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Diuretic mechanism and nephroprotective effect of bark aqueous extract of *Erythrophleum africanum* in wistar rat

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ABSTRACT:*Erythrophleum africanum* is a plant whose bark is used in traditional medicine as a diuretic and in the treatment of arterial hypertension.
Methods: In this study, the effect of an aqueous extract of bark *Erythrophleum africanum* (EAf) and diuretics was investigated in water-overloaded rats and electrolytes and biochemical parameters were assayed to detect its mechanism.
Results: The results showed that with EAf, the diuretic index was 1.93, the pH was 7.25, the sum of $Na^+ + Cl^-$ was 2.41, the Na^+ / K^+ ratio was 2.12 and the Cl- / ($Na^+ + K^+$) ratio was 0.81. Ca^{2+} and Mg^{2+} concentrations and creatine clearance increased significantly ($p < 0.01$) with EAf. These results are broadly similar to those for Furosemide.
Discussion: The bark extract of *Erythrophleum africanum* (EAf) therefore acts in a similar way to Furosemide, inhibiting sodium reabsorption by inhibiting the $Na^+/K^+/Cl^-$ transporter at the ascending branch of the loop of Henlé, and indirectly associated with urinary leakage of Mg^{2+} and Ca^{2+}. The extract is also thought to have nephroprotective properties. These results confirm the diuretic potential of *Erythrophleum africanum* leaves and justify its use in traditional medicine.

KEYWORDS:*Erythrophleum africanum*, nephroprotective, diuretic, electrolytes, biochemical parameters

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I. INTRODUCTION

Diuretic drugs are generally used clinically in oedematous states, such as congestive heart failure, hepatic cirrhosis, nephrotic syndromes and arterial hypertension. To be effective in correcting extracellular fluid volume, plasma volume and blood pressure, diuretics must induce natriuresis. Natriuresis can be achieved either by decreasing tubular sodium reabsorption or by increasing the load of filtered sodium, or by a combination of these effects[1]. This is because each segment of the nephron has a unique sodium entry mechanism and the ability to specifically inhibit this step[2-3].

However, pharmacological studies have shown that synthetic diuretics have numerous side effects (plasma acidosis, ionic imbalance, dehydration, hypotension, etc.) and cannot be administered in certain physiological situations such as pregnancy[4]. Although diuretics are effective in reducing fluid retention and hypertension, they can have adverse effects on renal function, in particular renal failure. Regular monitoring of renal function is therefore essential when using diuretics[5].

Currently, the WHO welcomes products from traditional pharmacopoeia as an alternative solution for treating diseases[6]. It is in this context that numerous studies have been carried out on plants with diuretic effects in order to identify their mechanism and verify their nephroprotective properties. The diuretic mechanism of *Saccharum officinarum*[7] and the nephroprotective properties of *Mimosa invisa*[8] and *Bridelia ferruginea*[9] have been studied.

We were interested in *Erythrophleum africanum* (Fabaceae), which is used in traditional medicine to treat a number of conditions including dental pain[10] and has diuretic and cardiotonic properties[11].

The mechanism and the nephroprotective effect of the bark extract of *Erythrophleum africanum* were verified by measuring electrolytes and biochemical parameters in the urine and blood of water-overloaded rats.

II. MATERIALS AND METHOD

2.1. Material

2.1.1. Plant

The plant material used consisted of *Erythrophleum africanum* (Fabaceae) bark harvested in the north-east of Ivory Coast in the Bounkani region, in Doropo in May 2023. The identification was carried out at the Floristic National Center of Felix HOUPHOUET-BOIGNY University (Ivory Coast) in comparison with the herbarium n°UCJ009361 of this center.

2.1.2. Animal

The animal material used consists solely of rats of the species *Rattus norvegecus* (Muridae), of the Wistar strain. They were reared under standard conditions of temperature, nutrition and atmospheric pressure at the UFR Biosciences of the Felix HOUPHOUET-BOIGNY University in Abidjan. The animals weighed between 126g and 200g and were used for toxicological and pharmacological tests. This study was conducted in accordance with the European directives of 24 November 1986 (86/609/EEC) and the decree of 19 April 1988 relating to animal experimentation in research[12].

