

Evaluation of Antidiabetic Activity of Aqueous Extract of Leaves from *Phragmanthera capitata* (Sprengel) S. Balle (Laurenthaceae) in Wistar Albino Rats

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Abstract: Diabetes mellitus is a public health problem affecting about 8.4% of people in the world and knows an alarming progression in sub-Saharan Africa and in Cameroon precisely. The current therapeutic strategies have prohibitive prices, are unavailable and have harmful side-effects. So, this work aimed at contributing to the valorization of medicinal flora and knowledge of traditional healers in Foumban, West-Cameroon, in the treatment of diabetes. An ethnopharmacological survey was conducted by consulting traditional healers of the District of Foumban. At the end of this investigation, *Phragmanthera capitata*, which was found to be the most active plant following preliminary tests, was selected for antidiabetic studies on three groups of rats: normal glycaemia, temporary hyperglycemia and diabetic. The first two groups were treated *per os*, with aqueous extract of leaves from *P. capitata*, with the doses of D2 (4 mL/kg) obtained from the traditional healers, D1 (half of D2), D3 (double of D2) and by glibenclamide (2.5 mg/kg). The rats of the last group were treated twice per day, with the same doses of extract and glibenclamide for 13 days and were sacrificed on the 14th day. Their blood was collected by cardiac puncture for the determination of biochemical and hematological parameters. Phytochemical screening was performed using standard methods. The administration of aqueous extract of *P. capitata* led to a hypoglycaemia effect in normal rats, a reduction of glycaemia in the temporary hyperglycaemia and diabetic rats. In addition, an improvement of lipid assessment, renal and hepatic parameters related to the installation of diabetes, as well as an immune-modulator effect of the extract were noted in diabetic rats treated for 13 days. Phytochemical screening indicated that the extract contains saponins, tannins, flavonoids, steroids and terpenes. This work along with findings justifies the traditional use of this plant in the treatment of diabetes mellitus.

Keywords: Diabetes, Ethnopharmacologic Survey, *Phragmanthera capitata*, Biochemical Parameters, Hematologic Parameters

1. Introduction

Diabetes formerly considered as an uncommon illness in sub-Saharan Africa became public health problem and knows an alarming progression [1]. According to the estimates, more than 21 million (5%) people were suffering from diabetes in 2014 and more than 41 million diabetics are expected in 2035 [1]. In Cameroon, the prevalence of diabetes rose from 1.5% in 1990 to 10% in 2014 and an

increase of 80% is expected by 2035 [2]. The socio-economic impact of diabetes is very important in most countries of sub-Saharan Africa and generates enormous health expenditures which do not cease growing. Diabetes and its consequences caused 481.000 deaths in Africa, of which 13.822 in Cameroon in 2014; 75% of these deaths occurred among people of less than 60 years old with a strong rate in the young people from 20 to 39 years old [3]. The burden of diseases related to diabetes and the reduction of the life

expectancy which results from this will in general slow down the economic growth of sub-Saharan Africa and the emergence of Cameroon in particular [4, 5].

The treatment consists of the insulin injection (diabetes of the type 1) or the administration of oral antidiabetics (type 2 diabetes) [6, 7]. The oral antidiabetics result in the standardisation of the glycaemia in less than 50% of cases. They do not have regressive effects on the established lesions and are not indicated in case of renal and hepatic insufficiencies as well as during pregnancy [7]. The cost of the treatment is considerable and is estimated at 108.9 USD per month in Cameroon [1]. Insulin capsule currently costs 3000 FCFA in Cameroon. The people suffering from diabetes and their family support almost the entire cost of the medical care [3]. Moreover, the supply in the population's drugs, the infrastructures and medical staff are insufficient [8]. Plants constitute a medical potential not only available at lower costs for the populations, but is also less toxic [9, 10]. Unfortunately, there are not enough scientific data on their effectiveness and harmlessness, making doubt of the effective role of these medicinal plants on diseases in general and diabetes in particular [2].

In addition, today, the knowledge of traditional healers is less transmitted and tends to disappear [11]. A simple manner to preserve the cultures, the knowledge and the plants which are dependent consists of developing this knowledge, test them to check and to validate their supposed effects and finally to valorize by integrating traditional medicine in the modern health care system [11, 12]. This work which falls under this strategy of WHO for traditional medicine aims at contributing to the valorization of medicinal flora and knowledge of traditional healers of the district of Foumban, West-Cameroon, in the treatment of diabetes.

2. Materials and Methods

2.1. Ethnopharmacological Survey

2.1.1. Description of the Area of Investigation

The ethnobotanical survey was conducted in three villages of the district of Foumban (Njissé, Njimbam I and Njimbam II), in the region of West-Cameroon. The district of Foumban covering a surface of approximately 418 km², is located between 5°43' and 5°43' Northern latitude and the 10°53'-10°55' of East longitude. The town of Foumban is the headquarters of Noun division and of the royal palace of Bamoun people [13]. This latter uses medicinal plant for its primary healthcare. In this area, traditional healers are famous to have a good knowledge on the use of medicinal herbs [13].

2.1.2. Data Collection

The ethnopharmacological investigation was conducted from July 15th, 2015 to October 17th, 2015 and included 27 traditional healers (Table 1). The data were collected through a semi-structured questionnaire making it possible to evaluate the knowledge about the disease and the plant, its use, the mode of preparation and the regulation

recommended by each questioned traditional healer. The traditional healers were also questioned on the means by which they lay out to diagnose the disease as well as the alternative measures in the event of failure of their treatment. The investigation was conducted in Bamoun language with the assistance of a local guide after obtaining the enlightened assent of the healers.

The collected data were registered in a Microsoft Excel worksheet. The value of use [14], a method of quantification which shows the relative importance of a plant species, was calculated according to the following formula: $UV = (n/N) \times 100$, where UV = value of use of a species, n = number of quotation of a species and N = full number of quotation. Following the application of this formula, the value of use of *Phragmanthera capitata* was found to be the highest and no study on its antidiabetic properties was found; reason why that plant was selected for thorough antidiabetic studies.

Table 1. Distribution of the healers questioned in the villages of the study area.

Villages	Number of healers questioned	Number of plants (receipts) listed
Njissé	11	16
Djimbam I	8	11
Djimbam II	8	8
Total	27	35

The listed plants were collected under the supervision of the traditional healers and were authenticated at the national herbarium of Cameroon by comparison with a reference sample preserved at the herbarium.

2.2. Evaluation of the Antidiabetic Properties of *Phragmanthera Capitata*

2.2.1. Preparation of the Extract and Doses of *Phragmanthera Capitata*

Phragmanthera capitata was collected from an avocado tree in February 2016. After harvest, the leaves were separated from the stem, then weighed and washed. The aqueous extract of the leaves of *Phragmanthera capitata* was prepared according to the instructions of the traditional healers by decoction in the following way: 125 g of well washed fresh leaves were added to 0.5 L of distilled water. The mixture was boiled during 15 min. After 30 minutes of cooling, the obtained solution was then filtered, to give approximately 250 mL of extract of greenish color. By considering this volume for a person of 70 kg (dose of the traditional healers), the dose of 4 mL/kg (D2) of body weight was administered to a group of rats. On the basis of this amount, the double amount of the traditional healers (D3) and the half of the amount (D1) were administered to two other groups of rats.

2.2.2. Breeding and Preparation of the Experimental Animals

The experimental animals were young albino rats (*Rattus norvegicus*) of Wistar variety and of both sexes. They were 3 to 4 months old and having an average weight of

182,666±21,960 g. They were obtained by breeding for 4 months at the animal house of the University of Dschang. The rats were randomly assigned to groups. The animals had free access to water and food whose composition was previously described [15].

