

Original Research Article

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## Diuretic Activity of the Aqueous Extract Leaves of *Crassocephalum crepidioides* (Asteraceae) in Rats

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### ABSTRACT

*Crassocephalum crepidioides* is a medicinal plant used in west and central Africa, not only as leguminous crop, but also to treat high blood pressure in particular. The objective of this work was to evaluate the diuretic activity of aqueous leaves extract of *C. crepidioides* in the treatment of high blood pressure as claimed by traditional healers. Rats both male and female were used to evaluate the diuretic activity. *C. crepidioides* was administered to rats at 25, 50, 75, 100 and 125mg/kg. The volume of urine was measured after 3, 6, 12 and 24h following there administration of the aqueous extract. The two positive control groups received Furosemide and Hydrochlorothiazide amiloride respectively, whereas the negative control group received distilled water (1ml/100g of body weight). The urinary and blood concentrations of creatinine, urea, ALAT, ASAT and electrolytes (Na<sup>+</sup>, k<sup>+</sup>, Cl<sup>-</sup>) were evaluated using a spectrophotometer. The significant increase of diuresis was accompanied by that of electrolytes excretion (Na<sup>+</sup>, k<sup>+</sup>, Cl<sup>-</sup>), food and water consumption, creatinine and urea excretions. The increase in elimination of Na<sup>+</sup>, k<sup>+</sup>, Cl<sup>-</sup> induced by the extract provoked the alkalization of urine. In the blood, a significant increase in the rates of electrolyte and urea, as well as a decrease in transaminase rate was noticed. However, no significant change was observed as far as the body weight and temperature, the pH of urine and the rate of creatinine serous in the animals treated with the aqueous extract of *C. crepidioides*. This work strongly indicated that *C. crepidioides* possesses a diuretic activity thus justifies its utilization by traditional healers for the cure of high blood pressure.

#### Keywords

*Crassocephalum crepidioides*,  
Diuretic and saluretic activities,  
Urinary volume,  
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### Introduction

Cardiovascular diseases (CVD) are diseases affecting the heart and blood vessels (veins and arteries). These diseases are the leading cause of death in the world. More people die

each year from cardiovascular diseases than from any other diseases<sup>1</sup>. An estimated 17.5 million deaths from cardiovascular diseases are responsible for 31% of total global mortality<sup>2</sup>. In CVD, there are several pathologies amongst which are; obesity, heart

rhythm disorders, aneurysm, hypertension (High blood pressure), infarction and many others. In addition to these pathologies, we also have risk factors that are classified into two categories: non-modifiable risk factors (gender, age, family history) and modifiable risk factors (hypertension, dyslipidemia, overweight, tobacco, alcohol, diabetes). High blood pressure is not only a disease, but it is also an important risk factor for the development of other cardiovascular diseases<sup>3</sup>.

High blood pressure is an abnormal rise in arterial wall pressure (systolic blood pressure  $\geq 140$  mmHg and / or diastolic blood pressure  $\geq 90$  mmHg)<sup>4</sup>. It is a cardiovascular disease system that has reached worrying proportions<sup>5</sup>. Recent data from the WHO indicate that nearly one billion people around the world suffer from this disease. Out of 972 million hypertensive patients, 34.3% come from developed countries and 65.7% from developing countries, as a result of inadequate hygienic practices<sup>6</sup>. Given these high statistics, it is therefore imperative to find treatments to counteract this great killer.

In general, the management of hypertension is a lifelong process<sup>7</sup>, the treatment reduces mortality and cardiovascular morbidity<sup>8</sup>. Six classes of drugs, called antihypertensives, are used for the modern treatment of hypertension among which diuretics are the oldest and best evaluated drugs. The expensive cost of these antihypertensive drugs, their undesirable side effects, as well as the increase in poverty are among other reasons that lead patients to abandon conventional health centers for the benefit of traditional medicine<sup>9</sup>. In order to promote the diversity of plant species of Cameroon, a set of questionnaires was given to the traditherapists of the Mifi Department, in the west region Cameroon in 2017. Following the survey, several plants were partly listed for the treatment of hypertension.

Considering the repeated use of *C. crepidioides* by several local healers, we decided to conduct research on this plant.

*Crassocephalum crepidioides* is a plant of the Asteraceae family, for which its leaves and stems are tender and fleshy mucilaginous, and are used not only in the treatment of cardiovascular diseases (e.g. arterial high blood pressure), but also as a vegetable in soups in the West and Central Africa. It is also indicated as an antibacterial plant<sup>10</sup>. It is a powerful antioxidant and protects against hepatotoxicity<sup>11</sup>. It has been shown to have anti-tumor activity<sup>12</sup>. The present study aims at evaluating the diuretic activity of the aqueous extract of *C. crepidioides* leaves in the treatment of hypertension as claimed by traditional healers and also to valorize the pharmacopeia and the flora Africa.

## **Materials and Methods**

### **Plant material**

*C. crepidioides* leaves were harvested from Bafoussam in the west region of Cameroon on June 30th 2017, and were identified at the National Herbarium of Cameroon (NHC) under registration number 20487/NHC.

### **Animal material**

Wistar rats strain (130 - 180 g) of two sexes, aged 2 to 3 months, originated from the Laboratory of Medicinal Plants, Health and Galenic Formulation of the Faculty of Sciences of the University of Ngaoundéré were used for this study. The living conditions of animals were in such a way that the environmental temperature was controlled at  $25 \pm 1.5^\circ\text{C}$ , while the relative humidity was  $45\% \pm 10\%$ . Animals had a free access to water and food. All animals were subjected to a surgery under the supervision of a veterinary surgeon from the School of

Veterinary Medicine, University of Ngaoundéré. Whereas the experimental procedure protocols were approved by the Institute of Animal Committee, the research was approved by the Animal Ethics Committee of the University of Ngaoundéré.

### **Drugs**

Furosemide (2 mg/kg) and Amiloride Hydrochlorothiazide (AHCT) (14 mg/kg) were used as reference pharmacological substances.

### **Preparation of the aqueous extract of the leaves of *C. crepidioides***

Fresh leaves of *C. crepidioides* were washed and a sample of 1kg was weighed and macerated in 1L of water at room temperature for 12hrs before the filtration process using a Wattman paper N° 3. A total volume of 1.60 L of extract obtained was lyophilized at -40°C to yield 26 g of powder that was stored at -20°C.

### **Phytochemical study**

Analytical tests for the identification of different group of metabolites in crude extracts of the leaves were made at the Institute of Medicinal Plants for Medicinal Research, Cameroon. The procedures described by Chen *and al*<sup>13</sup> were used for the detection of various chemical groups. In view of the identification of the chemical structure of the compounds responsible for the diuretic activity, preliminary tests of the phytochemical study were conducted. Briefly, Essential oils from the aqueous extract of *C. crepidioides* were extracted with hexane. These extracts were then stitched onto plates of thin layer chromatography on silica, the first disclosure was obtained by ultraviolet radiation (254 nm and 365 nm) and then with vanillin.

