

Evaluation of Diuretic Activity of Different Solvent Fractions of Methanol Extract of *Carissa edulis* Root Bark in Rats

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Abstract

Background: *Carissa edulis* is used traditionally for the treatment of HIV/AIDS symptoms, rheumatism, gonorrhoea, syphilis, rabies, malaria, epilepsy, chronic joint pain, and as a diuretic.

Objective: The present study investigates the diuretic activity of different solvent fractions of 80% methanol Soxhlet extract of *Carissa edulis* root bark in normal wistar rats.

Materials and methods: The volumes of urine output and urinary electrolyte were the parameters determined by oral administration of single doses of different solvent fractions of 80% methanol Soxhlet extract of *Carissa edulis* root bark at three dose levels (50, 500 and 1000 mg/kg) in normal wistar rats.

Results: The petroleum ether and n-butanol fractions showed no significant effect on the urine output and urinary excretion of K⁺ and Cl⁻ at all tested doses. Urinary excretion of Na⁺ was, however, affected by the petroleum ether fraction (p<0.002 at 1000 mg/kg) and n-butanol fraction (p<0.05 at 50 mg/kg; p<0.03 1000 mg/kg). The aqueous fraction significantly increased urine output in a dose dependent manner (p<0.005 at 50 mg/kg; p<0.001 at 500 and 1000 mg/kg). It also significantly increased urinary excretion of Na⁺ (p<0.006 at 500 mg/kg; p<0.001 at 1000 mg/kg) and Cl⁻ (p<0.05 at 50 mg/kg; p<0.001 at 500 and 1000 mg/kg) in a dose dependent manner. Moreover, the aqueous fraction produced no acute toxicity at the assayed dose, which was also consistent with previous results from mice model.

Conclusion: These findings collectively indicate that aqueous fraction exhibited significant diuretic activity, providing evidence, at least in part, for its folkloric use.

Keywords: Urine output; Urinary electrolyte; Acute toxicity

Introduction

Traditional medicine is used in every country in the world, and has been relied upon to support, promote, retain and regain human health for millennia [1,2]. Between 70% and 95% of citizens in a majority of developing countries, especially those in Africa, Asia, Latin America and the Middle East, rely primarily on traditional medicinal practices including the use of herbal remedies to address majority of their health-care needs and concerns [1,3,4].

Medicinal plants are used worldwide in the traditional management of some renal diseases and have a wide application as diuretic agents [5,6]. The diuretic activity of a number of plants used in ethnomedicine has been confirmed in experimental animal models [5]. The safety and efficacy of these plants for their claimed medicinal use, however, have not been extensively studied and remain to be in light of further investigation. The techniques of preparation employed by traditional healers are generally not standardized and in most cases do not comply with the requirement of good manufacturing practice [7].

Carissa edulis (Forssk) Vahl (syn. *Carissa spinarum* L.) (Apocynaceae) is a thorny shrub widespread in Africa, Australia, Vietnam, Yemen and India [8,9]. The plant bears sweet edible fruits, while its pungent root and leaf is used locally for a variety of medicinal purposes. These include the treatment of HIV/AIDS symptoms [10,11], tuberculosis [12], chest complaints, rheumatism, headache, gonorrhoea, syphilis, rabies, as diuretic [13], snake bite, evil eye, malaria [14], epilepsy, abdominal pain and chronic joint pain [15,16].

Some of these traditionally claimed uses of the plant were also scientifically reported by different scholars. Its root bark and leaf were reported to show different degree of antibacterial activity against different bacterial species [12,17,18]. It has also been previously reported that the aqueous extract from its root bark possesses

significant anti-herpes simplex virus activity *in vitro* and *in vivo* [19]. It is traditionally used in management of malaria. The *in vitro* study conducted showed that the methanol extract of the root bark has a mild antiplasmodial activity against some strains of *P. falciparum* [20,21]. In addition; its leaves were found to exhibit a mild hypoglycemic effect in streptozotocin induced diabetic rats [22].