2.1.3. Physiological and pharmacological substances

The pharmacological substances used in this study are essentially diuretic substances, in this case Furosemide 40 mg/kg B.W. (Sanofi Aventis, France), Aldactone 25 mg/kg B.W. (Pfizer pfe, France) and Esidrex 25 mg/kg B.W. (Norvartis pharma, Switzerland).

2.2. Methods

2.2.1. Preparation of the bark aqueous extract of *Erythrophleum africanum* (Fabaceae)

The bark of the plant was dried at room temperature in the shade for four weeks. After drying, they were crushed and then ground using a grinder at the pharmaceutical and biological sciences laboratory of the Felix HOUPHOUET-BOIGNY University (Abidjan, Ivory Coast). Next, 200 g of the powder obtained was mixed with 2 L of distilled water in a round-bottomed flask, then placed under stirring for 24 h. The homogenate obtained was filtered once through poplin cloth, then 3 times through cotton wool and finally 2 times through Whatmann n°2 filter paper. The filtrate was dried in a Med Center venticell oven at 70°C for 72 hours to obtain the burgundy-coloured of bark dry extract of *Erythrophleum africanum* (Fabaceae), which we called EAf.

2.2.2. Study of the mechanism of the aqueous extract of the bark of *Erythrophleum africanum* (Fabaceae) and diuretics

2.2.2.1. Experimental protocol

This study was carried out using the method described by Colot[13] and Sanogo[14]. Urinary volume was measured over 24 hours after the rats were placed in water overload and the test substances were administered orally.
To do this, the animals were fasted for 18 hours before the experiment with free access to water. Just prior to the experiment, 50 mL / kg B.W. of distilled water was administered to each rat. The control animals then received 2 mL of distilled water each. The animals in the other 4 (four) batches received EAf (1500 mg / kg B.W.), Furosemide (40 mg / kg B.W.), Aldactone (25 mg / kg B.W.) and Esidrex (25 mg / kg B.W.) at a rate of 2 mL / rat respectively. They were then immediately placed in individual metabolism cages and the urine produced over 24 hours was collected. The diuretic activity of the extract was determined using diuretic index values[15-16] (Table 1). The diuretic index is calculated according to the following formula:

$$\text{Diurétic index} = \frac{\text{Urinary volume mean of treated batch (mL)}}{\text{Urinary volume mean of Control (mL)}}$$

2.2.2.2. Evaluation of urine pH in rats

The effect of EAf and diuretics on urinary pH is determined by measuring the pH of urine collected 24 hours after treatment of the rats. A small quantity of urine was placed on a piece of pH paper. Finally, the paper is coloured to give a colour corresponding to the urinary pH value of each rat in each batch.

2.2.2.3. Electrolytes and biochemical parameters methods

The effect of EAf and diuretics on urinary excretion was measured by measuring creatinine, urea and Na+, Cl^-, K^+, Mg^{2+} and Ca^{2+} ions in the urine obtained 24 hours after treatment of the animals.
The urine was firstly filtered through cotton wool and then centrifuged at 3,000 rpm for 10 minutes to remove any residues. The supernatant was then recovered and the urinary levels of creatinine, urea and Na^+, Cl^+, K^+, Mg^{2+} and Ca^{2+} ions were measured directly using a spectrophotometer (Hycel PHF 104).
The results for these compounds are then multiplied by the 24-hour urine volume to obtain the daily urinary level of these electrolytes and compounds.
Blood is taken from the retro orbital vein in collection tubes. It is then centrifuged. Finally, creatinine measurement is based on the reaction with sodium picrate described by Jaffe[17] and modified by Fabiny and Ertingshausen[18]. The method for measuring urea is based on the work of Fawcett and Scott[19] and is based on the hydrolysis of urea into ammonia and carbon dioxide in the presence of water and urease.