2.2.3. Induction of Diabetes

Permanent diabetes mellitus was induced by intra-peritoneal injection of single dose of alloxan monohydrate (150 mg/kg) [16], to one night fasting rats. For this purpose, the aqueous solution of alloxan was prepared right before the injection. After injection of the solution of alloxan, the rats had free access to food and a diluted solution of glucose during one night in order to avoid the hypoglycemic shock. Three days after injection of the alloxan, the establishment of diabetes mellitus in rats was verified by the measurement of the glycaemia. The animals having presented a glycaemia higher than 333 mg/L were in permanent hyperglycaemia [17] and were selected for the continuation of work.

2.2.4. Effect of Aqueous Extract of *Phragmanthera Capitata* in the Normal Glycaemia Rats

In order to check the assertion of the healers according to which *Phragmanthera capitata* can be consumed without risk by a normal subject, five batches of 5 rats each, after fasting for 18 hours were randomly constituted and treated as follows:

- The rats of batch 1, negative control received distilled water (10 ml/kg);
- The rats of batch 2, positive control received in single administration, the solution of glibenclamide (2.5 mg/kg);
- The rats of batches 3, 4 and 5 received in single administration, respectively the doses D1, D2 and D3 of the aqueous extract of *P. capitata*. Before administration of the test substances, the basic glycaemia of each rat was determined. After administration of the test substances in single amount, blood samples were collected at an interval of 1 hour within a period of 4 hours [17] in order to determine glycaemia.

2.2.5. Effect of Aqueous Extract of *Phragmanthera Capitata* in Temporary Hyperglycaemia Rats

To determine the capacity of extract to reduce post prandial glycaemia, 5 batches of 5 rats (male and female) each was randomly made up. At time t-30 min (30 min before the administration of the overload of glucose), blood sample was collected in order to measure the basic glycaemia. The rats were then cramed:

- With distilled water (10 ml/kg) for batch 1, the negative control;
- With a solution of glibenclamide (2.5 mg/kg) for batch 2, positive control;
- With the aqueous extract of *Phragmanthera capitata*, D1, D2 and D3 (4 mL/kg) respectively for the rats of batches 3, 4, 5.

Blood samples were collected at an interval of 30 min within a period of 4 hours in order to determine the glucose

level in each sample.

2.2.6. Effect of Aqueous Extract of *Phragmanthera Capitata* in the Diabetic Rats

In order to determine the antidiabetic activity of the extract, six batches of 5 rats (male and female) each were randomly constituted and the animals were treated by cramming each morning at 8 AM and the evening at 6 PM during 14 days:

- The rats of batch 1, neutral control (not diabetic) and those of batch 2, negative control received distilled water (10 ml/kg);
- The rats of batch 3, positive control received a solution of glibenclamide (2.5 mg/kg);
- Those of batches 4, 5 and 6 respectively received D1, D2 and D3 doses.

Treatment was stopped on the 7th day after the return of glycaemia to normal and continued again on the 8th day. Blood samples for the determination of glycaemia were collected at an interval of 1 hour within a period of 5 hours on the first day of treatment, then every day (twice a day) within the first week and finally every two days until the 14th day.

2.2.7. Determination of the Glycaemia Level

Glycaemia was measured by a single drop of blood obtained from the caudal end of the animals (venous blood) using a glucometer with reactive strips (Accu Check Active) Rock manufactured in April 2016 and of batch number 24649031. This drop of blood obtained by puncture of the tail after excision was deposited on the active zone of a strip. The reading of the glycaemia was made automatically 5 seconds after and the result expressed in mg/dL of blood.

2.2.8. Determination of Some Hematological and Biochemical Parameters

i. Blood Sample Collection

On the last day of the cramming, the animals were subjected to 12 hours fasting (food and water), after what the animals were anaesthetized by the chloroform vapors and dissected. The blood of each animal was collected by cardiac puncture using a syringe and put in two tubes of which one with anticoagulant (EDTA) and the other without anticoagulant. The blood contained in EDTA tubes was used directly for the determination of the hematological parameters. The blood collected in the tubes without anticoagulant was left at rest during 3 hours in ice-cold water then centrifuged at 3000 g during 20 min. The obtained supernatant (serum) was collected using a micropipette, transferred in the labelled eppendorf tubes and stored at -30°C until the moment of the determination of the biochemical parameters [15].

ii. Determination of Hematological Parameters

The hematological parameters were measured using an impedance Coulter automat.

iii. Determination of the Biochemical Parameters

Serum parameters namely total cholesterol, total triglycerides, total proteins, creatinine, urea, Glutamate Oxalo-acetate transaminase (ASAT) and Glutamate Pyruvate transaminase (ALAT) were measured by enzymatic methods using commercial kits TECO DIAGNOSTICS (USA) for the first 5 parameters and Points Scientific (Belgium) for the last 2 parameters as described by the manufacturer. This was done using a spectrophotometer of Genesys brand.

2.3. Phytochemical Analysis of the Crude Extract of *Phragmanthera Capitata*

The phytochemical characterization of the extract was performed based on standard methods [18].

2.4. Statistical Analysis

The values of the parameters were expressed as average \pm standard error to average. After variance analysis (ANOVA and ANCOVER), comparison of averages between various batches of rats was carried out based on the test of Waller-Duncan using SPSS software version 20.0. Values of $p < 0.05$ were considered statistically significant.

3. Results

3.1. Ethnopharmacological Investigation

3.1.1. Demographic Characteristics of Healers, Perceptions About Causes and Symptoms of Diabetes

27 traditional healers, of male (89%) and female (11%) sexes, from 45 to 85 years old and illiterates for the majority (93%) were surveyed. They were of Moslem confession (100%).

Most healers (96%) used the term «ya'a chouka'a» (disease of sugar) to qualify the diabetes. According to the

latter, «ya'a chouka'a» appears in children at low age (4%), in overweight persons (80%) and mystically in other persons (6%). Other healers (11%) think that the exaggerated consumption of food such as corn fufu and palm oil favours the disease. The latter recommend their patients to avoid this food during the treatment. The questioned traditional healers use the following indications to diagnose the disease: abundant emission of urines, foaming and yellow color (61%), an intense thirst (16%), urine of diabetics attracts ants because of the sugar it contains (8%). Finally, others (15%) refer to the diagnoses of doctors and require a control of sugar rate at the hospital during treatment.

3.1.2. Principal Information Collected During the Ethnopharmacological Investigation

The species of listed plants gathered by families is presented in Table 2. It appears that 35 species of plants belonging to 25 botanical families are used by the healers of Fouban for the treatment of diabetes. Tree families contain more than two species of listed plants: Asteraceae (14.81), Apiaceae (11.11), Myrtaceae (7.41) and Zingiberaceae (7.41). Among the listed species, 5 are used in mixture and the 30 others are used alone. The most represented species used alone are: *Allium sativum* (8.57), *Aloe barbadensis* (8.57), *Phragmanthera capitata* (8.57), *Allium cepa* (5.71), *Mangifera indica* (5.71), *Catharethus roseus* (5.71), *Vernonia amygdalina* (5.71), *Persea americana* (5.71) and *Eucalyptus globulus* (5.71). The posology varies from 1 to 3 glasses per day in 1 to 3 times. The route of administration indicated by the healers is oral. The parts of the plant used in the various preparations are variable and are used in a fresh or dry state. The listed modes of preparation are: decoction, maceration, the infusion and powder. These various modes often depend on the part of the plant used, but the frequencies in the ethnopharmacological uses are variable.