The aqueous extracts of different parts of the plant were reported to show almost equivalent and at times better analgesic activity in comparison with aspirin [23]. The traditional use of *Carissa edulis* (*C. edulis*) as anticonvulsant is supported by studies conducted on animal models. The aqueous extract and hydro-alcoholic fractions of the plant's root bark have shown comparatively appreciable anticonvulsant activity [24,25].

The chloroform extract of the aerial part of the plant shows the highest diuretic effect. On the other hand, the aqueous extract exerts a significant decrease in the arterial blood pressure at a dose of 200 mg/kg while the petroleum ether extract produces the highest decrease in heart rate at the same dose [26]. The methanol extracts of root parts of

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the plant shows different degree of diuretic activity. Among the extracts, the root bark soxhlet extract is found to have highest diuretic activity on rats [13].

A number of compounds were identified from different parts of *C. edulis*. The aerial (leaf) part of the plant was reported to possess phenolic compounds, tannins, glycosides, terpenes, sterols [17], chlorogenic acid -1-ethylether-1-methylester, caffeic acid methyl ester, kaempferol, rutin, pinitol, β -amyrin, lupeol, stigmasterol glucoside, β -sitosterol, and β -sitosterol glucoside [26], 3-*O*-acetyl chlorogenic acid, along with four known flavonol glucosides including, kaempferol 3-*O*- β -D glucopyranoside, quercetin-3-*O*- β -D glucopyranoside, rhamnetin-3-*O*- β -D glucopyranoside and isorhamnetin-3-*O*- β -D glucopyranoside from ethyl acetate fraction and isorhamnetin-3-*O*- β -D glucopyranoside-(2" \rightarrow 1")-rhamnopyranoside, Caredulis, 1-{1-[2-(2 hydroxypropoxy) propoxy] propan-2-yloxy} propan-2-ol and (+) butyl-*O*- α -L-rhamnoside were isolated from butanol fraction [27]. In the chloroform fraction of the root alkaloids, saponins, and terpenoids were detected; whereas the methanol fraction was found to contain alkaloids, saponins, glycosides, phenolic compounds and terpenoids [28]. The root bark ethanol, petroleum ether, chloroform and acetone fractions were found to contain alkaloids, steroid and resin [12]. So far there are no scientific reports concerning the diuretic activity of the plant's root bark methanol crude extract solvent fractions. The aim of the study was, therefore, to evaluate the diuretic activity of the solvent fractions of the methanol Soxhlet extract of *C. edulis* in rats.

Materials and Methods

Chemicals and reagents

Hydrochlorothiazide (Esidrex-Novartis pharma AG, Basel, Switzerland), Absolute Methanol (PARK, Scientific limited, Northampton, UK), Petroleum ether (Labort Fine Chem Pvt. Ltd., India), n-butanol (E Marck, Germany), chloroform (Contain, Fisher Scientific UK Limited, UK), sulfuric acid (E Marck, Germany), Olive oil, ammonium hydroxide (PARK Scientific Limited, Northampton, UK), ethyl acetate, acetic acid (Fluka Chemie GmbH, Switzerland), glacial acetic acid (Central drug house (P) Ltd., New Delhi, India), and ferric chloride hexahydrate (Reiedel-De Haën Ag Seelze- Hannover, Germany).

Plant collection and authentication

Fresh root bark of *C. edulis* was collected from Hawassa town located in Southern Ethiopia, 260 km away from Addis Ababa. The plant was identified and authenticated by Mr. Melaku Wondafrash a taxonomist at the National Herbarium, Addis Ababa University. A voucher specimen (#001) was deposited for future reference.

