of cardiovascular diseases

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ABSTRACT

Diuretics are commonly used in the management of cardiovascular diseases like hypertension. The study investigated the diuretic activity of ether and ethanol extracts of *E. cotinifolia* leaf in adult Wistar albino rats with furosemide and normal saline as positive and negative controls respectively. Four groups each with 6 healthy adult rats of either sex were used. Group I was dosed 5 ml of normal saline intragastrically. Group II were given 5 ml of 10 mg/kg b.wt. of furosemide. Group III were given 5 ml of 400 mg/kg b.wt. of ethanol extract. Group IV was given 5 ml of 400 mg/kg b.wt. of ether extract. The mean hourly total cumulative total urine output for each group was determined for 5 hours and was used to calculate the diuretic activity of both extracts. The pH and specific gravity of the urine output were determined using the pH urinalysis strips and the urine electrolytes; creatinine and urea/BUN levels were determined using COBAS INTEGRA 400 plus automated analyzer. Ether extract had the highest mean hourly cumulative total urine output of 17.80±0.36 ml with diuretic activity of 1.60 as compared to the 11.00±0.66 ml of furosemide after 5 hours and difference was statistically significant ($p \leq 0.05$) at all the time intervals of urine collection. Ether extract group had a pH of 7.0±0.36, specific gravity of 1.02±0.01, $[K^+]$ of 132.00±3.61 mMol/l, creatinine of 2158.00±5.29 µMol/l and urea/BUN of 484.40±6.38 mMol/l that were slightly higher as compared to that of furosemide. The $[Na^+]$ was 126.00±4.01 mMol/l and that of chloride $[Cl^-]$ was 144.00±3.10 mMol/l slightly lower as compared to furosemide and the difference was statistically significant ($p \leq 0.05$). The results conclude that *E. cotinifolia* leaves contains compounds with diuretic activity and hence its folklore use as a diuretic in the management of cardiovascular diseases like hypertension is justified.

KEYWORDS

Diuretic activity;
E.cotinifolia;
Albino rats;
Cardiovascular diseases.

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INTRODUCTION

Diuretics are group of drugs used in the management of cardiovascular diseases (CVD) like hypertension and their related complications through the process of dieresis[1]. Diuresis increases the formation and secretion of dilute urine. Diuretics reduces the blood volume and the work load of the heart of the individual and hence their application in the management of CVD. Cardiovascular diseases are a major health problem worldwide and among these include coronary heart disease, cerebrovascular disease (stroke), hypertension, heart failure and rheumatic heart disease[2-4]. Hypertension is a silent killer disease and the most common cardiovascular disorder affecting approximately 1 billion people globally and accounts for approximately 7.1 million deaths annually[2-4]. In Uganda, according to the Uganda Heart Institute records, there was a 500% increase in outpatient attendance due to heart related conditions over the past 7 years (2002-2009)[5] with the hypertension posing a major threat. The number of hypertensive patients in Uganda has been reported to be increasing steadily annually from 2772 in 2001 to 4778 in 2006[2,6,7]. Among the conventional drugs commonly used in the management of hypertension in Uganda, are the diuretics[1,8] but accessibility, cost and their associated side effects are a problem to the ordinary poor people in rural areas with poor health care service delivery and facilities. As a result some patients turn to use of traditional medicinal herbs with diuretic activities like *Euphorbia cotinifolia* that are considered by the local communities to be devoid of the problems of the conventional medicines. *E. cotinifolia* is a herb that is reported to have various medicinal uses including diuretic activity[9-11]. It belongs to the family of Euphorbiaceae[12]. It is a shrub of medium height with coppery-red and young shoots. Previous studies have reported that *Euphorbiaceae* species of plants contain various compounds such terpenes, flavonoids, phenolics and alkaloids which are the major classes of plant secondary metabolites that poses diuretic activities[13-15]. Flavonoids have been reported to be used by local communities and traditional herbalists in the management of hypertension[16]. Studies on *E. hirta* and *E. thymifolia* in the family *Euphorbiaceae*, that are locally used in Africa and Australia to treat various diseases such as hypertension and edema, showed that aqueous and ethanolic leaf extracts of these plants produced a time-dependent increase in urine output and hence a high diuretic activity[10,11,13]. The study investigated the diuretic activity of the ether and ethanol leaf extracts of *E. cotinifolia* and their effects on cumulative total urine output, the urine electrolytes (Na^+, K^+ and Cl^-) levels, urine pH, urine specific gravity, urea/BUN and creatinine levels in adult Wistar albino rats.

