Research Article

Effects of Ethanolic Fraction of Roots Barks of *Anogeissus leiocarpa* (DC.) Guill & Perr on Urine Output and Lipid Profile on Rats

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Accepted on: 23-02-2016; **Finalized on:** 31-03-2016.

ABSTRACT

The purpose of this study was to evaluate the diuretic activity of the ethanolic fraction of *Anogeissus leiocarpa* and its effect on the lipid profile in rats. The ethanolic fraction was prepared starting from the aqueous total extract by using the liquid-liquid chromatography or chromatography based on partition in two immiscible liquid phases. For the assessment of the diuretic activity, the ethanolic fraction of *A. leiocarpa* were administered by gavage at doses ranging from 100 to 500 mg/kg bw and furosemide® at a dose of 20 mg/kg bw. The urine of the animals was then removed every hour for five hours. Hourly, urine volume is measured and at the fifth hour each urine sample is taken for electrolyte dosage. Concerning the study of its effect on the lipid profile, the ethanolic fraction was administered by oral way to healthy rats during 28 days, and then animals' blood was collected at day 29 for determination of lipid parameters. The results of this study showed that the ethanolic fraction of roots of *Anogeissus leiocarpa* caused a significant urine increase production ($p < 0.01$ and $p < 0.001$). However, the dose-dependent increase in urinary electrolytes was not observed. Therefore this extract did not show a significant effect on the change on the lipid profile of the rats after 28 days of treatment ($p < 0.05$). This fraction is thus aquaretic but no diuretic. It stabilizes the lipid parameters; its use in the long term would not generate risks of cardiovascular pathologies.

Keywords: *Anogeissus leiocarpa*, lipid profile, diuretic activity, gavage.

INTRODUCTION

In developing countries, the occurrence of heart diseases increases rapidly[1]. The high levels of plasmatic lipids, mainly of total cholesterol (TC), triglycerides (TG) and LDL-cholesterol (LDL-c) and the reduction in the HDL-cholesterol level are responsible for the hyperlipidemia which is at the origin of the occurrence of the cardiovascular diseases, and atherosclerosis[2-4]. The predominant cardiovascular diseases associated to hyperlipidemia are arterial hypertension, ischemic heart disease, the cerebral vascular accidents, and coronary heart disease. They are among the leading causes of death and cause each year 60% of all deaths, of which 80% occur in poor and disadvantaged countries[5]. In addition, they occupy about half of the economic cost caused by the non transmissible diseases, about 250 billion dollars US[5]. The therapeutic management of these diseases remains still problematic in developing countries. While the orthodox health system well structured and highly developed offer adequate care, over than 80% of people in rural African communities still rely on indigenous medicine as a primary source of health care[6]. This is partly explained by the high cost associated to the orthodox health system and also the benevolent attachment of the autochthones peoples to their culture and their tradition[7]. Natural therapies and traditional medicine focus on plants. These are the subject of particular interest in the search for new bioactive molecules and today represent an immense tank of new potential medicinal compounds[8].

Anogeissus leiocarpa (CD) Guill and Perr is a combretaceae that has retained our attention. It is a tropical plant present in dry savannas, dry forests and in the sudano-sahelian to sudano-guinean galleries along rivers at the edge of the rainforest[9]. This plant is much known in central and western Africa, where it has a wide range of uses[10]. In northern Côte d'Ivoire, its roots are used in the treatment of various microbial infections[11,12].

The present study aims to evaluate the effect of the ethanolic fraction of roots barks of the plant on the diuretic activity and on the parameters of the lipid profile in rats.

MATERIALS AND METHODS

Material

Animal material

Rats male and nulliparous female and non pregnant aged 8 to 12 weeks old and weighing between 170 and 200 g on average were used.

Plant material

The plant material consists of roots bark of *Anogeissus leiocarpa*. These barks were collected in January 2013 in Kouto, a city located at 725 km in the north of Abidjan following an ethnobotanical survey carried out near traditional healers of the locality. These barks were dried out of the sun for two weeks before being ground into a fine powder by grinding. From the powder obtained after

spraying, the different extracts to be tested were prepared.

Methods

Preparation of ethanol fraction

The liquid-liquid partition chromatography or chromatography based on solute partition in two immiscible liquid phases was used. It required the use of three solvents of different polarity namely dichloromethane, ethyl acetate and ethanol.

