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Assessment of diuretic activity of hydro alcoholic extract of *Sechium edule* (Jacq.) SW. (CUCURBITACEAE) fruit in rat

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Abstract

This work aimed to assess the diuretic activity of the hydro alcoholic extract of *Sechium edule* fruit. Its effect on diuresis was evaluated by measuring the urinary volume of 24 h, while its site of action was determined by measuring the urinary pH and dosing urinary sodium and potassium. It was administered orally at doses 100, 200 and 400 mg/kg. Our results indicate that it increases the 24 h urinary volume from 3.7 ± 0.4 ml in control group to 5.2 ± 0.2, 7.3 ± 2. 3 and 8.4 ± 2.4 ml in the groups treated with the extract at doses 100, 200, and 400 mg/kg respectively ($p<0.05$). It also increases the natriuria and kaliuria. Natriuria increases from 0.54 ± 0.02 mEq/L in control group to 0.74 ± 0.02, 0.86 ± 0.04 and 1.16 ± 0.09 mEq/L in group treated with extract at doses 100, 200, and 400 mg/kg respectively ($p < 0.05$), while kaliuria increases from 0.35 ± 0.03 mEq/L in control group to 0.52 ± 0.02, 0.65 ± 0.03 and 0.92 ± 0.02 mEq/L in group treated with the extract at doses 100, 200, and 400 mg/kg respectively ($p < 0.05$). On the other hand, the extract does not affect the urinary pH which is equal to 8.616 ± 0.016 in control group, and 8.72 ± 0.02, 8.743 ± 0,05 and 8.793 ± 0.05 in groups treated with the extract (NS).

These results indicate that *S. edule* fruit possesses diuretic activity, acting on Henle loop or dilution zone. Phyto screening performed on the extract revealed the presence of polysaccharides, steroids, triterpenes, alkaloids, phenolic compounds and flavonoids.

Keywords: *Sechium edule*; Fruit; Diuresis; Natriuria; Kaliuria

1. Introduction

Diuretics are used as a first treatment in patients with edema associated with liver cirrhosis, heart and kidney failure or hyperkalemia and hypertension because they increase the excretion of sodium, potassium and reduce blood volume. Several guidelines throughout the world put diuretics in their list as one of hypertension treatments with angiotensin-converting enzyme (ACE) inhibitors, angiotensin receptor blockers and calcium channel blockers (CCB), especially in patients where hypertension is associated with extracellular volume expansion and salt retention. They are specifically recommended in elderly patients or with diabetes since they are prone to fluid retention or with isolated systolic hypertension and patients of African origin [1]. Since ancient times, medicinal plants have been used as a source of treatment for diverse pathology, particularly in developing countries, where majority of people depend on herbal medicines to treat various illnesses. A high number of plants are used as diuretics in mono or poly-herbal preparations mostly in the form of decoction. For example, the bark of *Mangifera indica* (mango, ANACARDIACEAE) [2], the leaves of *Mimosa pudica* (*Sleeping mimosa,* FABACEAE) [3], *Taraxacum officinale* (dandelion, ASTERACEAE) [4], *Allium sativum*

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(garlic, LILIACEAE) [5], *Anthocleista djaloensis* (GENTIANACEAE) [6], and *Mystroxylon aethiopicum* (CELASTRACEAE) [7]. *Sechium edule* belonging to the family CUCURBITACEAE, also known as christophine or chayote is commonly used as food, but the decoction prepared from its roots or fruits are also used in traditional medicine for hypertension management. Scientific research has made evidence of the anti-hypertensive activity of those parts of this plant [8, 9].

The historical usage of the fruits of this plant as a diuretic has not been confirmed pharmacologically, the present study was conducted to evaluate the diuretic effect of the hydroalcoholic extract obtained from the fruits of this plant.

2. Materials and methods

2.1. Plant material

The fruits of *Sechium edule* were collected from Antsiranana, in the northern part of Madagascar in March 2024. They were thoroughly washed with tap water and dried under shade at room temperature for two months. The dried fruits were ground to powder using an electric grinder Crompton®.