2.2.3. Study of the mechanism of the bark aqueous extract of *Erythrophleum africanum* (Fabaceae)

The mechanism of the bark aqueous extract of *Erythrophleum africanum* was determined using the ratios between the levels of the various ions excreted (Na^+, Cl^- , K^+ , Mg^{2+} and Ca^{2+}) and the pH according to the methods described by Welu[16]**(Table 2)**. The sum of Na^+ and Cl^- is used to assess the salidiuretic activity of a product[20]. The Na^+ / K^+ ratio is used to classify substances according to their degree of sodium excretion in relation to potassium. The Cl- /(Na^+ + K^+) ratio is used to estimate carbonic anhydrase inhibition[21-22-23].

Table No.-1: Diuretic potential of extracts according to diuretic index values[15-16]

DIURETIC INDEX	**DIURETIC POTENTIAL**
> 1,50	Potential
1,50 > diuretic index > 1,0	Mean Potential
1,0 > diuretic index > 0,72	Low Potential
< 0,72	no potential

Table No.-2: Diuretic activity according to excreted ion values[21-22-23].

RAPPORT	**INTERPRETATION**
$Na^+ + Cl^-$ (Control) < $Na^+ + Cl^-$(traité)	Salidiuretic product
1 < Na^+ / K^+< 2	Satisfactory natriuretic product
2 < Na^+ / K^+< 10	Natriuretic product promoting urinary elimination of Na+ without excessive loss of K+.
Na^+ / K^+> 10	Potassium-sparing product
Cl^- / (Na^+ + K^+) < 0,8	Carbonic anhydrase inhibitor
0,8 < Cl^- / (Na^+ + K^+) < 1	Product does not inhibit carbonic anhydrase

2.2.4. Statistcal analysis

The GraphPad *Prism* 5.01 computer programme (San Diego CA, USA) was used for the statistical analysis of the results. The results were processed by analysis of variance (Anova), followed by Dunnett's multiple comparison test. The difference between two values is considered significant for ($p<0.005$). Values are

presented as the mean followed by the error on the mean (M ± ESM). This software was used for statistical processing of the various parameters. GraphPad *Prism* version 5.01 (San Diego CA USA) was used to plot the diagrams.

III. RESULTS

3.1. Effects of EAf and diuretics on diuretic index, $Na^+ + Cl^-$ sum, Na^+ / K^+ ratio and $Cl^- /(Na^+ + K^+)$.

To clarify the mechanism of EAf, we determined the following parameters: diuretic index, pH, $Na^+ + Cl^-$ sum, Na^+ / K^+ ratio and $Cl^- / (Na^+ + K^+)$ (Table I). The diuretic index obtained in the urine of rats treated with EAf was higher than that of the control, as were Furosemide, Aldactone and Esidrex. The pH of rats treated with EAf was basic, as was that of Furosemide, Aldactone and Esidrex. The $Na^+ + Cl^-$ sum of 2.41 mEq / L for the batch treated with the extract was higher than that of the control, as was that of the diuretics. The Na^+ / K^+ ratio in the urine of rats treated with EAf was between 2 and 10, similar to that of Furosemide and Esidrex. Finally, the $Cl^- / (Na^+ + K^+)$ ratio obtained with EAf was between 0.8 and 1 as in the batches treated with Furosemide, Aldactone and Esidrex.

Table No.-3: Effects of EAf compared with the diuretics on the diuretic index, the sum of $Na^+ + Cl^-$, the Na^+ / K^+ and $Cl^- /(Na^+ + K^+)$ ratios.