To carry out this chromatography, 20 g of aqueous extract of *A. leiocarpa* prepared according to the method described by Guédé-Guina[13] are added in 100 mL of a mixture of distilled water-solvent volume by volume (v/v).

The whole were homogenized for 24 hours at 27 ± 2°C, using a magnetic stirrer type IKAMAG-RCT. After decantation in a separator funnel, a residual aqueous phase and an organic phase was obtained.

This operation is repeated 4 times in a row and the collected organic fractions were collected and concentrated under vacuum at 60°C using a rotary evaporator (Büchi R110 Brand, Type MKE 6540/2) and then dried in an oven at 45°C.

The lower aqueous phase was then recovered and extracted with the given solvent polarity. The ethanolic fraction was used for the experiments.

Animal preparation

The rats were placed in metabolic cages 48 hours before the experiment to reduce stress effects.

Evaluation of urine output

Thirty (30) rats were fasted with free access to water for 18 hours. The animals were pretreated with physiological saline (NaCl 0.9%) at an oral dose of 15 mL/100 g bw, to impose a uniform water and salt load[14].

Forty five (45) minutes later, the rats were randomly divided into five groups of 6 rats each and treated orally as follows:

Lot 1 (Control): 1 mL of distilled water,

Lot 2 (Reference): 1 mL of 20 mg/kg bw of furosemide®,

Lot 3: 1 mL of 100 mg/kg bw of ethanolic fraction,

Lot 4: 1 mL 300 mg/kg bw of ethanolic fraction,

Lot 5: 1 mL of 500 mg/kg bw of ethanolic fraction.

Immediately after administration the rats were placed individually in metabolic cage and the cumulative urine production was determined hourly intervals for 5 h.

Determination of the parameters:

At the 5th hour urine was collected and the volume of urine excreted was measured. Then sodium (Na^+), potassium (K^+) and chloride (Cl^-) levels of urine were determined using a biochemical analyzer type CYANStart controller according to the manufacturer's instruction manual with appropriate reagents.

Using the data obtained for electrolytes, the saliuretic indices for Na^+, K^+, and Cl^-; aldosterone secretion index (Na^+/K^+); thiazide diuretic index (Na^+/Cl^-); carbonic anhydrase inhibition index [Cl^-/ (Na^++K^+)] were computed[15].

Determination of index

Indexes were determined using formulas:

Diuretic Index=Mean urine volume of test/Mean urine volume of control.

Lipschitz value= Mean urine volume of test/Mean urine volume of standard.

Effect of the ethanolic fraction of *Anogeissus leiocarpa* on lipid profile on healthy rats.

Twenty four (24) rats aged two to three months and weighing 170 to 200 g on average were used. The rats were divided into four lots of six according to their weight. Rats of lot 1 (control) received daily and individually by gavage 1 mL of distilled water for 28 days.

Rats of lots 2, 3 and 4 received respectively by gavage 100; 300 and 500 mg/kg bw of extract daily for 28 days in an amount of 2 mL per 100 g body weight.

The 29th day, or the day after the last day of treatment, blood is taken by amputation of the tail for the biochemical analyzes.

Statistical Analysis

Analyzes and graphical representations of data were performed using GraphPad Prism version 5.00 for Windows software (GraphPad Software, San Diego California USA, www.graphpad.com). The values expressed are the average of three experiments accompanied by the standard error of the mean (Mean ± SEM). Statistical analysis of the data were performed with one-way analysis of variance (ANOVA) followed by Dunnett's multiple comparisons test. The difference was considered statistically significant if $p < 0.05$.

RESULTS

Effect of ethanolic fraction on urine output in rats

The results of the effect of the ethanol fraction of *A. leiocarpa*, distilled water and furosemide® on urine output are shown in table 1.

At doses of 100, 300 and 500 mg/kg bw, ethanolic fraction causes the urinary excretion during the five hours of experimentation. Every hour, the ethanolic fraction of the plant produced diuresis which appeared to be a function of doses. From the 1st to the 5th hour, furosemide® at a dose of 20 mg/kg bw and the fraction at doses ranging from 100 to 500 mg/kg bw induce significant diuresis compared to control group ($p < 0.05$, $p < 0.001$). From the 4th to the 5th time urine output of lots 3

to 5 are not significantly different to reference ($p < 0.05$). Dose of 500 mg/kg bw of the fraction causes more rapid urinary excretion as furosemide® (urine volume greater than 1 mL in the first hour). Figure 1 reports the evolution of urinary excreted volumes at the end of five hours of experimentation.

