Phytochemical Profiling And Evaluation Of Diuretic Properties In Root Extracts Of *Fadogia Pobeguinii* Pobeg. (Rubiaceae).

Mbaïndogoum Koundambaye^{1,2,5}, Fidèle Mahoudo Assogba^{2, 4}, Djidenou Ahoton², Placide Mahougnan Toklo^{2, 4}, Camille Fernand Houndjo², Achille Loconon Yemoa³, Eléonore Chikani Yayi Ladekan², Yaya Mahmout⁵, Joachim Djimon Gbenou ^{2,4}.

¹Département De Physique Et Chimie. Faculté Des Sciences Et Techniques/Université De Sarh. B.P 105, Sarh,Tchad

²Laboratoire De Pharmacognosie Et Des Huiles Essentielles, Faculté Des Sciences Et Techniques. Université d'Abomey-Calavi, 01 B.P. 188 Cotonou, Bénin

³ Unité De Recherche En Chimie Analytique Et d'Analyse De Médicaments. UFR Pharmacie. Faculté Des Sciences De La Santé / Université d'Abomey-Calavi, 01 B.P. 188 Cotonou, Bénin.

⁴ Laboratoire KABA De Chimie Et Applications, Université Nationale Des Sciences, Technologies, Ingénierie Et Mathématiques, B.P. 486 Abomey Sogbo Aliho, Bénin.

⁵Laboratoire De Recherche Sur Les Substances Naturelles, Faculté Des Sciences Exactes Et Appliquées, Université De N'Djaména. BP 1027 N'Djaména, Tchad

Abstract:

Background: Fadogia pobeguinii is a suffrutex used by Chad population for treat some ailments including hypertension.

Objective: Assess the diuretic potential of this plant's extracts and determine the classes of compounds it closes. **Materials and Methods:** Female rats were randomly divided into three groups of six. In phase one, rats in the control group received a 0.9% NaCl aqueous solution at 25 ml/kg, while the test groups received 50 mg/kg, 100 mg/kg or 200 mg/kg of aqueous or ethanolic extract of root material. In the subsequent phase, a 2% solution of Tween-80 in a saline solution at 25 ml/kg was administered to the controls, while the test groups received the DCM fraction (1.81 mg/kg), AcOEt fraction (1.51 mg/kg), MeOH fraction (20.10 mg/kg) and BuOH fraction (0.60 mg/kg) of aqueous extract. The reference was furosemide at 10 mg/kg. For each group, the volume of urine excreted and its pH were measured at the end of the sixth hour, followed by the concentration of electrolytes. The parameters diuretic index, diuretic activity and the Na⁺/K⁺ ratio were then calculated. Various compounds were identified in the extracts not only by phytochemical screening but also by HPLC.

Results: Aqueous and ethanolic extracts were found to induce significant levels of diuresis (p < 0.05) in comparison to the saline control (0.9%), with the respective Na⁺/K⁺ ratios recorded as 2.14 and 2.20. Methanolic fraction of aqueous extract (1.81 mg/kg) induced a greater degree of diuresis (p < 0.05) than furosemide (10 mg/kg) and exhibited a Na⁺/K⁺ ratio as 2.33. The compounds responsible for this activity are believed to be flavonoids, tannins and saponosides families.

Conclusion: the both aqueous and ethanolic extract of *F*. pobeguinii roots have been demonstrated to possess diuretic and natriuretic properties in rats.

Key Word: Fadogia pobeguinii, hypertension, sodium potassium ratio, HPLC, phytochemical.

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I. Introduction

High blood pressure, also known as hypertension, is characterised by a sustained systolic blood pressure of 130 millimeters of mercury (mm Hg) or more and/or a diastolic blood pressure of more than 80 mm Hg¹. Over time, persistent elevated blood pressure is associated with cardiovascular and cerebral problems. High blood pressure is a major public health problem in sub-Saharan Africa. Its prevalence varies from 16% to 40% of people over the age of 18 years, and the age-standardised mortality rate for hypertension is 150 per 100,000 people, compared with a global average of 125 per 100,000². According to Fourcade and Touze (2011)³. hypertension in this region has specific characteristics, such as a low level of renin activity and a sodium-dependent nature.

