



Evaluation of diuretic properties from *Elaeis guineensis* Jacq. (Arecaceae) leaves aqueous extract in Wistar rat

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ABSTRACT

The main aim of the present investigation was to appreciate the diuretic and natriuretic activities and the toxicity from *Elaeis guineensis* leaves aqueous extract (EGLAE). After oral administration of the aqueous extract at doses of 67.5 mg/kg, 135 mg/kg and 202.50 mg/kg (body weight) to Wistar rat, the volume of urine excreted and the quantity of ions (Na^+ , K^+) are respectively determined in stepped up test-tube and by ionic spectrophotometrically measuring. The results show that the urine of Wistar rat has a pH between 6.1 and 8.6 with an average of 7.35 ± 1.25 . Diuresis basic after six hours is 7.8 mL. A significant diuretic activities (157.16%, 169.25%) are observed in Wistar rat treated respectively with 135 mg/kg of EGLAE and furosemide (as reference drug, 20 mg/kg) and a modest diuretic activity (146.32%) with a dose of 202.50 mg/kg of EGLAE. Its natriuretic activity was 2.34 at same dose of 135 mg/kg against 2.52 for furosemide (20 mg/kg) and 0.75 for distilled water. The dose of 135 mg/kg is the best in terms of diuretic and natriuretic activities. IC_{50} of larval toxicity of aqueous extract on *Artemia salina* has evaluated to be 1.710 mg/mL.

Key words: *Elaeis guineensis*, diuretic activity, Wistar rat, toxicity

INTRODUCTION

Of all tropical diseases, malaria is the first endemic that strikes more than three hundred million of people in the world. It kills a year, more than two million patients, mostly children under ten years [1]. In Benin, as in most malaria-endemic countries, malaria is the leading diseases and represents, in 2009, 43.1% of medical consultations. The incidence rate of malaria, all forms combined (serious and simple), was 15.2% in 2009 [2]. In the same way, high blood pressure is now recognized as a public health problem worldwide because of its frequency and its cardiovascular complications. Over a quarter (26.4%) of the world's adult population is hypertensive and an estimated 7 to 8 million patients die each year [3]. Although herbal medicine stay in Benin, traditional, popular and ancient. Scientific research in recent years has considered this area as priority. In Benin flora, several species of plants are indicated in the treatment of malaria and high blood pressure; *Elaeis guineensis* leaves aqueous extract concentrate was used as antimalarial "API PALU". Much work in traditional medicines have helped highlight the diuretic and natriuretic properties of several plant species. "Nitrokoudang" recipe used in Mali for the treatment of high blood pressure was evaluated for its diuretic properties [4]. The tea "adomassin" consisting of a mixture of 10 medicinal plants used by the people of Benin in the treatment of several diseases including malaria and arterial hypertension was also evaluated [5]. These properties fall dramatically into play in the treatment of arterial

hypertension[6]. Indeed, they lead to urinary excretion of water and sodium in the blood. This contributes to a decrease in blood volume and therefore a decrease in high blood pressure [4]. Drug-induced diuresis is also beneficial in many life-threatening disease conditions such as congestive heart failure, nephritic syndrome renal failure, and pregnancy toxemia [7]. Since diuretic effect of *Elaeis guineensis* leaves usually used in Benin's folk medicine has never been experimentally confirmed. In this vision we intend to evaluate, in the present study, the claimed diuretic and natriuretic activities of leaves hot water extract of *Elaeis guineensis*, Guinea-Congolese species, cultivated or spontaneous [8,9] in Wistar rats.

EXPERIMENTAL SECTION

2-1 - Material

2-1-1 - Plant Material

Elaeis guineensis leaves were collected in Porto-Novo (kandévié) in July-August 2013 and then dried for several days in the laboratory sheltered from the sun and then ground. It was identified and authenticated. A Voucher specimen (Ifangni: Adjakidje 4190) was deposited in the National Herbarium of Abomey Calavi University. The powder thus obtained was used to obtain the hot water extract.

2-1-2 - Animal material

Wistar rats either sex weighing between 150 and 250 g procured from the Animal House of Human Biology Unity of Health Science Faculty of Abomey Calavi University were used for the study and fed on corn bran, issues of cereals, cakes (soybean-cotton-palm), premix acids, amines, Grobel toxin bind, Limestone, dicalcium phosphate and rice bran and water *ad libitum*. Prior to the experiment the animals were acclimatized to the laboratory conditions for 7 days. They were randomly allocated to treatment groups and kept in metal cages of standard dimensions and housed under temperature $29 \pm 1^\circ \text{C}$, humidity $75 \pm 5\%$ and dark light cycle (12 h – 12 h). *Artemia salina* shrimp larvae were used to evaluate larval toxicity.