Table 2. Principal information collected during the ethnopharmacological investigation of plants used in the district of Fouban for the treatment of diabetes mellitus.

Family of plant	Scientific name	n	Vernacular name (common name)	Associated plants	Preparation	Posology for an adult of 70 kg	Authentication
Alliaceae	<i>Allium cepa</i> L.	2	Kaah (Onion of the village)	Lemon	Slice 3 large onions. Boil within a period of 10 min in 1 liter of water.	1 glass in the morning 1 glass at midday 1 glass in the evening.	C: Westphal N° 10091 H: 42791/HNC
Aloaceae	<i>Aloe barbadensis</i>	3	(Alovera)	/	Put 3 leaves in 1 liter of water within a duration of 30 minutes	1 glass in the morning 1 glass at midday 1 glass in the evening.	C: Dang D. N° 124 H: 18676/SRF-CAM
Anacardiaceae	<i>Mangifera indica</i> L.	2	Chim mango (Mango tree)	/	1 handful in 1 liter of water and boil within a period of 15 minutes	1 glass in the morning 1 glass at midday 1 glass in the evening.	C: Dang D. N° 104 H: 18646/SRF-CAM
Annonaceae	<i>Annona muricata</i> L.	1	Choua-choua (corossolier)	/	1 handful in 2 liters of water and boil	1 glass in the morning 1 glass in the evening.	C: Dang N° 162 H: 18681/SRF-CAM
	<i>Centella asiatica</i> (L) Urb.	1	(violettes marron or cochlearia)	<i>Costus lucanusianus</i>	Boil 1 handful of leaves in 2 liters of water	1 glass in the morning 1 glass at midday 1 glass in the evening.	C: Letouzey R. N° 5667 H: 742/ SRF-CAM
Apiaceae	<i>Daucus carota</i>	1	Carrot	/	Consume like vegetable	At will	C: Dang N° 514 H: 25579/HNC
	<i>Petroselinum crispum</i> Nym.	1	(parsley)	/	Put 1 handful in water at night	1 teacup in the morning 1 teacup in the evening	C: Dang D. N° 374 H: 25583/HNC
Apocynaceae	<i>Catharanthus roseus</i> (L) G.	2	(Periwinkle of Madagascar)	/	2 handfuls in 1.5 liter of water. Boiling the	1 teacup morning 1 teacup at midday 1	C: Leuwenberg A. J. M. N° 9519

Family of plant	Scientific name	n	Vernacular name (common name)	Associated plants	Preparation	Posology for an adult of 70 kg	Authentication
	Don.				mixture within a time limit of 15 min	teacup in the evening	H: 43386/HNC
Asteraceae	<i>Ageratum conyzoides</i> L.	2	Mejocktefeu (King of grasses)	Lemou mecheu-cheu (Lemon)	1 handful in 1 liter of water	1 glass in the morning 1 glass in the evening.	C: Leuwenberg A. J. M. N° 7505 H: 21155/SRF-CAM
	<i>Bidens pilosa</i> L.	1	Kouamdou	/	Boil 1 handful in 1.5 L within a period of 20 min	1 glass in the morning. 1 glass in the evening	C: Letouzey R. N° 3417 H: 4871/ SRF-CAM
	<i>Tridax procumbens</i> L.	1	yère	/	1 handful in 2 liters of water	1 glass in the morning 1 glass in the evening	C: Swarbrick N° 276 H: 33080/HNC
	<i>vernonia amygdalina</i> Delile	2	Voup (Dolé)	/	1 handful to have 1 glass of water	1 glass in the morning 1 glass at midday 1 glass in the evening	C: Letouzey R. N° 14607 H: 38383/HNC
Brassicaceae	<i>Brassica oleracea</i> L.	1	(Cabbages)	/	Press to have 1 glass	1 glass in the morning 1 glass at midday 1 glass in the evening.	C: Westphal N° 9988 H: 427418/HNC
Caesalpinaceae	<i>Senna occidentalis</i> (L.) Link	1	(Forgery kinkéliba)	/	Boil 1 handful of root in 1.5 L of water within a period of 30 min.	1 glass in the morning 1 glass at midday 1 glass in the evening.	C: Letouzey R. N° 6476 H: 7854/SRF-CAM
Caricaceae	<i>Carica papaya</i> L.	1	Dom pare (Wild papaw tree)	/	Boil 2 to 3 roots in 1.5 L of water within a period of 15 to 20 min.	1 glass in the morning 1 glass at midday 1 glass in the evening.	C: Betti J. L. N° 243 H: 66 220/HNC
Cucurbitaceae	<i>Citrullus Lanatus</i> (Thumb.) Matsum. & Nakai	1	melon (Watermelon)	/	Soak a fruit in 2 L of water or consume raw seed	/	C: Westphal N° 9249 H: 42461/HNC
Fabaceae	<i>Phaseolus vulgaris</i> L.	1	Koun (Bean)	/	1 handful in 2 liters of water	3 teacups per day	C: Westphal N° 8947 H: 42584/HNC
Lamiaceae	<i>Ocimum gratissimum</i> L.	1	(wild basil)	/	1 handful of leaves in 1.5 L of water during 30 minutes	1 glass in the morning 1 glass at midday 1 glass in the evening	C: Manning S. D. N° 654 H: 57748/HNC
Lauraceae	<i>Persea americana</i> Mill.	2	Chim Péya'a (avocado tree)	/	Slice 2 cores. Soak in 1 liter of water then let ferment for 2 days	1 glass in the morning 1 glass in the evening	C: Manning S. D. N° 891 H: 57756/HNC
Liliaceae	<i>Allium sativum</i> L.	3	(Garlic of the village)	/	Slice 1 to 2 pods. Add 1 liter of water and boil the mixture during 5 minutes	3 teacups per day within a period of 9 months.	C: Westphal N° 10019 H: 44810/HNC
Mimosaceae	<i>Tetrapleura tetraptera</i> (Schum. et Thom.) Taub.	1	(4 sides)	/	Clean and soak 1 fruit in 1.5 L of water	1 glass in the morning 1 glass at midday 1 glass in the evening.	C: Sonke B. N° 481 H: 65877/HNC
Moringaceae	<i>Moringa oleifera</i> L.	1	(Moringa)	/	1 coffee spoon of leaves in 1 glass of water or 3 grains each morning and evening	1 glass in the morning before eating 1 glass in the evening after the meal	C: Leuwenberg A. J. M. N° 9497 H: 49178/HNC
Myrtaceae	<i>Eucalyptus globulus</i> Labill.	2	Chim booner (Eucalyptus)	/	Boil 1 handful in 1 liter of water	1 glass in the morning 1 glass in the evening.	C: Letouzey R. sn H: 477/SRFCM
	<i>Psidium guajava</i> L.	1	Chim kouopeu (Guava tree)	/	Boil 1 handful of leaves in water to obtain 1 liter of medicine	1 goblet in the morning 1 goblet at midday 1 goblet in the evening	C: Mbamba N° 1010 H: 65619/ HNC
Poaceae	<i>Zea mays</i> L.	1	Gbom (corn)	/	1 handful in 1 glass of water or 1 spoon of powder in 1 glass of water	3 to 4 teacups per day before eating	C: Dang D. N° 81 H: 18625/SRF-CAM
Rosaceae	<i>Rubus rigidus</i> Sm. Var. camerounensis R. Let.	1	(wild mulberry tree)	/	Boil 1 handful of leaves in water to obtain 1 liter of medicine	1 glass in the morning 1 glass at midday 1 glass in the evening.	C: Jacques Félix-H N° 8457 H: 25649/HNC
Rubiaceae	<i>Nauclea latifolia</i> Sm.	1	Nguèn	/	Boil 3 to 4 pieces in 2 liters of water. Add 3 to 4 pieces of corossolier	½ glass in the morning ½ glass at midday ½ glass in the evening for child. 1 glass in the morning	C: Biholong M. N° 438 H: 42156/HNC