Treatment is therefore based on thiazide diuretics and calcium channel blockers, combined with dietary measures, including reducing sodium intake.

In Chad, as in a number of other African countries, it is estimated that up to 80 per cent of the population relies on traditional medicine for primary health care⁴. In addition to cultural reasons, the prohibitive cost of pharmaceuticals, lack of access to health infrastructure and shortage of qualified health workers are driving these Africans to practice traditional medicine. In the case of arterial hypertension, the traditional therapeutic approach is essentially based on plants. However, it is still very poorly documented.

Fadogia pobeguini, a suffrutex from the Rubiaceae family, is used in southern Chad to treat high blood pressure, malaria and erectile dysfunction. In northern Nigeria, the plant is used for its aphrodisiac properties. For this reason, Ohiomokhare *et al*⁵ have assessed the effects of crude extract of roots on field stimulation of intramural nerves. To the best of our knowledge, this work is the first to investigate the diuretic properties of extracts from the roots of this plant.

II. Material And Methods

Plant collection:

Fadogia pobeguinii roots were collected in their natural environment on november 1st, 2020 from three stands on the right bank of the Chari River, on the border of the town of Sarh in the Moyen-Chari region of Chad. The geographical coordinates of these stands are as follows N 09°07'36.9" E 018°28'43.2", N 09°07'37.1" E 018°28'44.6" and N 09°07'37.0" E 018°28'44.3". The roots were sorted, cleaned and then dried at laboratory temperature in accordance with the recommendations given by Sofowora⁶. After drying, they were ground and sieved. A fine powder was obtained.

A whole plant specimen has also been deposited in the Benin National Herbarium with the accession number AA6708/HNB.

Extraction:

Two extracts were prepared from the plant raw material.

Aqueous extract of *Fadogia pobeguinii* (AE) was obtained by putting 100 g of powder in 1 liter of distilled water and then boiling for 30 minutes.

Ethanolic extract of *Fadogia pobeguinii* (EE) was obtained by maceration of 100 g of powder in 1 liter of the mixture EtOH/H₂O (60:40; v/v) for 72 hours.

The solutions obtained were filtered with Whatman paper and then concentrated by means of rotavapor under vacuum and oven at a maximum temperature of 50 °C. Extraction yields are 12.5 and 6.2 % respectively for AE and HE. These extraction processes were repeated until the desired quantities of extracts were obtained for the biological tests.

Fractionation of aqueous extract of Fadogia pobeguinii

Aqueous extract was brought into contact successively with dichloromethane, ethyl acetate, methanol and butanol in the ratio (1:10; w/v). For each solvent, the whole extract-solvent assembly is stirred for 24 hours before being filtered. The evaporation of the solvents being carried out under the same conditions as when obtaining the extracts. The yields obtained with respect to plant material are 0.22 % for dichloromethane fraction (DCM fraction), 0.19 % for ethyl acetate fraction (AcOEt fraction), 2.50 % for methanol fraction (MeOH fraction) and 0.08 % n-butanol fraction (BuOH fraction), giving a total yield of the fractionation of 3%.

Diuretic and saluretic effects of extracts and fractions

The activity was assessed using the methods ^{7, 8}.

Experimental setup of the *in vivo* test

Seventy-six female albino Wistar rats weighing $(160 \pm 25 \text{ g})$ were obtained from the animal facility of the Faculty of Health Sciences, University of Abomey-Calavi, Benin. They were grouped into 13 lots, and the following treatments were administered to the different lots:

- Lot 1 served as the control group for extracts, receiving 0.9% NaCl at a dose 25 ml/kg;
- Lot 2 served as the control group for fractions, receiving 2% Tween-80 in NaCl 0.9% at a dose 25 ml/kg;
- Lot 3 was treated with furosemide (Lasilix) from Sanofi-Aventis France at a dose of 10 mg/kg body weight (F10);
- Lot 4 was treated with the aqueous extract at a dose of 50 mg/kg body weight (AE50);
- Lot 5 was treated with the aqueous extract at a dose of 100 mg/kg body weight (AE100);
- Lot 6 was treated with the aqueous extract at a dose of 200 mg/kg body weight (AE200);
- Lot 7 was treated with the ethanolic extract at a dose of 50 mg/kg body weight (EE50);
- Lot 8 was treated with the ethanolic extract at a dose of 100 mg/kg body weight (EE100);