Parameters / **Group**	**Diuretic index**	**pH**	**$Na^+ + Cl^-$**	**Na^+ / K^+**	**$(Cl^- / Na^+ + K^+)$**
Control	1	6,75	1,22	1,51	
EAf (1500 mg / kg B.W.)	1,93	7,25	2,41	2,12	0,81
Furosemide (40 mg / kg B.W.)	2,50	7,5	3,17	3,28	0,8
Aldactone (25 mg / kg B.W.)	1,75	7,33	1,8	10,25	0,91
Esidrex (25 mg / kg B.W.)	2,15	7,5	2,44	2,53	0,87

3.2. Effect of EAf and diuretics on urinary calcium excretion by rats

Figure 1 shows the concentration of calcium in the 24-hour urine of rats overloaded with water and treated with the extract or diuretics. The level of calcium in the urine of rats treated with the extract or reference substances increased significantly ($P < 0.001$) compared with that of the control. In fact, calcium levels in batches of rats treated with EAf, Furosemide, Aldactone and Esidrex increased by 259.25, 196.29, 159.25 and 177.77% respectively.

3.3. Effect of EAf and diuretics on urinary magnesium excretion by rats

Magnesium levels in the 24-hour urine of rats treated with the extract or reference substances increased significantly ($P < 0.001$) in all batches compared with the control batch with the exception of Esidrex. Magnesium in the urine of rats treated with EAf, Furosemide and Aldactone increased by 619.74, 1186.62 and 5040.12% respectively. **Figure 2** shows the concentration of magnesium in the 24-hour urine of rats overloaded with fluid and treated with the extract or the reference diuretics.

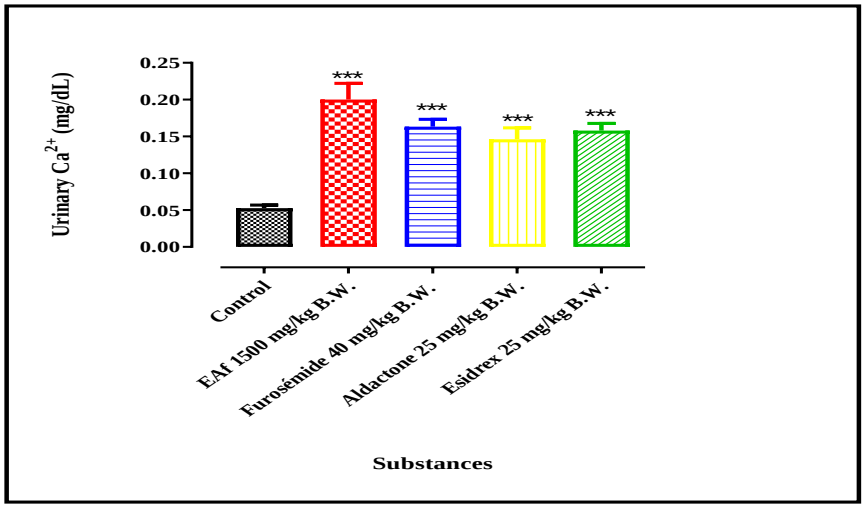

Figure No 1: Calciuria in water-overloaded rats treated with extract or diuretics compared with control

Values are expressed as mean ± SEM (n=4). Analysis was performed by applying. ANOVA followed by Dunnett test