Table 2 shows the values of diuretics indexes computed from the cumulative volumes of urine from the 5th hour.

The diuretic index or diuretic action at doses of 100, 300 and 500 mg/kg bw respectively of 3.22, 3.50 and 3.70 are more than 3 times higher than the diuretic index in the control group.

Lipschitz values compared to furosemide® (reference) show that the fraction at doses of 100, 300 and 500 mg/kg bw exert respectively 81, 88 and 93% of diuretic activity.

Table 1: Effect of the ethanol fraction of *A. leiocarpa* on urine excretion in rats.

Lots	Urine volume (mL)					
	0h	1h	2h	3h	4h	5h
Control	0±0	$0.23\pm0.19^{\#\#\#}$	$0.53\pm0.32^{\#\#\#}$	$0.53\pm0.32^{\#\#\#}$	$0.70\pm0.20^{\#\#\#}$	$0.90\pm0.40^{\#\#\#}$
Reference	0±0	$1.10\pm0.53^{***}$	$2.18\pm0.60^{***}$	$2.83\pm0.67^{***}$	$3.10\pm0.70^{***}$	$3.57\pm0.30^{***}$
Lot 3	0±0	$0.10\pm0.10^{\#\#\#\,ns}$	$1.00\pm0.50^{ns\#\#\#}$	$1.90\pm0.35^{***\#\#}$	$2.17\pm0.51^{***ns}$	$2.90\pm0.10^{***ns}$
Lot 4	0±0	$0.33\pm0.33^{**ns}$	$1.60\pm0.10^{***\#}$	$2.20\pm0.34^{***ns}$	$2.60\pm0.20^{***ns}$	$3.15\pm0.46^{***ns}$
Lot 5	0±0	$1.33\pm0.49^{***ns}$	$1.93\pm0.13^{***ns}$	$2.5\pm0.33^{***ns}$	$2.87\pm0.20^{***ns}$	$3.33\pm0.61^{***ns}$

Each value represents mean ± S.E.M of 6 rats. $^{***}P < 0.001$, $^{**}P < 0.01$: significant difference compared to control group (distilled water treated), $^{\#}P < 0.05$, $^{\#\#\#}P < 0.001$: significant different compared to reference (furosemide® treated); ns: no significant at $p < 0.05$.

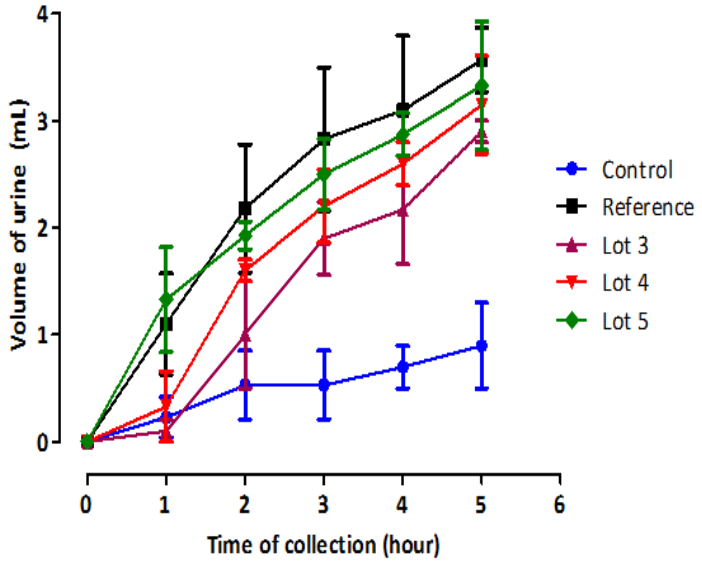

Figure 1: Time course of diuresis in rats treated with different doses of ethanolic fraction of *A. leiocarpa,* distilled water and furosemide®.

