



Assessment of the Diuretic Activity of *Petroselinum Crispum* Aqueous Leaves Extract in Wistar Rats

Virginie Atto¹, Koffi Bruno Koko¹, Emilie Bohila Lou Irie Kamo², Brou Andre Konan^{1,*} and Jacques Yao Datte¹

¹Laboratory of Biology and health, Training and Research Unit (UFR) Biosciences, Felix Houphouët-Boigny University, 22 BP 582 Abidjan, Cote d'Ivoire.

²Institut Pasteur Cote d'Ivoire, 01 BP 490 Abidjan 01.

Corresponding author: Brou Andre Konan (e-mail: akonanb@yahoo.fr).

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Abstract: **Objective:** This research work aims to contribute to the valorization of plants used in traditional medicine to improve populations health. It was particularly a question of evaluating the therapeutic effectiveness of the aqueous extract of leaves of *Petroselinum crispum* (Apiaceae), a plant traditionally used for its diuretic effect. **Methods:** Five groups of four 18h-fasted rats and under water overload using a 0.9% NaCl saline solution were treated separately with the saline solution, Furosemide (Furo 40 mg/kg) and 3 increasing doses of *Petroselinum crispum* aqueous leaves extract (PcAE 500, 100 and 1500 mg/kg). The quantities of urine collected at 2 h intervals for 24 h determined, electrolytes and biochemical markers of the kidney were measured. **Results:** PcAE led to a dose-dependent increase in urinary excretion. At a dose of 1500 mg/kg, it induced, 24 hours after administration, a statistically significant increase in urinary excretion compared to control rats having received only the saline solution (34.99 ± 8.46 ml/kg). The urinary excretion produced was statistically similar to that of Furo 40 mg/kg. This excretion was gone with an elimination of Na^+ and K^+ . Also the determination concentration of creatinine and urea in the urine revealed that the plant extract did not disrupt the renal function during treatment. **Conclusion:** *Petroselinum crispum* aqueous leaves extract has an diuretic and natriuretic activity similar to that of the furosemide. This activity would be conferred by the presence of alkaloids and flavonoids.

Key Words: *Petroselinum crispum*, Furosemide, Urinary excretion, Diuretic

I. INTRODUCTION

The diuresis is desired or even forced in the treatment of pathologies requiring the elimination of excess water [1]. Diuretics are the appropriate therapeutic tools for the treatment of high blood pressure and edema in certain conditions. The use of diuretics for the treatment of oedematous diseases is only formal and sufficient when the patient suffers from edema caused by hypervolemia [2]. In case of uncomplicated high blood pressure, the combination of thiazide and a potassium sparer at a low-dose is required [2]. The hypotensive and antihypertensive effects of diuretics are based on their actions, combined or not, on the cardiovascular system and on blood volume [3]. Diuretics are pharmacological substances that increase natriuresis and lead to an increase in isoosmotic sodium urinary excretion. The mechanism and the action sites of diuretic are based on their pharmacological classes [4]. In many developing countries, the populations mostly use the medicinal plants for their health needs [5]. The preponderance of traditional medicine is partly justified by the inadequacy between national health coverages, and the Health need of the populations. Traditional medicine is

also admitted by almost the african populations because of the Sale Traditionally Improved Drugs in the street and the high cost and side effects of synthetic drugs [6].

However, it relevant to notice that the use of a medicinal plant, whether it is reputable or not, is not totally safe and should be done following studies that have defined the context and how to use this plant. The natriuretic response of diuretic plants depends on the administered dose, the nature of the phytochemical products extracted from the plant, the method of extraction and the physiological state of the patient [7].

This work was undertaken to evaluate the scientific basis for the use of *Petroselinum crispum* (Apiaceae) as a diuretic in the treatment of hypertension. To confirm or refute the diuretic effect, the nature of the phytochemical products extracted from the plant was determined. The urinary volumes induced by different doses of *Petroselinum crispum* aqueous leaves extract was also measured.