****: p<0,001 compared to Control*

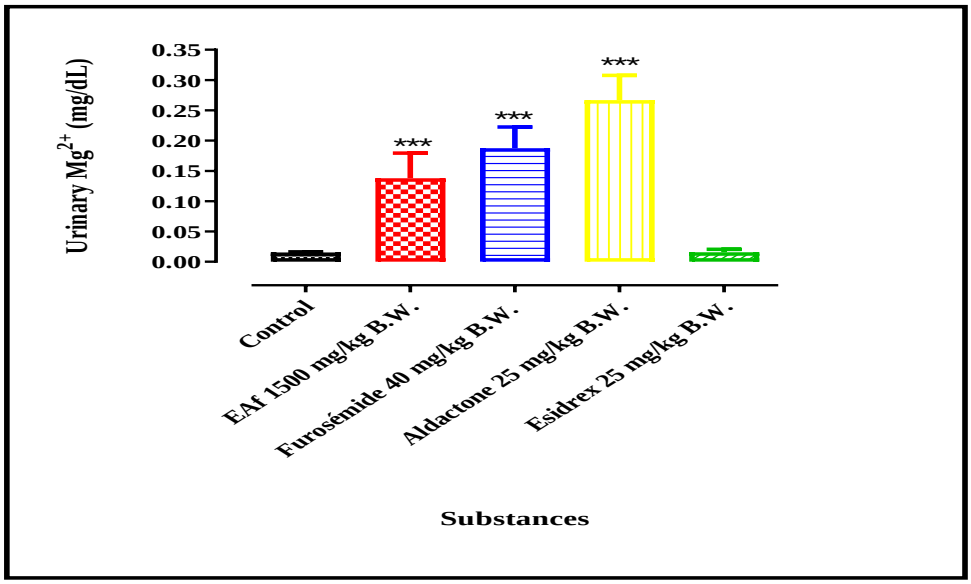

Figure No 2: Concentration of urinary magnesium in water-overloaded rats treated with the extract or diuretics compared with the control.

Values are expressed as mean ± SEM (n=4). Analysis was performed by applying. ANOVA followed by Dunnett test

****: p<0,001 compared to Control*

3.3. Effects of EAf and diuretics on renal function in rats

3.3.1. Effects of EAf and diuretics on urinary and plasma creatinine and urea levels in rats

The urinary urea levels of rats treated with EAf and Furosemide, Aldactone and Esidrex increased significantly ($p < 0.01$; $p < 0.001$) and plasma urea levels decreased significantly ($p < 0.01$; $p < 0.001$) compared to the control. Similarly, animals treated with the extract, Furosemide, Aldactone and Esidrex had very significantly (p

< 0.01; p < 0.001) increased urinary creatinine and decreased significantly (p < 0.01; p < 0.001) the plasma creatinine levels compared with the control. The results of urine and plasma creatinine and urea determinations are shown in **Table 4.**

3.3.2. Effects of EAf and diuretics on creatinine clearance in rats

The graph in **Figure 3** shows the different variations in clearance obtained in the treated batches 24 hours later. Creatinine clearance in the control batch was 0.34 ± 0.02 mL / min, 24 hours after receiving distilled water. Creatinine clearance increased significantly (P < 0.001), 24 hours later in urine with all substances administered compared with the control batch. Changes in clearance were 238.23, 307.35, 355.88 and 211.76% respectively with EAf, Furosemide, Aldactone and Esidrex.

3.3.3. Effects of EAf and diuretics on the ratio of urine urea to plasma urea in rats

In the control group, the ratio of urine urea to serum urea was 27.61 ± 1.41. This ratio did not vary significantly (p > 0.05) between EAf, Furosemide and Esidrex. This ratio did not vary significantly (p > 0.05) between EAf, Furosemide and Esidrex. Only Aldactone significantly (P < 0.01) increased this ratio by 110.4% compared with the control batch. The graph in **Figure 4** shows the variations in the ratio of urine urea to plasma urea in the different batches.

Table No.-4: Effects of EAf and diuretics on urinary and plasma creatinine and urea levels

Biochemical parameters	**Urea**		**Creatine**	
Substances	Urinary (mg / dL)	Plasmatic (mg / dL)	Plasmatic (mg / dL)	Urinary (mg / dL)
Control	104,6 ± 3	10.63 ± 0.11	0.031 ± 0.003	2.28 ± 0.13
EAf (1500 mg / kg B.W.)	225,2 ± 5 ***	6.62 ± 0.26**	0.07 ± 0.005 *	7.56 ± 0.66***
Furosémide (40 mg / kg B.W.)	280,8 ± 3***	7.74 ± 0.09**	0.098 ± 0.001***	5.87 ± 0.31 ***
Aldactone (25 mg / kg B.W.)	315,3 ± 1.9***	6.54 ± 0.26***	0.064 ± 0.004 *	8.68 ± 1.15***
Esidrex (25 mg / kg B.W.)	193,8 ± 1**	8.23 ± 0.66**	0.11 ± 0.01***	4.79 ± 0.14 **