Preparation of extract

Extraction and fractionation of the scraped, washed, air dried under shade and powdered plant material was done. About 1200 g of the powder was extracted with 80% methanol using Soxhlet extraction method. The extract was first filtered with a cotton gauze then again using Whatman filter paper No.1, concentrated under reduced pressure using rotavapor (Buchi, Rotavapor R-210/215, Switzerland) and then dried on water bath (at 40°C) to obtain a brownish gummy methanol extract of 135 g (11.25% w/w yield).

The extract was then suspended in 500 ml of warm distilled water. About 50 ml of the suspension was shaken at a time with equal volume of petroleum ether in a separatory funnel. Aqueous residue was re-shaken two times using the same volume of petroleum ether and the resulting fractionate (PE_i) was collected in a volumetric flask. The

aqueous residue (AQ_i) was then shaken with n-butanol in the same manner as petroleum ether to obtain the n-butanol fraction (nB_i). The PE_i and nB_i were concentrated under reduced pressure using rotavapor (Buchi, Rotavapor R-210/215, Switzerland) and then on water bath (at 40°C) to obtain 18.5 (13.7% w/w yield) and 23 g (17.0% w/w yield) respectively. These fractions were stored in refrigerator (-4°C) until used for the experiment. The aqueous fraction (AQ_i) was freeze dried using lyophilizer (Labconco, 12 L Console Freeze Dry 230 v-60 (7754040) Freeze Dry System, USA) and the resulting 49 g (36.3% yield) of the gummy brownish sample was kept in a desiccator until used for the experiment.

Phytochemical screening

Phytochemical screening of the fractions was performed using standard procedures as described by Tiwari et al. as well as Trease and Evans [29,30].

Animals

Adult male Wistar rats (133-284 g) and female Wistar rats (200-250 g), bred in Ethiopian Public Health Institute were used for the experiment. They were kept for a week in a controlled environment (12 hours light-dark cycle and temperature of 23 \pm 3°C) prior to pharmacological studies. The animals had free access to tap water and standard laboratory animal feed. All animals in the study were handled and cared for in accordance with the internationally accepted standard guidelines for use of animals [31].

Screening for diuretic Activity

Diuretic activity was determined following the slight modification of methods used by Vogel et al. [32]. Adult male Wistar rats weighing between 132-284 g were randomly divided into five groups for each fraction, (n=8). Group I received the vehicle (NS (aqueous fraction), 2% TW-80 in NS (n-butanol fraction) and 5% TW-80 in NS (petroleum ether fraction)) and served as negative control. Group II received the standard drug hydrochlorothiazide (10 mg/kg) and served as positive control. The test groups were group III, IV, and V which received increasing doses of the solvent fractions at 50 mg/kg, 500 mg/kg and 1000 mg/kg, respectively. The different doses of the fractions and the standard drug were dissolved in their respective vehicles immediately prior to use and administered orally after a period of overnight fasting with water provided *ad libitum*. The maximum volume administered was 25 ml/kg. Immediately after administration, each rat was placed individually in a labeled metabolic cage fitted with graduated test tube and a wire mesh. The volume of urine excreted for the next 5 hour was collected in test tube was determined and was stored in a refrigerator (-4°C) until electrolyte content analysis was carried out.

The parameters used to compare the test substances to the standard drug were diuretic action, diuretic activity, urinary excretion, saluretic index and Na⁺, K⁺ and Cl⁻. The volume of the urine excreted in 5 hours of the study period was expressed as the percent of the liquid (NS) administered giving rise to a measure of "urinary excretion" independent of animal weight (Formula 1). The ratio of urinary excretion in the test group to urinary excretion in the control group was used as a measure of the diuretic action, which also measure degree of diuresis for the given dose of a drug (Formula 2). As the diuretic action is prone to variability, a parameter known as diuretic activity was calculated. To obtain diuretic activity and the diuretic action of the test substance was compared to that of the standard drug in the test group (Formula 3) [33,34].