Family of plant	Scientific name	n	Vernacular name (common name)	Associated plants	Preparation	Posology for an adult of 70 kg	Authentication
Rutaceae	<i>Citrus medica</i> L.	1	Le mou'n mecheuche (Lemon tree)	/	Boil 1 handful of leaves in 2 litres of water during 15 minutes	1 glass at midday 1 glass in the evening for adults. 1 teacup before eating	C: Gayum H. N° 1 H: 65106/HNC
Solanaceae	<i>Solanum aculeastrum</i> Dunal	2	Choupeka (ox aubergine)	<i>Erigeron floribundus</i>	3 to 4 barks in 1 liter of water	1 glass in the morning 1 glass in the evening.	C: Dang D. N° 345 H: 25878/HNC
	<i>Solanum melongena</i> L.	1	(Aubergine)	/	Consume like vegetable	At will	C: Swarbrik N° 2264 H: 34757/HNC
Taraxacum	<i>Taraxacum officinal</i> (L.) Weber ex F. H. Wigg.	1	Tooth of lion (dandelion)		Judge the quantity of leaves for 1.5 liter	1 teacup in the morning 1 teacup at midday 1 teacup in the evening within a period of 2 weeks	C: Dang D. N° 315 A H: 25627/HNC
Urticaceae	<i>Urtica dioica</i>	1	Mba-mba (orti)		25 g in ½ L of water for infusion (root)	1 teacup in the morning 1 teacup at midday 1 teacup in the evening within a period of 2 weeks	C: Dang D. N° 305 A H: 25617/HNC
Laurentiaceae	<i>Phragmanthera capitata</i> (Sprengel) S. Balle	3	(mistletoe)	/	Boil 1 handful of leaves in 1.5 L of water within a period of 15 min	1 glass in the morning 1 glass at midday 1 glass in the evening.	C: F. R. Thollon H: MNHN-P-P00560770
Zingiberaceae	<i>Aframomum angustifolium</i>	1	Longoza	<i>Costus lucanusianus</i>	Boil 1 handful of leaves and 2 barks in water to have 1.5 liter of medicine	1 glass in the morning 1 glass at midday 1 glass in the evening	C: Dang D. N° 219 A H: 25927/HNC
	<i>Zingiber officinale</i> Roscoe	1	Ginger (didja)		For 1.5 L, triturate 2 roots + ½ citron	1 glass in the morning 1 glass in the evening.	C: Westphal N° 10107 H: 43146/HNC

C: identification of collector; n: frequency of appearance of the plant for ethnopharmacological use, H: record number from the herbarium.

3.1.3. Frequency of the Parts Used

Figure 1 presents the frequencies of the various parts of the plants used for the various preparations in the study zone. This reveals that the leaves constitute the most used part with an approximate percentage of 45%; followed by the fruits (15%), the roots (11%) and the barks (9%). Seven percent (7%) of the healers use the whole plant instead of being interested only in one part.

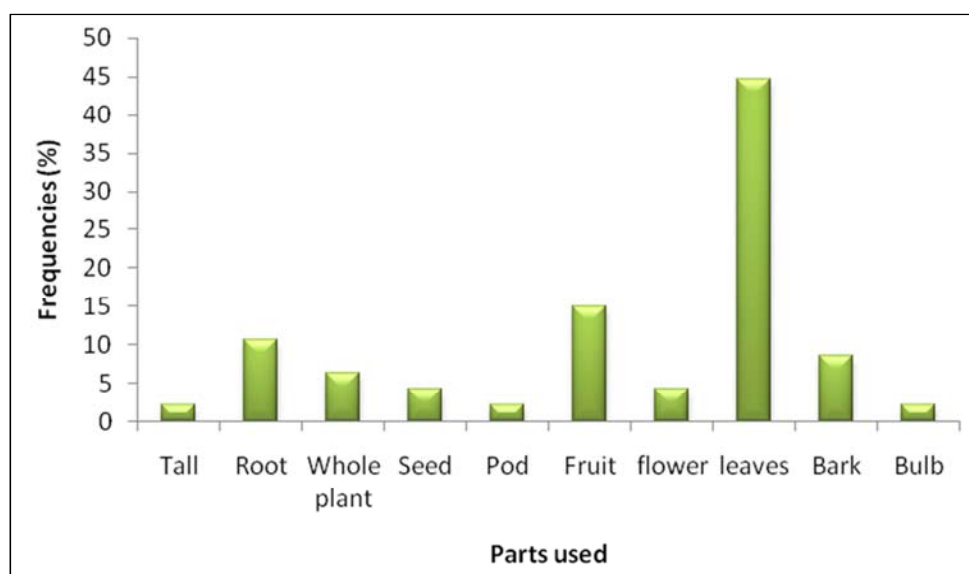


Figure 1. The frequencies of the different parts of the plants used by the traditional healers to prepare the antidiabetic drug.

3.1.4. Mode of Preparation of the Plants by the Healers

The frequencies of the various modes of preparation are presented by the diagram of Figure 2. It appears that the most used mode of preparation by the healers is decoction (51.28%) followed by maceration (28.21%).

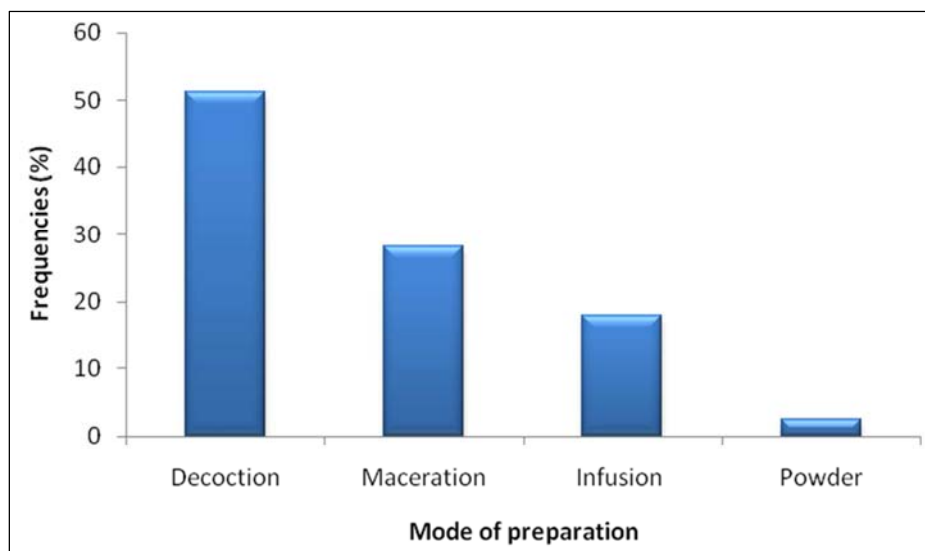


Figure 2. Frequency of the various modes of preparation of the antidiabetic drug.

3.2. Antidiabetic Properties of *Phragmanthera Capitata*

3.2.1. Effect of the Extract in the Normal Glycaemia Rats

Figure 3 below summarizes the effects of various doses of the extract of *Phragmanthera capitata* on the glycaemia of normal rats. This reveals that in treated animals in general and with the extract of *Phragmanthera capitata* particularly, the glycaemia of the rats drops gradually and significantly over time. The hypoglycaemiant effect of the glibenclamide is very significant between 1h00 and 2h00.

