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Evaluation of diuretic activity of hydro ethanolic extract of *Passiflora edulis f edulis* (PASSIFLORACEAE) leaves in rat

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Abstract

Passiflora edulis Sims is widely used in traditional medicine in many countries worldwide for the treatment of diverse diseases due to its scientifically demonstrated antitumor, anti-inflammatory and antihypertensive effects; however, no preclinical studies have been done on the diuretic activity of the hydro ethanolic extract from P. edulis leaves in rats. The objective of this work was to study the diuretic activity of the above-mentioned extract in rats. Oral administration of the extract at doses 75, 150 and 300 mg/kg increases diuresis which is equal to 3.3 ± 0.20 ml in the control animals, versus 4.56 ± 0.34, 5.7 ± 0.10 and 7.96 ± 0.37 ml in the animals treated with the extract ($p < 0.05$). Natriuresis of the animals treated with the extract increases to 0.91 ± 0.02, 1.07 ± 0.07 and 1.24 ± 0.23 mEq/L respectively, versus 0.77 ± 0.06 mEq/L in the control group ($p < 0.05$), while kaliuresis increases from 0.31 ± 0.01 mEq/L in the control group to 0.36 ± 0.02, 0.55 ± 0.04 and 0.88 ± 0.05 mEq/L in the animals treated with the extract ($p < 0.05$). Administration of extract does not affect the urine pH which is equal to 8.13 ± 0.51 in the control animals, versus 8.65 ± 0.47, 8.81 ± 0.5 and 8.83 ± 0.48 in the animals treated with the extract (NS). These results indicate the diuretic effect of hydro ethanolic extract of *Passiflora edulis* leaves.

Keywords: Diuretic; Kaliuresis; Natriuresis; Rats

1. Introduction

From time immemorial, medicinal plants have been used as a source of treatment for various disorders, especially, in developing countries, where many people depend on herbal medicines to treat illnesses such as edematous disorders, hypertension and low urine output. *Passiflora edulis f edulis* is one of the plants largely used in the area where we have conducted an ethnobotanical survey in the high plateau of Madagascar with the leaves decoction used for its diuretic and antihypertensive virtue. Diuretics have been used for decades in the treatment of hypertension. Its efficacy has been demonstrated in numerous clinical trials [1, 2]. Diuretics are drugs that increase the renal excretion of Na^+ and water by inhibiting sodium reabsorption in different parts of nephron. Their ability to alter long-term sodium balance induces important hemodynamic changes and results in a reduction in peripheral vascular resistances to sustain reduction in blood pressure [3]. Diuretics acting in the proximal tubule interferes non-competitively with both the luminal and cellular carbonic anhydrase enzymes resulting in impairment of Na^+, $HCO3^-$, and water reabsorption, causing loss of $HCO3^-$ ions in the urine that leads to alkaline diuresis. Loop diuretics reduce sodium chloride reabsorption in the thick ascending limb of the loop of Henle. This is achieved by inhibiting the Na^+-K^+-$2Cl^-$ carrier in the luminal membrane of this segment, thereby reducing the reabsorption of sodium and chloride. Medicines in this class are powerful diuretics increasing natriuresis and kaliuresis. Diuretics acting on the proximal part of the convoluted tubule of nephron inhibit the electroneutral Na^+-Cl^- cotransporter located on the apical membrane of the early segment of the distal convoluted

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tubule. Those diuretics increase natriuresis, chloruresis and kaliuresis. Diuretics of the distal part of convoluted tubule and collecting ducts (both cortical and medullary) are referred to as aldosterone-sensitive distal nephron; ENaC (epithelial sodium channel) inhibitors also act on this segment. Diuretics of these groups reduce kaliuresis. Osmotic diuretics increase intra luminal osmolarity in the renal tubules without affecting electrolyte balance, whereas aquaretics are substances that act directly by only affecting the excretion of water by inhibiting ADH [4, 5]. Various extracts, fruit juice and isolated compounds from *P. edulis* have shown a wide range of health effects and biological activities such as antioxidant, anti-hypertensive, anti-tumor, antidiabetic, hypolipidemic activities, and so forth [6]. However, the historical usage of *Passiflora edulis* leaves as a diuretic has not been confirmed pharmacologically. With the local people at where we have conducted our ethnobotanical survey indicating that the decoction of the leaves of this plant is very effective in the treatment of hypertension and urinary tract problems, the present study was conducted to explore the diuretic effect of the hydroalcoholic extract prepared from the leaves of *P. edulis*.

2. Material and methods

2.1. Chemicals and Reagents

Chemicals and solvents used in this study were of analytical grade purchased from Sigma-Aldrich Company (St Louis, Mo, USA).