Control: distilled water, Reference: 20 mg/kg bw of furosemide®; lot 3: 100 mg/kg bw; lot 4: 300 mg/kg bw; lot 5: 500 mg/kg bw of ethanolic fraction of A. leiocarpa

Table 2: Variation of diuretic index and Lipschitz value computed from cumulative volumes of urine to the fifth hour

Lots	Urine output at 5th hour (mL)	Diuretic Index	Lipschitz Valeur	Appreciation of the diuretic activity
Control	0.90±0.40	1	-	
Reference	3.57±0.30	3.96	1	Good
Lot 3	2.90±0.10	3.22	0.81	Good
Lot 4	3.15±0.10	3.50	0.88	Good
Lot 5	3.33±0.61	3.70	0.93	Good

The diuretic activity is good if diuretic index is greater than 1.50; moderate if between 1 and 1.5; low if it is between 0.72 and 1 and zero if it is less than 0.72.

Table 3: Effect of ethanolic fraction of roots barks of *A. leiocarpa* on 5th h urinary electrolyte

Lots	Urinary electrolyte level (mmol/L)			Saliuretic index			Na^+/K^+	Na^+/Cl^-	Cl^-/Na^++K^+
	Na^+	K^+	Cl^-	Na^+	K^+	Cl^-			
Control	126.00 ± 4.82^{ns}	22.87 ± 5.32^{ns}	$143.70\pm9.06^{\#\#\#}$	1.00	1.00	1.00	5.51	0.88	0.97
Reference	$136.70\pm5.78^{*}$	26.13 ± 1.96^{ns}	$166.00\pm2.52^{***}$	1.08	1.14	1.16	5.23	0.82	1.02
Lot 3	$121.30\pm6.88^{ns\#\#}$	22.38 ± 2.40^{ns}	$135.00\pm11.93^{ns\#\#\#}$	0.96	0.98	0.93	5.42	0.90	0.94
Lot 4	$111.30\pm7.670^{**\#\#\#}$	21.83 ± 3.29^{ns}	$122.30\pm3.53^{***\#\#\#}$	0.88	0.95	0.85	5.09	0.91	0.92
Lot 5	$108.00\pm10.07^{***\#\#\#}$	$20.20\pm1.50^{ns\#}$	$118.70\pm5.93^{***\#\#\#}$	0.86	0.88	0.83	5.35	0.90	0.93

Each value represents mean ± S.E.M of 6 rats. $^{***}P<0.001$, ** P<0.01: significant difference compared to control group (distilled water treated). $^{\#}P<0.05$, $^{\#\#\#}$ P <0.001: significant different compared to reference (furosemide® treated); ns: no significant at p < 0.05.

Table 4: Effect of daily administration of ethanolic fraction of roots barks of *A. Leiocarpa* for 28 days on rats' serum lipid profile

Serum lipid parameters (g/L)	Tested doses of ethanolic fraction (mg/kg bw)			
	0	100	300	500
CT	0.80±0.00	0.83 ± 0.04^{ns}	0.82 ± 0.00^{ns}	$0.79\pm0.00^{**}$
TG	0.92±0.20	0.90 ± 0.16^{ns}	0.92 ± 0.18^{ns}	0.97 ± 0.52^{ns}
HDL-c	0.61±0.01	0.60 ± 0.04^{ns}	0.58 ± 0.03^{ns}	0.61 ± 0.00^{ns}
LDL-c	0.10±0.02	0.09 ± 0.00^{ns}	0.08 ± 0.01^{ns}	0.12 ± 0.03^{ns}

Each values represents mean ± S.E.M of 6 rats. Statistical comparisons are made on the lines. ** P <0.01: significant difference compared to control group (distilled water treated); ns: no significant at p < 0.05.

TC: Total cholesterol (g/L); TG: Triglycerides (g/L); HDL-C: HDL-cholesterol; LDL-C: LDL-cholesterol (g/L).

Effect of ethanolic fraction on urinary electrolyte

Urine samples collected at the 5th hour were analyzed, and Na^+, K^+ and Cl^- levels were determined. Table 3 records these electrolytes levels and saliuretic indices values. The aldosterone secretion index (Na^+/K^+); thiazide diuretic index (Na^+/Cl^-); carbonic anhydrase inhibition index [Cl^-/ (Na^++K^+)] computed are also reported in this table.

The tested doses of the fraction did not show a significant variation on the urinary levels of K^+ compared to the control. Urinary levels of Na^+ and Cl^- of the treated lots with doses of 300 and 500 mg/kg bw of fraction are significantly lower compared to control group (p <0.01 and p <0.001) and reference group (p <0.001). The extract at a dose of 100 mg/kg bw does not cause significant variations in the levels of the three electrolytes compared to control group (p<0.05). The saliuretic index for Na^+, K^+ and Cl^- were dose-dependent. They decreased when the dose increased. The saliuretic index of Na^+ was 0.96, 0.88 and 0.86, that of K^+ to 0.98, 0.95 and 0.88 and that of Cl^- to 0.93, 0.85 and 0.83 respectively for the doses of 100, 300 and 500 mg/kg bw.