II. MATERIALS AND METHODS

A. PLANT MATERIAL AND PREPARATION OF THE AQUEOUS LEAVES EXTRACT

Fresh leaves of *Petroselinum crispum* (Apiaceae) were collected in May 2022 at Adjame (Abidjan, Cote d'Ivoire). The leaves have been identified as those of *Petroselinum crispum* (Apiaceae) at the National Floristic Center (CNF, UFR-Biosciences, Felix Houphouet-Boigny University) in comparison of the CNF's herbarium numbered UCJ019001.

The extraction process was implemented according to the method described by some authors [8], [9]. The fresh leaves were washed and dried at room temperature ($28 \pm 2^\circ\text{C}$). Dried leaves were converted into powder by using a laboratory blender. The powder (100 g) was macerated 24 hours in one liter of water. The mixture was filtered (Whatman $n^\circ 1$) for 3 times until complete exhaustion. The filtrate was concentrated under reduce pressure using a rotary evaporator (Buchi R110, type MKE 6540/2) at a temperature of 60°C and dried in a drying oven at $50 \pm 5^\circ\text{C}$. The concentrated extract obtained (PcAE) was stored at 4°C until it orally administered to the rats. The different concentrations of PcAE to be tested were prepared extemporaneously by dilution in NaCl 0.9 % saline solution.

B. ANIMALS AND ETHICAL CONSIDERATION

Twenty male rats weighing 160-280 g were used to demonstrate the diuretic activity of PcAE. These animals were all from the Laboratory of Biology and Health, Felix Houphouet-Boigny University, Abidjan, Cote d'Ivoire. The rats daily received ad libitum water and food for rabbit (pellets) made by IVOGRAIN. The rats were housed in a metabolic cages and maintained under standard laboratory conditions (temperature $25 \pm 2^\circ\text{C}$) with dark and light cycle (12/12 h). Animals were treated according to the good laboratory practices [10]. The experimental protocols were conducted in accordance with the protocols for the protection of experimental animals of the European Council on Legislation 2012/707 [11].

C. DRUGS USED

Furosemide (Lasilix, Sanofi-Aventis, France), a loop diuretic was used as the reference diuretic drug to assess the diuretic activity of *Petroselinum crispum* aqueous leaves extract (PcAE). The concentrations of drugs (PcAE and Furosemide) to be tested were prepared extemporaneously by dilution in NaCl 0.9 % saline solution.

D. PHYTOCHEMICAL STUDY OF PETROSELINUM CRISPUM AQUEOUS LEAVES EXTRACT

The detection of the phytochemical compounds is based on the specific chemical reactions that they induce [12]. These different tests were carried out with a solution of PcAE obtained by dissolving 5 g of PcAE in 50 mL of distilled water [13], [14].

E. MEASUREMENT OF DIURETIC ACTIVITY OF PETROSELINUM CRISPUM AQUEOUS LEAVES EXTRACT

The assessment of the diuretic activity of PcAE was performed in vivo in rat using the method described by Kau et al. [15]. Five groups of 4 rats fasted for 18 hours and overloaded at 50 ml/kg with 0.9% NaCl saline solution were used. Groupe 1 served as normal and the others groups served as test animal.

The group 1 received the 0.9% NaCl saline solution. Group 2 received furosemide (Furo 40 mg/kg) while groups 3, 4 and 5 were treated with PcAE at 500, 100 and 1500 mg/kg, respectively. Animals were placed individually in metabolic cages. The rats' urine was then collected and measured every two hours and accumulated for twenty-four hours in each group.