### **Diuretic activity**

A preliminary screening was carried out to select "well urinated" rats. In the process, 1 ml /100g body weight of distilled water was orally administered to each rat. Treated rats were individually placed in metabolic cages, to undergo the collection of urine that was measured after every 6 hours. All animals that excreted more than 40% of the volume of distilled water administered were selected, while those that excreted less were excluded<sup>14</sup>. After selection, 30 rats were divided into 6 groups of 5 rats, each of which was then individually placed in metabolic cages under acclimation for 7 days.

Furthermore, the aqueous extract of *C. crepidioides* leaves was administered to the rats at doses 75, 100 and 125 mg/kg of body weight using an esophageal probe. Animals in the control group were treated with distilled water (negative control), Furosemide (2 mg / kg) and Amiloride hydrochlorothiazide (14 mg/kg) (positive controls). All treated animals were individually placed in metabolic cages and submitted to a special urinary collection device. The urinary volume was determined at 0hr, 3hrs, 6hrs, 12hrs and 24hrs after administration of the single dose of the extract or reference drugs. All experimental procedures were approved by the institute of Ethical committee of Department of Biological Science of the University of Ngaoundéré (ECDBSUN 15/01/2015/UN/FS/DSB).

### **Parameters**

Treated animals were sacrificed by decapitation. Blood was collected in the heparinized EDTA tubes. The serum was separated by centrifugation at 3000 rpm for 15 minutes and then stored at -20°C for the determination of the biochemical parameters such as creatinine, urea, sodium ion,

potassium ion, chloride ion, ALAT and ASAT.

The urinary concentrations of sodium and potassium ions were determined using the photometric flame (Jenway, PFP 7) as described by the standard method<sup>15</sup>. Creatinine (Creat), urea, and electrolyte concentrations in the serum and urine samples were evaluated using a bidirectional mass spectrophotometer. (Secomam RS 232C, France)<sup>16</sup>. In animals treated with the extract and reference substances, urinary osmolarities and natriuresis were measured during the diuretic response, particularly when at the maximum excretion rate.

The osmolarity of plasma and urine samples was measured by cytometry using an osmometer (Knauer). Osmolar clearance (Cosm) was determined from plasma osmolality (POSM), urinary osmolarity (Uosm) and urine flow (V) according to the following formula:  $\text{Cosm} = \text{Uosm} \cdot \text{V} / \text{POSM}$ . The GFR (glomerular filtration rate) was assessed by creatinine clearance (CreatC). The amount of  $\text{Na}^+$  and  $\text{K}^+$  was calculated as a parameter for the saluretic activity. The ratio of  $\text{Na}^+ / \text{K}^+$  was calculated for the natriuretic activity. To estimate the carbonic anhydrase inhibition activity, the ratio of  $\text{Cl}^- / (\text{Na}^+ + \text{K}^+)$  was calculated<sup>17</sup>.

### **Measurement of Body Temperature**

The body temperature was measured using a digital thermometer. The sensor tip of the thermometer was introduced and held for 2 minutes in the anus of the animal. Animals with a body temperature between 35-38°C were selected while those with a temperature below or above this range were eliminated. This temperature was measured before and after 24hrs of administration of the aqueous extract.

### **Statistical analysis**

Results were expressed as mean  $\pm$  standard deviation. The comparison of the averages was made using the ANOVA one-way test followed by the Krustal-Wallis test. The variance analysis software was Statgraphics Centurion XV. The difference was either not significant for  $P > 0.05$ , or significant for  $P < 0.05$ . Excel software was used in the plotting of graphs.

### **Results and Discussion**

#### **Phytochemical study**

Phytochemical screening performed on crude extracts with methanol and ethyl acetate revealed the presence of several primary and secondary metabolites such as fatty acids, sugars and carotenoids, Athraquinones, glycosides, anthracene, saponins, alkaloids, tannins, Coumarins. Phenolic compounds including triterpenes, volatile oils and sterols are also present in the extract. The presence of flavonoids and alkaloids is remarkable. The result of the phytochemical studies is represented in Table 1. Four fractions were collected and analyzed to find out the presence of alkaloids, tannins, flavonoids, and Saponins. The fractions were eventually pooled into four and numbered A, B, C, and D based on their predominant phytochemical reactions as shown in Table 1. These initial observations and findings suggest that the aqueous extract of leaves of *C. crepidioides* contains several chemical compounds which have biological potential activity hence deserves further investigation.

#### **Effect of the aqueous leaves extract of *C. crepidioides* on the effective doses on urinary volume**

A single dose-response administration of the aqueous extract of *C. crepidioides* (25, 50, 75,

100 and 125 mg/kg) showed a significant increase ( $P < 0.05$ ) in the volume of urine 24 hours later. However, the urinary excretion was dose dependent (Figure 1). Screening of leaves extract doses showed a significant ( $p < 0.05$ ) increase in the volume of urine excreted at 3hrs, 6hrs, 12hrs and 24hrs after administration. At the dose of 100 mg/kg, the volume of urine increased by 106.1%. The highest dose (125 mg/kg), induced 115.8% increase in the volume of urine of the treated group. Only 36.33% overload was eliminated in the negative control group, whereas HCTZ eliminated 76.65 % and furosemide 87.87% of urine after 12hrs ( $P < 0.05$ ) of administration. During the experiment, water consumption was significantly ( $P < 0.05$ ) increased in the rats subjected to plant extract treatment (from  $22.8 \pm 2$  and  $25.8 \pm 2.8$  mL/day), respectively for doses of 100 and 125mg.kg, and those treated with reference drugs ( $14.2 \pm 4.6$  mL/day for furosemide and  $13 \pm 4.2$  mL/day for amiloride) compared to distilled water treated rats.

#### **Effect of the aqueous leaves extract of *C. crepidioides* on the lag time of urinary excretion**

The lag time significantly decreased in animals treated with aqueous extract at 100 mg/kg (42.5%), 125 mg/kg (50%), as well as in animals which were administrated with standard pharmacological drugs (55%) compared to the control group (Figure 2). The overload eliminated after 1hr by rats treated with the three highest doses of *C. crepidioides* aqueous extract tested (75, 100, and 125 mg/kg) were 110%; 42.8% and 56.7% respectively whereas HCTZ eliminated 51.04% furosemide 61.87% and control 18.31 % ( $P < 0.05$ ) (Figure 2). The extract displayed a dose-dependent decrease as the first urination latency in 17.23 min ( $P < 0.05$ ), 15.35 min ( $P < 0.01$ ), and 13.22 min ( $P < 0.01$ ) at doses 75, 100, and 125 mg/kg. Negative

control group average first urination latency was 45, 21 min, furosemide 20.66 min ( $P < 0.01$ ), and HCTZ 22.45 min ( $P < 0.05$ ) (Figure 1). All treatments induced at least a 3-fold increase in the overload elimination compared with negative control group after 60 s. Only 30.54% overload was eliminated in the negative control group, whereas HCTZ eliminated 85.34 % and furosemide 90.66% ( $P < 0.05$ ).