MATERIALS AND METHODS

Study design and setting

An experimental study investigated the diuretic activity of the ether and ethanol extracts of *E. cotinifolia* leaf and their effects on cumulative total urine output, the urine electrolytes (Na^+, K^+ and Cl^-) levels, urine pH, urine specific gravity, urea/BUN and creatinine levels in adult Wistar albino rats with furosemide and normal saline as controls. The study was conducted using standard methods and procedures. The experimental animals were procured from the Department of Pharmacology and Therapeutics, Makerere University College of Health Sciences.

Processing and extraction

The fresh leaves of *E. cotinifolia* were collected using standard methods[17]. The herb was taxonomically identified by a botanist and the voucher specimens were deposited at the Makerere University herbarium for future reference. The leaves were cleaned with distilled water and then air-dried to constant weight. The dry leaves were pounded into a coarse powder using a mortar and pestle. Sequential extraction was then done using ether and 96% ethanol, respectively. About 300 g of the powder in an erlenmeyer flask was soaked in 500 ml of ether for 4 days with occasional shaking to facilitate extraction of the active compounds from the plant material. The mixture was then decanted and filtered using Whatman No.1 filter paper using a buchner funnel and a suction pump. The residue was then air-dried in the shade for 3 hours in preparation for the ethanol extraction in the same way as for the ether extraction. The ether and ethanol were recovered from the extracts using a Heidolph rotary evaporator (BÜCHI Rotavapor R-205 model) to obtain the dry ether and ethanol leaf extracts that were used for the biological bioassays.

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Study animals

Twenty four healthy adult Wistar albino rats of either sex, weighing between 80 to 150 g were used in the study. The rats were housed in standard environmental conditions (temperature 25°C; photoperiod approximately 12 hours of natural light per day; relative humidity of 50 to 55%) in order to acclimatize them before the experiment.

Study animal treatment

All rats were deprived of water and food for 12 hours prior to the study. Their bladders were then completely emptied by gentle compression of the pelvic area and gently pulling their tails. Each rat was then intragastrically administered 5 ml of 0.9% sodium chloride or normal saline using the intra-gastric tube. After 30 minutes, their bladders were emptied as above to obtain the basal urine output. The amount of urine was measured using a measuring cylinder. After the basal urine output study, the animals were then allowed to feed for about 5 hours, after which they were deprived of food and water for another 12 hours in preparation for the diuretic activity study. Rats were randomly grouped into 4 treatment groups each with 6 animals based on the Lipschitz method[18]. Rats were then put in their respective group metabolic cages in preparation for the diuretic activity study. The 4 groups of animals were treated accordingly with group I given 5 ml/animal of normal saline (negative control). Group II was given 5 ml of the 10 mg/kg b.wt. of furosemide, a diuretic drug (positive control). Group III was given 5 ml of 400 mg/kg b.wt. of ethanol extract. Group IV was given 5 m of 400 mg/kg b.wt. of ether extract.

Preparation of the dose of ether and ethanol extracts of *E. cotinifolia*

The dose of ether extract was prepared by dissolving 1000 mg of the extract with three drops of dimethylsulfoxide (DMSO) and then topped up with normal saline to produce a concentration of 1000 mg / 5 ml (200 mg/ml). The dose of ethanol extract was prepared by dissolving 1000 mg of the extract in 5 ml normal saline to produce a concentration of 1000 mg / 5 ml (200 mg/ml). The dose of furosemide was prepared by dissolving 100 mg of the drug in 1 ml of normal saline to produce a concentration of 100 mg/ml. The rats were each dosed 5 ml of 400 mg/Kg b.wt. of ethanol extract for treatment group III and ether extract treatment group IV. Group II rats were dosed 5 ml of the 10 mg/kg b.wt. of furosemide.