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

$$\text{Diuretic activity} = \frac{\text{Diuretic action of test group (DA}_t\text{)}}{\text{Diuretic action of standard drug (DA}_f\text{)}} \text{ (Formula 2)}$$

$$\text{Diuretic activity} = \frac{\text{Diuretic action of test group (DA}_t\text{)}}{\text{Diuretic action of standard drug (DA}_f\text{)}} \text{ (Formula 3)}$$

Determination of urinary electrolyte content

Na⁺, K⁺ and Cl⁻ concentration of urine output of the fractions, negative control and positive control were determined using Ion Selective Electrode (ISE) analysis (Roche, Electrolyte Analyzer AVL 9181, USA). Dilutions of the samples were made as required to bring electrolyte content of the samples to be in the range that can be determined by the analyzer [35].

Determination of electrolyte content of the fractions

Each dose level (50, 500 and 1000 mg/Kg) of the nB_f and AQ_f were suspended in distilled water to form 5 ml formulation in different concentrations (2, 20 and 40 mg/ml) respectively. The concentration of Na⁺, K⁺ and Cl⁻ were determined in the formulation by using method stated in section 2.7. The electrolyte content of PE_f was not determined at present study due to the fact that the electrolyte analyzer uses only aqueous solution [35].

Evaluation of onset and duration of action of the most active fraction

The most active fraction at the most effective dose was administered orally to eight rats, and urine output difference was recorded every hour for the next 5 hours. The negative and positive controls were given orally NS (0.9% NaCl) and HCT (10 mg/kg) respectively.

Acute toxicity

The study was carried out by limit test in accordance with OECD 423 guideline [36]. Six female Wistar rats of weighing between 200-250 g were divided into two groups: control group that received NS and a test group that received a limit dose of 5000 mg/kg of the aqueous fraction orally. The animals were deprived of food for 18 hours with free access to water.

Immediately after administration, the animals were carefully observed continuously for the first 4 hour for any overt signs of toxicity and death and then for the next 24 hours. Thereafter, they were kept under close observation up to 14 days to monitor the presence of any signs of morbidity or mortality. The weight of each animal was recorded at the 1st, 7th, and 14th day of administration to verify any weight change that might have occurred. Finally, after cervical dislocation, the rats were dissected at the 14th day to observe gross pathology of the vital organs such as liver and kidney.

Statistical analysis

Results were expressed as mean ± standard error of mean (S.E.M). The results were analyzed using SPSS statistics Software (version 20). Statistically significant differences between treatment groups were evaluated by analysis of variance (one-way ANOVA) followed by Tukey's multiple comparison test. Probabilities less than 0.05 (p<0.05) were considered to be significant.

Results

Phytochemical screening

The various phytochemical screening tests performed on the solvent fractions of the crude methanol Soxhlet extract of *C. edulis* root

bark revealed the presence of secondary metabolites such as tannins, saponins, terpenoids and cardiac glycosides (Table 1).

Results of urinary output and urinary electrolyte excretion

Petroleum ether fraction: As shown in Table 2, the petroleum ether fraction slightly increased urinary output at doses of 1000 mg/kg. The diuretic activity, however, was not significant. At the doses of 50 and 500 mg/kg, the fraction reduced urine output as compared to the negative control.

As shown in Table 3, the PE_f of methanol extract of the plant increased urinary excretion of Na⁺, K⁺, and Cl⁻ at all doses except at 500 mg/kg. However, significant increase in excretion of Na⁺ occurred at 1000 mg/Kg.

n-Butanol fraction: As shown in Table 4, nB_f of methanol extract of the plant showed an increase in excretion of the administered NS at the dose of 50 and 1000 mg/kg. The increase in the mean percent of urine excretion, however, was not significant. The fraction showed slight inhibitory effect on the mean percent excretion of the administered normal saline at the medium dose.

As shown in Table 5, the nB_f of the methanol Soxhlet extract of the plant showed significant increase in urinary excretion of Na⁺ at all doses except at 500 mg/kg. Although increase in excretion was not significant, the fraction also seems to increase urinary excretion of K⁺ and Cl⁻ at all dose levels except at 500 mg/kg.