The aldosterone secretion index decrease of 1.63%, 7.62% and 2.90% respectively for doses of 100, 300 and 500 mg/kg bw.

The carbonic anhydrase inhibition index decreased respectively at the same doses of 3.10%, 5.15% and 4.12%. In contrast to the both indexes, the thiazide diuretic index increased to 2.27%, 3.41% and 2.27% respectively at doses of 100, 300 and 500 mg/kg bw.

Effect of ethanolic fraction on serum lipid profile

Table 4 shows the effect of the ethanolic fraction of *A. leiocarpa* on lipid profile in healthy rats for 28 days by oral administration of single dose per day. As shown, at the end of 28 days of treatment, there was any significant effect seen of all tested doses of the fraction on plasma levels of TG, LDL-C and HDL-c compared to control (p <0.05). However, only the high dose of 500 mg/kg bw significantly reduced TC level compared to control (p <0.01).

DISCUSSION

This study was carried out, in one hand, to examine the diuretic potential of the ethanolic fraction of *A. Leiocarpa* on hydrated rats and, in the other hand, to evaluate its long-term effect on plasma lipid profile by oral way.

Concerning the diuretic activity assessment, conscious rats using hydrated diuretic model were used. This is reliable, sensitive validated and well recognized model for the assessment of diuretic potential of pharmacophores[16]. The results showed that the different fractions doses tested provoked urine output in a dose-dependent manner. That suggests that the effect was genuinely intrinsic and casual, and results are not due to nonspecific actions[14]. For the highest dose (500 mg/kg

bw) urine output started to increase one hour after administration of the extract, which is extremely fast and lasted throughout the study (until the fifth hour). This action profile indicates rapid absorption which is therapeutically desirable[17]. Although the increase in urine output was fast and strong, urine was not hypernatremic nor hyperkalemic nor hyperchloraemic (natriuresis and kaliuresis of lots treated with doses of 300 and 500 mg/kg bw were significantly lower than the control lot at p <0.01, while the chloruresis was not significantly altered at p <0.05). Low saliuretics index of Na^+, K+ and Cl- confirm this analysis. These observations collectively suggest that the ethanolic fraction did not act as a loop diuretic. Indeed, loop diuretics such as furosemide® cause increase in urinary Na^+, K+ and Cl- levels by inhibiting the $Na^+/K^+/2Cl^-$ co-transporter in the thick region of the ascending limb of loop of Henle[18]. They compete with Cl^- cotransporter, and thus block the reabsorption of salt.

The fraction did not increase aldosterone secretory index (Na^+/K^+) of urine suggesting that the increased urine output by the fraction is different from potassium sparing diuretics. Potassium sparing diuretics act on the distal tubule of the loop of Henle by antagonising the aldosterone hormone and increasing the Na^+/K^+ ratio. Potassium sparing diuretics act on the distal tubule of the loop of Henle by antagonising the aldosterone hormone and increasing the Na^+/K^+ ratio[19]. Aldosterone opens the sodium and potassium channels, and stimulates Na^+/K^+-ATPase. It allows the reabsorption of salt and potassium excretion. If his action is blocked, sodium epithelial channel and the potassium channel is closed, the pump Na^+/K^+- ATPase is less active, Na^+ is not reabsorbed and potassium cannot any more leave in the tubular lumen (sparing of potassium) with decrease in urinary potassium level and a risk of increasing K^+ level in extracellular medium.

The extract did not increase urinary electrolytes. In addition, there was a non significant increase thiazide diuretics index. As shown these results, we can suggest that the observed increase in urinary excretion is not thiazide type mode of action. Thiazide type of diuretics increase thiazide diuretic index and simultaneously increase urinay Na^+ and K^+ levels by inhibiting the Na^+/Cl^- symporter co-transporter in the distal convoluted tubule of nephron[20] by competing for Cl^- binding sites and increasing excretion of Na^+ by inhibiting Na^+ reabsorption[21].

Poverty chlorides in urine and the decrease in the carbonic anhydrase inhibition index exclude the enzymatic activity of carbonic anhydrase in the distal convoluted tubule[20] in the induction of diuresis.

