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Research Article

Diuretic activity assessment of an aqueous extract of *Hibiscus sabdariffa* (Malvaceae) leaves on *Wistar rats*

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Abstract

Hibiscus sabdariffa (Malvaceae) is a plant known as a diuretic. We undertook to evaluate the therapeutic effectiveness of this plant. Twenty *Wistar* rats aged between 6 and 8 weeks and weighing between 150 and 250 g were divided into five homogeneous batches of four rats. Batch 1 received only the saline overload, NaCl (50 ml/kg). Immediately after the saline overload, batches 2, 3, 4 and 5 received orally, furosemide, the standard diuretic (40 mg/kg bw), or single doses of 500, 1000 and 1500 mg/kg bw of the aqueous extract of *H. sabdariffa* leaves, respectively. The results showed that, 24 hours after the administrations, the urinary volumetric excretions of 123.06±11.07; 66.11± 6.19; 112.22±4.36 and 61.39±10.28% respectively. The increases in urinary volumetric excretion induced by the extract were statistically significant compared to the control rats (34.99±8.46%). The extract at a dose of 1000 mg/kg bw and furosemide showed statistically similar values of urinary volumetric excretion. This urinary excretion was accompanied by an elimination of Na^+ and K^+ ions similar to furosemide. The urinary measurement of creatinine and urea also revealed that the extract did not interfere with normal renal function during treatment. This study shows that the aqueous extract of *H. sabdariffa* leaves has diuretic and natriuretic activity similar to that of furosemide. This activity could be attributed to the presence of alkaloids, steroids and flavonoids, compounds known for their diuretic effects.

Keywords: *Hibiscus sabdariffa*, Furosemide, urinary excretion, diuretic

INTRODUCTION

The use of plants occupies an important place in the healthcare of many populations around the world.[1] This is partly due to socio-cultural habits and partly to the wealth of organic compounds of therapeutic interest that plant species contain.[2,3] As a result, 80% of the world's population treat their health problems with pharmacopoeia products.[4] This practice was reinforced by the census of african pharmacopoeia plants, which intensified after independence.[5] This survey showed that many traditional medicine plants are used to treat arterial hypertension (AH). Hypertension is a chronic non-communicable disease characterised by an increase in blood pressure (BP) above the normal range. This condition, which is common throughout the world, is the cause of numerous cardiovascular complications including strokes.[6] According to[7], the number of people suffering from hypertension worldwide could reach 1.56 billion by 2025. According to the Abidjan Heart Institute, Côte d'Ivoire recorded a prevalence rate of 38% in 2017. Apart from the modern treatment, people tend to use plants from the pharmacopoeia, particularly plants with diuretic properties.[8] However, few scientific studies have been carried out on a large number of plant species used to treat hypertension. Among these plants, *Hibiscus sabdariffa* (Malvacae) is a plant used in traditional medicine to treat a number of pathologies such as microbial infections and hypertension. This work was undertaken with a view to developing medicinal plants and establishing scientific basis for the use of *H. sabdariffa* (Malvacae) as a diuretic in the treatment of hypertension.

MATERIALS AND METHODS

Materials

Animal

Wistar rats, *Rattus norvegicus* (Muridae), weighing between 150 and 250 g were used. These animals are from the Animal PhysiologyLaboratory house at a temperature between 28±3°C with a 12-hour light/dark cycle.They were fed *ad libitum* with standard pellets produced and sold by IVOGRAIN® in Abidjan, Côte d'Ivoire, and had free access to water. The experimental protocols were followed in accordance with the protocol for the protection of experimental animals of the European Council legislation 2012/707 (EU, 2012).[9]

Plant

These are *Hibiscus sabdariffa* (Malvaceae) leaves, locally known as "dah". The leaves were collected from a field in Agboville, capital of the Agneby-Tiassa Region (Côte d'Ivoire). The plant has been identified and registered at the National

FloristicCentre at the Félix Houphouët-Boigny University in Cocody under the number: LLCJ011904.

Physiological solution and chemical

Solution of Sodium Chloride (NaCl, 9‰) was used to prepare all the solutions administered orally to the rats. The standard diuretic used was Furosemide, a loop diuretic.