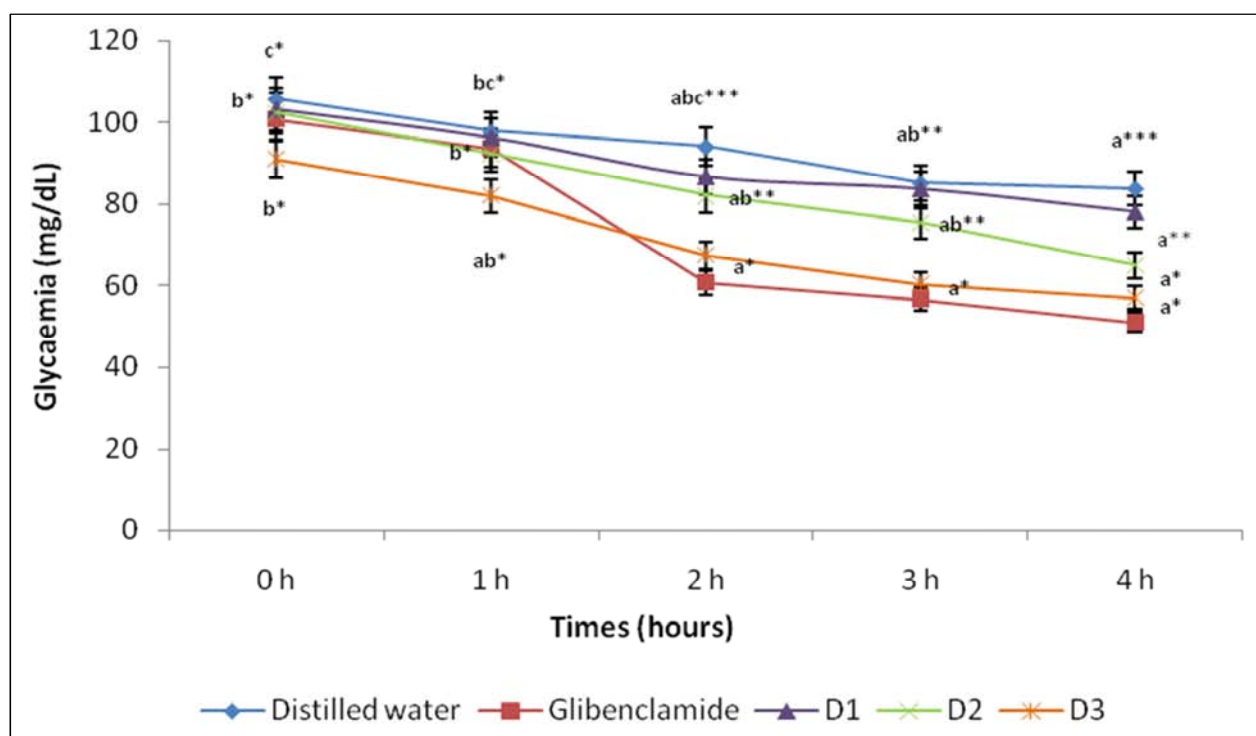


Figure 3. Evolution of glycaemia during 4 hours following the administration by oral way of three doses of *P. capitata* extract.

The values of the glycaemia related to the same curve and affected by same letter, just as those related to the same column and affected by same number of stars are not statistically significant with the threshold of 5%. N= 5 repetitions.

3.2.2. Effect of the Extract in Temporary Hyperglycaemic Rats

Figure 4 below presents the effects of various doses of the extract of *Phragmanthera capitata* on temporary hyperglycaemia caused by oral overload of glucose (4g/kg) in the pretreated rats. Thirty minutes after administration of glucose, hyperglycaemia whose level is variable according to the substances and doses with which animals are pretreated

was observed. This level is more important in the negative control rats (distilled water), thus showing an anti-hyperglycemic effect of the aqueous extract of *Phragmanthera capitata*. This hyperglycemia is stabilized between 30 and 60 min, then falls in the animals pretreated with the plant extract as a function of doses. The effect of D3 dose is almost similar to that of glibenclamide (reference drug).

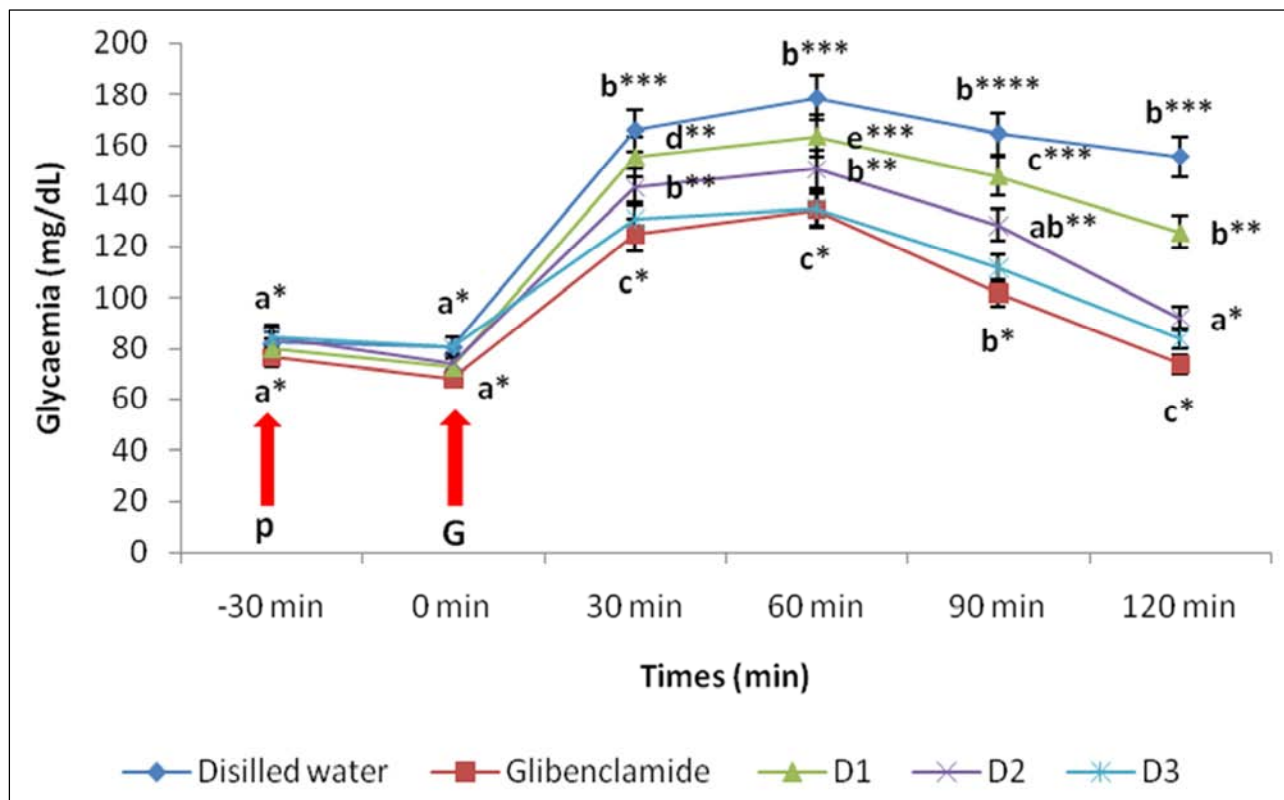


Figure 4. Variation of glycaemia in the temporary hyperglycaemia rats treated with three doses of *P. capitata* extract.