2.2. Preparation of extract

The extract of the fruit powder was made using the cold maceration technique. Two hundred grams of the powder was soaked with a mixture of ethanol-distilled water (60:40) at room temperature, for 3 days. The extract was filtered using muslin cloth and Whatman® grade 1 filter paper. The marc was discarded, while the filtrate was centrifuged at 3000 rpm for 10 minutes. The supernatant was evaporated to dryness using rotative evaporator Evapotec® and stored in a desiccator until used for the experiment. Qualitative phytochemical investigation of the extract was carried out to determine the presence of secondary metabolites according to the technique of Fong *et al* (1977) [10].

2.3. Experimental animals

Both male and female Wistar rats aged 6–10 weeks, with a weight range of 260–300 g, inbred in the animal house of the Laboratory of Pharmacology and Cosmetology, Sciences Faculty, University of Antananarivo, Antananarivo, Madagascar, were used for the experiment. They were housed under standard environmental conditions at 22 ± 2°C and 12 h/12 h light/dark cycle. The animals were allowed free access to tap water and laboratory animal food LFL 1420. Each rat was placed in an individual metabolic cage 24 h before the onset of the experiment for adaptation.

Rats were randomly assigned into 4 groups (negative control and three test groups) comprising 6 rats per group. Negative control rats were treated with the vehicle used for reconstitution of the aqueous extract, distilled water (DW), and the three test groups were treated with 100, 200, and 400 mg/kg of the hydroalcoholic extract administered orally in a volume of 10 mL/kg. All animals were subjected to fasting overnight with free access to water before experiment [11].

2.4. Evaluation of diuretic activity

Rats had 50 mL/kg of water orally to impose uniform water load. Rats were divided into 4 groups comprising 6 animals per group. Immediately after administration of water in control group and the extract in the three treated groups, the animals were placed in individual metabolic cages. Then, urine of 24 h was collected and its volume was measured. During the time of urine collection, no food or water was made available to the rats.

Urine volume was recorded, diuretic Index was calculated and compared between the three different doses and with the controls. Diuretic Index was calculated using the following formula [11]:

$$\text{diuretic index} = \frac{\text{urinary volume of animals treated with extract}}{\text{urinary volume of control animals}}$$

2.5. Electrolyte level analysis

The concentrations of Na+ and K+ in the collected urine were determined using a flame photometer (Systonic®), whereas pH was directly determined on fresh urine samples using a pH meter (PHS-3C, PHS-25 ®). The ratio of electrolytes Na+/K+ was calculated to evaluate the activity of the extract [12].

2.6. Statistical analysis

All the values are expressed as mean ± standard error of the mean ($\bar{x}$ ± s.e.m). Statistical analysis was preformed using one-way ANOVA followed by Student's 't' test, p value of less than 0.05 was considered statistically significant.

3. Results

3.1. Extraction yield

Maceration of 200 g of dry fruit powder in ethanol-water (60:40) at room temperature for 72 hours gives 32.64 g of dry extract, which corresponds to 16.32 % of extraction yield. Phyto screening performed on this extract reveals the presence of high quantity of phenolic compounds and alkaloids, mild quantity of flavonoids, steroids, and triterpenes while saponins and glycosides are present in low quantity.

3.2. Effect of the extract on diuresis

The hydroalcoholic extract of *Sechium edule* fruit produces diuresis which appeared to be a function of dose. The urinary excretion of *Sechium edule* fruit extract is higher compared to the control group which is equal to 3.7 ± 0.4 ml versus 5.2 ± 0.2, 7.3 ± 2.3 and 8.4 ± 2.4 ml in the groups treated with the extract at doses 100, 200 and 400 mg/kg respectively ($p<0.05$) (Figure 1). The diuretic index values equal to 1.40, 1.97 and 2.27 for the respective doses of 100, 200 and 400 mg/kg show that the values of the high doses 200 and 400 mg/kg are superior to 1.5, indicating a high diuretic potential of *Sechium edule* fruit extract.

