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Phytochemical screening and acute toxicity assessment of leaves from *Piliostigma thonningii* (Fabaceae), a plant used in traditional medicine against diabetes in Côte d'Ivoire

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Abstract

In Côte d'Ivoire, the prevalence rate of diabetes is 9.6%. This rate is high and worrying. However, there is no satisfactory therapy for this pathology described as a "silent killer". It is therefore essential to find an alternative through the heritage of plant resources used in traditional medicine. The decoction of *Piliostigma thonningii* leaves is frequently used by Ivorian traditional therapists. This study focuses on this plant species by performing a phytochemical screening and evaluating the acute toxicity of aqueous extracts from the plant's leaves. In laboratory, the phytomedicine was explored for its pharmacodynamic effects. After fasting the mice for 12 hours, the stock solution of 200 mg/ml was administered by gavage at a volume of 0.6 ml per 20 grams of body weight. The toxicological study showed that the aqueous extract from leaves of *Piliostigma thonningii* did not show toxic effect on the animals. The phytochemical screening of the tested sample in Pharmacodynamics revealed various chemical groups: alkaloids, coumarins, flavonoids, polyphenols, quinones, saponosides and catechic tannins. Polyphenols, alkaloids and flavonoids are believed to be responsible for the anti-diabetic activity. This preliminary verification, based on pharmacological and phytochemical tests, confirms the traditional therapeutic use of the tested plant.

Keywords: Côte d'Ivoire, phytochemical, pharmacodynamics, plant, *Piliostigma thonningii*

Introduction

The interest of traditional medicine is increasing considerably all over the world. According to the World Health Organisation (WHO), this medicine could be defined as all combination of knowledges and practices, whether explicable or not, used to diagnose, prevent or eliminate a physical, mental or social disease and which may be based exclusively on ancient experience and observations transmitted from generation to generation, orally or in writing ^[1]. Traditional human pharmacopoeia is still very present in African societies. Indeed, nearly 80% of the population use traditional medicine and remedies from the traditional pharmacopoeia as a first line of defence to deal with health problems ^[2]. In Côte d'Ivoire, the seroprevalence of diabetes is 9.6% while the disease is without definitive cure in the country as everywhere in the world ^[3]. The use of phytomedicines therefore remains an alternative to be encouraged. Thus, ethnomedicinal investigations carried out in the country by several authors on anti-diabetic plants revealed the frequent use of decoction of leaves from *Piliostigma thonningii* ^[4]. However, studies devoted to phytochemical and toxicity of the plant are rare in Côte d'Ivoire. This study is part of this approach. It performs a phytochemical screening to determine the chemical groups responsible for the anti-diabetic activity and evaluates the acute toxicity of the aqueous extract from leaves of *Piliostigma thonningii* in order to prevent possible intoxications.

Materials and methods**Materials****Plant Material**

The plant material concerns leaves of *Piliostigma thonningii*.



Fig 1: Leafy stem of *Piliostigma thonningii*

Animal material

The animals used for the experiment are male mice aged 8 to 9 weeks. These animals weighed between 150 and 350 g.

Study method

Plant identification

The plant was identified at the National Floristic Center (CNF) of Félix HOUPOUËT-BOIGNY university in Abidjan, Côte d'Ivoire.

Organization of rats used for experiments

The rats were placed in plastic cages in the laboratory at a temperature of 20-22 °C, 12 hours of light and 12 hours of darkness during 7 days. The cages contained wood shavings which were changed every 3 days. We divided them into 6 homogeneous batches of 10 mice:

Batch 01: Control mice receiving distilled water.

Batch 02: Treated mice receiving *Piliostigma thonningii* at 200 mg ml⁻¹.

Batch 03: Treated mice receiving *Piliostigma thonningii* at 100 mg ml⁻¹.

Batch 04: Treated mice receiving *Piliostigma thonningii* at 66.66 mg ml⁻¹.

Batch 05: Treated mice receiving *Piliostigma thonningii* at 50 mg ml⁻¹.

Batch 06: Treated mice receiving *Piliostigma thonningii* at 40 mg ml⁻¹.

The animals were fasted for 18 hours before administration of the extract by gavage. They were deprived of food but not water.