Determination of diuretic activity

The diuretic activity of the plant extracts was determined according to Lipschitz et al. method (1943). Immediately after administration of the dosage to rats, they were kept in the metabolic cages at room temperature (approximately 25°C) and neither food nor water was provided during the experiment. The cumulative urine was collected in a pan at the bottom of the metabolic cage and measured using a measuring cylinder at an interval of an hour for 5 hours. - The urine remaining in the bladder after every hour was expelled by pulling gently at the base of the tail to ensure that the urinary bladders of the animals were completely drained before the next hour urine collection. At the end of the 5 hours, the total cumulative urine output for each group was also noted and recorded. The animals were taken out of the metabolic cages and put back in their normal cages and then provided with food and water as normally. Using the total cumulative urine output the urinary excretion (UE), diuretic action and the diuretic activity of ethanol and ether extracts of *E. cotinifolia* leaf were estimated according to Gujaral et al. (1955) method. The urine pH, specific gravity, urine electrolytes, urea/BUN and creatinine levels were also determined.

Determination of urinary excretion (UE), diuretic action and diuretic activity of ethanol and ether extracts of *E. cotinifolia*

The diuretic activities for each of the ethanol and ether extracts of E. cotinifolia leaf were determined using the Gujaral et al. (1955) formulae as below:

Urinary excretion (UE) = Total urinary output x 100 / Total liquid administered

The ratio of urinary excretion in test group and control group was denoted as diuretic action, which is the measure of the degree of diuresis as shown below:

Diuretic action = UE in test group / UE in control group

Then, Diuretic activity = Diuretic action of extract / Diuretic action of furosemide (control)

The diuretic activity was interpreted according to the method of Gujaral et al. (1955) as very good (above 1.50), moderate (between 1.00 to 1.50), mild (between 0.72 to 1.00) and poor (less than 0.72).

Determination of pH and specific gravity of the cumulative total urine

The pH and specific gravity of the total cumulative urine output of all groups were determined using urinalysis strips and the results were recorded.

Determination of total cumulative urine output electrolytes levels, urea/BUN and creatinine levels

The electrolytes levels (K^+, Na^+ and Cl^-), urea/BUN and creatinine levels in hourly cumulative urine output of all groups were determined by using automated analyzer machine (Roche Cobas Integra® 400 Plus, Hoffmann-La Roche Ltd) and the results were recorded.

Statistical data analysis

Since the study involved the collection of hourly total urine voided for all the 6 rats in each group (not as individual rat) and it was that total urine which was analyzed for the different parameters, therefore the data was presented as an observation of the total amount of the parameters analyzed for the total 6 rats in each group. However, the draw back of this study was that the difference in the total urine output and urine parameters between the control and the test group could not be compared statistically.

Ethical considerations

The research work was approved by the Faculty of Medicine Higher degrees, Research and Ethics committee of Makerere University Institution Review Board (IRB). All necessary ethical issues and animal rights were considered throughout the experimental study. Experiments were conducted in accordance with the internationally accepted principles for laboratory animal use and care[19,20].