2-2 – Methods

2-2-1 - Obtaining *Elaeis guineensis* leaves aqueous and Flavonoid extracts

Elaeis guineensis leaves aqueous extract (EGLAE) was prepared by decocting 50 g of leaves powder in 500 mL boiling distilled water for 30 min. The resulting mixture is then filtered on Watman paper ($\varnothing = 185 \text{ mm}$). Then, one part of the filtrate was subjected to evaporation in a rotary evaporator (Büchi R 400 brand) at 40°C . Thereafter, the extract thus obtained was lyophilized by first freezing at -70°C in a deep freezer for 12 h and then dried in freeze-dryer.

For flavonoid extract, the second part of the collected filtrate was extracted with n-hexan (2 x 200 mL), dichloromethane (2 x 200 mL), ethyl acetate (2 x 200 mL) successively. The obtained extracts were evaporated by a rotary evaporator (Büchi R 400) at 40°C . The extraction was repeated twice.

The required doses were taken and reconstituted in distilled water before oral administration.

2-2-2 – Preliminary phytochemical Analysis

Phytochemical screening which is a qualitative chemical analysis based on color reactions and precipitation of the major groups of chemical compounds in plants [10] was carried out to find out the phytoconstituents present in the *Elaeis guineensis* leaves aqueous extract.

For flavonoid extract (flavex) identification and analysis, the stationary phase was 10 x 20 HPTLC Silica gel 60 F254 (Merck ©) Sigma glass support plate when the mobile phase was ethyl acetate/formic acid/water (81/12.5/6.5). Flavonoids extract of *E. guineensis* leaves sample was deposited in bands of 5 mm and 10 mm a part at the fixed volume of $10 \mu\text{L}$ at 1 mg/mL of flavonoids extract. Migration distance was 80 mm and the revelator used to be successively NP/NEU reagents and PEG for reading visual.

2-2-3 - Therapeutic doses of aqueous extract of *Elaeis guineensis* leaves

The extract concentrate antimalarial API PALU consists essentially of aqueous extract of *Elaeis guineensis* leaves. Taking this medicine by adults is 2 to 3 tablespoons every 3 hours, eight times a day. In our study, we considered that an adult takes one tablespoon is equal to 15 mL of the suspension in a single daily dose. In comparing this volume to an adult of average weight 60 kg, the dose of API PALU consumed per kg body weight per day is 0.25 mL. The volume of 15 mL of the suspension is dried to obtain the mass estimated to 4.05 g once daily or approximately 67.50 mg/kg once daily. So, one, two and three tablespoons of API PALU correspond to doses of solids which are respectively 67.50, 135.00 and 202.50 mg/Kg/day taken once in a 60 kg adult. This allowed us to obtain the values of the three doses of *Elaeis guineensis* leaves aqueous extract used in this study.

Aqueous extract efficacy dose was 135 mg/kg, the correspondant flavonoids extract (flavex) dose was 9.76 mg/kg because EGLAE yielded 15.22% and flavonoids extract's 1.1%.

2-2-4 - Composition of experimental groups and animal preparation

In the in vivo diuretic and natriuretic activities, we used animals weighed between 150-250 g. Wistar rats were fasted for 18 h before each test but with free access to tap water only and then were giving an oral loading of saline water. Groups of five animals were formed: group 1 (diuresis base): 50 mL/kg of distilled water body weight; group 2 (control): 25 mL of distilled water per kilogram of body weight; group 3 (reference): 20 mg/kg of furosemide body weight;

Groups 4, 5, 6 and 7 respectively received doses 67.5, 135 and 202.5 mg/kg body weight of the aqueous extract and 9.76 mg/kg of flavonoids extract.

2-2-5 - Determination of diuresis base

The experimental protocols have been approved by Benin Institute of Applied Biomedical Science Ethical Committee. Before determining the diuretic and natriuretic activities, diuresis basic animal was measured by oral administration of distilled water at 50 mL/kg bw. Urinary excretion was measured 6 h after administration.