Aqueous fraction: As shown in Table 6, the AQ_f of *C. edulis* root bark increased excretion of administered normal saline significantly at all doses tested in dose-dependent manner. The fraction showed a diuretic activity of about 78% of HCT at 1000 mg/kg.

The AQ_f of methanol soxhlet extract of the plant significantly increased urinary excretion of Na⁺ and Cl⁻ at all doses administered except at 50 mg/kg. The fraction also showed significant kaliuresis at doses of 50 and 500 mg/kg (Table 7).

Electrolyte content of the fractions: (Table 8) Electrolyte content of nB_f and AQ_f methanol Soxhlet extract of *C. edulis* root bark.

Onset and duration of action of the most active fraction at

Test for	Aqueous fraction	n-butanol fraction	Petroleum ether fraction
Tannin	+	+	+
Saponin	+	+	+
Flavonoid	-	-	-
Terpenoid	+	-	-
Cardiac glycosides	-	+	+

Key: (+) = present, (-) = absent

Table 1: Phytochemical screenings of methanol Soxhlet extract fractions of *C. edulis* root bark.

Treatment	dose (mg/kg)	cumulative urine Volume (ml)	% of saline excreted	Diuretic action	Diuretic activity
5% TW-80 in NS (Control)	--	1.25 ± 0.25	24.47	1	--
HCT	10	6.45 ± 0.59	131.28	5.36	1
PE _f	50	1.29 ± 0.11	22.86	0.93	0.17
	500	0.66 ± 0.13	15.14	0.62	0.12
	1000	1.63 ± 0.09	29.96	1.22	0.23

Each value represent the mean ± SEM of eight rats. ^a p < 0.001

Table 2: Effect of PE_f of methanol Soxhlet extract of *C. edulis* root bark on urine volume in normal rats.

Treatment	Dose (mg/kg)	Electrolyte concentration of urine (mmol/L)			Na ⁺ /K ⁺	Na ⁺ /Cl ⁻	Cl ⁻ /Na ⁺ +K ⁺
		Na ⁺	K ⁺	Cl ⁻			
5% TW-80 in NS	—	85.40±11.03	106.60±18.89	128.55±6.26	0.80	0.66	—
HCT	10	297.30±21.14 ^a	204.00±24.63 ^c	203.13±28.61	1.46	1.46	0.41
PE _f	50	130.24±23.75	121.97±25.40	156.00±14.89	1.07	0.83	0.62
	500	87.65±7.37	107.15±15.90	127.58±15.93	0.82	0.69	0.65
	1000	180.13±11.34 ^b	181.45±15.44	193.48±20.16	0.99	0.93	0.54

Each value represent the mean + SEM of eight rats. ^a p< 0.001, ^b p< 0.002, ^c p< 0.02.

Table 3: Effect of PE_f of methanol Soxhlet extract of *C. edulis* root on urinary electrolyte excretion in normal rats.

Treatment	dose (mg/kg)	cumulative urine Volume (ml)	% of saline excreted	Diuretic action	Diuretic activity
2% TW-80 in NS (Control)	--	1.13 ± 0.23	18.64	1.0	--
HCT	10	2.69 ± 0.31 ^a	52.67	2.38	1.0
PE _f	50	1.06 ± 0.13	19.74	0.94	0.39
	500	0.84 ± 0.12	17.45	0.74	0.31
	1000	1.47 ± 0.54	22.64	1.30	0.55

Each value represent the mean ± SEM of eight rats. ^a p< 0.01

Table 4: Effect of nB_f of methanol Soxhlet extract of *C. edulis* root bark on urine volume in normal rats.