RESULTS

The results of the hourly cumulative urine output, total cumulative urine output, the diuretic activity, urine pH, specific gravity, urine creatinine, urea/BUN and urine electrolytes (K^+, Na^+ and Cl^-) concentrations after the 5 hours of dosing the animals are tabulated in TABLE 1 and 2. In all the 4 treatment groups, there was a steady increase in the mean hourly cumulative total urine output but the increase was observed more in the group treated with the ether extract that produced 17.80±0.36 ml of urine in 5 hours. This was followed by the ethanol extract with 13.00±0.41 ml in the same period of time and was statistically significant ($p \leq 0.05$). The mean total hourly urine output for both the ether and ethanol extracts were higher as compared to the normal saline and furosemide treatment groups and were statistically significant ($p \leq 0.05$) (TABLE 1 and Figure 1). There was also a steady increase in the diuretic activity of both the ether and ethanol extracts of *E. cotinifolia* but the increase was more with the ether extract at 5 hours with the diuretic activity of 1.60 as compared to that of ethanol extract with an index of 1.16 and results of diuretic activities were comparable to the reference diuretic drug (TABLE 1). The ether and ethanol extracts also showed to have a long duration of action as compared to the furosemide and normal saline treatment groups observed in the study (Figure 1). The mean total cumulative urinary pH of the ether extract and furosemide were 7.00±0.36 and 6.50±0.50 respectively, slightly lower than that of normal saline and ethanolic extract that had a pH of 9.00±0.50 and were statistically significant ($p \leq 0.05$) except for the ether extract. There was no difference in the mean total cumulative urinary specific gravity for all the 4 different treatment groups. The mean total cumulative urine electrolytes were varying. The mean [K^+] concentrations of Normal saline was higher (>150.0±3.37 mMol/l) as compared to that of furosemide, ether and ethanol extracts with [K^+] levels of 107.20±1.53 mMol/l, 132.00±3.36 mMol/l and 126.04±2.52 mMol/l respectively and were statistically significant ($p \leq 0.05$) as compared to the furosemide group. The mean [Na^+] concentrations also were higher with the normal saline treatment group (189.30±2.65 mMol/l), followed by the ethanol extract with 181.00±3.52 mMol/l, then furosemide with 172.10±3.51 mMol/l and lastly with ether extract with 126.03±4.01 mMol/l and the ether extract electrolyte concentrations were statistically significant ($p \leq 0.05$) as compared to the furosemide group. The mean [Cl^-] concentrations of the ether extract was the lowest of the 4 different treatment groups with 144.02±3.10 mMol/l and were statistically significant ($p \leq 0.05$) as compared to furosemide group. The ether extract had the highest mean creatinine concentrations of 2158.00±5.29 µMol/l as compared to the other 3 treat-

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ment groups and were statistically significant ($p \leq 0.05$) as compared to the furosemide group. The mean total cumulative urine output levels of urea/BUN in the normal saline was higher (640.90±8.87 mMol/l), followed by the 484.40±6.38 mMol/l of ether extract, then 310.50±4.12 mMol/l of the ethanol extract and lastly 282.00±5.67 mMol/l of furosemide and were statistically significant ($p \leq 0.05$) as compared to the furosemide group (TABLE 2).

DISCUSSION

The results showed a higher hourly cumulative urine output and diuretic activity of both the ether and ethanol leaf extracts with the effect observed more with the group that was dosed with the ether extract. The diuretic activity of a drug or substance is considered to be good if it is above 1.50, moderate if it is in the range of 1.00-1.50, little if is between 0.72-1.00 and poor if it is less than 0.72[21]. The observed diuretic activity of the herb could be attributed to by the presence of ter-

TABLE 1 : Diuretic activity of the ether and ethanol extracts of *E. cotinifolia* leaf in Wistar albino rats

Drug activity	Drug/extract	Time interval (Hours)					
		*0	1	2	3	4	5
*Mean hourly total cumulative urine output ± SD (ml)	Normal saline	0.00	2.50±0.05	6.50±0.55	9.50±0.30	11.50±0.71	12.00±0.50
	Furosemide	0.00	5.00±0.32	7.50±0.25	8.00±0.31	8.50±0.36	11.00±0.66
	p-value		*0.0032*	*0.03*	*0.05*	*0.0006*	*0.0086*
	Ethanol extract	0.00	5.00±0.4	7.50±0.25	9.00±0.31	10.00±0.50	13.00±0.41
	p-value		*0.29*	*0.84*	*0.04*	*0.02*	*0.03*
	Ether extract	0.00	3.30±0.21	9.80±0.53	10.80±0.25	12.30±0.28	17.80±0.36
	p-value		*0.003*	*0.02*	*0.0005*	*0.01*	*0.0038*
Diuretic action	Furosemide	0.00	2.01	1.15	0.84	0.74	0.93
	Ethanol extract	0.00	2.01	1.15	0.95	0.87	1.08
	Ether extract	0.00	1.33	1.51	1.14	1.07	1.48
Diuretic activity	Ethanol extract	0.00	1.00	1.00	1.13	1.18	1.16
	Ether extract	0.00	0.66	1.31	1.35	1.45	1.60
Diuretic activity reference	Good : >1.50; Moderate: 1.00-1.50; Little: 0.72-1.00; Poor: <0.72 (Gujaral et al., 1955)						