In the present study, the fraction failed to show an increase in urinary electrolytes (in terms of Na^+, Cl^- and K^+ levels), aldosterone secretion index, thiazide secretion index, and carbonic anhydrase inhibition index spite of marked and significant increase in urine volume.

These findings indicate that the increase in urine output may be due to an aquaretic action common to many phytodiuretic agents[22] rather than a true diuretic action. Our results agree with those of Shenoy[23] and Ellepola[17]. *A. leiocarpa* is used in traditional treatment of arterial hypertension[24,25]. Its antihypertensive effect has been demonstrated by these authors. Our results show that the mechanism of this antihypertensive activity would not pass by removal of salt through urine. Indeed, Ouedraogo[25] showed that the antihypertensive effect of the aqueous extract of the plant would imply a vasodilatory effect.

It is interesting to note that several plants claimed as diuretics in traditional medicine are aqueretic rather than diuretics[26,23]. Aquaretics mediates their action by increasing the volume of urine via enhanced blood flow in the kidneys, thereby raising the glomerular filtration rate[27] or by initial vasodilatation or by inhibition of tubular reabsorption of water and anions[28].

The thinking here is that these herbs act on the glomerulus (unlike conventional diuretic drugs which act further along the nephron) to increase water excretion from the body, but their effect on electrolytes such as sodium and potassium is largely neutral. Aquaretics may work by causing dilation of glomerular arterioles, thereby increasing glomerular filtration rate. In other words, aquaretics act by increasing fluid loss from the body in a physiological manner, by increasing the formation of primary urine[29]. Mechanism of action of diuretics can be explained otherwise.

There are three sub-types receptors of Arginine vasopressin (AVP) or ADH located in different tissues of the body which are known as V1a, V1b, and V2. Of these, V2 receptors are mainly found in the collecting tubules of kidney and are involved in free water reabsorption[30,31]. Aquaretics antagonize AVP effect by acting via V2 receptors and thereby retard the free water absorption[32].

Their indication could be extended to states hyponatremia, or more likely restricted to hyponatremia of SIADH (syndrome of inappropriate secretion of antidiuretic hormone), congestive heart failure and possibly decompensated cirrhosis[33].

Currently, beside the synthetic diuretics such as tolvaptan, lixivaptan and conivaptan natural diuretic herbs including *Solidaginis virgaurea, Betulae folium, Ononidis radix, Graminis rhizoma* are therapeutically used[32,27].

The evaluation of the effect of the ethanolic fraction of *A. leiocarpa* on the lipid profile was carried out. Assessment of plasma lipid profile is required for the state of wellbeing of every individual as cardiovascular diseases and coronary heart diseases are silent, serial killers of our age[34].

HDL-c and LDL-c fraction associated with total cholesterol (TC) determinations are used for the assessment of

cardiovascular risk and lipid in the liver exploration. The triglyceride level is useful in assessing the atherothrombotic risk, but also, in case of increase, the risk of acute pancreatitis.

The normal rats treated with the ethanolic fraction showed no significant difference in the lipid profile compared to normal control group except the TC of animals treated with a dose of 500 mg/kg bw which was significantly ($p<0.01$) decreased. According to many studies, LDL-c is considered the most dangerous among the serum lipids, and the oxidation of LDL-c leads to its increased penetration of arterial walls[35]. Moreover, elevated LDL-c levels play a crucial role in the development of atherosclerotic lesions that progress from fatty streaks to ulcerated plaques[36]. Thus, serum LDL-c levels are used as the basis for initiating and monitoring the treatment of patients with elevated blood cholesterol levels[37].

Indeed, if the hepatic synthesis or degradation of plasma lipids are not stimulated, the rates of these parameters do not undergo any observable change[38].

CONCLUSION

In view of these results, we may suggest firstly that the ethanolic fraction *A. leiocarpa* causes an increase in urine output, this effect is aquaretic rather than saliuretic. On the other hand, this fraction does not modify the parameters of the lipid profile, and thus may be used by people over a long period without risk of disorders related to plasma lipids.

Acknowledgement: The authors thank the management of the Ecole Normale Supérieure (ENS) in Abidjan (Côte d'Ivoire) and Mr Ahoussi Corneille for his technical assistance.

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Source of Support: Nil, **Conflict of Interest:** None.