#### **Effect of the aqueous leaves extract of *C. crepidioides* on cumulated urine volume excreted and pH**

Figure 3 shows the changes on the cumulated volume of urine excreted and pH by rats 24hrs later, following treatment with various doses of *C. crepidioides* aqueous extract, administration of an equivalent volume of distilled water (negative controls), or treatment with one of the two diuretic drugs used as positive controls (furosemide, HCTZ). There was a significant increase ( $p < 0.05$ ) in the cumulated volume of urine excreted in 24hrs in animals treated with the plant extract (100.2%, 106.12% and 115.82% respectively for 75, 100 and 125 mg/kg) compared to the negative control group. In rats administered with the reference pharmacological substances, a significant increase in urinary volume (122.04 % and 50.97% respectively for furosemide and AHCT) was observed. The pH values ( $6.6 \pm 0.1$ ) of urine of rats treated with the extract of *C. crepidioides* were higher than the control group  $6.2 \pm 0.2$ ). However, the pH values ( $6.7 \pm 0.1$ ) of urine of animals treated with the extract were lower than that of rats treated with pharmacological substances ( $7.0 \pm 0.1$ ) (Figure 3).

#### **Effect of the aqueous leaves extract of *C. crepidioides* on the rectal temperature after 24hrs**

It was noticed that, 24 hours after treatment, the initial and final rectal temperatures varied



slightly and insignificantly ( $p > 0.05$ ). The difference between the initial and final temperatures at different doses compared to the control group (ED) showed no significant difference ( $p > 0.05$ ) (Figure 4).

### **Electrolyte excretion**

#### **Effects of *C. crepidioides* on cumulative urinary excretion of sodium ions ( $\text{Na}^+$ )**

During the experiment, the significant increase in urinary volume ( $p < 0.05$ ) and urinary rate of sodium excretion was dose dependent (387.56%, 423.56% and 482.46% respectively for 75, 100 and 125mg/kg per day compared to the control) (Figure 5). The Amiloride hydrochlorothiazide (14 mg/kg) induced a significant increase in sodium ions excretion ( $90.23 \pm 3.11$  mEq/kg/24h) compared to the control group ( $30.56 \pm 3.22$  mEq/kg/24h). Like Amiloride hydrochlorothiazide, Furosemide also induced a significant increase ( $180.4 \pm 1.13$  mEq/kg/24h) compared to the control group ( $30.56 \pm 3.22$  mEq/kg/24h).

#### **Effects of *C. crepidioides* on cumulative urinary excretion of potassium ions ( $\text{K}^+$ )**

Figure 6 below indicates that the aqueous leave extract of *C. crepidioides* caused a significant increase ( $p < 0.05$ ) in urinary excretion of potassium ions after 24hrs, of 13.43%, 99.41% and 139.56% respectively at the doses of 75, 100 and 125 mg/kg compared to the control group.

Urinary excretion of potassium ions increased from  $17.23 \pm 2.68$  mEq/kg/24h in control group to  $25.33 \pm 3.33$  mEq/kg/24h at dose 125 mg/kg. whereas, animals treated with furosemide also showed a significant increase in urinary excretion of potassium ions ( $37 \pm 1.21$  mEq/kg/24h) compared to the control group ( $17.23 \pm 2.68$  mEq/kg/24h) (Figure 6).

#### **Effects of *C. crepidioides* on cumulative urinary excretion of chloride ( $\text{Cl}^-$ )**

Aqueous extract of *C. crepidioides* showed a significant increase in the excretion of chloride ions. In figure 7 it was noticed that the urinary excretion of chloride ions increased significantly and proportionately to the concentrations of the extract of 233.33%, 255.35% and 338.96% respectively at the doses of (75, 100 and 125 mg/kg) compared to the negative control group.

This significant increment reached a 443.03% rate in animals treated with furosemide and 192.6% in the group treated with the AHCT compared to negative control.

Excretion of chloride ions increased from  $23.92 \pm 2.35$  mEq/kg/24h in negative control to  $104.99 \pm 3.5$  mEq/kg/24h at the dose of 125 mg/kg (Figure 7).

#### **Effect of the aqueous leaves extract of *C. crepidioides* on the natriuretic, saluretic and CAI activities in urine**

During the experiment, the natriuretic activity ( $\text{Na} + \text{K} +$ ) ( $p < 0.05$ ) in rats treated with aqueous extract at 75mg/kg (156.06%), 100mg/kg (221.33%) and 125mg/kg (187.86%) showed a significant increase compared to the negative control group (distilled water). In animals treated with furosemide and amiloride, there was a significant increase ( $p < 0.05$ ) in natriuretic activity from 94.14% to 143.09% compared to control group.

As far as the saluretic activity is concerned, the increment was significant ( $p < 0.05$ ) from 332.17%, to 352.1% and 395.6% with equivalent doses of 75, 100, 125 mg/kg respectively compared to animals group treated with distilled water. In positive control group (animals treated furosemide and

AHCT), the saluretic activity was also significantly increased from 450.7% to 110.35% for furosemide and amiloride compared to control group (distilled water).

The aqueous leaves extract at doses of 75, 100 and 125 mg/kg resulted in a significant decrease ( $p < 0.05$ ) in the CAI activity by 35.5%, 35.5% and 28% respectively, compared to the control group (distilled water). The administration of furosemide (7%) and amiloride (5.17%) indicated a significant decrease ( $p < 0.05$ ) in CAI activity in treated animals compared to the untreated group.

The natriuretic and saluretic index was significantly ( $p < 0.05$ ) elevated in the rats treated with the aqueous leaves extract and in rats treated with reference drugs compared to animals treated with distilled water (index = 1.00). On the other hand, there was no significant decrease ( $p > 0.05$ ) in CAI index within animals treated with the aqueous leaves extract and the reference pharmacological substances compared to the control group (CAI index = 1) (Table 2).

### **Effect of the aqueous leaves extract of *C. crepidioides* on the kidneys functioning index**

Table 3 below shows the effect of the aqueous leaves extract of *C. crepidioides* on the kidneys functioning index. The significant increase in creatinine in urine of animals treated with furosemide was 180.80%, and 112.6% in those animals treated with amiloride compared to that of the control group. Similarly, the doses of 100 and 125 mg/kg indicated a significant increase of 22.22% and 204.7% respectively compared to the negative control.