Treatment	Dose (mg/kg)	Electrolyte concentration of urine (mmol/L)			Na ⁺ /K ⁺	Na ⁺ /Cl ⁻	Cl ⁻ /Na ⁺ +K ⁺
		Na ⁺	K ⁺	Cl ⁻			
2% TW-80 in NS	—	27.63±4.49	221.23±33.42	131.88±32.44	0.12	0.21	—
HCT	10	191.38±49.07 ^a	261.65±32.24	240.38±18.92	0.73	0.80	0.53
nB _f	50	162.70±34.53 ^b	280.65±19.33	221.63±28.26	0.58	0.73	0.50
	500	146.05±31.29	207.60±23.87	179.75±41.24	0.70	0.81	0.51
	1000	172.25±24.27 ^c	243.90±14.03	250.00±33.08	0.71	0.69	0.60

Each value represent the mean ± SEM of eight rats. ^a p< 0.008, ^b p< 0.05, ^c p< 0.03.

Table 5: Effect of nB_f of methanol Soxhlet extract of *C. edulis* root bark on urine electrolyte excretion in normal rats.

Treatment	dose (mg/kg)	cumulative urine Volume (ml)	% of urine excreted	Diuretic action	Diuretic activity
NS (Control)	--	0.69 ± 0.10	14.73	1.0	--
HCT	10	5.07 ± 0.34 ^a	109.39	7.35	1.0
AQ _f	50	1.63 ± 0.05 ^b	35.61	2.36	0.32
	500	2.63 ± 0.13 ^a	44.48	3.81	0.52
	1000	3.93 ± 0.17 ^a	66.75	5.70	0.78

Each value represent the mean ± SEM of eight rats. ^ap< 0.001, ^bp< 0.005.

Table 6: Effect of AQ_f methanol Soxhlet extract of *C. edulis* root bark on urine volume in normal rats.

Treatment	Dose (mg/kg)	Electrolyte concentration of urine (mmol/L)			Na ⁺ /K ⁺	Na ⁺ /Cl ⁻	Cl ⁻ /Na ⁺ +K ⁺
		Na ⁺	K ⁺	Cl ⁻			
NS (Control)	—	9.33 ± 1.45	106.48 ± 10.86	12.50 ± 2.99	0.09	0.75	—
HCT	10	278.14 ± 21.67 ^a	129.47 ± 9.26	263.86 ± 6.72 ^a	2.15	1.05	0.65
AQ _f	50	35.63 ± 11.83	204.95 ± 16.16 ^c	107.25 ± 11.76 ^c	0.73	0.33	0.45
	500	124.38 ± 37.38 ^b	216.38 ± 42.18 ^d	184.10 ± 24.10 ^a	0.57	0.68	0.54
	1000	250.43 ± 20.53 ^c	126.03 ± 16.01	223.14 ± 31.1 ^a	1.99	1.12	0.59

Each value represent the mean ± SEM of eight rats. ^ap< 0.001; ^bp< 0.006; ^cp< 0.04; ^dp< 0.02; ^ep< 0.05.

Table 7: Effect of AQ_f of methanol Soxhlet extract of *C. edulis* root bark on urinary electrolyte excretion in normal rats.

most effective dose: As shown in Table 9, AQ_f (1000 mg/kg) produced significant diuresis (p<0.03) starting from the 2nd hour that also extended till 4th hour in comparison to the negative control. The onset of the diuretic activity of the AQ_f was observed to be slower than that of HCT. The duration of action of the fraction was 3 hours.

Acute toxicity: The animals showed no signs of toxicity in the first 4 and 24 hours of observation period of the administration of the AQ_f. Moreover, they did not show any gross pathological change (color, size and texture) on their vital organs such as liver and kidney as compared to that of the control group.

Discussion

In this study, the PE_f and nB_f of the methanol Soxhlet extract of

the plant did not increase urine output significantly at the dose levels employed. It suggests that most of the less polar components of root bark of the plant may not have diuretic activity. It showed slight inhibitory effect on the urinary output at the dose of 500 mg/kg perhaps due to the presence of different components which might interact with each other.