Methods

Preparation of the aqueous extract of *Hibiscus sabdariffa* leaves

100 g of fresh *Hibiscus sabdariffa* leaves were washed in distilled water, ground using a blender and boiled for 15 minutes in one (1) litre of distilled water. The decoction was filtered twice on cotton wool and twice on Whatman filter paper. The filtrate obtained was dried in an oven set at 50°C for 48 hours. After drying, a fine water-soluble powder weighing 10g was obtained. This was the aqueous extract of *H. sabdariffa* leaves codified "EAHS". EAHS was used for pharmacological studies on diuresis and natriuresis.

Phytochemical study of the aqueous extract of *Hibiscus sabdariffa* leaves

The detection of the phytochemical compounds was based on the principle that they induce chemical reactions in the presence of appropriate reagents.[10] These tests were carried out using the analytical techniques described in previous works. [11, 12,] For these tests, a solution of the aqueous extract was prepared by dissolving 5 g of the aqueous extract of *H. sabdariffa* leaves in 50 ml of distilled water.

Experimental design of the diuretic activity studyof the aqueous extract of *Hibiscus sabdariffa* leaves

The diuretic activity of aqueous extract of *Hibiscus sabdariffa* leaves was determined using the method as follows.[13] Rats weighing between 150 and 250 g, without pre-treatment, divided into five (5) batches of four (4) were each placed in a metabolic cage. The animals were fasted 18 hours before the experiments start. EAHS was dissolved in a normal saline solution (NaCl, 0.9%) to obtain the concentrations required for oral administration.

The administration of the extract immediately followed that of the saline solution overload (50 ml/kg)administered as shown below.

- Batch 1: The rats received only saline solution overload and were considered as control group

- Batch 2: The rats received saline solution overload and were treated with furosemide (40 mg/kg bw)

- Batch 3: The rats received saline solution and were treated with EAHS (500 mg/kg bw)

- Batch 4: The rats received saline solution and were treated with EAHS (1000 mg/kg bw)

- Batch 5: The rats received saline solution and were treated with EAHS (1500 mg/kg bw)

The urine of the rats was collected; the quantity was measured every two hours and accumulated for twenty-four hours in each group. The diuretic activity was calculated as follows[14]

$$UE\ (ml/kg) = \frac{UV\ (ml)}{P\ (kg)}$$

With, **UE** = urine excretion, **UV**= urine volume and **P**= weight of the animal.

$$UVE\ (\%) = \frac{VE\ (ml)}{VA\ (ml)} * 100$$

With, **UVE** = urinary volumetric excretion, **VE**= volume excreted and **VA**= volume of the test substance administered.

$$DI = VE_{Treated}/VE_{Control}$$

DI: diuretic index and **VE:** volume excreted

$$SI = UEC_{Treated}/UEC_{Control}$$

SI: salidiuretic indexand **UEC:** urine electrolyte concentration

$$NI = UC_{Na+}/UC_{K+}$$

NI: natriuretic index and **UC:** urine concentration

Statistical analysis

The statistical analysis of the results and the graphical representation of the data were carried out using Graph Pad Prism 7 software (San Diego, California, USA). Statistical differences between the results were determined using analysis of variance (ANOVA) followed by the Turkey-Kramer multiple comparison test, with a significance level of $p<0.05$. All values are presented as mean ± SEM (Standard Error on the Mean).

RESULTS AND DISCUSSION

Results

Phytochemical study of the aqueous extract of *Hibiscus sabdariffa* leaves

Table I shows the results of the phytochemical screening of the aqueous extract of *Hibiscus sabdariffa* leaves (EAHS). It revealed the presence of several chemical compounds including sterols, polyterpenes, polyphenols, flavonoids, saponosides, alkaloids, gallic tannins and alkaloids. On the other hand, the absence of quinonic compounds and catechic tannins in EAHS was noted.

Table I: Phytochemical screening of the aqueous extract of *Hibiscus sabdariffa* leaves.