Creatinine clearance significantly decreased ( $p < 0.05$ ) and was dose-dependent at 75mg/

kg (28.02%), 100mg/kg (200%) and 125mg.kg (542.8%) compared to the control group. This was also observed in animals treated with furosemide and amiloride, which have a creatinine clearance of  $0.021 \pm 0.02$  and  $0.018 \pm 0.019$  mL/min respectively, compared to  $0.9 \pm 0.01$  mL /min in animal treated only with distilled water as the control.

The glomerular filtration rate (GFR) significantly decreased ( $p < 0.05$ ) by 148.4% for furosemide and 238% for AHCT compared to control. The aqueous extract of *C. crepidioides* caused a significant decrease ( $p < 0.05$ ) of the glomerular filtration rate by 91.56% at 75mg/kg, 341.6% at 100mg/kg and 783.3% at 125mg/kg, and was dose-dependent compared to the control group.

The amount of filtrated urea increased significantly ( $p < 0.05$ ) in furosemide-treated animals (33.08%) and in those administrated with AHCT (30.8%) compared to animals treated with distilled water. In the same vein, the plant extract at 125 m /kg resulted in a significant ( $p < 0.05$ ) increase in the amount of filtrated urea (17.58%) compared with distilled water treated animals.

The Uosm decreased significantly compared to the control rats ( $P < 0.05$ ), with percentages of 79.08%, 72.81%, and 56.35% respectively for extract of *C. crepidioides* doses of 75, 100 and 125 mg/kg, 15.20% for furosemide and 24.68% for AHCT.

Furosemide and AHCT significantly increased ( $P < 0.05$ ) Cosm by 43.46% and 60.87% respectively compared to the control group. Cosm of rats treated with *C. crepidioides* doses of 75 and 100 mg/kg decreased significantly ( $P < 0.05$ ) by 6.91% and 2.23% respectively compared to control group. The dose of 125 mg/kg did not show any significant increase in Cosm.  $C_{H_2O}$

significantly increased by 16.35%, 19.98% and 23.63% respectively at the highest dose of extract (125 mg/kg), AHCT and furosemide (Table 3).

**Effect of the aqueous extract of the leaves of *C. crepidioides* on biochemical parameters of blood**

Several biochemical parameters were evaluated in the blood of rats treated with aqueous leaves extract of *C. crepidioides*, reference pharmacological substances (furosemide and AHCT) and in animals treated with distilled water (Table 4). The concentrations of Na +, K + and Cl- ions increased significantly (p < 0.05) at all aqueous leaves extract doses.

The animals treated with the furosemide and AHCT equally show a significant increase (P < 0.05) in creatinine and urea levels (P < 0.05) (28.57% and 4% for the furosemide; 25.39% and 12% for the AHCT) compared to the control test.

There was a significant increase in the rate of urea in the blood from 41%, 42.8% and 43 % respectively for the doses of 75, 100 and 125 mg/kg compared to the control test. In contrast, no significant change in the rate of creatinine was observed in the animals that were treated with an aqueous extract.

**Effect of the aqueous leaves extract of *Crassocephalum crepidioides* on transaminase levels in blood and urine**

Table 5 below shows the effect of the aqueous leaves extract of *C. crepidioides* on the amount of ALAT and ASAT in the blood and in urine. The aqueous extract at all doses resulted in a significant decrease (p < 0.05) of serum transaminase levels (317.02%, 318.07% and 320.12% for ALAT and 146.22%, 150.12% and 150.1%) for ASAT, respectively at doses 75, 100 and 125mg/kg) compared to the control animals. In urine, no significant difference was observed in the level of transaminase between the aqueous extract treated-rats and the distilled water treated-rats.

**Table.1** Phytochemical screening Result, quantitative analysis and reactions of the four fractions

Phytochemicals	Indication of leaves extract	Content (%)	Fractions			
			A	B	C	D
Anthocyanin	+	0.693±0.012	-	-	-	-
Glycoside	+	0.312±0.025	-	-	-	-
Flavonoid	+++	16.210±0.162	+++	-	-	-
Alkaloid	++	11.530±0.187	-	++	-	-
Phenol	++	0.457±0.035	-	-	-	-
Steroids	++	0.374±0.033	-	-	-	-
Tannins	+	0.268±0.048	-	-	-	++
Saponins	++	5.345±0.057	-	-	++	-

+++, presence of bioactive compound in very high concentrations;

++, presence of bioactive compound in high concentrations;

+, presence of bioactive;

-, absence of bioactive compound



**Table.2** Natriuretic, saluretic and CAI activities in the urine

	Doses (mg / kg)	Natriuretic activity (Na <sup>+</sup> /K <sup>+</sup> )	Saluretic activity (Na <sup>+</sup> + Cl <sup>-</sup> )	CAI activitic (Cl <sup>-</sup> / (Na <sup>+</sup> +K <sup>+</sup> ))	Natriuretic index	Saluretic index	CAI index
control	ED	2,39 ± 0,1	57,28 ± 1,76	0,61 ± 0,63	1.00	1.00	1.00
	75	6,12 ± 6,92*	247,55 ± 1,5*	0,45 ± 0,58*	2.56*	4,32*	0,73
extract	100	7,68 ± 0,50*	258,97 ± 0,51*	0,45 ± 1,21*	3.21*	4,52*	0,73
	125	6,88 ± 11*	283,57 ± 3,04*	0,48 ± 1,03*	2.87*	4,95*	0,78
FURO	5	4,64 ± 0,78*	315,48 ± 3,98*	0,57 ± 0,19*	1.94*	5,5*	0,93
AHCT	14	5,81 ± 0,43*	167,63 ± 2,63*	0,58 ± 0,35*	2.43 *	2,92*	0,95

Each value represents the mean ± SEM, n = 5. (\*P < 0.05) significant difference compared to the control group (ED = Distilled Water). FURO = Furosemide, AHCT = Amiloride Hydrochlorothiazide, CAI = Inhibition of Carbonic Anhydrase

**Table.3** Effect of the aqueous leaves extract of *C. crepidioides* on the kidneys functioning index

Dose (mg / kg)	control	75	100	125	Furosemide	AHCT
Creatinine (mg/dl)	0,63±0,31	0,32±0,04	0,77±0,38*	1,92±0,35*	1,77±0,05*	1,34±0,05*
Creat C (ml/min)	0,9±0,01	0,703±0,75*	0,30±0,13*	0,14±0,11*	0,021±0,02*	0,018±0,019*
GFR (ml/min)	1,59±0,41	0,83±0,90*	0,36±0,16*	0,18±0,14*	0,64±0,07*	0,47±0,07*
Urea (mg/dl)	17,12±2,33	14,42±9,05	18,24±4,87	20,13±5,59*	22,81±1.7*	22.4±1.3*
Uosm (mOsmol/kg)	197±9	110±11*	114±15*	126±12*	171±12*	158±16*
Cosm (mL/min)	0.046±0.005	0.043±0.012*	0.045±0.012*	0.048±0.017	0.074±0.021*	0.066±0.011*
CH <sub>2</sub> O (mL/min)	0.055±0.011	0.054±0.012	0.056±0.018	0.064±0.016*	0.068±0.011*	0.066±0.013*