2-2-6 - Determination of the diuretic activity

Diuretic activity was estimated using the methods according to authors [4, 11-13]. The principle is to take the measurement of urinary excretion in Wistar rats being saline overload. 50 mL/kg of a solution of NaCl 1.8% were administered to Wistar rats before the various treatments without forgetting the three respective doses of aqueous extract: 67.5; 135 and 202.5 mg/kg furosemide 20 mg/kg and distilled water. After treatment, five Wistar rats from the same group were placed in the metabolic cages (1 per cage). For each group, the following parameters were measured: the latency (onset of the first drop of urine after the animals was placed in the metabolic cage), the volume of urine excreted and the urine pH 6 h after administration. Excreted Urinary Volume (EUV) was given by the formula: $EUV = VE/VA \times 100$ (VE = volume excreted, VA = volume administered); EUV values <80% = antidiuretic activity, EUV range 80-110% = no activity, EUV range 110-130% = low activity, EUV range 130-150% = moderate activity and $EUV \geq 150\%$ = important activity [11].

2-2-7 - Determination of the saludiuretic activity

The principle is the influence of a diuretic on the natriuresis and kaliuresis in animals placed in aqueous overload. We worked on the following groups: - Group 8 (control) treated with distilled water (25 mL/kg); - Group 9 treated with furosemide (20 mg/kg); - Groups 10, 11, 12 and 13 each received a dose of 67.5; 135 and 202.5 mg/kg of *Elaeis guineensis* leaves aqueous extract and 9.76 mg/kg of flavonoids extract. The substances were orally administered. Immediately following, distilled water at a dose of 50 mL/kg was administered to animals. After treatments, animals from the same group were placed in the metabolic cages (1 per cage). Urine is collected for 4 hours. The urinary concentrations of sodium and potassium ions were determined using a Spectrophotometer Brand Erbalyte and saludiuretic activity is expressed in value of the ratio $[Na^+]/[K^+]$.

2-2-8- Toxicity tests on shrimp larvae

The test is performed on larvae of brine shrimp (*Artemia salina* Leach) using previous author's methods [14-18]. For the experiment, *Artemia salina* eggs are incubated in seawater collected in the Atlantic Ocean. After 48 h incubation, the eggs hatch into young larvae (*Artemia nauplii*) are collected using a Pasteur pipette. Then prepared a series of solutions of test samples at varying concentrations and the progressive serial dilution technique using salt water and 16 larvae introduced into each solution. All solutions and even control solutions containing no active ingredients are left to stir for 24 hours. Counting under a microscope the number of surviving larvae in each solution after 24 hours incubation is used to evaluate the toxicity of the solution. In case of death is observed in the control medium, the data are corrected by Abbott's formula: % Death = $[(\text{test-control})/\text{control}] \times 100$. Data (dose response) were log-transformed and the LC50 value is determined by linear regression.

2-2-9- Statistical Analysis

The results are expressed as mean \pm standard error of mean (SEM). Statistical processing was done on samples in independent series using ANOVA with software STATISTICA version 5.5. The results are considered statistically at probability level of $P < 0.05$.

RESULTS AND DISCUSSION

Our study is aimed on the assessment of diuretic and natriuretic activities of *Elaeis guineensis* leaves hot water extract. The extraction yielded 15.22%. This rate is significantly lower than a nether obtained which was 19% [4]. This difference lies in the nature of plants to extract. Some chemical constituents of leaves are water extractable. The

preliminary phytochemical analysis (table 1) allowed to identify alkaloids, tannins, flavonoids (flavones), leucoanthocyanes, saponins, triterpenoids, steroids and reducing compounds. These results are similar to those obtained [4] unlike the following: alkaloids, triterpenoid and steroid and on the aqueous extract of the leaves of *E. guineensis* [6].

Table 1 : Phytochemical composition of *Elaeis guineensis* leaves aqueous extract (EGLAE)

| Chemical compounds class | Test | Test Result |
|--------------------------|---|---------------|
| Alkaloids | General test: Dragendorff reagent Extraction: Mayer reagent | + + |
| Gallic tannins | Saturation of Na acetate + a few drops of FeCl ₃ , 1% | +++ |
| Cathechic tannins | Stiasny reagent | +++ |
| Flavonoids | Shinoda reagent (cyanidine reaction) | + (Flavons) |
| Anthocyanes | Adding some drops of HCl 5% to 1 mL of decocted + alcalinisation (with drops of ammoniac 50%) | - |
| Leucoanthocyanes | Shinoda reagent (chlorhydric alcohol) | + |
| Quinonic derivates | Born-Trager reaction : Concentrated HCl Diluted HCl | - - |
| Saponosides | Foam index (FI) of diluted aqueous decoction (positive if FI ≥ 100, meaning foam height ≥ 1 cm) | ++(FI > 1 cm) |
| Triterpenoids | Liebermann-Buchard reaction (acetic anhydride-sulfuric acide 50:1) Kedde reaction (dinitrobenzoic acid 2% in ethanol + NaOH (1 N) 1:1) | + + |
| Steroids | RAYMOND reaction (Dinitrobenzene 1% in ethanol + NaOH 20%) Grignard reaction (soaked paper with picric acid 5%) | + + |
| Cardenolids | Viscosity study (in absolute ethanol) | - |
| Cyanogenic derivates | Ammoniac at 25% : hight fluorescence | - |
| Mucilages | Chloroform + ammoniac : red ± hight coloration | - |
| Coumarins | Extraction, elution with toluen + AcEt (97/3), revelation with sulfuric anysaldehyde or vanilin | + |
| Anthracenic derivates | | + |
| Essentiel Oils | | +++ |