The urinary volumes thus collected made it possible to evaluate the diuretic power of the substances studied After measuring the quantities of urine collected, some electrolytes (Na^+ , K^+ and Cl^-) and kidney biochemical markers (creatinine and urea) were measured using an HITACHI Chemistry auto-analyser. And the urinary excretion and natriuretic index of drugs were assessed by the following methods [15];

$$\text{UE (ml/kg)} = \frac{\text{UV (ml)}}{\text{W (kg)}},$$

where, UE is urinary excretion, UV is urinary volume and W is rat weight.

$$\text{NI (mmol/ml)} = \frac{\text{UC Na (mmol)}}{\text{UC K (mmol)}},$$

where, NI is natriuretic Index, UC Na is Na^+ urinary concentration and UC K is K^+ urinary concentration.

F. DATA ANALYSIS

The values were expressed as mean with standard error of the mean ($m \pm \text{sem}$). The data were evaluated by analysis of variance followed by Tukey-Kramer with GraphPad Instat software (Microsoft, San Diego, California, USA) method. The graphical representations of data were performed by the GraphPad Prism 8 software (Microsoft, San Diego, California, USA). The difference between the averages is considered statistically significant when $p < 0.05$.

III. RESULTS

A. PHYTOCHEMICAL COMPOUNDS OF PETROSELINUM CRISPUM AQUEOUS LEAVES EXTRACT

The phytochemical screening carried out on PcAE revealed the presence of sterols, polyterpenes, polyphenols, saponosides, flavonoids, quinonnic compounds, alkaloids, and catechic tannins. However, the absence of gallic tannins in PcAE was noted (Table 1).

Phytochemical compounds	Tests or reagents	Results
Sterols and polyterpenes	Liebermann	+
Polyphenols	Ferric chloride	+
Flavonoids	Cyanidin	+
Saponosides	Vigorous agitation	+
Quinonic compounds	Borntraeger	+
Alkaloids	Dragendorff	+
	Bouchardat	+
Catechic tannins	Stiasny	+
Gallic tannins	Hydrochloric acid	-

TABLE 1: Phytochemical compounds of *Petroselinum crispum* aqueous leaves extract

B. EFFETS OF THE STUDIED DRUGS ON THE URINARY EXCRETION

PcAE-induced urine volume was dose dependent after two hours. Indeed, PcAE at 500, 1000 and 1500 mg/kg induced respective urinary volumes of 1.3 ± 0.4 ; 1.9 ± 0.29 and 2.3 ± 0.45 ml and giving in the same order urinary excretions of 7.14 ± 0.4 , 10.55 ± 0.27 and 13.85 ± 0.07 ml/kg. These values compared to those of the control group (UV = 1.49 ± 0.68 ml and UE = 9.31 ml/kg) indicated a non-significant difference ($p > 0.05$) for all doses of PcAE.

With a urinary volume of 7.86 ± 1.2 ml or a urinary excretion of 30.83 ± 0.33 ml/kg, Furo 40 mg/kg showed highly significant effects compared to the control group having received the NaCl 0.9 % saline solution (Figure 1a).

After four hours, only PcAE 1500 mg/kg induced an increase in urinary volume (5.07 ± 0.32 ml) which was statistically significant at $p < 0.05$ and greater than that of the control (2.53 ± 0.87 ml). At this dose of PcAE 1500 mg/kg, urinary excretion was estimated at 30.52 ± 5.11 ml/kg. A highly significant increase was observed with furosemide at the same time. Furo 40 mg/kg made it possible to record a urinary volume of 11.7 ± 1.36 ml corresponding to a urinary excretion of 45.89 ± 2.08 ml/kg (Figure 1b).

At the end of ten (10) hours, EAPC at 1000 and 1500 mg/kg initiated a significant ($p < 0.05$) and very significant ($p < 0.01$) increase in urinary excretion compared to the control, respectively. The urinary volume excreted at 1000 mg/kg was 4.73 ± 0.64 ml and 26.14 ± 4.14 ml/kg for the urinary excretion. Concerning PcAE 1500 mg/kg, the values were 6.62 ± 0.63 ml for the urinary volume and 39.85 ± 1.8 ml/kg for the urinary excretion. So PcAE 1500 mg/kg induced the highest urinary excretion (Figure 1c).