***0 hours was baseline**

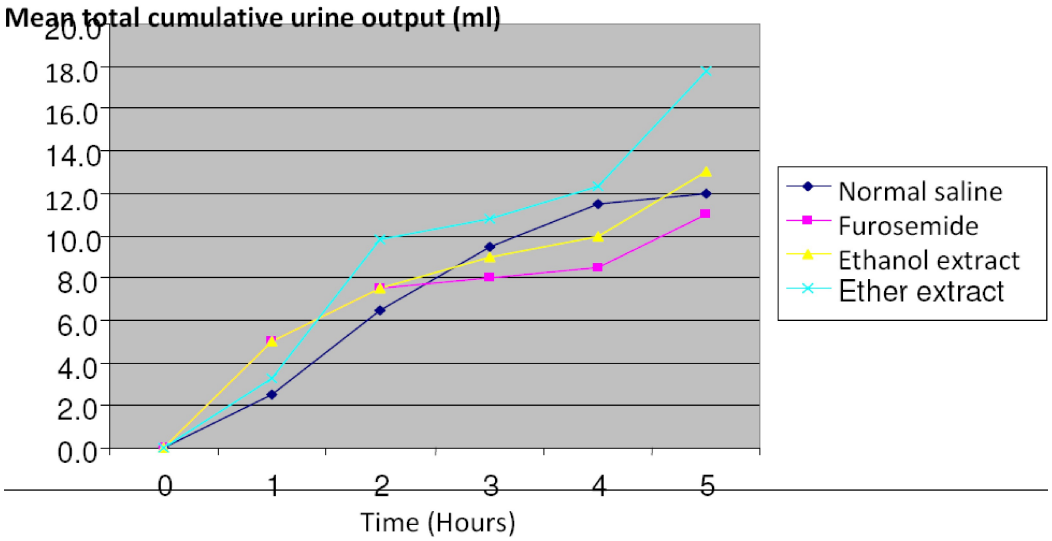

Figure 1 : Mean total cumulative urine output of the wistar albino rats after dosing with ether and ethanol leaf extract of *E. cotinifolia* during the 5 hours of observation

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TABLE 2 : Effect of ether and ethanol leaf extracts of *E. cotinifolia* on the mean total cumulative urine pH, specific gravity, urine electrolytes, urea/BUN and creatinine concentrations after 5 hours of dosing

Drug	pH± SD	Specific gravity± SD	K^+ ± SD (mMol/l)	Na^+ ± SD (mMol/l)	Cl^- ± SD (mMol/l)	Creatinine ± SD (µMol/l)	Urea/BUN ± SD (mMol/l)
Normal saline	9.00±0.45	1.02±0.02	>150.00±3.37	189.00±2.65	222.00±7.51	1768.20±10.32	640.90±8.87
Furosemide	6.50±0.50	1.02±0.01	107.00±1.53	172.00±3.51	216.00±3.06	1918.40±4.63	282.00±5.67
p-value	*0.044*	*0.84*	*0.002*	*0.043*	*0.12*	*0.0006*	*0.0001*
Ether extract	7.00±0.36	1.02±0.01	132.00±3.61	126.00±4.01	144.00±3.10	2158.00±5.29	484.40±6.38
p-value	*0.35*	*0.98*	*0.012*	*0.009*	*0.0001*	*0.0002*	*0.0004*
Ethanol extract	9.00±0.50	1.01±0.06	126.00±2.52	181.00±3.52	207.00±2.00	1638.90±2.63	310.50±4.12
p-value	*0.027*	*0.34*	*0.0009*	*0.14*	*0.103*	*0.00021*	*0.032*