Chemical compounds of total brut extract of EGLAE. The phytochemical analysis was performed as described in Methods section. (+++) too high, (++) high (+) low: indicates the presence of the compounds in the plants; (-) indicates the absence of compound in plants

Reducing compounds are used to treat cough and tannins are used against diarrhea and bleeding in tighter tissues [18]. The hemostatic effect was attributed to the presence of tannins [7, 19]. Flavonoids are potent diuretics and especially those of type flavanones [19]. But the phytochemical analysis of leaves of *Elaeis guineensis* reveals the presence of flavones (table 1). This could explain the modest diuretic activity and the “tray effect” of our hot water extract containing flavonoids like flavons at high dose of 202.50 mg/kg and the low activity of flavonoids extract at therapeutic dose of 9.76 mg/kg. By analyzing the mechanism by which flavonoids act, it be find that the protective effects and dilating blood vessels and their ability to make the blood fluid may be due to flavonoids [19, 20]. They argue that the elimination of toxins is a prerequisite for promoting weight loss [20].

Treatment with EGLAE at 135.00 mg/kg compared to furosemide was given the largest ($p < 0.01$) with EUV diuretic activities of 157.16% and 169.25% respectively (Table 2).

Table 2 : Effect of oral administration of *Elaeis guineensis* leaves aqueous extract on urine volume and diuretic index in Wistar rats (n = 5)

| Treatments | Dose/kg | VANaCl (mL) | VE/6h (mL) | EUV (%) | Diuretic Activity |
|-----------------|-----------|-------------|---------------|---------|--------------------|
| Distilled Water | 25.00 mL | 16.34 | 13.60 ± 5.81 | 83.54 | No activity |
| Dose 1 | 67.50 mg | 19.53 | 24.15±8.72*** | 123.65 | Low activity |
| Dose 2 | 135.00 mg | 13.91 | 21.86±6.55** | 157.16 | Important activity |
| Dose 3 | 202.50 mg | 12.54 | 18.35 ± 5.72* | 146.32 | Moderate activity |
| Flavex | 9.76 mg | 7.56 | 9.57± 1.66 | 126.58 | Low activity |
| Furosemide | 20.00 mg | 11.34 | 19.20± 4.32** | 169.25 | Important activity |

The comparison was made from distilled water (control group), D1 = 1 dose, D2 = 2 doses, D3 = 3 doses. Flavex = flavonoids extract, NS: No significant difference, * significant difference, **moderately significant difference, *** very significant difference,

This result agrees with that which showed furosemide exerts diuretic effect on the loop of Henle, which makes its a diuretic [21]. Dose of 67.50 mg/kg of *Elaeis guineensis* leaves aqueous extract has low diuretic activity while those 202.5 mg/kg lead to moderate ($p < 0.05$) diuretic activities with 146.32% of urinary volume in six hours (Table 2). Sodium elimination was significant ($p < 0.05$), while potassium was spared in animals treated with aqueous extract of *Elaeis guineensis*. The natriuretic activity of the aqueous extract was 2.21 at a dose of 67.50 mg/kg and 2.34 for 135 mg/kg against 2.12 for flavonoids extract (9.76 mg/kg) and 2.52 for furosemide (20 mg/kg) and 0.75 in Wistar rats in the control group, treated with distilled water (Table 3).