Preparation of the crude aqueous extract of the leafy stems of *Piliostigma thonningii*

The total aqueous extract of the leafy stems of *P. thonningii* was prepared following the method recommended in traditional medicine which is decoction [5]. For this experimental study, the methods were adapted to the reality of the Pharmacognosy laboratory of Félix Houphouët-Boigny university. For this, we boiled 1000 g of leafy stem drug added in 6 litres of distilled water, in a suitable vessel (metal pot), for 30-45 min [6]. Crude extracts were obtained, from the aqueous decoctate of the drugs from *P. thonningii*. The freshly harvested drug was previously dried in the shade [7]. To obtain the aqueous decoctate, the resulting solution was filtered and the filtrate was evaporated under the Rotavapor at a temperature of 60 °C. The dry extract obtained is the aqueous crude extract of the leafy stems of *P. thonningii*. This extract was subjected to identification reactions by

precipitation and staining. The operation was repeated several times in order to obtain a sufficient quantity of extract for the experiments. After 2 days, the crystals obtained were pulverised using a porcelain mortar and pestle. The fine powder collected: 33.39 g constitutes the total dry extract. This is stored in a sterile glass jar in a refrigerator, hermetically sealed. The maximum concentration corresponding to a concentration at the limit of solubility of each extract was sought.

Phytochemical screening

For this experience, 0.5 litre of the decoctate obtained was used. The phytochemical screening was carried out of this aqueous phase by means of the usual reactions for the various organs. The phytochemical screening was performed according to the method already described by several authors [8-9].

Search for sterols and polyterpenes

The chemical test set up by Liebermann allowed to bring out the chemical groups. We evaporated to dryness, without carbonized the residue, in a capsule on the sand bath, 5 ml of the solution. The residue was subsequently dissolved in 1 ml of acetic anhydride and the resulting solution was decanted into a test tube. Finally, we added 0.5 ml of sulfuric acid in the test tube and observed the solution. The sight of a purple ring at the interphase, turning to blue and then green, indicated a positive reaction.

Search for polyphenols

The reaction with ferric chloride (FeCl₃) made it possible to characterize the polyphenols. We mixed a drop of alcoholic ferric chloride solution (2%) with 2 ml of each solution. Ferric chloride reacts with polyphenolic derivatives and let appearing a blackish blue, more or less dark green color, indicating the presence of polyphenols.

Search for flavonoids

The search for flavonoids was carried out from the reaction to Cyanidin. It consisted in evaporating to dryness in a capsule, 2 ml of each solution before cooling them. The residue is taken up in 5 ml of a half hydrochloric alcohol. The solution is decanted into a test tube, in which we have added 2-3 chips of magnesium and observed a release of heat. A pink-orange or sometimes purplish color was obtained. Finally, we added 3 drops of isoamyl alcohol, which intensifies the coloring when flavonoids are present.

Search for tannins

The search for catechetal tannins was carried out using Stiasny's reagent. Five (5) ml of each extract was evaporated to dryness. After adding 15 ml of Stiasny's reagent to the residue, the mixture was kept in a water bath at 80 °C for 30 min. The observation of a precipitate in large flakes characterized the catechetal tannins. For the gallic tannins, we filtered the previous solution (5 ml of extract and 15 ml of Stiasny's reagent). The filtrate was collected and saturated with sodium acetate. The addition of 3 drops of FeCl₃ would cause the appearance of an intense blue-black color, a sign of the presence of gallic tannins.

Search for free or combined quinone substances

The detection of free quinone substances was performed with the reagent of Bornsträegen. For the combined quinone substances, we carried out a preliminary hydrolysis. The

experiment consisted in hydrolyzing the solutions to characterize all the quinone substances, like the free quinone substances and the compound quinone substances. For that, we evaporated 2 ml of each solution to dryness in a capsule and triturated the residue in a fifth into 5 ml of hydrochloric acid. Thereafter, we kept the solution obtained in a boiling bath-water for 30 min. The process led to cooling, extracting the hydrolysate with 20 ml of chloroform in a test tube, collecting the chloroform phase in another tube and adding 0.5 ml of half-diluted ammonia. The sight of a color ranging from red to purple indicated the presence of quinones.

Search for alkaloids

The characterization of the alkaloids was established from the reagent of DRAGENDORFF (reagent to potassium iodobismuthate) and that of Burchard (iodine-iodide reagent). We evaporated to dryness in a capsule, 6 ml of each solution, taken up the residue in 6 ml of alcohol at 60 °C and distributed the alcoholic solution in 2 test tubes. After this step, we added 2 drops of DRAGENDORFF reagent to the first tube and 2 drops of BURCHARD reagent to the second tube. In the first tube, the sight of a precipitate or an orange coloration indicated the presence of alkaloids. In the second tube, the observation of a precipitate or a white cream coloration was evidence of a positive reaction.

