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Diuretic activity assessment of an aqueous extract of *Zanthoxylum gilletii* (Rutaceae) stem bark in rats

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ABSTRACT

Recent analyses have highlighted *Zanthoxylum gilletii* richness in phytochemical compounds known for their essential role in renal and cardiovascular function. This study aims to evaluate the phytochemical composition, acute oral toxicity, and diuretic activity of an aqueous extract of *Zanthoxylum gilletii* stem bark (EA*Zg*). The acute toxicity test was performed according to OCDE guidelines No. 423, with doses of 300, 500, and 2000 mg/kg BW. The aqueous extract (100, 200, and 300 mg/kg BW) was administered orally to rats to study the dose-response effect. Next, the dose of 200 mg/kg BW of EA*Zg* was used for comparison with a group receiving furosemide (20 mg/kg BW) and a control group. The time to first urination was recorded, and urine volume was collected over 24 hours for each group of rats and urine's electrolytes determined. Aqueous extract of *Zanthoxylum gilletii* stem bark (EA*Zg*) contain flavonoids, polyphenols, saponines, sterols and triterpenoids. At the single dose of 2000 mg/kg, revealed no lethal effects. The results highlighted progressive and significant increase in urinary excretion and a reduction of the time of the first urination in treated rats at 200 mg/kg BW of EA*Zg* and furosemide at 20 mg/kg BW) compared to control group ($p < 0.05$; $p < 0.01$). Excretion of sodium in furosemide group, and potassium in both groups, also significantly ($p < 0.05$; $p < 0.01$) increased with the low kaliuresis observed in EA*Zg* group ($p < 0.01$; $p < 0.05$). These results highlight its potentials for the development of new therapeutic agents for renal and cardiovascular deseases.

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INTRODUCTION

Cardiovascular diseases (CVDs) are the leading cause of mortality worldwide and disproportionately affect low and middle income countries (WHO, 2023). In sub Saharan Africa, hypertension has emerged as the most prevalent non communicable disease and a major modifiable risk factor for stroke, heart failure, and edematous complications (Seedat *et al.*, 2018; Minja *et al.*, 2022). In Côte d'Ivoire, hypertension prevalence reaches 30–40% among adults, frequently progressing to congestive heart failure associated with chronic oedema (WHO, 2023; Ekra Kouadio *et al.*, 2021). Given the limitations of conventional antihypertensive therapies, medicinal plants have attracted growing scientific attention as potential sources of diuretic and cardioprotective agents. *Zanthoxylum gilletii* (Z. *gilletii*) a Rutaceae species widely distributed across tropical Africa. Phytochemical analyses of have uncovered its rich composition of bioactive secondary metabolites like flavonoids, alkaloids, and terpenoids, which are known to play key roles in renal and cardiovascular functions (Mbula *et al.*, 2022; De Lange Jacobs *et al.*, 2020). Nonetheless, experimental evidence regarding its diuretic effects remains scarce, making further investigation into this property's potential to manage hypertension and fluid retention conditions highly pertinent.

MATERIALS AND METHODS

Plant collection and identification

The stem bark of *Zanthoxylum gilletii* was collected in the South of Ivory Coast in December 2020. Authentication was carried out by the botanical experts of the CNF (National Floristic Centre) of the Félix Houphouët-Boigny University under the number UCJO 16133.

Animals

Rattus norvegicus strain, females (nulliparous and non-pregnant) and males, healthy young adult, aged 8–12 weeks, albino rat weighing 200-250 g were used. They were obtained from the Vivarium (animal house), Ecole Nationale Supérieure (ENS), and Abidjan, Côte d'Ivoire.

Animals housed in metabolic cages and maintained under standard laboratory conditions (T°= 25 ± 2°C) with dark and light cycle (12/12 h). They were allowed free access to standard dry pellet diet and water *ad libitum*.