Twenty-four hours (24 h) after the administration of drugs to the rats, the urine volume collected was 7.5 ± 0.58 ml and 13.1 ± 2.1 ml for PcAE 1500 mg/kg and Furo 40 mg/kg, respectively. In comparison to the urinary excretion of the control group (34.99 ± 8.46 ml/kg), PcAE 1500 mg/kg induced a significative increase of the urinary excretion (45.13 ± 0.58 ml/Kg). In comparison to the furosemide, the urinary excretion induced by PcAE 1500 mg/kg was very similar to that induced by the furosemide. Indeed, urinary excretion induced by Furo 40 mg/kg was estimated at 51.38 ± 2.8 ml/kg (Figure 1d).

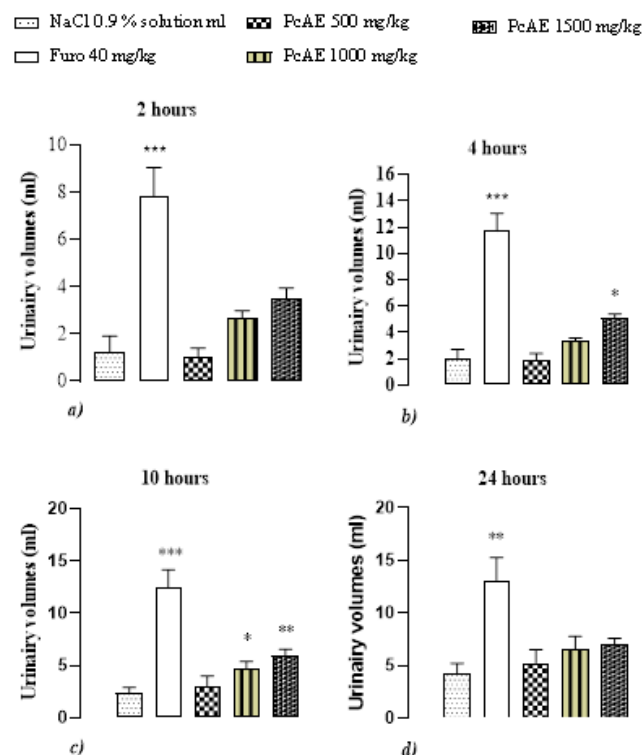


FIGURE 1: Effects of test substances on urinary excretion in rats

C. EFFECTS OF TEST DRUGS ON THE URINARY ELECTROLYTES IN RATS

The urine electrolyte concentrations measured are reported in Table 2. All doses of PcAE promoted Na^+ elimination compared to the control group, which had an estimated Na^+ elimination of 213.33 ± 2.08 mmol/l. However, the statistical analysis has revealed a non-significant difference ($p > 0.05$). Indeed, for PcAE at 500, 1000 and 1500 mg/kg, Na^+ elimination toward the urine was 246.67 ± 26.31 , 239.33 ± 18.15 and 228 ± 18.03 mmol/l, respectively. Furo 40 mg/kg and all PcAE doses did not cause a significant decrease ($p > 0.05$) of the natriuresis in comparison to the control group (213.33 ± 2.08 mmol/l).

The plant extract (PcAE) at any dose induced a non-significant increase at $p > 0.05$ in potassium excretion compared to the control group (29.56 ± 6.94 mmol/l). Urinary potassium measured at the doses of 500, 1000 et 1500 mg/kg of PcAE were respectively 38.7 ± 10.71 , 42.27 ± 9.25 , 34.43 ± 6.39 mmol/l.

In opposition to EAPC, Furo 40 mg/kg caused a significant decrease ($p < 0.05$) of the kaliuresis (13.16 ± 1.94 mmol/l) compared to that induced by the saline solution in the control group. Regardless of the test substance administered, the urine chlorine values determined were similar to those promoted by the saline NaCl 0.9 % solution (control group). Thus the chlorine excretions in the urine were 201.67 ± 8.5 ,

200.33 $\pm$ 5.51, 228.67 $\pm$ 24.54, 214 $\pm$ 12.53 and 203 $\pm$ 9.64 mmol/l for the saline solution, Furo 40 mg/kg and three doses of the plant extract (PcAE 500, 1000 and 1500 mg/kg), respectively.