Each value represents the mean ± ESM, n = 5. \* P < 0.05 significant difference compared to control (ED = Distilled Water). Cl Crea = Creatinine Clearance, DFG = Glomerular Filtration Rate, AHCT = Amiloride Hydrochlorothiazide, mEq = milliequivalent

**Table.4** Effect of the aqueous extract of the leaves of *Crassocephalum crepidioides* on biochemical parameters of blood

	Doses (mg/kg)	creatinine (mg.dl)	urea (mg.dl)	Na <sup>+</sup> (mEq/L)	K <sup>+</sup> (mEq/L)	Cl <sup>-</sup> (mEq/L)	Posm (mOsmol/kg)
control	ED	0,63 ± 0,31	2,5 ± 0,14	22,39 ± 2,72	5,02 ± 0,51	13,85 ± 1,61	252.12±21.21
	75	0,92 ± 0,3	2,91 ± 0,73*	60,17 ± 0,40*	17,46 ± 0,53*	78,72 ± 0,82*	257.23±2.23*
extract	100	0,64 ± 0,27	2,93 ± 1,02*	59,5 ± 0,63*	18,59 ± 0,55*	65,68 ± 1,52*	262.43±31.43*
	125	0,58 ± 0,028	3,57 ± 1,03*	54,62 ± 0,84*	17,72 ± 0,75*	68,88 ± 1,52*	267.62±42.67*
FURO	5	0,81 ± 0,25*	2,6 ± 0,14*	39,95 ± 1,6*	9,94 ± 1,39*	15,09 ± 0,51*	268.82±32.84*
AHCT	14	0,79 ± 0,08*	2,8 ± 0,14*	29,23 ± 2,33*	7,84 ± 0,39*	10,59 ± 0,83*	267.35±44.57*

Each value represents the mean ± ESM, n = 5. \* P < 0.05 significant difference compared to control (ED = Distilled Water), AHCT = Amiloride Hydrochlorothiazide, mEq = milliequivalent

**Table.5** Effect of the aqueous leaves extract of *C. crepidioides* on the amount of ASAT and ALAT in the blood and in urine

		Control		Extract (mg/kg)	
		ED	75	100	125
urine	ASAT	46,66 ± 0,82	44,744 ± 1,1	42,733 ± 0,34	40,644 ± 1,72
	ALAT	59,74 ± 1,71	48,016 ± 0,15	48,595 ± 0,24	46,732 ± 1,8
Blood	ASAT	92,20 ± 3,72	37,445 ± 0,04*	36,859 ± 1,17*	36,853 ± 1,84*
	ALAT	183,20 ± 3,72	43,93 0,9*	43,748 ± 0,53*	43,606 ± 1,67*

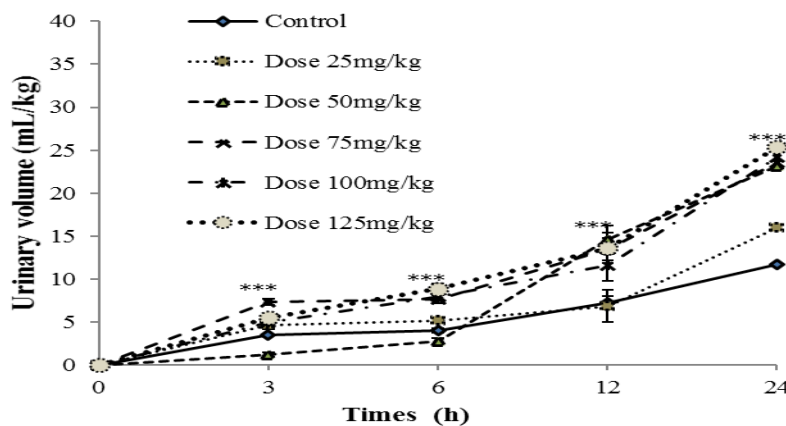
Each value represents the mean ± ESM. \* P < 0.05 significant difference compared to control (ED = Distilled Water). ALAT = Alanine Aminotransferase, ASAT = Aspartate Aminotransferase

**Table.6** Effect of the aqueous leaves extract of *C. crepidioides* on the urine output index and electrolytic excretion index in 24 hours of urine collection

Drugs (mg/kg)	Diuretic index	Na <sup>+</sup> index	Cl <sup>-</sup> index	K <sup>+</sup> index	n
Control	1.00	1.00	1.00	1.00	5
75	2.002	5.29	3.22	1.74	5
Extract	100	2.06	5.58	1.74	5
	125	2.15	6.01	3.83	2.07
Furosemide (5 mg/kg)	2.22	6.07	4.88	3.13	5
HCTZ (14 mg/kg)	1.50	3.25	2.56	1.34	5

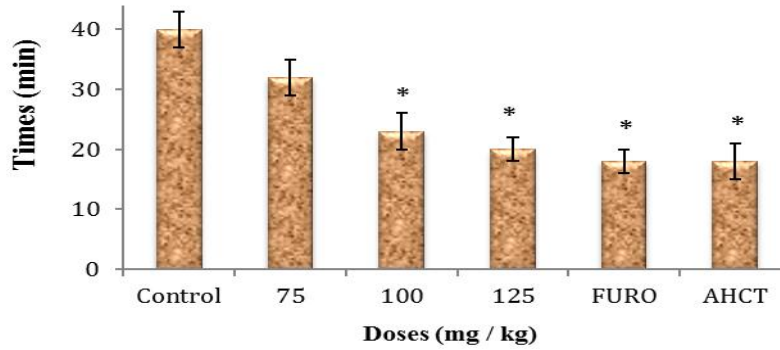
n is the number of animals used in each group; diuretic index: urine volume of test group/urine volume of control group. Na<sup>+</sup> index, sodium excretion in test group/sodium excretion in control group. K<sup>+</sup> index, potassium excretion in test group/potassium excretion in control group. Cl<sup>-</sup> index, chloride excretion in test group/chloride excretion in control group