In saline primed rats, 50, 500 and 1000 mg/kg of aqueous fraction caused a significant increase in urine output beginning from the 3rd hour of administration as compared to the negative control. In comparison, a single dose of HCT induced a brisk and significant diuresis within 60 minutes of administration. The delay in the onset of diuresis with the AQ_f may indicate that its diuretic activity is probably mediated via secondary organic metabolites. The diuretic activity of AQ_f lasted beyond the study period suggesting that the fraction has

Extract.	Dose (mg/kg)	Electrolyte concentration of aqueous fraction (mMol/L)		
		Na ⁺	K ⁺	Cl ⁻
nB _f	50	<1	<4.5	<1
	500	189	11.7	<1
	1000	336	25.2	<1
AQ _f	50	<1	11.5	<1
	500	<1	70.6	<1
	1000	<1	108.2	<1

Table 8: Electrolyte content of nB_f and AQ_f methanol Soxhlet extract of *C. edulis* root bark.

Treatment	Dose (mg/kg)	urine volume (ml)				
		1 st hr	2 nd hr	3 rd hr	4 th hr	5 th hr
NS	—	0.00 ± 0.00	0.56 ± 0.62	0.19 ± 0.29	0.22 ± 0.43	0.00 ± 0.00
HCT	10	0.47 ± 0.53 ^a	1.28 ± 0.62 ^b	1.34 ± 0.60 ^c	0.94 ± 0.51 ^d	0.97 ± 0.43 ^c
AQ _f	1000	0.00 ± 0.00	0.50 ± 0.40	0.78 ± 0.25 ^d	1.00 ± 0.55 ^a	0.16 ± 0.19 ^c

Each value represent the mean ± S.E.M of eight rats. ^a p<0.02; ^b p<0.05; ^c p<0.001; ^d p<0.03.

Table 9: Onset and duration of action of AQ_f (1000 mg/kg) as compared to NS and HCT (10 mg/kg).

a slow clearance. Interestingly, the diuretic activity of AQ_f was dose dependent indicating that this effect is intrinsic, genuine and possibly receptor mediated [37].

The increase in diuresis induced by AQ_f was reflected in similar manners in urinary ionic excretion. It significantly increased excretion of urinary electrolytes (Na⁺ and Cl⁻) in a dose dependent manner. Although the fraction significantly increased urinary excretion of K⁺ at two lower dose levels, it showed decrease in excretion of the electrolyte at the highest dose tested. The ratio of Na⁺/K⁺ was calculated for aldosterone secretory index (natriuretic activity) [34]. The natriuretic ratio values greater than 2.0 indicate favorable natriuretic activity [38]. The AQ_f however, did not increase the Na⁺/K⁺ ratio. This observation suggests that AQ_f is not acting as a potassium-sparing diuretic. Potassium-sparing diuretics are usually very weak, have slow onset of action, cause urine alkalization and increase the urinary Na⁺/K⁺ ratio [39,40].

The urine of AQ_f treated rats was both markedly hypernatremic and hyperkalemic. Further, AQ_f increased the urinary Na⁺/Cl⁻ ratio (thiazide secretory index) [34]. Collectively, these observations suggest that AQ_f may act by thiazide like mode of diuretic action. Thiazide type of diuretics elevate thiazide secretory index, simultaneously increase urinary Na⁺ and K⁺ levels at least by 50-60% by inhibiting the Na⁺/Cl⁻ co-transporter in the distal convoluted tubule of the nephron [41].

HCT and thiazide like diuretics have additional carbonic anhydrase inhibitory action in proximal tubule [42]. The ratio Cl⁻/Na⁺ + K⁺ is calculated to estimate carbonic anhydrase inhibition. Carbonic anhydrase inhibition can be excluded at ratios between 1.0 and 0.8. With decreasing ratios, slight to strong carbonic anhydrase inhibition can be assumed [43]. Thus, the present study indicates that AQ_f might have slight inhibitory property on carbonic anhydrase enzyme in renal tubules.