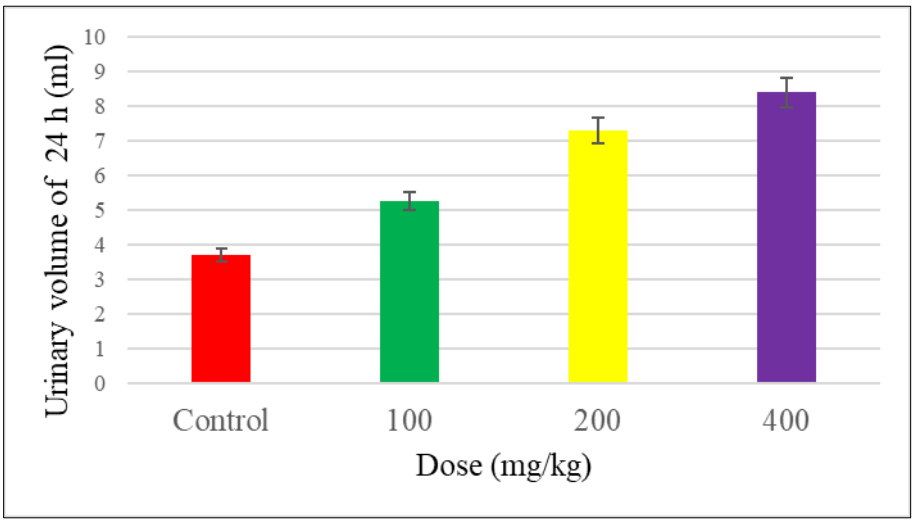

Figure 1 24 h urinary volume of the rats of control group ( ) and those treated with *Sechium edule* fruit extract, administered orally, at doses 100 ( ), 200 ( ) and 400 mg/kg ( ), after water load of 50 ml/kg (m ± sem; n = 6; $p < 0.05$)

3.3. Effect of the extract on natriuria and kaliuria

The results of analysis for the electrolyte content (Na+, K+) of the urine samples collected over the 24 hours are presented in Table 1. A significantly increased sodium loss was observed in rats treated with the extract at all doses as compared to the control group. The natriuria for control group is equal to 0.543 ± 0.023 mEq/L versus 0.74 ± 0.02, 0.863 ± 0.044 and 1.16 ± 0.09 mEq/L in animals treated with *S. edule* fruit hydro alcoholic extract at doses 100, 200 and 400 mg/kg ($p<0.05$).

Potassium loss is also observed in all doses compared to the control group. Kaliuria of control group is equal to 0.35 ± 0.028 mEq/L, versus 0.52 ± 0.02, 0.65 ± 0.03 and 0.92 ± 0.02 mEq/L in animals treated with *S. edule* fruit extract at doses 100, 200 and 400 mg/kg ($p<0.05$).

Table 1 also shows the Na+/K+ ratio of the extract at three different dose levels. The value of Na+/K+ ratios at 100 (1. 42), 200 (1.32), and 400 (1.26) are all between 1 and 2. These results indicate that *Sechium edule* fruit hydroalcoholic extract is a good natriuretic.

Table 1 Na+, K+ and Na+/K+ values

Doses (mg/Kg)	Na+ (mEq/mL)	K+ (mEq/mL)	Na+/K+
0	0.543 ± 0.023	0.35 ± 0.028	1.55
100	0.74 ± 0.02	0.52 ± 0.02	1.42
200	0.86 ± 0.04	0.65 ± 0.03	1.32
400	1.16 ± 0.09	0.92 ± 0.02	1.26

3.4. Effect on urine pH

The urinary pH was measured, and results show that there is no significant difference between the urinary pH of control group and those treated with the extract. The values are 8.616 ± 0.016 in control group, and 8.72 ± 0.02, 8.743 ± 0.05 and 8.793 ± 0.05 in the groups treated with the extract at doses 100, 200 and 400 mg/kg respectively (N.S) (Figure 2).