- Lot 9 was treated with the ethanolic extract at a dose of 200 mg/kg body weight (EE200);
- Lot 10 was treated with the DCM fraction at a dose of 1.81 mg/kg body weight;
- Lot 11 was treated with the AcOEt fraction at a dose of 1.51 mg/kg body weight;
- Lot 12 was treated with the MeOH fraction at a dose of 20.10 mg/kg body weight;
- Lot 13 was treated with the n-BuOH fraction at a dose of 0.60 mg/kg body weight;

The animals were placed in a metabolic cage in the laboratory at a temperature of approximately $25^{\circ}C \pm 2$ and humidity of approximately $65\% \pm 5$. They were deprived of food and water for 18 hours prior to the experiment. Each of the extracts (fractions) was dissolved in NaCl 0.9% solution (2% Tween-80 in 0.9% NaCl solution) and administered orally to the animals at a dose of 25 ml/kg.

The volume of urine excreted at the 6th hour and the pH of the urine were measured for each lot. The collected urine was immediately stored at 4°C for determination of Na⁺ and K⁺ using a flame spectrophotometer.

In order to evaluate the diuretic activity of these extracts and to compare it with that of furosemide, the following values were calculated after the experiment:

- The urinary excretion (UE) is a ratio of total urinary output by total liquid administered

UE = Total urinary output*100/Total liquid administered (formula 1)

- The diuretic index (DI) is the ratio of urinary excretion in test group by urinary excretion in the control group Diuretic index = UE of treatment group/UE of control group (formula 2)

- The diuretic activity is the ratio of the diuretic action of the extracts in the test group by that of the standard drug.

Diuretic activity = DI of test group/ DI standard drug (formula 3)

Phytochemical screening of extracts and fractions.

The chemical reactions of staining and precipitation in tube allowed to highlight the presence or absence of the classes of compounds in extracts and fractions by using Houghton and Raman method⁹.

Qualitative analysis using High Performance Liquid Chromatography HPLC/DAD

HPLC-DAD (Hitachi VWR 5430) was used for identifying some phenolic compounds in extracts.

Sample preparation

Aqueous extract, ethanolic extract and 11 phenolic compounds (Gallic acid, Chlorogenic acid, Caffeic acid, Ferulic acid, Rutin, Quercetin, Luteolin, Isorhamnetin, Rhamnetin, Chrysin, flavone purchased from Sigma-Aldrich Germany) presented in figure 1 were prepared at 1 mg/ml in methanol, then filtered using nylon membranes (0.45 μ m, Millipore, Milford, MA, USA) before injection.

HPLC-DAD conditions

 $10 \ \mu$ L of each standards and extracts was injected. The elution time is 45 minutes. Separation of the compounds was carried out using a AcclaimTM 120 C18 column (100 mm x 4, 6 mm, particle size 5 μ m) at temperature of 25 °C. The flow rate of the gradient elution was 1 ml/min with a binary mobile phase. Mobile phase was constituted with a solution of 1% phosphoric acid, pH=3.78 (solvent A) and methanol (solvent B). The analysis of phenolic compounds began with 20% of solvent B then increased to 50% from 0 to 20 min. From 20 to 25 min, then 25 to 30 min, solvent B increased respectively from 50 to 70, then 70 to 80. Solvent B decreased to 20% from 30 to 35 min and remained constant from 35 to 45 min. Phenolic compounds were detected at the wavelength of 280 nm.

Identification of peaks

The retention time (Rt) is therefore recorded for each compound and then the constituents of the extracts separated under the same conditions as the standards. The identification of phenolic compounds in extracts was done on the basis on retention time (Rt) in comparison with analytical standards. The absolute retention time of a compound in an extract is assumed to be identical to that of the reference compound with a tolerance of $+/-0.1\min^{10, 11}$.

Statistical analysis:

Statistical analysis of the data was performed with one-way analysis of variance (ANOVA) followed by Tukey post hoc multiple comparison test. A p-value lower than ($\langle \rangle$ 0.05 was considered significant. The GraphPad Prism 5 software was utilized for this purpose.