2.2. Plant Material

Fresh leaves of *Passiflora edulis* were collected in March 2019 from Antsirabe, about 175 km southwest of Antananarivo, Madagascar. The plant was authenticated by taxonomist, at the Botany Department of "Parc Botanique et Zoologique de Tsimbazaza" (PBZT) of Antananarivo, and a voucher specimen was deposited at the Herbarium, for future reference. To prepare the extract used in this work, the leaves were dried under shade. The dry leaves were ground into powder and macerated in ethanol-water (60:40) solvent at room temperature for 3 days. The macerate was filtered with a cotton gauze then through Whatman qualitative grade 1 filter paper, and the filtrate centrifuged at 3000 rpm for 10 minutes. The supernatant was concentrated in a rotary evaporator (Buchi, Rotavapor R-210/215, Switzerland), under reduced pressure.

2.3. Experimental Animals

Healthy Wistar rats of either sex of 8-10 months, weighing 200-300 g were used for the experiments. All animals were bred at the animal house of the Pharmacology Department, Faculty of Sciences, University of Antananarivo. The animals were fed with a standard animal diet and had water *ad libitum*. Handlings of the animals was approved by the ethics review board of the Sciences Faculty (Reg. N° ECFS-0124/10).

2.4. Determination of Diuretic Activity

All animals were subjected to fasting with free access to water for 12 hours before test. They got water load of 50 ml/kg, by oral route and were randomly assigned into 4 groups of 6 animals per group. After 30 minutes of water load, the animals of control group were treated orally with the vehicle used for reconstitution (10 mL/kg of distilled water) while the rest received the extract at doses 75, 150 and 300 mg/kg. Dose selection was based on data obtained from preliminary tests. Immediately after administration of products, rats were placed in individual metabolic cages.

Urine excreted in 24 h was collected and centrifuged at 7000 rpm for 10 minutes. Supernatant volume was measured, pH and urine concentration of Na^+, K^+ were determined. pH was directly determined on fresh urine samples using a pH meter (Pierron®), while Na^+, K^+ were dosed using flame spectrophotometer (Systonic®). The ratio of urinary excretion in the test group to urinary excretion in the control group was used as a measure of diuretic action of the extract, while ratio Na^+/K^+ was calculated to evaluate natriuretic activity of the extract [7].

$$\text{Diuretic index} = \frac{\text{urinary excretion of treated group}}{\text{urinary excretion of control group}}$$

According to Kau *et al*. (1984), the diuretic index (DI) indicates the diuretic activity of a product:

DI < 0.72	No potential diuretic activity
0.72 < DI < 1.0	Low potential diuretic activity
1.0 < DI < 1.5	Mild potential diuretic activity
1.5 < DI	High potential diuretic activity

On the other hand, the ratio Na^+/K^+ indicates the natriuretic activity of the extract:

$1< Na^+/K^+<2$	natriuretic product
$2< Na^+/K^+<10$	natriuretic product without excessive potassium loss
$Na^+/K^+ >10$	potassium sparing product

2.5. Statistical analysis

Results are expressed as means ± SEM. All statistical analyses were performed using SPSS version 25. Statistically significant differences among groups were evaluated by one-way ANOVA followed by Student "t" test. Statistical significance was set at $p<0.05$.

3. Results

3.1. Effect on Urine Volume

Administered orally, the hydro ethanolic extract of *P. edulis* leaves increases diuresis in rat. From the lowest dose of 75 mg/kg, the extract produced a significant difference in urine volume compared to the control group. The 24 h urinary volume of the treated groups increases in a dose dependent manner. It is equal to 3.3 ± 0.20 ml in the control animals, versus 4.56 ± 0.34, 5.7 ± 0.10 and 7.96 ± 0.37 ml in the animals treated with the extract at doses 75, 150 and 300 mg/kg respectively ($p < 0.05$) (Figure 1). After calculation, the diuretic index is equal to 1.38, 1.72 and 2.41 respectively for the doses 75, 150 and 300 mg/kg. With a value superior to 0.72 for the lowest dose and superior to 1.5 for the highest dose, these results indicate that *P. edulis* leaves extract possesses high potential diuretic activity.