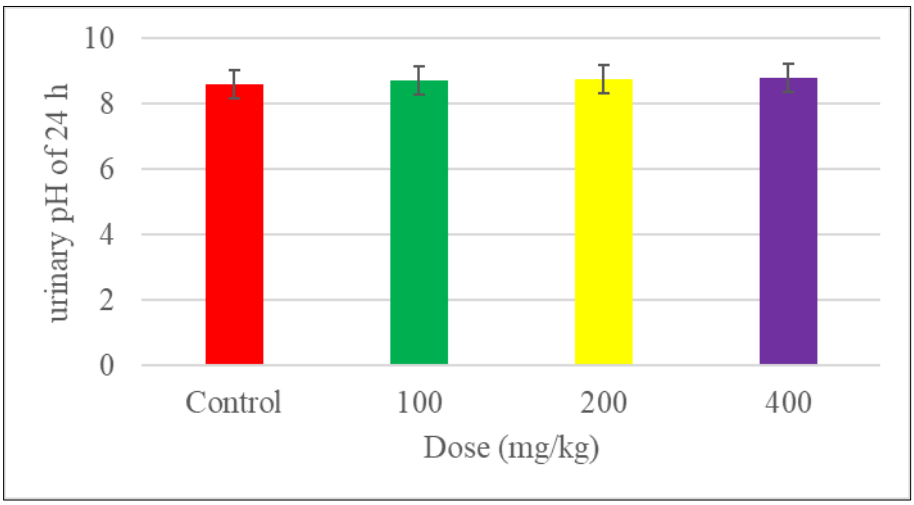

Figure 2 Urinary pH of 24 h of animals in control group ( ) and those treated with *Sechium edule* fruit hydroalcoholic extract, administered orally, at doses 100 ( ), 200 ( ) and 400 mg/kg ( ), after water load of 50 ml/kg (m ± esm; n = 6; N.S)

4. Discussion

According to the results of ethnopharmacological survey that we have conducted in the northern part of Madagascar, the decoction of the fruit of *Sechium edule* is used to enhance urinary emission in person with oliguria. Since its diuretic activity has not been proven scientifically, this work was undertaken to verify this traditional use. This study reports the diuretic and natriuretic effects of the hydroalcoholic extract of *Sechium edule* fruit.

In view of urine output, this extract produces a significant increase in diuresis which is dose dependant. Considering its diuretic index, superior to 1.5, this extract possesses a high diuretic potential [12]. The increase in Na+ output and Na+/K+ ratio value indicates the inhibition of sodium reabsorption in the renal tubule. Or according to the urinary pH values obtained, there is no significant difference between the control group and the 3 treated groups. These results indicate that *Sechium edule* fruit extract does not act in the proximal tubule, because diuretics acting on this part of tubule inhibits the carbonic anhydrase and increases the urinary pH, which is not the case here [13]. On the other hand, renal K+ excretion is affected by distal Na+ delivery. Our results show that *Sechium edule* fruit extract increases the potassium output. These results indicate that it is not a sparing potassium diuretic, because those diuretics acting on distal convoluted tubule inhibit the renin angiotensin system and reduce the potassium secretion [14]. In this study, the increase in excretion of K+ (kaliuretic effect) of the extract might be due to high concentration of the Na+ delivered in the distal convoluted tubule. According to our results, this extract does not spare potassium and does not act in the distal convoluted tubule. On the other hand, the value of the ratio of concentration of excreted Na+ and K+ is between 1 and 2 and it does not increase as the dose of the extract increases. These results indicate that the extract is a good natriuretic with lesser hypokalaemia side effect, which is a good point for a long-term use [15].

The diuretic activity dose-dependent effect of the extract indicates that this effect is intrinsic, and possibly receptor-mediated. According to our results, it does not act on proximal convoluted tubule neither on distal tubule, we suggest that it might act in the thick ascending limb of the loop of Henle, or in the diluting zone. To elucidate its site of action, evaluation of Cl^- output would help. But with the present results, the empirical use of *Sechium edule* fruit as diuretic is justified. For the time being, according to our results, even though it has less hypokalaemia side effect, its use should not be chronic because its kaliuretic activity implies complications. But it is interesting if it acts on the diluting zone as thiazide like, as it is the case of isoquercitrin, a flavonoid isolated from *Withania aristata* [16,17].

5. Conclusion

From the data of volume, electrolyte, and urinary pH analysis, it is plausible to assume that the plant's diuretic activity is by inhibiting the tubular reabsorption of water and electrolytes. This study provides further evidence that *Sechium edule* fruit possesses a diuretic activity as claimed in its traditional use.

Compliance with ethical standards

Disclosure of conflict of interest

The authors declared no conflict of interest.

Statement of ethical approval

This study obtained approval from the Scientific and Ethical Committee of the Sciences faculty of the University of Antananarivo, Antananarivo, Madagascar. The animals were treated with care throughout the study period and were kept in a well-controlled area according to the National Guidelines for the Care and Use of Laboratory Animals

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