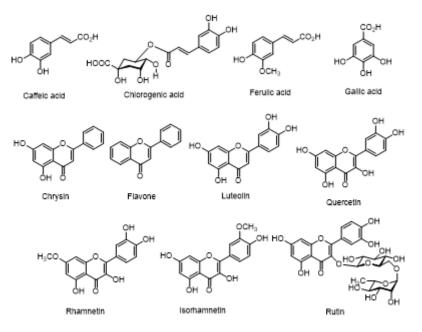


Figure 1. Chemical structure of phenolics used as reference compounds

III. Result

Urinary output and urinary electrolyte excretion caused by extracts and fractions

The urinary excretions caused by administration of AEFP and EEFP at 50, 100, and 200 mg/kg body weight and the concentrations of excreted electrolytes are shown in table 1.

Table 1.	Effect of the AF	E and EE of <i>F</i>	nohegui	<i>nii</i> on the uring	e volume and	urinary elect	rolytes in rats
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Treatment	Urinary excretion (%)	Diuretic index	Diuretic activity	Na ⁺ (mEq/L)	K^+ (mEq/L)	Na ⁺ /K ⁺
NaCl 0.9%	27.64 ± 2.60	1.00 ± 0.09	-	135.33 ± 3.77	119.67 ± 4.37	1.13
F10	102.61 ± 4.82^{a}	3.39 ± 0.16	1.00 ± 0.05	$196.83 \pm 7.33^{\mathrm{a}}$	125.83 ± 7.77	1.57
AE50	84.26 ± 7.29^{ab}	3.06 ± 0.27	0.81 ± 0.07	$132.66 \pm 5.00^{\text{ b}}$	71.66 ± 7.55 ^{ab}	1.85
AE100	114.45 ± 3.64 ^{abc}	4.14 ± 0.13	1.23 ± 0.13	160.33 ± 5.39 ^{abc}	75.33 ± 6.05 ^{ab}	2.14
AE200	$109.71 \pm 2.94^{\rm ac}$	3.97 ± 0.10	1.06 ± 0.03	154.83 ± 5.42 abc	70.97 ± 5.71 ^{ab}	2.20
EE50	$57.81\pm7.50~^{abcdef}$	2.09 ± 0.27	0.56 ± 0.07	135.78 ± 8.86^{bde}	72.00 ± 12.76 ^{ab}	1.93
EE100	69.90 ± 4.68 abcdef	2.53 ± 0.67	0.67 ± 0.04	146.00 ± 6.96^{bcd}	74.67 ± 3.44 ^{ab}	1.96
EE200	78.37 ± 3.41 ^{abde}	2.83 ± 0.12	0.75 ± 0.03	$170.68\pm8.04~^{abcefg}$	81.15 ± 6.70 ^{ab}	2.10

Notes: Cumulative values are expressed as mean \pm standard error of mean (n=6)

The administration of aqueous or ethanolic extracts produced a greater volume of urine than the saline solution. AE100 induced the greatest volume of urine, exceeding that of F10 (p < 0.05). Consequently, AE100 exhibited the highest diuretic activity, with a Lispcht index of 1.23 ± 0.13 . A comparison made between AE50 with EE50,

AE100 with EE100 and AE200 with EE200 shows that the aqueous extract induced a higher urine volume than the ethanolic extract.

F. pobeguinii extracts demonstrated maximal natriuretic effects at the AE100 and EE200 doses, exceeding the effect of 0.9% saline. However, these Na⁺ excretions remained significantly lower (p < 0.05) than those induced by furosemide at a dose of 10 mg/kg. All the K⁺ concentrations were lower compared to those induced by saline administration. The urinary K⁺ content of the saline solution did not differ significantly from that of F10. The Na⁺/K⁺ ratios were found to be 2.14 and 2.10 for AE100 and EE200, respectively.

Urinary output and urinary electrolyte excretion caused by fractions:

From AE 100, 1.81 mg/kg of the DCM fraction, 1.51 mg/kg of the AcOEt fraction, 20.10 mg/kg of the MeOH fraction and 0.60 mg/kg of the BuOH fraction were obtained and administered to the rats. Table 2 summarises the findings.