Aqueous extract of stem bark of *Zanthoxylum gilletii*

To prepare the aqueous extract 100g of powder of stem bark of *Zanthoxylum gilletii*, milled in a micro mill (Culatti® MFC, Allemagne), are boiled for 30 minutes in 1 liter of distilled water. The resulting decoctions is filtered twice through absorbent cotton and then once through Wattman filter paper. The filtrate was collected in a flask and then evaporated at 60°C, using a rotary evaporator (Büchi) and oven-dried at 50 ± 5°C. After drying, a fine water-soluble powder was provided represents the aqueous extract of stem bark of Z. *gilletii* (EA*Zg*). This product is used for toxicity tests and pharmacological studies on urinary excretion in rats.

Reference drug

Furosemide (Lasilix 20 mg, Cp), a diuretic drug which acts potent loop diuretic, was used as the reference drug. It was dissolved in distilled water just before to administration.

Phytochemical analysis

Phytochemical analysis of the aqueous extract of stem bark of *Zanthoxylum gilletii* (EA*Zg*) were identified by the reactions in tube (Towanou *et al.*, 2023).

Acute toxicity test

The Acute toxicity test was conducted following the guidelines outlined in the OCDE no 423, (OCDE, 2001). Six females, *Rattus norvegicus* (rats), aged 8–12 weeks were used. The dose of the sample administered was 300, 500 and 2000 mg/kg body weight (BW). Animals are fasted 18 hours before the experiment. Rats were treated according to good laboratory practices. The doses of 300, 500 and 2000 mg/kg of aqueous extract of stem bark of Z. *gilletii* and distilled water as a single dose (1 ml) orally using oral feeding needle. Animals were observed

individually for first 30 min, then for the first 24 h, with special attention given during the first 4 h, and daily thereafter, for a total of 14 days to observe toxicity signs like changes in skin and fur, eyes, mucous membranes, respiratory, circulatory, autonomic and central nervous systems and for behavioural pattern.

Pharmacological studies

Dose-response activity of EAZg on urinary excretion

The protocol used is that of Kau *et al.* (1984). Overall, 45 wistar strain rats aged 8–12 weeks weighing 200-250 g were used. They were divided into six groups and placed individually in metabolic cages. After the induction of the fluid overload (50 ml/kg BW), animals received drug or distilled water :

Groups 1: Rats were orally administered 2mL of distilled water.

Groups 2-3: Rats were orally administered 100, 200, 300, 400 and 500 mg//kg BW of EA*Zg* respectively.

Groups 4: Rats were orally administered 5 ml/kg BW of Furosemide 20 mg/kg (FURO 20), dissolved in distilled water, as a reference drug.

Groups 5: Rats were orally administered 5 ml/kg BW of distilled water (negative control group).

Comparative effects of furosemide and EA*Zg* on urinary excretion on urinary excretion in rats

Three groups of five rats were composed and distributed i.e., rats in group 1 (control group) received 2 ml of distilled water whereas Groups 2, and 3 received 200 ml/kg BW of EA*Zg* (EA*Zg* 200) and 20 mg/kg BW of furosemide (FURO 20 mg/kg BW), respectively. The urine volume were collected each 2 hours during 24 hours from each group of rats was recorded.

All experimental protocols were conducted in accordance with the protocols for the protection of experimental animals of the European Council on Legislation 2012/707.

Analytical procedures

At the end of the 24 h period, urine samples were collected and aliquoted into Eppendorf tubes. Urinary concentrations of Electrolytes (Na^+, K^+ and Cl^- ions) were evalued using the photometric. The colorimetric and kinetic technique allows for the measurement of creatinine and urea respectively (Janssens, 2015).

Statistical analysis

GraphPad Prism 8.4.3 Software (La Jolla, CA, USA) was used to carry out statistical analysis of the data and graphical representations. The significance of differences observed between concentrations was assessed using analysis of variance (ANOVA) followed by the Tukey-Kramer test. The data and results expressed as mean ± SEM (standard error of mean). The results were considered statistically significant when $p < 0.05$.

RESULTS

Phytochemical study of the aqueous extract of stem bark of *Zanthoxylum gilletii*

The phytochemical screening revealed the presence of sterols, polyterpenes, polyphenols, flavonoids and saponins in stem bark of *Zanthoxylum gilletii* (Table 1).