Table 3: Results of urinary electrolytes from Wistar rats after treatment (n = 5).

| Treatments | Concentration of ions (mmol/L) | | | [Na ⁺]/[K ⁺] ratio |
|---------------------|--------------------------------|----------------------------|-----------------|---|
| | Na ⁺ | K ⁺ | Cl ⁻ | |
| Distilled water | 17.24 ± 7.69 | 22.82 ± 4.63 | 11.12 ± 7.14 | 0.75 ± 0.45 |
| 67,50 mg/kg (D1) | 42.40± 8.69** | 22.17 ± 7.33 ^{NS} | 40.12 ± 4.98 | 2.21 ± 1.27 |
| 135,00 mg/kg (D2) | 46.85±4.22*** | 20.02± 5.79 ^{NS} | 38.44 ± 8.54 | 2.34± 0.94 |
| 202,50 mg/kg (D3) | 35,40 ± 4.22** | 20.79 ± 7.59 ^{NS} | 37,31±7.33 | 1.70 ± 0.49 |
| Flavex (9.76 mg/kg) | 32.22± 1.2** | 15.2± 1.1 ^{NS} | 9.77± 1.53 | 2.12± 0.09 |
| Furosemid (20mg/kg) | 48.56±0.26*** | 19.22 ± 1.61 ^{NS} | 40.18 ± 12.53 | 2.52 ± 0.43 |

The comparison was made from distilled water (control group), D1 = 1 dose, D2 = 2 doses, D3 = 3 doses. Flavex = Flavonoids extract, NS: No significant difference, * significant difference, **moderately significant difference, *** very significant difference

The normal value for [Na⁺]/[K⁺] ratio is reported to be 2.05 – 2.83 [11]. The concentration of aldosterone is found to be dependent of [Na⁺]/[K⁺] ratio. If the [Na⁺]/[K⁺] ratio falls below the normal in plasma, the aldosterone secretion will be decreased and if ratio rise above the normal, the aldosterone secretion will be increased [12]. Significant increase in Na⁺ ion excretion ($p < 0.05$) was observed in aqueous extract treated animals but it was less than the furosemide controls and high than flavonoids extract treated animals. These diuretic and natriuretic effects of *Elaeis guineensis* leaves aqueous extract can be beneficial in the treatment of certain cases of arterial hypertension in that they act by urinary excretion of part of water and sodium in the blood. This will result a decrease in blood volume and therefore a drop in blood pressure [4]. Furthermore the K⁺ ion which was not excreted is the major cation of the middle intracellular and its gradient of both sides of the cell membrane is mainly determining transmembrane electrical potential influencing the excitability of tissues such as nerves and muscles [22]. However the results show that for 135.00 mg/kg, there is a high excretion of sodium ($p < 0.01$) and potassium retention. While the aqueous extract of leaves of *Elaeis guineensis* can be used for one hand to maintain the electrochemical composition of the intracellular medium and also treat high blood pressure at this dose. The pH of the urine collected is obtained between 6.1 and 8.6 with a mean of 7.35 ± 1.25. This confirms that urinary pH values are in previous work between 4.6 and 7.8 [23]. The difference between pH values may be due to the nature of animals used for testing or animal diets. The effective dose of aqueous extract of leaves of *Elaeis guineensis*, looks of diuretic and saluretic results 135 mg/kg. As diuretic therapy may lead to number of life threatening electrolytic disorder and toxicities, so safety profile studies are carried out following a sub chronic administration of extracts. This amplifies the heterogeneous array of diuretic curatives available for safe and effective treatment of edema and cardiovascular diseases [24].

Toxicity tests performed on *Artemia salina* gave an LC50 = 1,710 mg/mL for *Elaeis guineensis* leaves aqueous extracts. This test evaluated primary toxicity of plant extracts [25], cyanobacteria toxins [26], detection of fungal toxins [27], and other biological activities [25,28] reflected the sensitivity of shrimp larvae to EGLAE and by extension that of the human species. In fact, this test expresses a good correlation between the cytotoxic activity of shrimp larvae and cell 9PS and 9KB (human carcinoma nasopharyngeal) on the one hand, cells A-549 lung

carcinoma and HT-29 cells of colon carcinoma on the other [29]. According to LC50 value, *Elaeis guineensis* leaves aqueous extracts can be without toxicity

CONCLUSION

Elaeis guineensis leaves aqueous extract has from 135 mg/kg an important diuretic activity. This extract therefore contains active ingredients that give the diuretic action. The aqueous extract of leaves of *Elaeis guineensis* also promotes the elimination of certain ions such as Na⁺, Cl⁻ and K⁺ retention. It then has anatriuretic activity. The effective dose of aqueous extract of leaves of *Elaeis guineensis*, looks diuretics and natriuretic results is 135 mg/kg and it be due to the presence of flavonoids in synergy action with another constituents in EGLAE. It has an LC₅₀ equal to 1,710 mg/mL, therefore can be without toxicity.

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