Values with different superscript letters are significantly different for p < 0.05 ^a against controle (NaCl 0.9%) ^b against the standard (F10), ^c against AE50, ^d against AE100, ^e against AE200, ^f against EE50, ^g against EE100.

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Treatment	Urinary excretion (%)	Diuretic index	Diuretic activity	Na ⁺ (mEq/L)	K ⁺ (mEq/L)	Na ⁺ /K ⁺
2% Tween-80 in 0.9% NaCl	30,27 ± 3.79	1.00 ± 0.28	-	133.83 ± 5.11	120.83 ± 2.86	1.07
F10	102.61 ± 4.82^a	3.39 ± 0.16	1.00 ± 0.05	196.83 ± 7.33^{a}	125.83 ± 7.77	1.57
DCM fraction 1.81 mg/kg	26.92 ± 5.94^{b}	0.89 ± 0.20	0.26 ± 0.06	167.33 ± 9.27 ^{ab}	114.51 ± 6.18	1.46
AcOEt fraction 1.51 mg/kg	34.37 ± 3.50^{b}	1.13 ± 0.12	0.33 ± 0.03	$191.08\pm7.86^{\mathrm{ac}}$	$71.67\pm5.68^{\ abc}$	2.68
MeOH fraction 20.10 mg/kg	109.95 ± 2.23^{ab}	3.63 ± 0.07	1.07 ± 0.02	181.33 ± 12.71 ^{ab}	$78.23\pm8.62^{\ abc}$	2.33
BuOH fraction 0.60 mg/kg	38.38 ± 2.43^{ab}	1.27 ± 0.08	0.37 ± 0.02	200.98 ± 5.77^{ace}	$72.58\pm4.90~^{abc}$	2.77

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Table 2. Effect of the AEF	r and its fractions on the urme	volume and urinary electrolytes in rats

Notes : Cumulative values are expressed as mean \pm standard error of mean (n=6)

Values with different superscript letters are significantly different for p < 0.05 ^a against controle (0.9% NaCl) ^b against the standard (F10), ^c against DCM fraction 1.81 mg/kg, ^d against AcOEt fraction 1.51 mg/kg, ^e against MeOH 20.10 mg/kg.

The urine output recorded after six hours showed that the DCM and AcOEt fractions did not induce significantly different quantities of urine than the saline solution (p < 0.05). The MeOH fraction produced the largest volume of urine, which was even greater (p < 0.05) than that induced by F10. This diuretic activity is 1.07 times higher than that of furosemide.

Each of the fractions showed greater Na^+ excretion than saline. The BuOH fraction was found to have the highest Na^+ concentration, exceeding that induced by furosemide at 10 mg/kg. Conversely, the K^+ concentrations induced by the fractions were found to be lower than those of saline and furosemide, with the MeOH fraction inducing the greatest excretion of K^+ , which was not significantly different from the BuOH. The Na⁺/K⁺ ratios were found to be 2.33 and 2.77 for the MeOH and BuOH fractions, respectively.

Effect on urine pH

The effects of the aqueous and ethanolic extracts on rat urine pH are illustrated in figure 2, while the effects of the fractions are illustrated in figure 3.

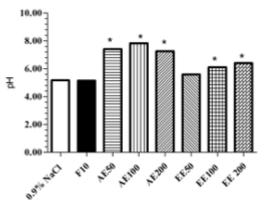


Fig 2. Urinary pH of rats treated with extracts, furosemide 10 mg/kg (F10) and 0.9% NaCl. Notes: Values with superscript * are significantly different for p < 0.05 against controle (0.9% NaCl)

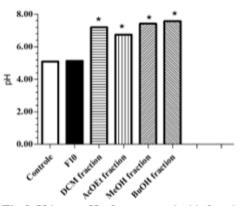


Fig 3. Urinary pH of rats treated with fractions from AE, furosemide 10 mg/kg (F10) and 2% Tween-80 in 0.9% NaC1 (controle). Notes: Values with superscript * are significantly different for p < 0.05 against controle (2% Tween-80 in 0.9% NaC1 0.9% NaC1)

AE50, AE100 and AE200 produced slightly alkaline urine with a significantly increased pH (p<0.05) compared to urine from rats treated with saline (pH=5.10) and F10 (pH=5.14). AE100 induced strong alkalinisation of the urine (pH=7.83). EE50, EE100 and EE200 induced acidic urines with the highest by EE200 (pH=6.40).