Table 1. Phytochemical screening of the aqueous extract of stem bark of Zanthoxylum gilletii

Secondary metabolites		Reactant/Tests	Results
Polyphenols		$FeCl_3$	+
Flavonoids		Cyanidin	+
Alcaloids		Dragendorff; Bouchardat	-
Tanins	Catechic	Stasny	–
	Gallic	Acetate of sodium and ferric chloride	–
Saponosids		Physical	+
Quinons		Borntraeger	–
Sterols and Triterpenoids		Lieberman	+

(+) : presence of Secondary metabolites

(-) : absence of Secondary metabolites

Acute toxicity of the aqueous extract of stem bark of *Zanthoxylum gilletii*

The acute oral toxicity assessment of AEZg administered to female Wistar rats revealed no mortality or observable signs of toxicity throughout the 14-day observation period. Animals treated with

doses up to 2000 mg/kg body weight showed no abnormalities in behavior, feeding activity, salivation, diarrhea, bleeding, body weight, or general appearance compared with the control group. These findings indicate that the aqueous extract possesses a favorable safety profile under the conditions of this study and may be considered relatively non-toxic at the tested doses (Table 2).

Table 2. Acute toxicity of AEZg administered per os to female wistar rat

Observation period	30 to 60 minutes			One day			14 Days		
				Distilled water or AEZg doses (mg/kg)					
Toxic symptoms /mortality	Control	500	2000	Control	500	2000	Control	500	2000
Cotorsion /isolation	No	No	No	No	No	No	No	No	No
Salivation / diarrhea	No	No	No	No	No	No	No	No	No
Bleeding	No	No	No	No	No	No	No	No	No
Feeding perturbation	No	No	No	No	No	No	No	No	No
Body weight variation	No	No	No	No	No	No	No	No	No
Mortality	No	No	No	No	No	No	No	No	No

No: absence of toxic symptoms or mortality, Yes : presence of toxic symptoms or mortality

Pharmacological effects of the aqueous extract stem bark of *Zanthoxylum gilletii*

Dose-response effect of a EA*Zg on urinary excretion*

Fig. 1 present results of dose-response activity of EA*Zg* oral administration (100, 200 and 300 mg/kg BW) and negative control. Oral administration of the aqueous of EA*Zg* increased the urinary flow in a dose-dependent manner. Maximum urinary volume was observed at 200 mg/kg (4.37 ± 0.60 ml), exceeding the control (3.2 ± 0.88 ml; p< 0, 05) but lower than furosemide (7.53 ± 0.48 ml). No signs of dehydration were observed in the rats after 24 hours.

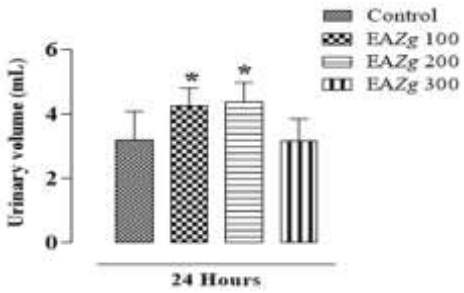

Fig. 1. Dose-response effect of aqueous extract of stem bark of *Zanthoxylum gilletii* on urinary excrétion of rats
Each value represents mean ± S.E.M; n=5. Abbreviations: EAZg 100, 200 and 300, aqueous extract of stem bark of *Zanthoxylum gilletii* 100, 200 or 300 mg/kg of Body Weigth; FURO 20, furosemide 20 mg/kg

Comparative effects of aqueous extract of stem bark of *Zanthoxylum gilletii* and furosemide on urinary excretion in rats

Effect on the time to first urination

Furosemide 20 mg/kg BW and EA*Zg* 200, showed the minimal times of first urination 34.75 ± 3.82 and 48.75 ± 9.32 min respectively as compared to the control group (78 ± 13.29 ; min $p < 0.001$, $p < 0.05$) (Fig. 2).