Compounds researched		Test or reagents	Results
Sterols and polyterpenes		Liebermann	+
Polyphenols		Ferric chloride	+
Flavonoids		Cyanidine	+
Saponosides		Foam test	+
Quinonic compounds		Borntraäeger	-
Alkaloids		Dragendorff	+
		Bouchardat	+
Tannins	Catechic tannins	Stiasny	-
	Gallic tannins	Hydrochloric acid	+

(+) : Presence of the compound; **(–)** : Compound not present

Diuretic activity of the aqueous extract of *Hibiscus sabdariffa* leaves in rats

Effect of the aqueous extract of *Hibiscus sabdariffa* leaves on the rats' urine excretion

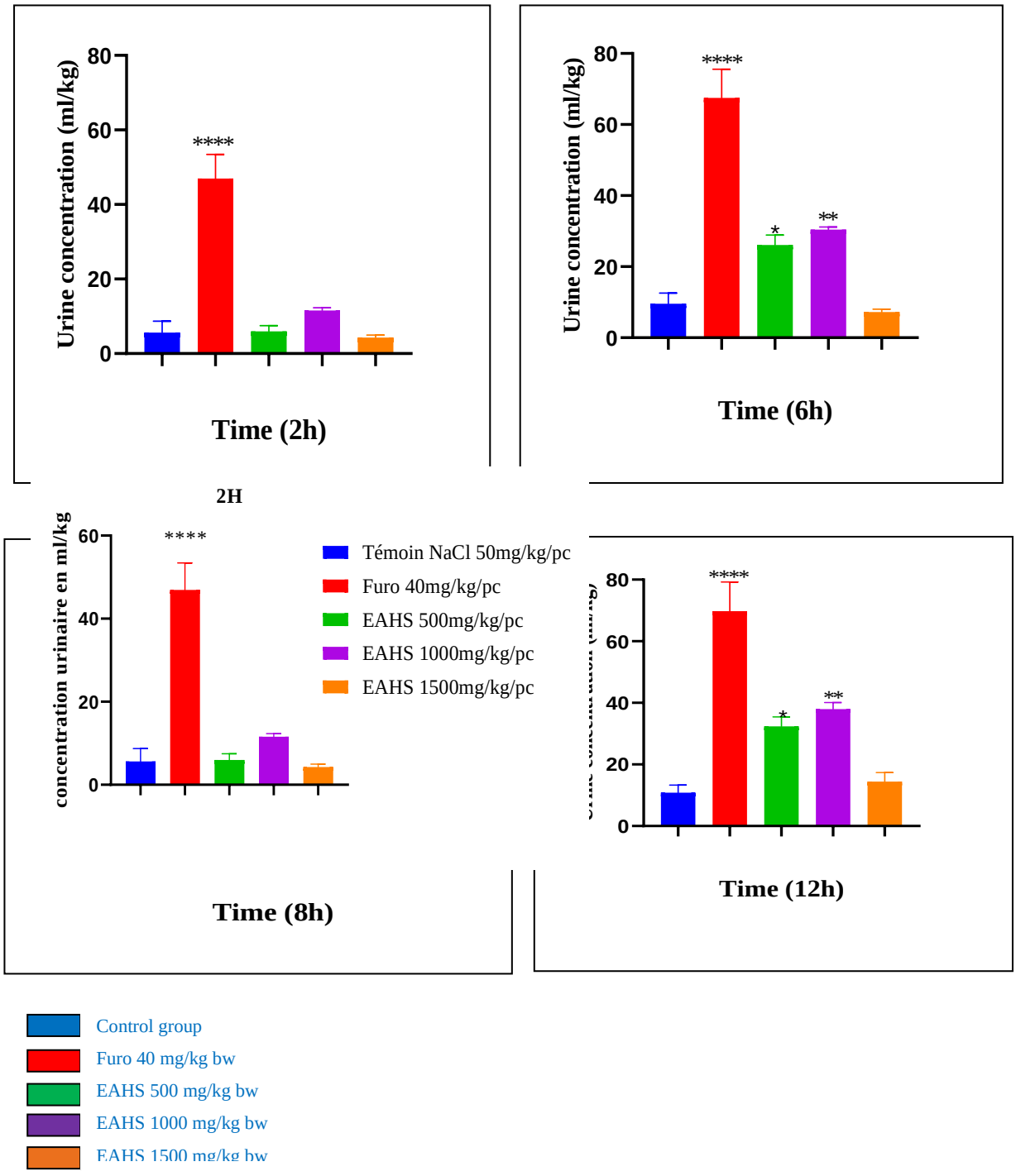
The results showed that, after two hours (2h), the urine volumes collected in the rats batches treated withthe extract different doses were not significantly different ($p>0.05$) to the control (5.63±3.1 ml/kg). However, the urineexcreted volumeobtained with furosemide (46.94±0.47ml/kg) was much higher ($p<0.0001$) than those obtained in the control batch. After six (6), eight (8) or 12 hours of experimentation, the dose of 500 and 1000 mg/kg bw doses of EAHS induced significant ($p<0.05$) and very significant ($p<0.001$) increases in urine excretion compared with the control respectively. For 500 mg/kg bw of EAHS, this increase was 259.33% ; 306.67% and 157.07% for urine excretions of 34.46±0.74 ; 39±0.76 ; 43.06±1.47 ml/kg at 6, 8, 12 h respectively. As for 1000 mg/kg bw, the extract increased by 172.05 ; 208.86 and 126.21% for urine excretions of 26.09 ± 3.82 ml/kg ; 29.62 ± 3.58 and 37.89 ± 3.17 ml/kg for the same hours. 1000 mg/kg bwof EAHs bw produced the highest urine excretion, 12 hours after administration. The dose of 1500 mg/kg/pc induced urine excretion that was statistically similar ($p<0.05$) to that of the control group. This was true throughout the experiment (Graph 1).