Search of saponosides

The saponosides were detected by assessing the abundance of foams after stirring plant extract solutions. We put 15 ml of each extract into a test tube, stirred vigorously for 10 seconds and let to calm for 10 min. The persistence of the foam at a height of 2 to 3 cm, exhibited of the presence of the saponosides.

Acute oral toxicity

The concentrations of the aqueous crude extract (3 liters) were prepared on the principle that the concentrations to be administered should be related to the body weight of the rats, thus the doses were expressed as mg/kg body weight. Then, 4 g of dry extract of the leafy stems of *P. thonningii*, were used for the preparation of the phytomedicine, with a maximum concentration of 200 mg ml⁻¹. From this stock solution, different dilutions were made to obtain concentrations of: 1; 1/2, 1/3, 1/4 and 1/5, corresponding to doses of: 200; 100; 66; 50 and 40 mg ml⁻¹, respectively. The different batches of animals were treated at different doses against the control receiving distilled water.

Gavage of mices

After fasting the animals for 12 hours, the stock solution (200 mg ml⁻¹) was administered by gavage to the different batches, according to the methodology. Gavage was performed using an intubation cannula with a slightly curved tip. This operation required the use of a volume of 0.6 ml per 20 grams of body weight. The dose of phytomedicinal to be administered is then expressed in mg kg⁻¹/vo body weight. Overall, volumes of 0.57 to 0.9 ml were administered to the animals, depending on their body weight. The different concentrations calculated above correspond to the following doses: 6000; 3000; 2000; 1500 and 1200 mg kg⁻¹/vo of body

weight. After administration of the extract, the animals were returned to their metal cages where they could access the pellets. They were observed immediately and then every 30 minutes for 8 hours on the first day and once a day for 48 hours. During this period, clinical signs (agitation, lack of appetite, motor difficulties and dyspnoea) and the number of dead animals were recorded. The toxicity parameters evaluated in our study were: (i) the maximum tolerated dose (MTD); (ii) the 100% lethal dose (LD100) and (iii) the 50% lethal dose (LD50). The maximum tolerated dose (MTD) represents the maximum dose that does not kill any animal when the extract is administered. The 100% lethal dose (LD100) is the lowest dose that kills all animals. The calculation of the DL 50 is made from the following formula:

$$DL_{50} = DL_{100} \cdot \frac{\sum(a \times b)}{n} [10].$$

LD50: Lethal dose 50%; LD100: Lethal dose 100%; a: average of the sum of the deaths between two successive doses; b: difference between two successive doses; n: average of the number of animals used per batch. Six toxicity classes were used to assess the significance of the LD50 values obtained (Table I).

Table 1: Toxicity class [11]

Index or class of toxicity	Commonly used term	Toxicological parameter (LD50)
1.	Extremely toxic	DL ₅₀ ≤ 1 mg kg ⁻¹
2.	Highly toxic	1 mg/kg ≤ DL ₅₀ ≤ 50 mg kg ⁻¹
3.	Moderately toxic	50 mg/kg ≤ DL ₅₀ ≤ 500 mg kg ⁻¹
4.	Slightly toxic	500 mg/kg ≤ DL ₅₀ ≤ 5 g kg ⁻¹
5.	Nearly toxic	5 g/kg ≤ DL ₅₀ ≤ 15 g kg ⁻¹
6.	Relatively harmless	DL ₅₀ ≥ 15 g kg ⁻¹

Results of the tri-phytochemical and toxicological study

Tri-phytochemical study

The tri phytochemical Characterisation tests, carried out on a single fraction of natural substances, gave the results that are shown in Table 2. These results indicate that the different groups of compounds found in the plants are polyphenols, flavonoids, quinone substances, alkaloids according to Burchard's reagent, alkaloids according to Dragenbdorff's reagent and saponosides. The tests for sterols and polyterpenes are negative in the extract. This is also the case for gallic tannins.

Experimental toxicological characteristics

The results of the toxicological parameters are recorded in Table 3. The doses administered (1200 to 6000 mg kg⁻¹ bw/vo) were harmless for the mice. No mice died in the batches formed. The 100% lethal dose (LD100) is therefore zero. The maximum tolerated dose (MTD) is 6000 mg kg⁻¹ bw/vo, the highest dose administered to animals. According to the Karber and Berhens formula, the LD50 is zero over 48 hours of observation. There was therefore no dose-response effect. It should be noted that the doses obtained in the laboratory are very concentrated compared to those of traditional therapists.