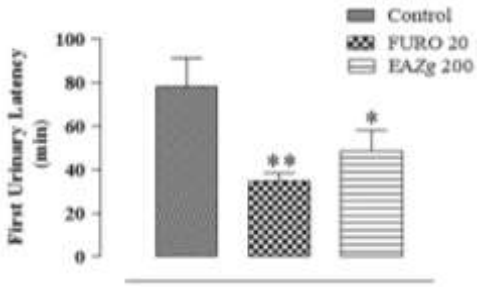

Fig. 2. Effect of aqueous extract of stem bark of *Zanthoxylum gilletii* on the time to first urination of rats
Each value represents mean ± S.E.M; n=5. Abbreviations: EAZg 200, aqueous extract of stem bark of *Zanthoxylum gilletii* 200 mg/kg of Body Weigth; FURO 20, furosemide 20 mg/kg

Effect of aqueous extract of stem bark of EAZg on urinary volume

Urinary excretion (ml/kg) volumetric induced by EA*Zg* and furosemide obtained every two hours during 24 hours, is treatment-dependent and had the different kinetics (Fig. 3).

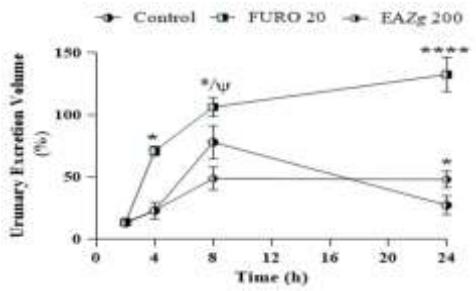

Fig. 3. Evolution of urinary excretion measured after EAZg and furosemide administration on rat

Urine output are measured every two hours for 24 hours. Each value represents mean ±S.E.M; n=5. Abbreviations: EAZg 200, aqueous extract of stem bark of *Zanthoxylum gilletii* 200 mg/kg of Body Weight; FURO 20, furosemide 20 mg/kg of Body Weight

Indeed, administration of EA*Zg* (200 mg/kg) and FURO (20 mg/kg) resulted in EUV values of 23.13 ± 1.04% and 70.93 ± 3.64%, respectively, at four hours, compared to 22.89 ± 6.82% in the control group ($p < 0.05$). These EUV values increased progressively after eight hours for the different treatments (48.08 ± 6.63%; 132.5 ± 13.77%; 27.37 ± 7.59%; $p < 0.001$).

At this stage, with EA*Zg* (200 mg/kg) the EUV max, is reached at 48.75 ± 9.32% compared to 106.4 ± 7.63% and 78 ± 13.29% in the FURO (20 mg/kg) group and the controls, respectively. The EUV max, is obtained at 24 hours in rats treated with FURO 20 mg/kg (132.5 ± 13.77%; $p < 0.001$).

Effect on electrolytes output

Urinary Na^+ concentrations determined in rats from the EAZg (200 mg/kg BW) and FURO (20 mg/kg BW) groups were significantly higher ($p<0.05$) than those in the control group. The respective concentrations recorded were 151.2 ± 6.44 mmol/L (EAZg 200 mg/kg BW), 183.9 ± 4.03 mmol/L (FURO 20 mg/kg BW) versus 138.1 ± 18.39 mmol/L for the control group, as shown in Fig. 4A.

Fig. 4B shows that the K^+ concentration obtained on rats treated with FURO (20 mg/kg BW) and EA*Zg* (200 mg/kg BW) was significantly higher than that obtained with control ($p < 0.01$; $p < 0.05$). The values obtained are 35.39 ± 3.78 mmol/L; 91.91 ± 4.99 mmol/L; 55.13 ± 3.42 mmol/L respectively for FURO (20 mg/kg BW), EA*Zg* (200 mg/kg BW) and the control group.