The values of glycaemia related to the same curve and affected by same letter, just as those related to the same column and affected by same number of stars are not statistically significant with the threshold of 5%. N= 5 repetitions. P: administration of Product, G: administration of glucose.

3.2.3. Effect of the Extract on the Hyperglycaemia of the Diabetic Rats

The effect of *Phragmanthera capitata* extract on glycaemia of diabetic rats is presented in Figure 5. Glycaemia of neutral and negative control rats remains constant in the course of time of experimentation. The effect of D1 dose is low since glycaemia of rats treated at this dose remained very high until the end of experiment. On the other hand, D2 and D3 doses caused a significant

and progressive fall of glycaemia of rats during the first week. The effect of D3 dose was comparable to that of glibenclamide at the tested dose. On the evening of the 7th day, animals were not treated and their glycaemia increased significantly in rats treated with glibenclamide and in a non significant way in rats treated with the D3 dose. Glycaemia then reduces with the resumption of treatment on the 8th day.

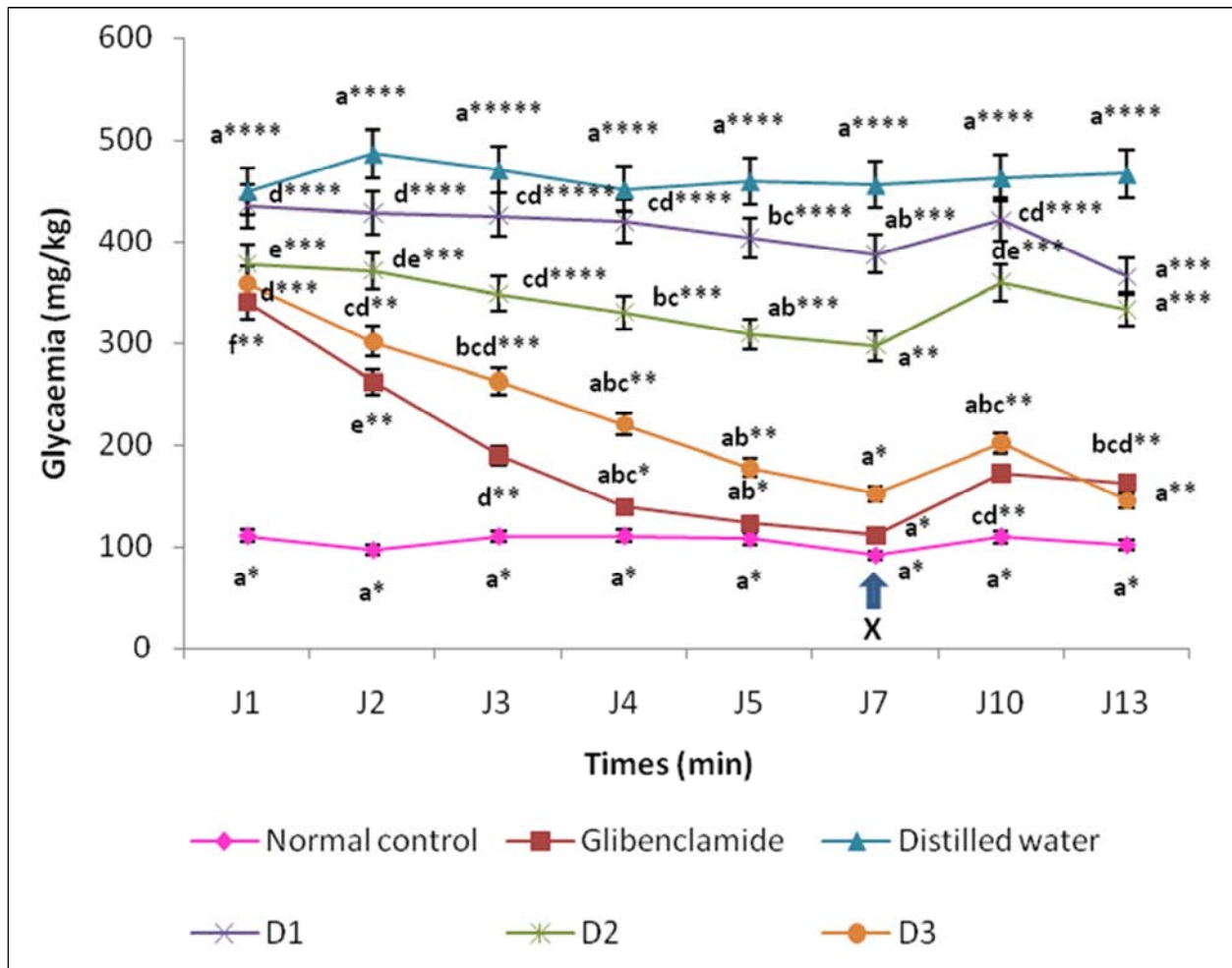
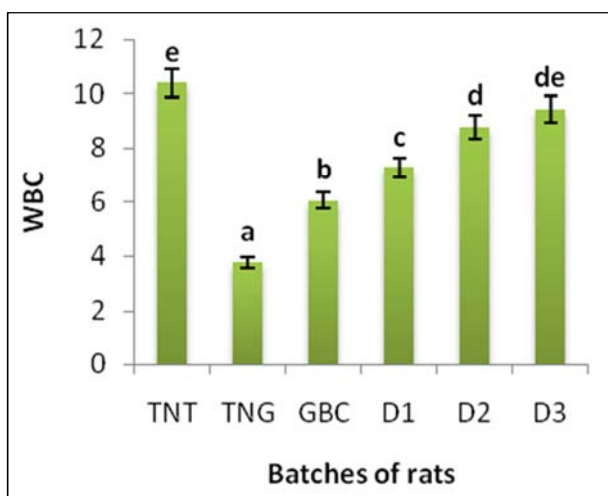


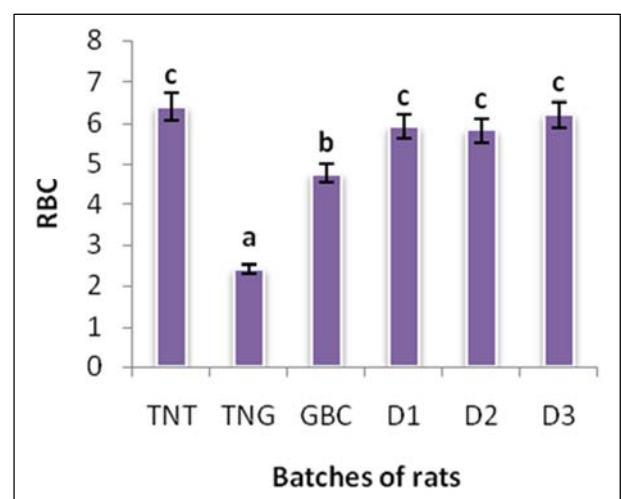
Figure 5. Evolution of glycaemia of diabetic rats during two weeks following daily administration of *P. capitata* extract.

The values of glycaemia related to the same curve and affected by same letter, just as those related to the same column and affected by same number of stars are not statistically significant with the threshold of 5%. N= 5 repetitions. J = days, X = stop of the treatment the evening and recovery the 8th day.