Table 2: Different groups of compounds highlighted

Chemical groups	Sterols and Polyterpenes	Polyphenols	Flavonoids	Tannins		Quinones	Alkaloids	Saponins
				Cat	Gal			
Reaction of the extract of <i>P. thonningii</i>	-	++	++	+	-	++	+	+

Table 3: Mortality rate of mice after gavage of aqueous extract of *Piliostigma thonningii* leaves

Parameters evaluated	Batch 1 (Control)	Batch 02	Batch 03	Batch 04	Batch 05	Batch 06
Substance administered by gavage	Distilled water	Extract of <i>P. Thonningii</i>	Extract of <i>P. Thonningii</i>	Extract of <i>P. Thonningii</i>	Extract of <i>P. Thonningii</i>	Extract of <i>P. Thonningii</i>
Initial concentration (mg/ml)	0,6 ml/20g	200	100	66,66	50	40
Corresponding dose (mg/kg b.w./v0)	30 ml/kg	6000	3000	2000	1500	1200
Number of mice per batch	10	10	10	10	10	10
Number of dead mice, 48 h after gavage of the phytomedicine	0	0	0	0	0	0
Mortality (%)	0	0	0	0	0	0

Discussion

Phytochemical study of the extracts

We proceeded by a primary validation of traditional medical practices, by searching, for the plant which was the subject of pharmacodynamic study, the chemical groups which would make it possible to explain the antidiabetic effect. The phytochemical screening revealed different chemical groups: alkaloids, flavonoids, polyphenols, quinones, saponosides and catechin tannins. In a similar phytochemical study, alkaloids, flavonoids, phenols, saponosides, steroids, tannins and terpenes were characterised [12]. The preliminary study shows the presence of 4 elements: flavonoids, terpenoids, tannins and saponosides [13]. Similar research notes that *P. thonningii* contains sterols and terpenes [14]. This study does not come to the same result. The country, the place where the samples were taken and the season could possibly explain these observed differences. Among the chemical compounds revealed, the alkaloids would be incriminated in the anti-diabetic activity of *P. thonningii*. The presumed activity of the extract would also be due to the synergistic presence of alkaloids, tannins, flavonoids and saponosides.

Acute toxicity study

The experiments we carried out show that the leaf extract appears to be harmless to mice at 6000 mg kg⁻¹/vo. The ivorian species seems to be free of any acute toxicity under the conditions of our experiment. In Kenya, however, the plant showed remarkable toxicity. Further experiments showed that in rats, administration of the ethanolic extract resulted in mortality; the estimated LD50 was 3000 mg kg⁻¹/bw [15]. In Nigeria, the plant showed mild toxicity with an LD50 > 5000 mg kg⁻¹ intravenously. The aqueous extract was found to be non-toxic orally [16]. Similar acute toxicity study reported that the plant extract had an estimated LD50 of 3807, 89 mg kg⁻¹ [17]. Overall, there are conflicting results for this species. This discrepancy in acute toxicity seems to be related to the country of origin and various related factors. The DMT (6000 mg kg⁻¹/vo) is much higher than the daily dose (8498 mg kg⁻¹ /vo) recommended by traditional therapists. The aqueous extract of *P. thonningii* offers an appreciable safety margin. This reassures us about the use of the phytomedicine prescribed by the traditherapist. *Piliostigma thonningii*, traditionally prescribed at a daily dose of 84.98 mg kg⁻¹/vo, is not toxic, which could explain the various traditional therapeutic uses of the plant.

Conclusion

The phytochemical screening showed that the therapeutic effect of the plant is due to various secondary metabolites (alkaloids, flavonoids, polyphenols, quinones, catechic

tannins and saponosides). Thus, its therapeutic effect would be due to these secondary metabolites, notably saponosides, flavonoids and polyphenols, known for their antioxidant properties. However, it is not excluded that alkaloids are responsible for the anti-diabetic activity. Indeed, alkaloids have a stimulating effect on hepatic glycogenogenesis. Our results, combined with literature reviews, have established relationships between the presence of chemical molecules and their anti-diabetic therapeutic virtues. Thus, the presence of saponins, flavonoids and catechic tannins in the extract could be responsible for its hypoglycaemic and anti-hyperglycaemic effects. The toxicity study was carried out on mice with the aqueous extracts. This extract did not show acute toxicity under the conditions of our experiments.

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