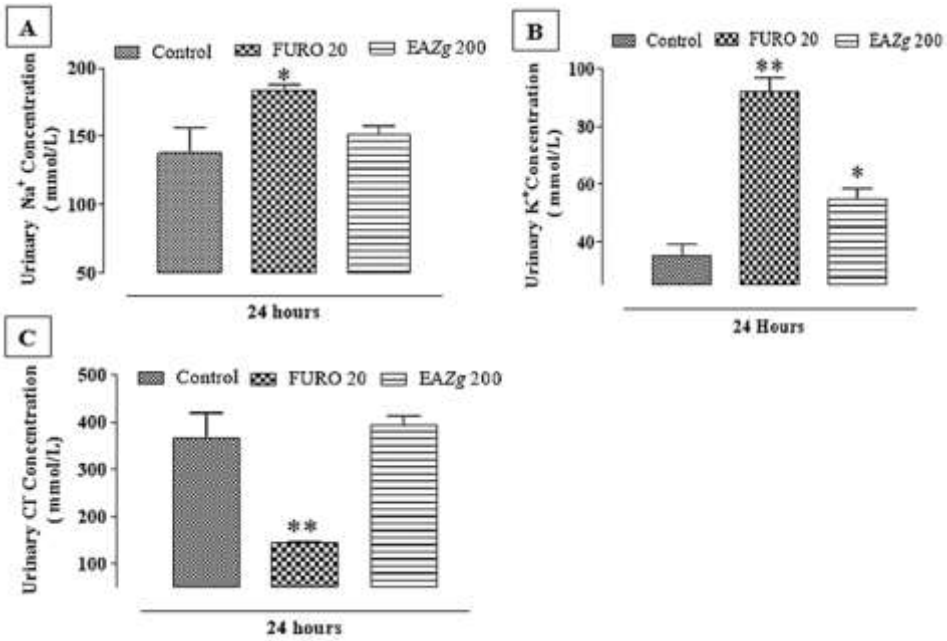

Fig. 4. Effect of aqueous extract of stem bark of *Zanthoxylum gilletii* on urinary output (A) sodium, (B) potassium and (C) chloride of rats at 24 hours

Each value represents mean ± S.E.M; n= 5. Abbreviations: EAZg 200, aqueous extract of stem bark of *Zanthoxylum gilletii* 200 mg/kg of Body Weigth; FURO 20, furosemide 20 mg/kg

Urinary Cl^- concentration recorded in rats treated with FURO (20 mg/kg BW) was significantly lower (145.1 ± 2.75 mmol/L) than that obtained in rats treated with EAZg 200 mg/kg BW (394.6 ± 19.46 mmol/L; $p < 0.01$) and that of the control group (365.4 ± 54.42 mmol/L (Fig. 4C).

DISCUSSION

The phytochemical screening of the aqueous stem bark extract of *Zanthoxylum gilletii* revealed the presence of polyphenols, flavonoids, sterols, polyterpenes, and saponosides, while tannins, quinones, and alkaloids were absent. These findings are consistent with recent studies by (Dzouemo *et al.*, 2022), who identified diverse bioactive metabolites such as alkaloids, coumarins, lignans, triterpenes, and steroids in the bark of *Z. gilletii*, confirming the chemical richness of this species.

Acute toxicity evaluation demonstrated that oral administration of doses up to 2000 mg/kg body weight did not induce mortality or clinical signs. EA*Zg* is thus classified in category 5 according to the OCDE Globally Harmonised Classification System, with an LD_{50} greater than 5000 mg/kg BW. This suggests that *Z. gilletii* is safe for pharmacological use, in line with recent reviews reporting no major adverse effects associated with its extracts (Mbula *et al.*, 2022).

Pharmacologically, the aqueous extract at 200 mg/kg induced significant urinary excretion, comparable to furosemide. This diuretic effect appears to be mediated through inhibition of sodium, chloride, and water reabsorption at the loop of Henle, similar to loop diuretics (Odlind *et al.*, 1984). The extract also reduced the time to first micturition and promoted electrolyte elimination, confirming its diuretic potential (Sadki *et al.*, 2010). Importantly, the low kaliuresis suggests a potassium-sparing effect, which is therapeutically advantageous compared to conventional loop diuretics.

These results align with findings from other medicinal plants with diuretic properties, including *Salvia scutellarioides* (Ramirez *et al.*, 2006), and *Spergularia purpurea* (Jouad *et al.*, 2001).

Zanthoxylum gilletii contains numerous phytochemicals of therapeutic interest. This species exhibits significant diuretic activity, comparable to that of furosemide, and a favorable safety profile. These results highlight its potential in regulating renal function and could be a source for the development of new therapeutic agents.

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