The natriuretic index represented by the Na^+/K^+ ratio was calculated. For all the test substances administered, the Na^+/K^+ ratio was greater than 5. That indicates a potassium-sparing effect. Indeed, these values were 7.22, 15.78, 6.45, 6.95 and 12.65 respectively for the saline solution (control group), Furo 40 mg/kg and PcAE at 500, 1000 and 1500mg/kg. The diuretic index value obtained with PcAE 1500 mg/kg was similar to that of furosemide used at 40 mg/kg (Table 2).

D. EFFECTS OF TEST DRUGS ON THE URINARY CONCENTRATIONS OF KIDNEY BIOMARKERS IN RATS

24 hours after oral administration of the substances to rats, urinary creatinine and urea levels were measured and presented in Table 3. The oral administration of PcAE decreased the creatinuria in dose-dependent manner. For PcAE at 500, 1000 and 1500 mg/kg, urinary creatinine levels were 7.50 $\pm$ 1.64, 5.01 $\pm$ 0.4 and 2.93 $\pm$ 0.75 mmol/l, respectively. Compared to the control (11.27 $\pm$ 0.85 mmol/l), all values obtained in rats treated with different doses of the plant extract showed a significant decreases at $p < 0.05$. The urinary creatinine excretion produced with the furosemide at the dose of 40 mg/kg was 2.34 $\pm$ 2.03 mmol/l. This was statistically similar to that obtained in rats treated with PcAE1500 mg/kg. The urea content has decreased significantly regardless of the test substance administered in comparison to the saline solution (control) that recorded a level of urea equal to 590.63 $\pm$ 91.09 mmol/l. The values determined were 192.86 $\pm$ 33.02, 369.71 $\pm$ 17.4, 141.29 $\pm$ 19.64, and 188.44 $\pm$ 4.25 mmol/l for Furo 40 mg/kg and three doses of PcAE (500, 1000 and 1500 mg/kg). PcAE 1500 mg/kg had a level of urea that was statistically similar to that observed with the furosemide, a reference diuretic substance used at 40 mg/kg.

IV. DISCUSSION

The phytochemical study carried out on the aqueous extract of the leaves of *Petroselinum crispum* (Apiaceae) has revealed the presence of sterols, polyterpenes, saponosides, quinonic compounds, alkaloids and catechetal tannins. On the other hand, the absence of gallic tannins was noted. Other studies carried out on the same part of the plant have revealed the presence of gallic tannins and the absence of sterol and polyterpene compounds [16].

This comparative difference in chemical groups could be explained by the type of solvent, the geographical location of the plant, the climate, and the extraction method and/or the harvest period. The study of the diuretic activity of EAPC has demonstrated a dose-dependent increase in urinary excretions. The dose of 1500 mg/kg/bw has caused the greatest urinary excretion. This excretion is similar to that of furosemide. Indeed, furosemide is a standard diuretic known

to have its onset of action one hour after oral administration and reaches its maximum effect in 2 to 3 hours [17].

EAPC, on the other hand has induced an increasing urinary excretions over time. Diuretics that mimic the effect of furosemide act on the $Na^+/K^+ cl^-$ pump at the loop of Henle [18]. They influence the dilution-concentration mechanism of the urine to promote significant diuresis. These diuretics may be antagonists to the action of antidiuretic hormone (ADH). Indeed, DHA is responsible for regulating the reabsorption of water from the filtrate into the renal collecting tubes in order to maintain the body's osmolality by inhibiting urinary excretion [19].