**Fig.1** Effect of the aqueous leaves extract of *C. crepidioides* on the urinary volume



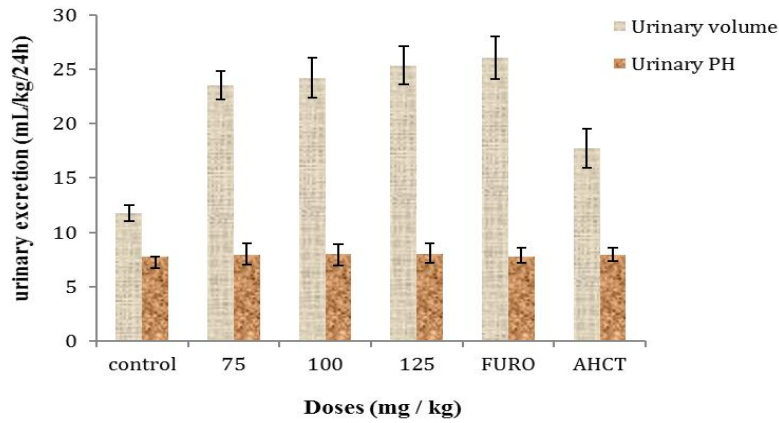
Values are means ± S.E.M., n = 5, \* significant difference compared to the control

**Fig.2** Overload eliminated and urination latency



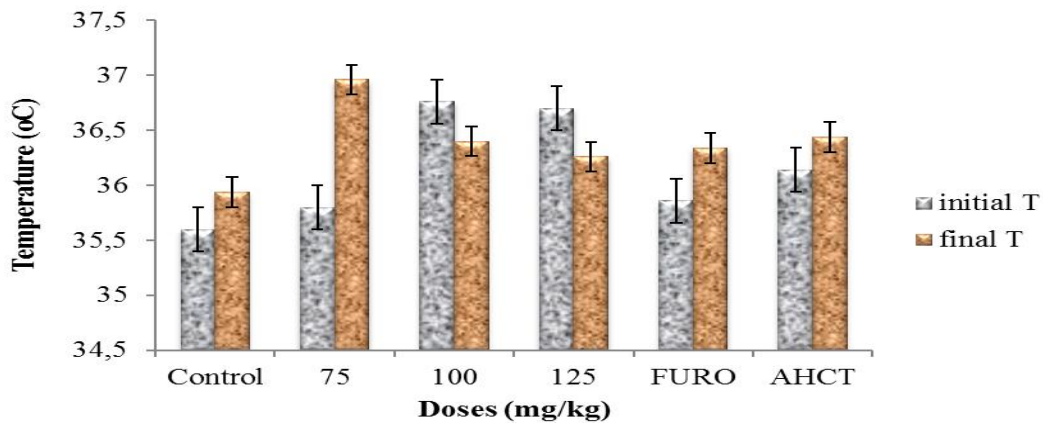
Each bar represents the mean  $\pm$  ESM. AHCT = Amiloride Hydrochlorothiazide. FURO= Furosemide

**Fig.3** pH and the cumulated urinary excretion volume at different time intervals

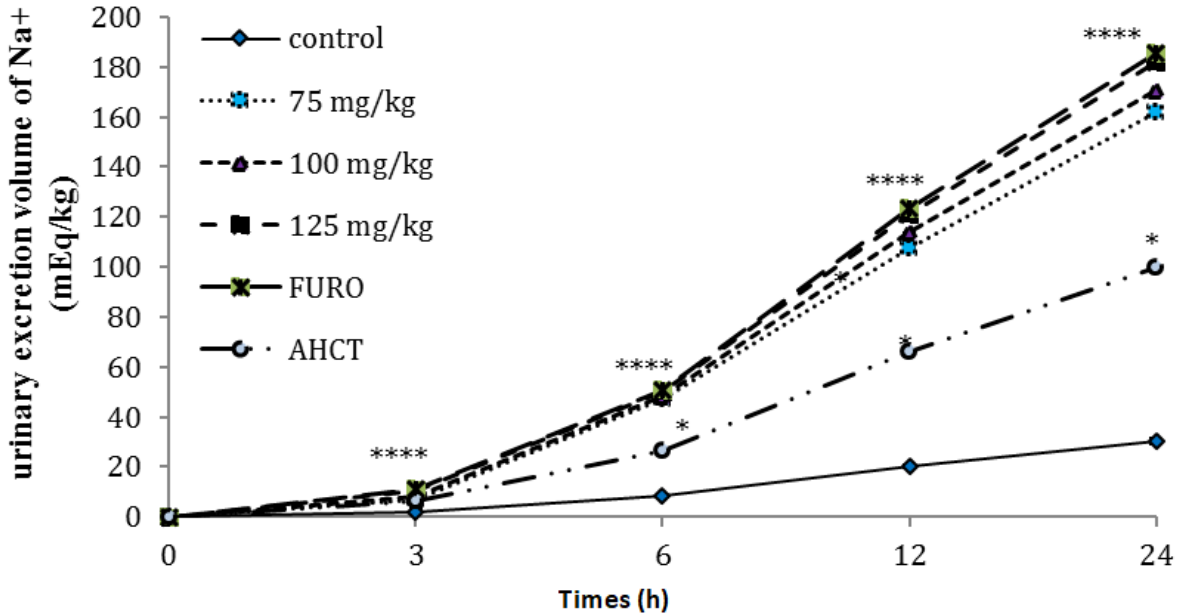


Each bar represents the mean  $\pm$  SEM, n = 5, AHCT. = Amiloride Hydrochlorothiazide; FURO = Furosemide. \* P < 0,05 significant difference compared to control (Distilled Water)

**Fig.4** Rectal temperature after 24 hours. Each bar represents the mean  $\pm$  SEM, n = 5. AHCT = Amiloride Hydrochlorothiazide, FURO = Furosemide