All fractions produced urine with pH values significantly higher than that of the saline solution. The AcOEt fraction produced an acidic urine (pH= 6.75). The other fractions gave slightly alkaline urines: the DCM (pH= 7.20), MeOH (pH= 7.42) and BuOH (pH= 7.58) fractions.

Phytochemical analysis of F. pobeguinii root extracts

Table 3 reports the families of compounds found in the extracts and fractions. The extracts contain the same families of compounds. Similarly, the MeOH and BuOH fractions of the AE contain the same families of compounds.

Phytochemicals	AE	EE	DCM fraction	AcOEt fraction	MeOH fraction	BuOH fraction
Alkaloids	-	-	-	-	-	-
Anthocyanes	-	-	-	-	-	-
Leucoanthocyanes	-	-	-	-	-	-
Anthracene derivates	-	-	-	-	-	-
Coumarines	+	+	+	+	-	-
Cyanogenic derivatives	-	-	-	-	-	-
Flavonoids	+	+	-	+	+	+
Quinones derivates	-	-	-	-	-	-
Mucilages	+	+	-	+	+	+
Reducing compounds	+	+	-	+	+	+
Saponins	+	+	-	+	+	+
Steroids	+	+	-	-	-	-
Triterpenoids	+	+	+	-	+	+
Catechic tannins	+	+	-	-	+	+
Gallic tannins	+	+	-	-	+	+
NT 4						

Table 3. Phytochemical constituents of extracts and fractions from

Notes: AE: aqueous extract; EE: ethanolic extract; +: present; -: absent

HPLC analysis revealed the retention times for the reference compounds, as shown in Figures 4, 5, 6, and 7 in table 4. The comparison of these retention times with those of the compounds eluted from the aqueous extract (Figure 8) and those from the ethanolic extract (Figure 9) indicated the presence of gallic acid, flavone, and rhamnetin in each extract (table 5).

S° n	Standard	Code	Retention
			time (min)
1.	Gallic acid	1	2.960
2.	Chlorogenic acid	2	4.820
3.	Caffeic acid	3	7.100
4.	Ferulic acid	4	11.333
5.	Rutin	5	15.827
6.	Quercetin	6	20.953
7.	Luteolin	Ø	23.400
8.	Isorhamnetin	8	24.400
9.	Rhamnetin	9	26.427
10.	Chrysin	10	28.360
11.	flavone	(11)	28.607

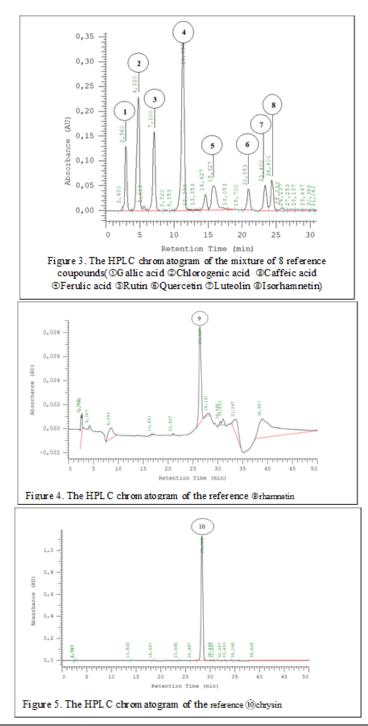
Table 4. Retention times and codes of the reference compounds

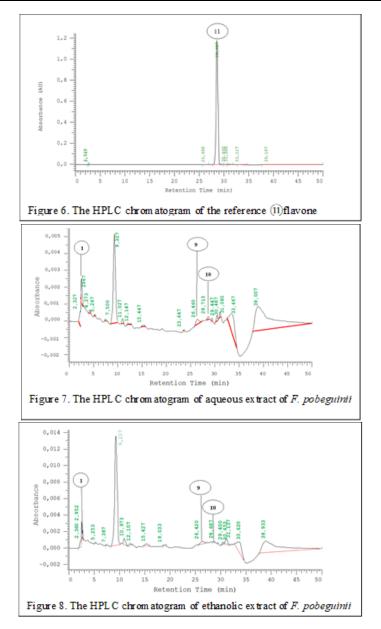
Table 5. Identification of polyphenols in aqueous and ethanolic extracts