An increment of urinary output in rats might result from high potassium content in the plant infusion [44]. Potassium overloading, which occurs when the kidney tubules are incapable of absorbing it, produces urinary excretion of the osmotic type [45]. Quantitative determinations of the electrolytes present in the AQ_f of *C. edulis* revealed the presence of high amount of K⁺. This suggests that diuretic activity of the fraction might seem to be an osmotic type, as K⁺ content of the extract was high to account for the diuretic activity.

The theory that the majority of the medicinal plants have a diuretic

activity only due to the presence of the potassium seems somewhat doubtful [46]. In fact, pharmacodynamic studies performed on medicinal plants emphasized that frequently no correlation exists between the diuretic activity observed and the K⁺ content of the extract [47]. It is also possible that AQ_f might manifest cumulative effect due to the presence of one or several secondary active metabolites [48].

The exact nature of the active principles responsible for the diuretic effects of the extracts of the plant is, so far, not known. However, the qualitative phytochemical analysis carried out with AQ_f, nB_f and PE_f revealed active phytochemical groups such as saponin, tannin and terpenoid and the previous studies have demonstrated that these groups are responsible for diuretic activity by exerting favorable effects on physiological processes of the kidney such as by increasing potassium sparing capacity, binding with adenosine A1 receptor associated with diuretic activity or possibly by inhibiting tubular reabsorption of water and accompanying anions [49,50]. Previous studies have revealed that sesquiterpene lactones, triterpenes [51], tannins, saponins and organic acids [52] have diuretic activities. So, one can suppose that the identified compounds in the most active fraction of *C. edulis* might be responsible, at least in part, for the observed diuretic activity and that they may act individually or synergistically.

The diuretic activity may be produced by stimulating vasodilatation thereby increasing renal blood flow [53], or by producing inhibition of tubular reabsorption of water and anions [54]. However, at present, we do not have evidence in favor or against the operation of such potential mechanism of diuresis.

The data of our study do not show specific mechanisms involved in the observed effects. The AQ_f appears to have multiple mode of diuretic action. This could be due to the several secondary metabolites present in the AQ_f that act synergistically or antagonistically to produce a resultant effect. This hypothesis was confirmed by Kaniyas et al. [45] who demonstrated that the diuretic action of the most active fraction should not be attributed exclusively to the presence of their potassium content but also to other constituents. Multiple mode of diuretic action is reported with some herbal medications [5].

Our results showed that aqueous fraction produced no acute toxicity at the assayed dose, as evidenced by the absence of mortality of the animals during the study period and no macroscopic alterations were detected in the viscera of exposed animals. Therefore, the LD₅₀ of the fraction was greater than 5000 mg/kg body weight. Our results are in agreement with the findings of previous studies on mice models [25,54].

The limit test is used to determine if the toxicity of a test substance is above or below the specified dose. In this test, three to six female animals, which are more sensitive than male, are given a single oral dose of 2, or 5 g/kg. The majority of test animals are rodents (mice or rats). Although the results of the limit test may not be precise, this form of testing is usually sufficient to determine range of dose(s) at which LD₅₀ of the tested substance is located [36].

Conclusion

The present study supports the ethnomedical use of *C. edulis* as diuretic agent. The AQ_f of the methanol Soxhlet extract of the root bark of the plant showed significant diuretic activity while the PE_f and nB_f of the extract did not show significant diuresis at the tested doses in rats. Also, the study showed that the AQ_f is safe and did not cause any signs of toxicity at test dose in rats. However, the chemical constituents responsible for its diuretic activity are currently not determined. The AQ_f is safe for Therefore, further investigations are required to isolate and identify the compounds present in the root bark extract which are responsible for exhibit these therapeutic activities.

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