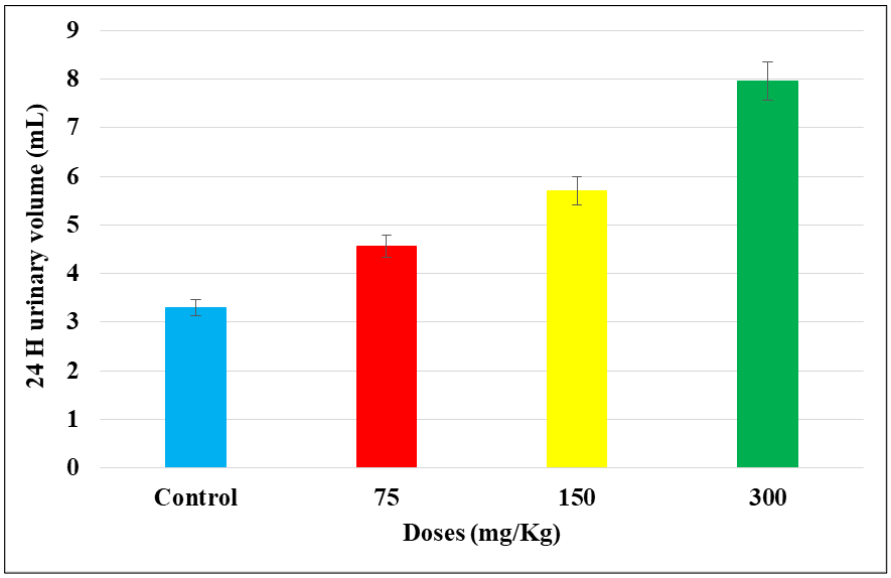

Figure 1 24 h urinary volume of the animals of control group and those treated with *P. edulis* leaves extract, administered orally, at doses 75, 150 and 300 mg/kg, after water load of 50 ml/kg (m ± SEM; n = 6; $p < 0.05$)

3.2. *P. edulis* leaves extract effect on Na+ and K+ excretion

P. edulis leaves extract, administered orally, caused significantly increased sodium and potassium excretion. Natriuresis of the animals treated with the extract at doses 75, 150 and 300 mg/kg increases to 0.91 ± 0.02, 1.07 ± 0.07 and 1.24 ± 0.23 mEq/L respectively, from 0.77 ± 0.06 mEq/L in control group ($p < 0.05$), while kaliuresis increases from 0.31 ± 0.01 mEq/L in the control group to 0.36 ± 0.02, 0.55 ± 0.04 and 0.88 ± 0.05 mEq/L in the animals treated with the extract at doses 75, 150 and 300 mg/kg respectively ($p < 0.05$). The Na^+/K^+ ratio decreases when the doses increase. It is equal to 2.53, 1.95 and 1.40 respectively for the doses 75, 150 and 300 ($p<0.05$). The decrease of the ratio value indicates the increase of K^+ excreted (Table 1).

Table 1 Effect of *P. edulis* leaves extract on Na^+ and K^+ excretion

Doses (mg/Kg)	Na^+ (mEq/mL)	K^+ (mEq/mL)	Na^+/K^+
75	0.91 ± 0.02	0.36 ± 0.02	2.53
150	1.07 ± 0.07	0.55 ± 0.04	1.95
300	1.24 ± 0.23	0.88 ± 0.05	1.40

3.3. *P. edulis* leaves extract effect on urinary pH

Urinary pH measurement revealed that *P. edulis* leaves extract does not modify the urinary pH compared to the control animals which is slightly alkaline (Figure 2). The urinary pH of the control animals is equal to 8.13 ± 0.51, versus 8.65 ± 0.47, 8.81 ± 0.50 and 8.83 ± 0.48 in the animals treated with the extract at doses 75, 150 and 300 mg/kg respectively (NS). These results indicate that *P. edulis* leaves extract does not affect the excretion of H^+.

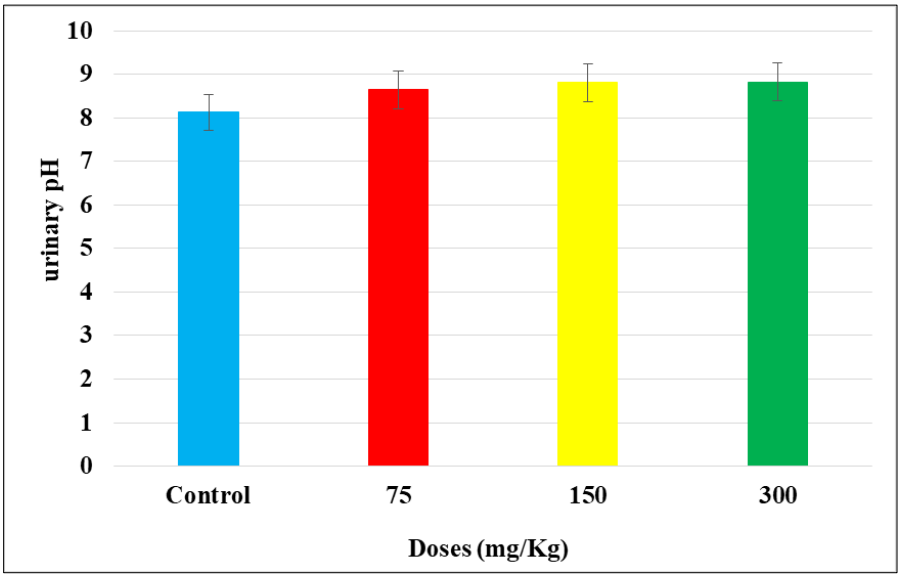

Figure 2 Urinary pH of 24 h of animals in control group and those treated with the extract, administered orally, at doses 75, 150 and 300 mg/kg, after water load of 50 ml/kg (m± SEM; n = 6; $p < 0.05$)