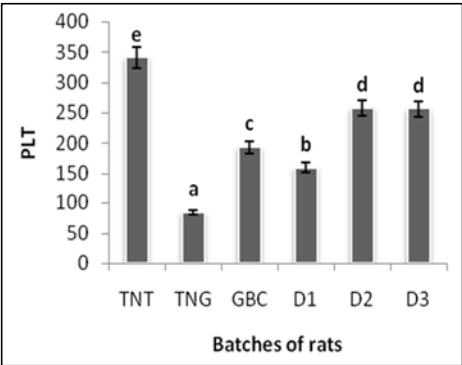
i. Effect of *Phragmanthera Capitata* Extract on the Hematological Parameters



(a)



(b)



(c)

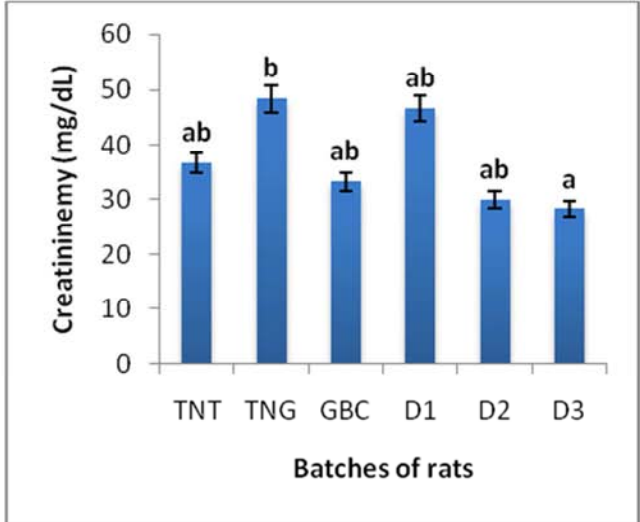
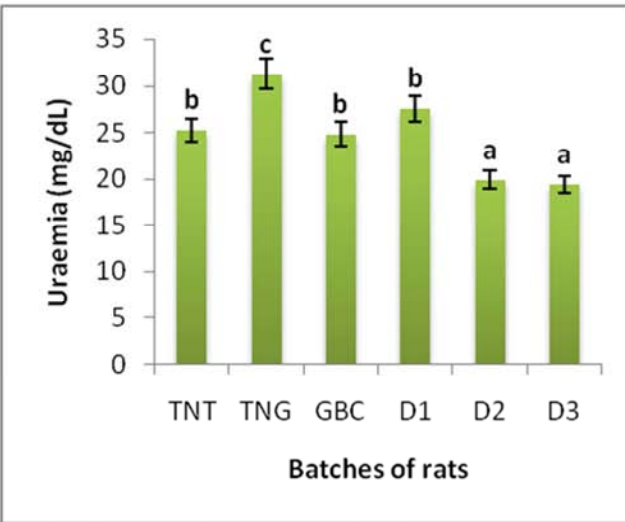
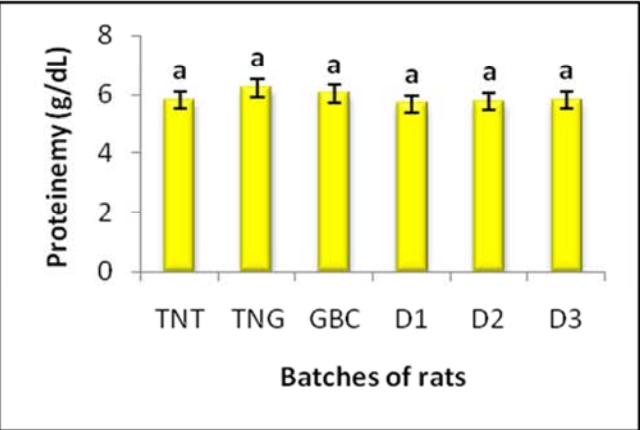
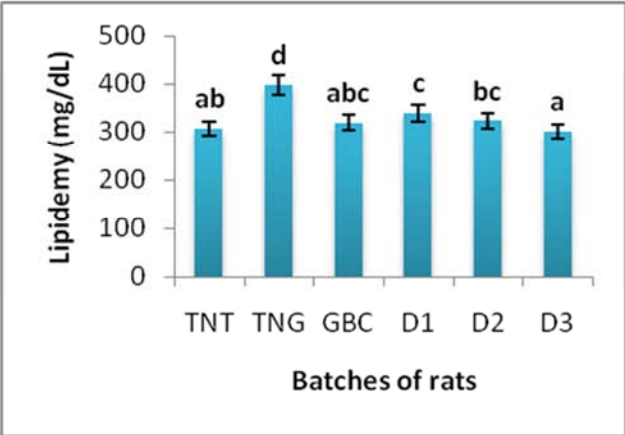
Figure 6. Hematologic parameters of the batches of studied rats.

(A): rate of white blood cell (WBC) ($10^3/\mu\text{L}$), (b): rate of red blood cell (RBC) ($10^6/\mu\text{L}$), (c): rate of platelets (PLT) ($10^3/\mu\text{L}$). TNT: normal control, TNG: negative control (distilled water), GBC: glibenclamide

The values of white blood cells, erythrocytes and platelets of various batches of diabetic rats are presented in Figure 6 below. This reveals that the diabetic state induced a leucopenia (significant fall of the rate of WBC), an anaemia (significant fall of the rate of RBC) and a thrombopenia (significant fall of the rate of PLT) of the negative control rats and would be the sign of hematopoiesis disturbance in these rats. The administration of the extract to the rats corrected these anomalies, depending on the doses.

ii. Effect of *Phragmanthera Capitata* Extract on the Serum Biochemical Parameters

For all the batches of the studied diabetic rats, the results of various types of serum biochemical parameters are presented in Figure 7.



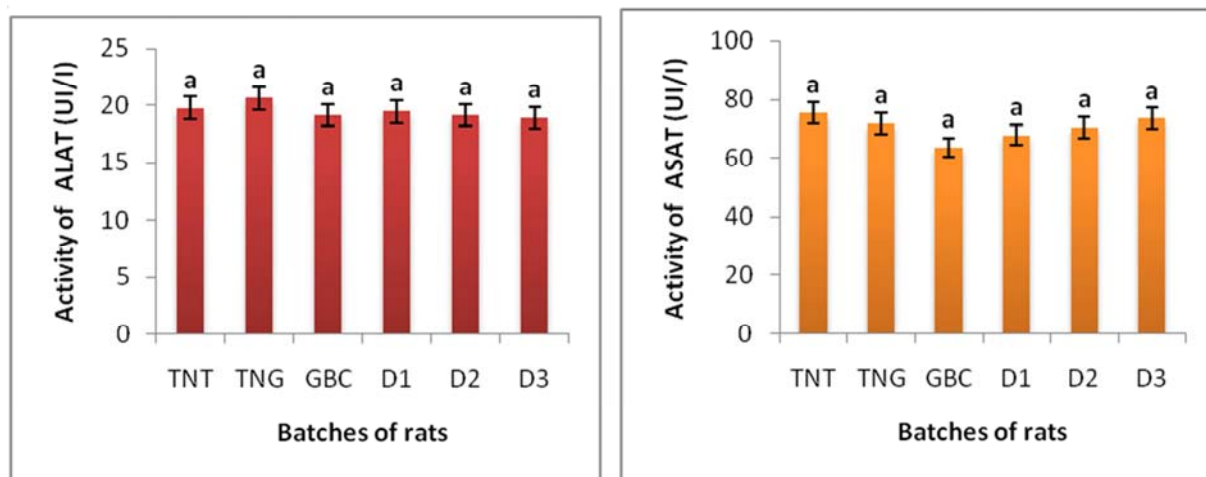


Figure 7. Biochemical parameters of the various batches of studied rats.