EAPC has also induced in urinary excretion of sodium and potassium similar to that caused by the furosemide. The increase of the diuresis by EAPC could be explained by the presence of compounds such as alkaloids, steroids. Indeed, the work of Patel et al. [20] on *Lepidium sativum* Garden Cress (Cruciferae) has shown that the diuretic effect of plants is due to the photochemical compounds they contain. On the other hand, the flavonoids contained in *Spergularia purpurea* extract are responsible for its diuretic activity. The natriuretic index represented by the Na^+/K^+ ratio was greater than 5 for all the doses of EAPC, suggesting that the extract had a potassium-sparing effect [21]. These results are similar to those obtained with alcoholic extracts of *Oxystelma esculentum* and *Hibiscus sabdariffa* promote the elimination of $Na^+/K^+ cl^-$ and make urine more alkaline [22].

EAPC would act at the level of the dilution zone. This urinary alkalization could be explained by the inhibition of HCO_3 reabsorption [23]. In addition, EAPC at the dose of 1500 mg/kg/bw has caused an urinary excretion of creatinine and urea that was similar to that 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 EAPC are similar to those induced by the aqueous extract of the leaves of *Spergularia purpurea* (Caryophyllaceae) [24]. These authors reported that this extract induced in an increase in glomerular filtration rate due to their effect on renal purification function.

V. CONCLUSION

The study of the diuretic activity of *Petroselinum crispum* aqueous leaves extract showed an dose-dependent increase in urinary excretions. The dose of 1500 mg/kg caused the greatest urinary excretion. This excretion was similar to that of furosemide. This study showed that *Petroselinum crispum* aqueous leaves extract has a diuretic and natriuretic activity. These results could militate in favour to the exploitation of this plant as a diuretic in the treatment of high blood pressure and oedema. However, further studies are still needed, including the consideration of determining the efficacy of the extract on experimentally induced hypertension.

Electrolytes	NaCl 0.9 % solution	Furo 40 mg/kg	PcAE		
			500 mg/kg	1000 mg/kg	1500 mg/kg
Na^+ (mmol/l)	213.33 $\pm$ 2.08	207.67 $\pm$ 2.31	246.67 $\pm$ 26.31	239.33 $\pm$ 18.15	228 $\pm$ 18.03
k^+ (mmol/l)	29.56 $\pm$ 6.94	13.16 $\pm$ 1.94*	38.27 $\pm$ 10.71	38.27 $\pm$ 10.71	34.43 $\pm$ 6.39
Cl ⁻ (mmol/l)	201.67 $\pm$ 8.50	200.33 $\pm$ 5.51	228.67 $\pm$ 24.54	214 $\pm$ 12.53	203 $\pm$ 9.64
Natriuretic Index (Na^+/k^+)	7.22	15.78**	6.45	6.95	12.65**

TABLE 2: Urinary electrolyte concentrations and Natriuretic Index induced by test drugs in rats

Biochemical marker	NaCl 0.9 % solution	Furo 40 mg/kg	PcAE		
			500 mg/kg	1000 mg/kg	(1500 mg/kg)
Creatinine (mmol/l)	11.27 $\pm$ 0.85	2.34 $\pm$ 2.03**	7.5 $\pm$ 1.64*	5.01 $\pm$ 0.04*	2.13 $\pm$ 0.75**
Urea (mmol/l)	590.63 $\pm$ 92	192.86 $\pm$ 33.02***	369.71 $\pm$ 17.4**	141.29 $\pm$ 19.64***	188.44 $\pm$ 4.25***

TABLE 3: Urinary concentrations of renal biomarkers induced by test substances in rats

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AUTHOR CONTRIBUTION

All authors contributed equally in the study. They made substantial contributions to the design of the study, the collection of the data as well as the preparation and analysis of the data. They also drafted the manuscript and gave final approval for its submission to the journal for consideration of publication.

CONSENT

It is not applicable.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research reported. Also, this research did not receive any specific grant from any funding agency in the public, commercial, or not-for-profit sector. It was funded by personal efforts of the authors.

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