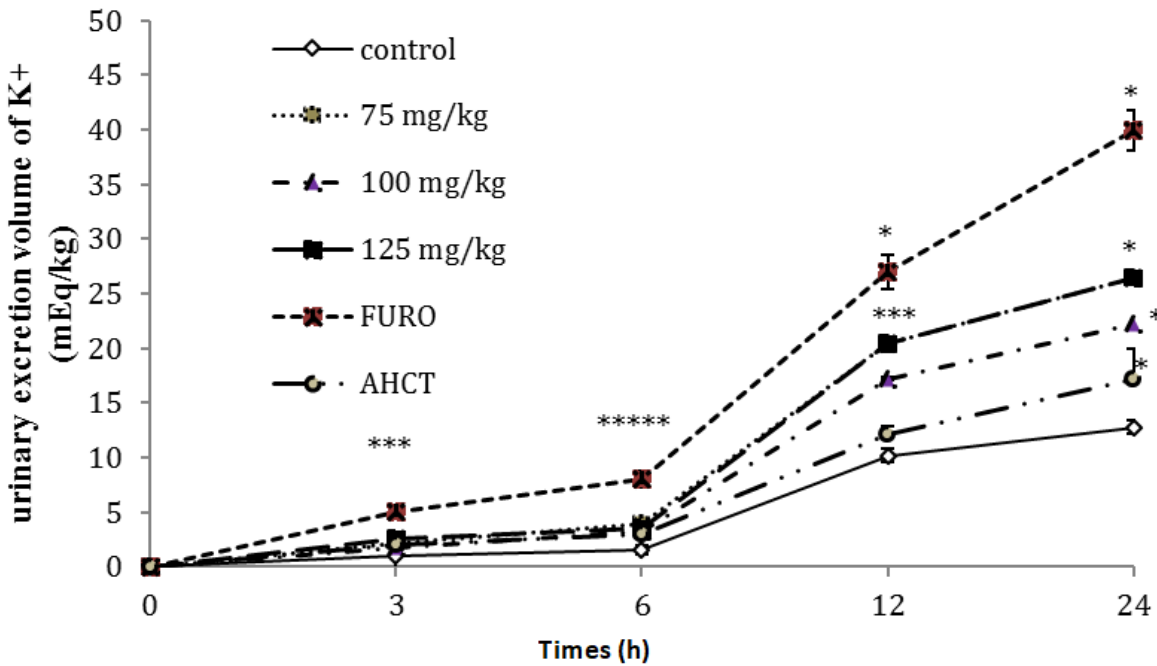


**Fig.5** Effect of *C. crepidioides* on cumulative urinary excretion volume of Na<sup>+</sup> at different time intervals.



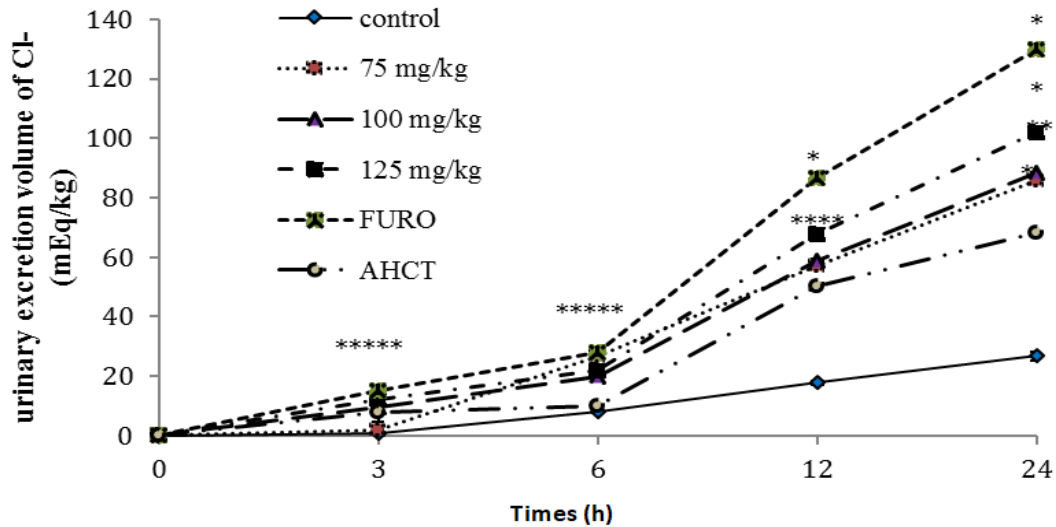
Each curve represents the mean  $\pm$  S.E.M., n = 5, AHCT = Amiloride Hydrochlorothiazide; FURO = Furosemide. (\* P < 0.05) significant difference from the control

**Fig.6** Effect of the aqueous leaves extract of *C. crepidioides* on the urinary excretion of K<sup>+</sup> at different time intervals.



Each curve represents the mean  $\pm$  SEM, n = 5, AHCT = Amiloride Hydrochlorothiazide; FURO = Furosemide. (\* p < 0,05) significant difference from the control

**Fig.7** Effect of the aqueous leaves extract of *C. crepidioides* on the urinary excretion of Chloride ions at different time intervals.



Each curve represents the mean  $\pm$  SEM, n = 5, AHCT = Amiloride Hydrochlorothiazide; FURO = Furosemide (\* p < 0,05) significant difference compared to the control

**Effect of the aqueous leaves extract of *C. crepidioides* on the urine output index and electrolytic excretion index in 24 hours of urine collection**

Diuretic index of the aqueous extract of *C. crepidioides* at doses of 100 and 125 mg/kg is lower than that of furosemide and higher than that of HCTZ (Table 6). The diuretic index passed from 1 in the control to 2.15 in rats treated with the dose of 125 mg/kg. The index of Na<sup>+</sup>, Cl<sup>-</sup> and K<sup>+</sup> increased with the dose of 125 mg/kg compared to the control group (Table 6).

The increase in water consumption observed during the experiment could be attributed to the diuretic power of the plant extract, which causes excessive excretion of urine, and consequently, an increase in water intake by animals. The urinary pH of 6.5 and 8 could be a proof that this plant consumed as vegetable could contribute to the diuretic effect that maintains the acid-base balance, bearing in mind that alkaline foods are generally fruits and vegetables<sup>18</sup>. The body temperature of

animals did not change during handling, reflecting the fact that *C. crepidioides* has no antipyretic activity. A similar result was obtained by Ntchapda *et al.*,<sup>19</sup>, who showed that the aqueous root bark extract of *Zanthoxylum heitzii* has no antipyretic activity in rats. The significant increase in urinary volume at all time frames for doses 75, 100 and 125mg/kg during animal screening indicated that these were the effective doses of the drug.

Whereas the increase in urinary volume after 3 hours of administration of extracts reveals the immediate effect of the plant extract, the one after 24 hours (100.2%, 106.1% and 115.82% corresponding to 75, 100 and 125mg.kg, and 124.3 and 51% for the Furosemide and Amiloride compared to the negative control are indications that the aqueous extract of *C. crepidioides* is quite diuretic. It speeds up and increases the elimination of the overloaded fluids. This increase in diuresis may be attributed to the presence of flavonoids and saponosides in the extract as claimed by Magharani *and al.*<sup>20</sup>.



These results are in agreement with those of Ntchapda *and al.*<sup>21</sup>, who had achieved an 82.52% increase in urinary excretion for 375mg/kg of aqueous extract of *Fucus glusoma*. It is also possible that the aqueous extract of *C. crepidioides* might have caused a cumulative effect of several active substances due to active secondary metabolites synthesis<sup>21</sup>. Furthermore, the increase in urinary excretion could be attributed to the direct effect of the transport of an active component, or the indirect modification of the oncotic and hydrostatic pressure within the peritubular capillaries<sup>22, 23</sup>.

In addition to the elimination of urine, there was a significant excretion ( $p < 0.05$ ) of Na<sup>+</sup> and K<sup>+</sup> ions compared to distilled water treated animals. It was noted that the aqueous leaves extract of *C. crepidioides* at dose 125 mg/kg qualitatively increased the urine electrolyte excretion (Na<sup>+</sup>, K<sup>+</sup> and Cl<sup>-</sup>), similar to furosemide which is known for its potential saluretic and diuretic effects.