Effect of aqueous extract of *Hibiscus sabdariffa* leaves on the urinary volumetric excretion (UVE)

The different extract doses produced, 24 hours after their administration, urinary excretions of 44.5±3.34 ml/kg, 57.41±3.33 ml/kg and 24.17±1.8 ml/kg respectively, corresponding to urinary volumetric excretion of 66.11±6.19%, 112.22±4.36% and 61.39 ± 10.28%. Compared with the control (34.99±8.46%), the extract induced a statistically significant increase in urinary volumetric excretion. The urinary excretion produced with furosemide during the same period was 73.38±11.8 ml/kg, corresponding to a urinary volumetric excretion of 123.06±11.07%. This urinary volumetric excretion increased very significantly compared with the control. The extract doseof 1000 mg/kg bw and furosemide showed statistical similar values of urinary volumetric excretion. In addition, 1000 mg/kg bw of AEHsproduced the highest diuretic activity. The diuretic index obtained was 2.9 at this dose extract, that was statistically similar to that obtained with furosemide (3.9) (Table II).

Effect of EAHs and furosemide on urine electrolyte excretion

In order to monitor urinary electrolyte (Na^+, K^+ and Cl^-)leakage, three electrolytes were measured 24 hours after the administration of the various substances (Table III). EAHs, at doses of 500 and 1500 mg/kg bw, produced a significant increase ($p<0.001$) in urine Na^+ excretion after 24 hours compared with the control (213.33±1.2 mmol/L). The Na^+ elimination was 227.33 ± 2.03 and 237 ± 2.08 mmol/L respectively for the extract doses of 500and 1500 mg/kg bw. However, in rats treated with 1000 mg/kgbw of EAHs and in those given the furosemide (40 mg/kg bw),the natriureses determined were comparable to those of the control batch. Urine Na^+ excretion concentrations were 220±3.61 mmol/L, 207.67±1.33 and 213.33±1.2 respectively. In addition, EAHs induced non-significant ($p>0.05$) increases in urinepotassium and chlorine excretion at all extract doses compared to the control group. The 1000 mg/kg bw dose of the extract produced the highest diuretic activity. In fact, the diuretic index obtained was statistically similar to that of furosemide.



Graph 1: Urine excretion induced by different doses of EAHS and furosemide.

Values are represented as M ± SEM; n = 4; *p<0.05; **p<0.01; ***p<0.001; ****p<0.0001

Table II: Urinary excretion (UE) and urinary volumetric excretion (EUV).