TNT: normal control, TNG: negative control (distilled water), GBC: glibenclamide, ASAT: Glutamate Oxalo-acetate transaminase and ALAT: Glutamate Pyruvate transaminase.

The results revealed a high rate of lipidemia and uraemia. The administration of *Phragmanthera capitata* extract caused a significant reduction of these parameters. Furthermore, proteinemia, creatininemia as well as the activity of transaminases ALAT and ASAT were comparable in all the batches of diabetic rats.

3.3. Phytochemical Composition of *Phragmanthera Capitata* Extract

Table 3 summarize the classes of compounds found in *Phragmanthera capitata* extract. The extract of the leaves of plant contains tannins, flavonoides, saponins, steroids and terpenes.

Table 3. Phytochemical composition of *P. capitata* extract.

Class of compounds	Results
Tanins	+
Flavonoides	+
Alkaloids	-
Saponines	+
Steroides	+
Terpenes	+

+: Presence; -: Absence

4. Discussion

On the whole, 35 species of plants were listed by 27 traditional healers, male being in majority. This male prevalence is explained by the fact that virtues of plants are ancestral knowledge which is generally transmitted from generation to generation mainly to the successor generally of the male gender [19]. Moreover, it is largely admitted that knowledge of medicinal herbs is a reserved domain of men in Africa [20]. Healers showed a good knowledge of the disease and have means to diagnose it. They admitted that the disease can be hereditary or result from a misuse of sugar by the body, the overweight, as well as the bad feeding habits being

risk factors.

The family of Asteraceae was strongly represented (14.81%) at the end of the investigation. This strong predominance of Asteraceae is explained by the fact that this family is one of the most represented plant families on earth [21, 22]. This representativeness was also observed following ethnopharmacological investigations on antidiabetic plants in Ivory Coast, Algeria, Benin and Morocco by [22-25] respectively.

Several species of plants are regularly used in preparation of phytomedicationss: *Allium sativum*, *Aloe barbadensis*, *Phragmanthera capitata*, *Allium cepa*, *Mangifera indica*, *Catharethus roseus*, *Vernonia amygdalina*, *Persea americana* et *Eucalyptus globulus*. A strong frequency of quotation of a plant during an ethnobotanical investigation is a good indicator of its effectiveness. One can thus think that, among these nine plants, the most represented could be effective in the treatment of diabetes mellitus [26].

The preparation of traditional drug is done primarily by decoction. The leaves are part mainly used in these preparations whose administration route is oral. The preponderance of decoction would be due to the fact that compared to infusion; it softens the hard parts of plants to allow a more complete extraction of active ingredients [27]. It is preserved longer than maceration which rather ferments quickly [27]. The prevalence of leaves would be explained by the fact that they are very abundant, generally available throughout the year, of easy accesses and collection as well as easy handling [22]. In addition, proper collection of leaves preserves plant from destruction [26, 28]. The oral administration of preparations would be explained by the fact that traditional healers know that the source of the disease is from the interior of the body [22, 25].

The administration of *Phragmanthera capitata* extract causes a significant fall of basic glycaemia of the normoglycemic, temporary hyperglycemic and diabetic rats. This extract contains saponins, flavonoides, tannins, steroids

and terpenes. It is clearly established that saponins [29] terpenes [30] and tannins [31] have antidiabetic properties. Some flavonoides like quercetins have the capacity of increasing secretion of insulin by pancreatic β cells [32]. Other flavonoides inhibit glucose carriers in the intestine or decrease expression of the genes which control neoglucogenesis and then reduce the degradation of glycogen [33-36]. Tannins would act at the cellular level by supporting the action of insulin [37]. One can thus think that compounds belonging to these various groups act individually or in synergy. The administration of *P. capitata* extract during 14 days in diabetic rats brought back glycaemia practically to the normal value on the 13th day of treatment. It is consequently possible that this extract inhibits the activity of digestive enzymes such as α -glucosidase or amylases. This inhibition thus reduces intestinal absorption of glucose coming from digestion of polysaccharides [38]. Lectines and viscotoxines present in *Viscum album*, the European mistletoe, are able to inhibit the activity of α -glucosidase [39]. *Phragmanthera capitata*, an African species of mistletoe, could contain the same type of substances. So, the extract could act by stimulating secretion of insulin by residual β cells or by potentiating the effects of insulin or by miming the effects of insulin. Indeed, the capacity of lectines to stimulate the release of insulin by isolated cells has been described previously [40]. The effect of the extract at D2 and D3 doses is comparable to that of glibenclamide (insulin-secreting hypoglycaemic sulphonamide). This latter binds to a specific receptor on the membrane of pancreatic β cells in the vicinity of potassium channel ATP depending and causes its closing. This involves a membrane depolarization of β cells with opening of the calcium channels voltage dependent and an impulse on Ca^{2+} thus starting by exocytosis of the granules of insulin secretion [41]. It would be difficult to specify the mode of action of *P. capitata* because the extract is made up of a mixture of compounds which can interfere or have synergistic action.

Leucopenia, anaemia and thrombopenia were observed in the untreated diabetic rats and would be the sign of a disturbance of hematopoiesis in these rats. The administration of the extract to rats corrected these abnormalities according to the administered doses better than the reference drug (glibenclamide). The previously studies showed that spathulenol, a sesquiterpene has an immunomodulator effects [42]. Terpenes, flavonoides and tannins contained in the extract would have reduced the hyperglycaemia of diabetic rats by activating the hematopoiesis. In addition, the administration of *P. capitata* extract produced a significant reduction in serum lipidemia, an important risk factor of diabetes. This hypolipidemic effect can also be the consequence of hypoglycaemic effect of the extract. Besides, a study showed the hypolipidemic effect of flavonoides, terpenes and phenolic compounds [36]. One can thus think that, by reducing glycaemia, flavonoides and terpenes contained in the extract reduced at the same time serum rate of lipids, by stimulating lipogenesis from glucose transported in the cells and by inhibiting lipolysis

[43]. A significant increase of urea concentration was noted in untreated diabetic rats sign of a renal dysfunction in these rats, resulting from toxicity of alloxan [44, 45]. Administration of the extract caused a significant reduction in uraemia thus showing a protective effect on kidney. The determination of the activity of transaminases ALAT and ASAT revealed a comparable activity in all the batches of studied rats showing that there was no hepatic toxicity due to alloxan or plant extract administration. The beneficial effect of the extract on kidneys and liver would be ascribed to its capacity to reduce glycaemia and oxidative stress induced by alloxan and/or hyperglycaemia. Indeed, the studies of [46] show that polyphenols and triterpenes found in the extract have the ability to neutralize free radicals resulting from oxidation of alloxan or formed during diabetes state.

5. Conclusion

The administration of *Phragmanthera capitata* extract to diabetic rats significantly decreased hyperglycaemia. The dose and the double dose of the extract used by traditional healers were effective against diabetes. These results thus justify the traditional use of this plant in the treatment of diabetes mellitus. But at this stage, the exact mechanism of action of the extract is not known. This will constitute one of our objectives in the next study.

Ethics Approval and Consent to Participate

All studies involving animals were conducted according to the ethical guidelines of the Committee for Control and Supervision of Experiments on Animals (Registration no. 173/CPCSEA, dated 28 January, 2000), Government of India, on the use of animals for scientific research.

Consent to Publish

Not applicable

Availability of Data and Materials

Not applicable

Competing Interests

The authors have no conflict of interest to declare.

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Authors' Contributions

CF is the field investigator and has drafted the manuscript.

J-RK designed the study and GSSN supervised field work. All authors read and approved the final manuscript.

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