4. Discussion

According to the results of the ethnobotanical surveys that we have conducted in the region of Antsirabe, Madagascar, decoction prepared from *P. edulis* leaves is used in traditional medicine to take care of hypertension, edema and urinary problem. After analyzing that information, we came up with the hypothesis that it might possess diuretic activity. Diuretics are mainly used to adjust the volume and composition of body fluids in a variety of disorders, including hypertension and edema. This work aimed to evaluate the diuretic activity of this plant. To reach our objective, a hydroethanolic extract was prepared from the leaves of this plant and administered orally to rats. The urinary volume of treated animals and control animals were compared, then the extracts' effect on electrolytes excretion was evaluated, especially Na^+, K^+ and H^+. With regards to urine output, the extract increases urine excretion in a dose-dependent manner. The effect turned out to be more significant at higher doses tested compared to the control, possibly due to increased concentration of active components. On the other hand, diuretic index value of *P. edulis* leaves extract is superior to 1 for the three doses that we administered, indicating a diuretic activity of the extract. It is even superior to 1.5 for the highest dose, which means it possesses a high potential diuretic activity [7]. These results indicate that the extract inhibits Na+ reabsorption in the tubule. In view of electrolyte composition of urine, the extract does not affect the urinary pH, which means, it does not affect the urinary excretion of H^+. We suggest that it does not act in the proximal tubule, because if it did, the pH would have increased compared to the control group [7]. It increases the urinary excretion of Na^+ and K^+ of the treated animals compared to the control group. Na^+/K^+ ratio was calculated for natriuretic activity. Values > 2 indicate a favorable natriuretic effect, whereas ratios >10 indicate a potassium-sparing effect [7]. Increased Na^+/K^+ implies more Na+ excretion than K^+, which is regarded a very good profile for diuretic agents.

However, *P edulis* extract decreased the Na^+/K^+ ratio, indicating that the plant has low natriuretic potential but a pronounced kaliuretic effect. Elevation of urinary excretion of K^+ in a dose dependent manner and the ratio Na^+/K^+ value inferior to 10 suggest that the extract does not spare the potassium, which suggest that it is not a distal convoluted diuretic [8, 9]. In addition, the ratio Na^+/K^+ decreases in a dose dependent manner when the dose increases. This confirms the hypothesis that the extract does not inhibit the reabsorption of Na^+ in the distal tubule, because the more Na^+ gets to the distal convoluted tubule, the more Na^+ is reabsorbed and the more K^+ is excreted in the distal convoluted tubule. Therefore, it is possible to presume that this extract acts by inhibiting Na^+ reabsorption either on the thick ascending branch of Henle loop or in the diluting zone. Previous studies have demonstrated that there are several compounds, such as flavonoids, saponins or organic acids which could be responsible for the plants' diuretic effects. The observed diuretic effect of those secondary metabolites could also be due to promoting vasodilatation in the afferent arterioles of the renal vasculature, thereby increasing the rate of glomerular filtration, which in turn promotes increased urine formation and electrolytes excretion [10]. The presence of flavonoids in *P. edulis* has been reported in literatures, and referring to those data, one can advance a hypothesis for the vasodilating effect of *P. edulis* extract [11, 12]. The present study supports the traditional use of *P. edulis* in Malagasy folk-medicine as a diuretic and anti-hypertensive agent. Its activity could be associated with the flavonoids that it contains. However, it is not evident to give the exact mechanisms of action of the extract based on the results obtained vis-a-vis the bioactive principles present in the extract. It could be used, though, as starting point for further investigation.

5. Conclusion

Collectively, the results of this study revealed that hydro ethanolic extract of *P. edulis* leaves possesses significant diuretic activity. Urinary pH and electrolyte analysis show that it might act as diuretic of the diluting zone or promoting vasodilatation in the afferent arterioles of the renal vasculature. This study thus substantiates this plant's traditional claim as a diuretic and anti-hypertensive agent.

Compliance with ethical standards

Disclosure of conflict of interest

The authors report no conflicts of interest for this work.

Statement of ethical approval

The experiments were conducted following the guidelines of the ethics committee of the Sciences Faculty, University of Antananarivo, Madagascar (Reg. N° ECFS-0124/10).

Funding Statement

No funding was received for this work.

Author Contributions

All authors made a significant contribution to the conception and design of the study, execution, acquisition, analysis, and interpretation of data.

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