	Substances				
Parameters	NaCl	Furo	EAHS 500	EAHS 1000	EAHS 1500
UE (ml/kg)	16.3±1.1	73.38±11.8***	44.5±3.45**	47.41±3.33**	24.17±1.8*
UVE (%)	34.99±8.46	123.06±11.07***	66.11±6.19**	112.22±4.36***	61.39±10.28**
DI	-	3.9	1.89	2.99	1.75

DI: Diuretic index; **EAHs:** Aqueous extract of *Hibiscus sabdariffa* leaves;**UE:** Urine excretion;**UVE:** Urinary volumetric excretion

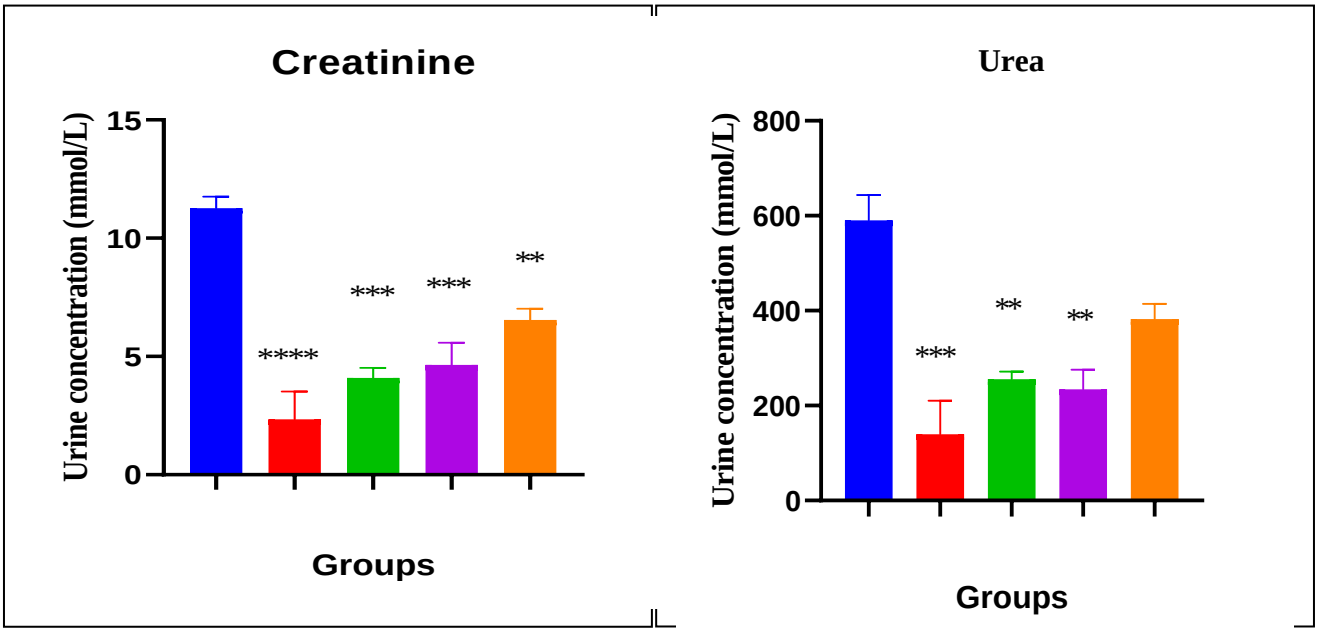
Table III : Urine electrolyte concentrations and urinary indices.

Parameters	Substances				
	NaCl	Furo	EAHS 500	EAHS 1000	EAHS 1500
Na^+ (mmol/L)	213.33± 1.2	207.67 ± 1.3	227.33 ± 2**	220 ± 3.61	237 ± 2.08**
K^+(mmol/L)	28.56 ± 4.01	13.17 ± 1.11**	30.53± 9.87	25.30 ± 0.92	40.40 ± 4.9
Cl^- (mmol/L)	201.68 ± 1	167 ±1**	219.33±1.8	205± 1.1	220.68 ± 2
SI	-	0.97	1.06	1.03	1.11
NI	7.47	15.77**	7.45	8.69	5.87

SI: salidiuretic index; **NI:** natriuretic index; **EAHS:** aqueous extract of *Hibiscus sabdariffa* leaves