Values are expressed as mean ± SEM (n=4). Analysis was performed by applying. ANOVA followed by Dunnett test

:p<0,01;: p<0,001 compared to Control*

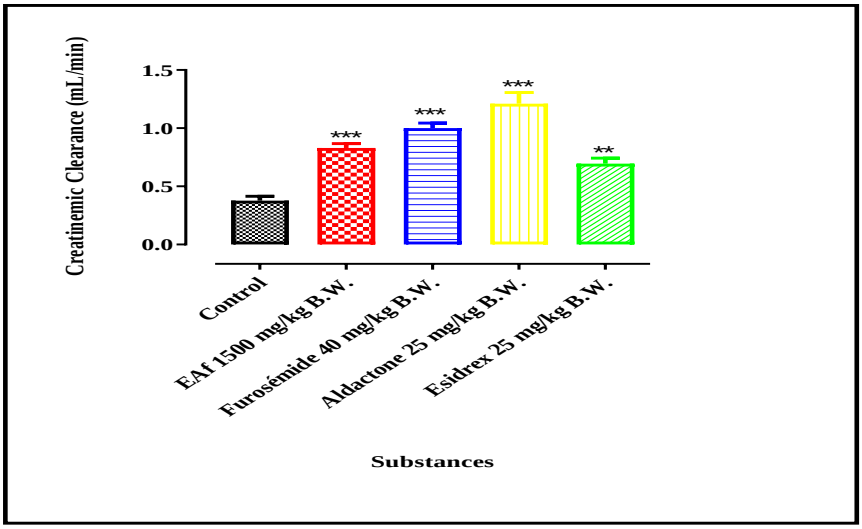

Figure No 3: Creatinine clearance of rats treated with extract or diuretics compared with the control.

Values are expressed as mean ± SEM (n=4). Analysis was performed by applying. ANOVA followed by Dunnett test

:p<0,01;: p<0,001 compared to Control*

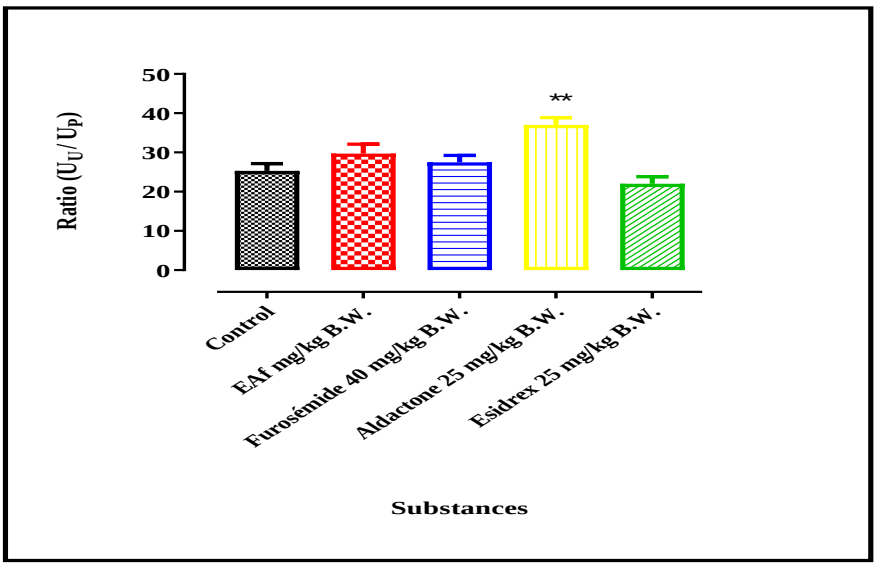

Figure No 4: Ratio of urine urea to blood urea in rats treated with extract or diuretics compared with the control.