Extract	Retention time (min)	Retention time of reference (min)	Identified compound
Aqueous	2.327		ni
	2.967	2.960	Gallic acid
	4.273		ni
	5.267		ni
	7.500		ni
	9.327		ni
	11.027		ni
	12.147		ni
	15.447		ni
	23.647		ni
	26.460	26.427	Rhamnetin
	28.713	28.607	Flavone
	29.447		ni
	30.487		ni
	31.080		ni
	33.467		ni
	39.001		ni
Ethanolic	2.360		ni
	2.952	2.960	Gallic acid

5.253		ni
7.383		ni
9.287		ni
10.973		ni
12.107		ni
15.427		ni
19.033		ni
26.420	26.427	Rhamnetin
28.687	28.607	Flavone
29.400		ni
30.453		ni
31.107		ni
33.620		ni
22.020		

Notes. ni: not identified, min: minutes





IV. Discussion

F. pobeguinii is a plant that has been employed by the population of southern Chad in the treatment of various pathologies, including hypertension. The present study aims to examine the diuretic properties of the roots of this plant. Two extracts were prepared from the roots of the plant. The first was an aqueous extract simulating the beverage prescribed by traditional health practitioners. The second was an ethanol macerate. The second is easier to preserve than the first and could be offered if it proves effective or better than the first.

Diuretics play an important role in the treatment of a number of diseases. In conditions such as liver cirrhosis and kidney disease, hyperkalemia, hypertension, heart failure or renal failure, diuretics increase sodium excretion and reduce blood volume ¹². In general, the value of the diuretic index is used to assess the diuretic properties of an extract (fraction or compound). Diuretic activity is considered good if the diuretic index is greater than 1.50, moderate if it is in the range [1.00; 1.50], mild if it is between [0.72; 1.00[and nil if it is less than 0.72 ^{7, 13}. For both extracts and all doses, the diuretic activity is good as the diuretic index is at least 2.09, indicating that they are potent diuretics Moreover, at the maximum level of effect of the extracts, the ratios of Na⁺/K⁺ electrolyte contents are a minimum of 2.10. These extracts exhibit natriuretic activity ^{14, 15}.

The presence of coumarins, reducing compounds, flavonoids, mucilages, saponins, triterpenoids and steroids has been reported in the genus *Fadogia*^{16, 17, 18, 19, 20}. Alkaloids appear to be absent, as opposed to their presence in *F. agrestis* and *F. Cienkowski*^{16, 20}.

The polyphenolic profile of the extracts encompasses an acid phenol, a flavone and a flavonol. Gallic acid has been demonstrated to possess salidiuretic properties in rats. It has been shown to neither interfere with

the urinary excretion of K⁺ ions nor inhibit the formation of CaOx crystals *in vitro* ^{21, 22}. The presence of gallic acid would therefore justify the diuretic and natiuretic effects observed in the extracts, which may be accentuated by the presence of certain saponins and triterpenoids known for their diuretic and natiuretic properties ^{23, 24}. Sa présence justifierait donc l'effet diuretique et natiurétique observé pour les extraits. Flavone and rhamnetin are thought to possess antioxidant and anti-inflammatory properties, with a resultant hypotensive/antihypertensive effect ^{25, 26, 27}. This lends credence to the utilization of this particular plant in folkloric medicine.

The aqueous extract was subjected to fractionation, which revealed that the methanolic fraction was both more aquaretic and natriuretic. This finding suggests that these extracts would not act in a synergistic manner. The study therefore suggests a potential for the isolation of active compounds, which is a fascinating avenue for further research.

V. Conclusion

The present study demonstrated that *Fadogia pobeguinii*, a plant traditionally employed in the treatment of hypertension in southern Chad, possesses diuretic and natriuretic properties when examined in an experimental animal model. The aqueous extract was found to possess a greater degree of diuretic potency in comparison to the ethanolic extract. Consequently, this experimental evidence may provide a scientific rationale for the widespread utilization of this plant.

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