Assessment of renal function

24 hours after the oral administration of the substances to the rats, te results of the levels of creatinine and urea excreted by urine route were measured and presented in Graph 2. In animals administered with furosemide (40 mg/kg bw), 500, 1000 and 1500 mg/kg bw of EAHs, creatinine values were 2.34±1.17, 4.10±0.41, 4.65±0.98 and 6.54±0.48 mmol/L respectively. Urea levels were 139.70±70.48, 255.50±16.01, 234.41±40.99 and 382.03±32.14 mmol/L respectively. Compared to the control, whose creatinine level was 11.27±0.49 11 mmol/L and urea 590.63±53.11 mmol/L, these values in treated animals showed high significant variations ($p<0.001$). These values decreased by 79.24%; 63.62%; 58.74%; 41.97% for creatininuria and by 70.63%; 56.74%; 60.31%; 35.32% for urine urea content with furosemide, EAHs 500, 1000 and 1500 mg/kg bw respectively.



Graph 2: Urine concentration of urea and creatinine after administration of different doses of EAHs and furosemide over 24 hours.

n =4; **p<0.01; ***p<0.001; ****p<0.0001

DISCUSSION

Phytochemical screening revealed the presence of polysterols, polyterpenes, polyphenols, flavonoids, saponosides, alkaloids and gall tannins. These results are comparable to those obtained from calyces.[14] These authors showed the presence of anthocyanins, polysterols and polyterpenes. A study of the diuretic activity of aqueous extract of *hibiscus sabdariffa* leaves in rats revealed an increase in urinary volumetric excretion (UVE) comparable to that of furosemide. Diuretics that mimic the effect of furosemide act on the $Na^+/K^+/Cl^-$ pump at the loop of Henle, and inhibit sodium and water reabsorption at the loop of Henle, as does furosemide.[15] They influence the urine dilution-concentration mechanism to encourage high diuresis. These diuretics mayantagonise the action of antidiuretic hormone (ADH). ADH is responsible for regulating the reabsorption of water from the filtrate in the renal collecting tubules in order to maintain the body's osmolarity by inhibiting urinary excretion. EAHs also causes urinary leakage of sodium and potassium. These urinary electrolyte excretions were greater than those caused by furosemide. The effects of EAHs are similar to those induced by the aqueous extract *Ficus exasperata* (Moraceae), Rosmarinus officinalis (Lamiaceae) and Centaurium erythraea (Gentianaceae).[16,17]The natriuresis and chloriuresis induced by EAHs are greater than those induced by furosemide. This would explain the relatively high salidiuretic and natriuretic properties of the studied extract. The urine potassium

concentration induced by EAHs was low, as was that induced by furosemide. This low potassium concentration suggests that potassium excretion is spared. This "potassium-sparing" property has also been reported in the diuretic effect of furosemide. EAHs is thought to contain a large amount of potassium, giving it the advantage of apotassium-sparing diuretic effect.[18] EAHs induces normal urine excretions of creatinine and urea similar to those of the control and relatively lower than those induced by furosemide. These different eliminations of urea and creatinine are thought to result from an increase in their glomerular filtration rate. The effects of EAHs are similar to those induced by the aqueous extract of *Spergularia purpurea* leaves (Caryophyllaceae).[19] These authors reported that this extract resulted in an increase in glomerular filtration rate, associated with an increase in creatinine clearance.The leaves of the *H. sabdariffa* plant have a diuretic effect. Not only do they promote better excretion of water and electrolytes (Na^+, Cl^-, K^+), but above all they increase glomerular filtration rate due to their effects on the renal purification function, as do *Ficus exasperata* (Moraceae) and *Bridelia ferruginea* (Euphorbiaceae). These diuretic plants are used as first-line treatments to moderate hypertension.[20,21]

CONCLUSION

The studies of the pharmacological effects of aqueous extract of *Hibiscus sabdariffa* leaves on diuresis have shown that it has diuretic and natriuretic activity comparable to that of furosemide, the reference diuretic. This activity could be attributed to the presence of alkaloids, steroids and flavonoids, compounds known for their diuretic effects. These results support the use of this plant as a diuretic in the treatment of high blood pressure.

Author's contribution:

All authors have contributed equally to the work

Conflicts of Interest:

The authors declare no conflicts of interest involved in this study

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