Values are expressed as mean ± SEM (n=4). Analysis was performed by applying. ANOVA followed by Dunnett test

*;**: p<0,01 compared to Control*

IV. DISCUSSION

The aim of this study was to identify the diuretic mechanism of an aqueous extract of *Erythrophleum africanum* (Fabaceae). This plant is used in traditional medicine to treat high blood pressure and heart failure, and as a diuretic. To gain a better understanding action of this extract, a salidiuretic study was carried out. This study was carried out by overloading rats with water in order to determine the concentration of ions excreted and the action site of this in the nephron. The aim of water overloading is to dilute the concentration of electrolytes in the internal liquid, that causing the reabsorption of this. According to Rasamindrkotroka[24], any salidiuretic

substance increases the excretion of electrolytes despite their reabsorption. The result indicates that the urine pH of rats treated with EAf and diuretics is basic, showing that the extract and diuretics promote the elimination of Na^+, K^+ and Cl^- and make the urine more alkaline[17].

The $Na^+ + Cl^-$ sum in the urine of the treated batch with EAf as well as that of the diuretics is higher than that of the control indicating that EAf could therefore be classified as a salidiuretic product. Indeed, according to Al-saikhan and Ansari[17], when this sum is greater than that of the control, the substance is considered as a salidiuretic.

The Na^+ / K^+ ratio of EAf is close to that of Furosemide and Esidrex. EAf could be classified as a natriuretic product promoting urinary elimination of Na^+ without excessive loss of K^+. Indeed, according to Welu[16], when the Na^+ / K^+ ratio of a substance tested is between 2 and 10, this substance can be considered as a natriuretic substance promoting urinary elimination of Na^+ without excessive loss of K^+.

Finally, the Cl- / ($Na^+ + K^+$) ratio is between 0.8 and 1 for EAf, as it is for the other substances. EAf could be classified as a non-inhibitor of carbonic anhydrase. According to Kebamo[20], when the Cl^- / ($Na^+ + K^+$) ratio of a substance tested is between 0.8 and 1, this product is not a carbonic anhydrase inhibitor.

Finally, measurement of Mg^{2+} and Ca^{2+} ions in the urine showed that EAf significantly increased urinary excretion of Mg^{2+} and Ca^{2+}, similar to that of Furosemide, compared with control. All this shows that EAf acts in the same way as Furosemide. Furosemide is a powerful diuretic that inhibits sodium reabsorption by inhibiting the $Na^+/K^+/Cl^-$ transporter at the ascending branch of the loop of Henlé, and this indirectly causes urinary leakage of Mg^{2+} and Ca^{2+} [25].

In order to assess the state of renal function in these animals at the end of the experiment, creatinine clearance and the ratio of urine urea to plasma urea were determined. These are clinical markers of renal function. According to the results we obtained, EAf significantly reduces creatinine levels while significantly increasing urinary creatinine. These results are similar to those obtained with *Bridelia ferruginea* (Euphorbiaceae), which increases urinary creatinine[26].

The ratio of urine urea to plasma urea increases very little, with a value of 31.40 ± 3.57, well above 10. When this value is below 10, organic renal failure is indicated[27].

EAf also significantly increases creatinine clearance. The aqueous extract of *Erythrophleum africanum* bark (Fabaceae) therefore has a considerable and beneficial effect on glomerular filtration. These effects are similar to those observed in rats treated with *Zea Mays* (Poaceae) extracts[28].

V. CONCLUSION

Our results show that the aqueous extract of Erythrophleum africanum bark (Fabaceae) promotes an increase in natriuresis, chloriuresis, kaliuresis and pH. It also increases urinary calcium and magnesium excretion, as does Furosemide. All this would confirm the potential diuretic effect of EAf and justify the use of Erythrophleum africanum (Fabaceae) as a diuretic and in the treatment of arterial hypertension.

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No

Disclosure

The author reports no conflicts of interest in this work.

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