The elimination of Na<sup>+</sup> by furosemide and the different aqueous leaves extract doses of *C. crepidioides* could be explained by the fact that these substances act on the various segments of the nephron<sup>24</sup>. It is therefore possible that the aqueous extract of *C. crepidioides* acts in a similar way as furosemide on the Na<sup>+</sup>, K<sup>+</sup>, Cl<sup>-</sup> co-transporters located on the ascending branch of Henle's loop of the nephron thus inhibiting the direct reabsorption of Na<sup>+</sup>, K<sup>+</sup>, Cl<sup>-</sup> through competition with the Cl<sup>-</sup> site of the Co-transporter. This will end up allowing an excretion of 20-25% of the amount of filtered Na<sup>+</sup><sup>25</sup>. The presence of the sodium pump between the tubular cells and the interstitial fluid prevents sodium from migrating to the tubular cell<sup>26</sup>. Urinary excretions of sodium and to a lesser extent potassium suggest that the extract inhibits the sodium pump located on the ascending branch of the Henle's loop<sup>27</sup>.

The increase in Na<sup>+</sup> excretion could also be the result of the inhibitory effect of our aqueous extract. The aqueous extract of *C. crepidioides* might have acted by inhibiting the angiotensin-converting enzyme, leading to a decrease in the secretion of aldosterone, thus promoting an increase in the urinary excretion of sodium<sup>28</sup>. In addition, increased sodium excretion had been shown to decrease the rate of glomerular filtration by increasing the Na<sup>+</sup> loading required for Na<sup>+</sup>/K<sup>+</sup> exchanges, or by stimulating these exchanges via the hyperaldosteronism it causes, and thus, reducing the blood mass<sup>14</sup>. The increase in natriuresis in response to acute treatment with the aqueous leaves of *C. crepidioides* partly explains the increase in diuresis<sup>29, 30</sup>.

The results of this study indicated a significant ( $p < 0.05$ ) increase in natriuretic activity at all extract doses, as well as the reference drugs compared to the negative control group. Studies by Ntchapda *and al.*<sup>17</sup> pointed out that when the ratio Na<sup>+</sup>/K<sup>+</sup> > 2, the extract had a significant natriuretic activity, causing a higher urinary excretion of sodium than that of potassium. Similar results were obtained by Sanogo *and al.*<sup>31</sup>, with the extemporaneous infusion of a recipe used in traditional medicine for the treatment of hypertension. The results obtained with the aqueous extract of *C. crepidioides* showed that the CAI > 0.8 thus the extract of the plant promotes a significant urinary excretion, as well as the saluretic activity in agreement to what was revealed by Ntchapda *and al.*<sup>19</sup>. These natriuretic, saluretic and CAI effects of *C. crepidioides* might be beneficial in the management of some cases of hypertension, as they act through urinary excretion of part of the water and sodium in the blood. This will result in a decrease in blood volume, and therefore a drop-in blood pressure, stipulated by Sanogo *and al.*<sup>31</sup> with a "Nitrokoundang" recipe for the treatment of hypertension.

Creatinine and urea were two markers of the kidney's role. Their metabolites which were the end products derived from protein metabolism had a generally constant concentration under normal conditions<sup>32</sup>. The urinary creatinine and urea concentration in animals treated with the extract increased significantly ( $p < 0.05$ ), or decreased significantly ( $p < 0.05$ ) in the blood, justifying the good functioning of kidneys in the elimination of metabolic wastes<sup>33</sup>. In fact, both creatinine and urea were eliminated from the blood through the process of glomerular filtration. In other words, the creatinine and urea concentrations in urine were ensured by the kidney. The determination of creatinine clearance is necessary to calculate the glomerular filtration rate, which decreases with the extract doses. This decrease may be due to increased  $\text{Na}^+$  excretion, which tends to reduce GFR by increasing the  $\text{Na}^+$  load available for  $\text{Na}^+/\text{K}^+$  exchange, which may further stimulate such exchange by hyperaldosteronism, causing the reduction of the blood volume<sup>34</sup>.

The biochemical parameters in the blood were higher in the animals treated with the extract and the reference substances compared to the control animals. However, the level of these parameters remains lower than the rate observed in the urine. This revealed that the aqueous extract not only causes a decrease in the blood volume, but also resulted in the elimination of electrolytes ( $\text{Na}^+$ ,  $\text{K}^+$  and  $\text{Cl}^-$ ), as well as elimination of metabolic wastes.

Increased serum transaminase levels had been reported to be caused by liver damage including hepatic cell necrosis, hepatitis, cirrhosis, hepatotoxicity of certain drugs and myocardial infarction<sup>33</sup>. Results showed that the aqueous extract of *C. crepidioides* significantly ( $p < 0.05$ ) decrease the level of blood transaminase compared to control group. This decrease might reflect the protective role of plant against hepatotoxicity.

The results obtained are in agreement with those of Aniya *and al.*<sup>11</sup>, who indicated that isochlorogenic acid, quercetin and kaempferol glycoside are active components of *C. crepidioides* with high free radical scavenging activity, a powerful antioxidant and a protector against hepatotoxicity induced by galactosamine (GalN) and lipopolysaccharide (LPS) or carbon tetrachloride ( $\text{CCl}_4$ ). This decrease could also be attributed to the presence of chemicals such as flavonoids, saponosides and alkaloids in the plant extract known for their antioxidant activity and protective hepatoprotection<sup>35</sup>. Similarly, the study conducted by Barrakat *and al.*<sup>36</sup> has shown that diuretics have antioxidant properties. The high concentration of transaminase in urine indicated that most of the body's wastes, including ASAT and ALAT are excreted in urine.

In conclusion, the results of the present study strongly suggest that *C. crepidioides* leaves' aqueous extract have potent and dose-response diuretic properties in acute use in rats. These findings justify at least partly the use of this extract in folk medicine for the treatment of hypertension. Future studies aimed at identifying the active principles accounting for these effects of *C. crepidioides* leaves' aqueous extract may lead to the discovery of a potent diuretic, with potentially antioxidant properties.

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### Availability of data and materials

The datasets supporting the conclusions of this article are presented in this main paper. Plant materials used in this study were identified at the Cameroon National Herbarium where voucher specimens are deposited.

### Authors' contributions

Ntchapda Fidèle supervised the study, improved the study mechanism and corrected the manuscript. Designed the study and was involved in the drafting and correction of the manuscript, analysis and interpretation of data.

Talla Ernest Rodrigue carried out the plant selection and collection and performed the experiments.

Bonabe Christian, Kemeta Azambou David Romain, Talla Emmanuel provided technical assistance in carrying out biochemical assays, contributed some reagents.

All the authors read the manuscript, critically revised it for important intellectual content and approved the final version of the manuscript.

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