penes, phenolics and alkaloids especially in the ether leaf extract that showed the highest diuretic activity of 1.60. It has also been reported that extraction with water and different selective polar solvents like ethanol, can be selective for compounds that are hydrophilic leaving out the lipophilic compounds. This could reduce the concentration of diuretic compounds present in leaves and hence explains why the urine output and diuretic activity was higher for the ether extract as compared to the ethanol extract and furosemide[10,11,13]. The results of the effect of the ether and ethanol extracts of *E. cotinifolia* on the mean pH, specific gravity, urea/BUN, creatinine, Na^+, K^+ and Cl^- ion concentration on the cumulative total urine showed that the mean pH in the ether extract and furosemide groups were 7.00±0.45 and 6.50±0.50 respectively, slightly lower than that of normal saline and ethanol extract that had a pH of 9.00±0.50 respectively. The ether and ethanol extracts showed a greater kaliuresis than the positive control group. The observed effect of the ether and ethanol extracts on the electrolyte concentrations shows a similar effects seen with the conventional diuretics used commonly in clinical practice during the management of cardiovascular diseases especially hypertension (Brunton et al., 2006). The diuretics are drugs that increase the rate of urine flow; however, clinically useful diuretics also increase the rate of extraction of Na^+ (natriuresis) that may be accompanied by excretion of K^+ (kaliuresis) and an accompanying anion, usually Cl^- (chlorouresis) (Brunton et al., 2006). The observed effects of the extracts of *E. cotinifolia* showed similar effects reported in studies on *E. hirta* and *E. thymifolia* in the family Euphorbiaceae where *E. cotinifolia* belongs, that are locally used in Africa and Australia to treat various diseases such as hypertension and edema[10,11,22,23]. And studies on the aqueous and ethanol leaf extracts of these plants produced a time-dependent increase in urine output and hence a high diuretic activity[10,11,22,23]. The sodium ions if not reabsorbed from the renal tubules, it increases the osmotic pressure within the renal tubules and attract more water within the lumen and hence promote the process of diuresis. Concerning electrolyte excretion, the ether extract produced the least sodium and chloride excretion as compared to the ethanol and the controls. Loop diuretics like furosemide inhibit the sodium-potassium-chloride co-transporter in the thick ascending limb of the kidney, hence leading to both diuresis and natriuresis (increased sodium loss) and increased loss of potassium and chloride ions in the urine as observed in the study[1,8]. Sodium chloride in the body is the major determinant of extracellular fluid volume and most clinical applications of diuretics are directed towards reducing extracellular fluids volume by decreasing the body sodium chloride content[10,11,13]. On this basis, it can therefore be deduced that furosemide is a superior diuretic to the ether extract, as it leads to greater natriuresis than the ether extract. However, the ethanol extract showed a superior diuretic activity to furosemide as it yields greater natriuresis but the ether extract of *E. cotinifolia* may possibly have another mechanism of action that lead to the highest total cumulative urine output observed in the study. Also previous studies of *E. hirta*, showed that, the electrolyte excretion was significantly affected by the plant extracts t[10,11,13]. The water extract increased the mean urine excretion of Na^+,

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K^+ and HCO_3^-. In contrast, the ethanol extract increased the excretion of HCO_3^-, decreased the loss of K^+ and had little effect on renal removal of Na^+[22]. The ether extract had the highest creatinine levels of 2158.00±5.29 µmol/l as compared to the other 3 treatment groups. The total cumulative urine output levels of urea/BUN in the Normal saline was higher (640.90±8.87 mmol/l), followed by the 484.40±6.38 mmol/l 0f ether extract, then 310.50±4.12 mmol/l of the ethanol extract and lastly 282.00±5.67 mmol/L of Furosemide (TABLE 2). The observed effects could therefore be attributed to by the presence of terpenes, phenolics and alkaloids in the ether and ethanol leaf extracts of *E. cotinifolia* that posses diuretic and electrolyte effects in the body and hence the use of the herb in the management of cardiovascular diseases like hypertension and edema by the local communities where access to healthcare services are a problem.

CONCLUSION

The ether extract of *E. cotinifolia* leaf exhibited the potent diuretic effects along with the natriuresis, kaliuresis and chlorouresis. These results have provided the evidence of the diuretic activities of *E. cotinifolia* leaf and support its use by the local communities in the management of cardiovascular diseases like hypertension.

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