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ANTIDIABETIC ACTIVITY AND SAFETY EVALUATION OF TWO CAMEROONIAN MEDICINAL PLANTS IN STREPTOZOTOCIN-RAT MODEL: *TETRAPLEURA TETRAPTERA* (FABACEAE) AND *IRVINGIA GABONENSIS*(IRVINGIACEAE)

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Abstract

Background: *Irvingia gabonensis* and *Tetrapleura tetraptera* are widely employed by traditional healersof the southern regions of Cameroon to treat diabetes. However. there is only limited or no scientific evidence on the real potential of these two medicinal plants to serve as alternative therapies. The present works thus aimed to evaluate the antidiabetic activity of the barks of *I. gabonensis* and fruits of *T. tetraptera.*

Methods: The antidiabetic potential of the four extracts was assessed by evaluating the hypoglycaemic activity through the oral glucose tolerance test (OGTT) in male Wistar albino rats and the subacute antidiabetic assay in streptozotocin-induced Wistar rat model. For the later. clinical markers (water and food intake, urine excretion), Fasting Blood Sugar FBS), the effects on some vital organs (liver and kidney), and lipid profile were considered. Safety was evaluated using the in vivo acute toxicity test in mouse model.

Results: Out of the four extracts prepared from*T. tetraptera*and *I. gabonensis*, and tested for their antidiabetic potential, only the hydroethanol extract of the stem bark of *I. gabonensis* showed promising hypoglycemic and anti-hyperglycemic activity from preliminary screening. Of the two doses of this extract considered for the subacute antidiabetic assessment, the dose of 500mg/Kg demonstrated the highest activity reflected in significant improvement in both clinical and biochemical markers with a 100% recovery rate after 21 days treatment of the STZ-induced diabetic Wistar rats. At this dose, the *I. gabonensis* extract was shown to also improve liver and kidney functions as well as mitigating dyslipidemia in diabetic rats. Overall, its pharmacological properties were above those of the reference drug metformin at 10mg/Kg dose. More interestingly, the acute toxicity study revealed a relative safety of this extract in mice.

Conclusion: The present work confirms the potential of the hydroethanolic extract of *I. gabonensis*to serve a source of new antidiabetic drugs. Further investigations are therefore envisaged.

Keywords: Type 2 diabetes, Streptozotocin, acute toxicity, *Irvingia gabonensis*,*Tetrapleura tetraptera*.

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Introduction

Diabetes mellitus is a group of metabolic diseases characterized by chronic hyperglycemia resulting from a defect in insulin secretion or insulin action or both associated abnormalities. It is manifested by symptoms such as polyuria. polyphagia. polydipsia. weight loss and blurred vision. It is diagnosed by a fasting blood glucose

level above 126 mg/dL and postprandial blood glucose above 200 mg/dL. There are four types of diabetes namely: type I diabetes. type II diabetes. gestational diabetes and other types of diabetes. Type II diabetes represents 90 to 95% of diabetic cases. but it is also the first cause of amputations in the world and the first cause of blindness [1]. In Cameroon. it is a public health problem and thus appeals to national and international actors to find concrete solutions to this disease [2]. Type II diabetes results from high calorie diet. poor lifestyle. overweight. obesity. advanced age. ethnicity and family ties which all lead to insulin resistance which in the long term will be added to an insulin-secretory deficit [3]. Type II diabetes has long been considered a disease of rich countries. But over time we see that it is becoming a global scourge.In 2021, there were 537 million diabetics in the world and it was expected to rise to 783 million in 2045 if nothing was done. It should also be noted that approximately 6.7 million adults (20-79) worldwide lost their lives in 2021 due to either diabetes or its complications. In Africa. there were 24 million diabetics during the same year and it was expected that it would rise to 55 million in 2045 if no predisposition was put in place. In addition. 306.000 African adults (20-79) lost their lives due to diabetes or its complications. Concerning Cameroon. which is our center of interest. in 2021 there were 620.800 diabetics with a prevalence of 4.8% (IDF. 2021). The management of type II diabetes requires regular exercise. quitting smoking. having a healthy diet and a normal weight. If the lifestyle change is not enough. drugs such as Metformin. insulin. DDP-4 inhibitors. SGLT2 inhibitors. GLP-1 agonists. Acarbose and many more are used. others that act not only as pancreatic replacements. insulin sensitizers but also digestive enzyme inhibitors (IDF. 2019). However the use of these drugs is accompanied by side effects such as severe hypoglycaemia and exaggerated weight gain. In addition to these side effects. the price of these drugs on the market is expensive especially for poor and indebted countries like Cameroon where the SMIG is 36.250 FCFA. knowing that a diabetic in Cameroon spends nearly 164.940.15 FCFA per year [1]. It is with this in mind that several studies have been carried out to be able to remedy this situation based on the practices initially carried out by the populations. which is that of the use of plants for treatment. which will allow the manufacture of drugs. traditional improved and also to identify new molecules in these plants that have antidiabetic activity that will be less expensive and have no side effects [4]. This is how we went to two parts of plants generally used by Cameroonian populations to treat diabetes. Namely the bark of *Irvingia gabonensis* and the fruit of *Tetrapleura tetraptera*. from which the patient drinks a glass of the decoction morning noon and evening until recovery [5, 6]. Omonkhua et al. (2014) showed the hypoglycemic activity of the fruits of *Tetrapleura tetraptera* induced by injection of streptozotocin. From all the above, it emerges that no study has yet been carried out to demonstrate the antidiabetic activity of these plant parts on a type II diabetes profile.

Material and Methods

Plant sample collection and processing

The fruits of *Tetrapleura tetraptera* commonly called 4-sided spices were harvested in the Nyabibete village of Zoetele, DJA & LOBO Division, South Region, Cameroon. They were then identified at the National Herbarium of Cameroon described as follows: From the family of legumes-Mimosoïdae. in comparison with the material of Betti Jean de Lagarde N ° 304 of the specimen of the collection of Herbarium N ° 66344 /HNC. The barks of *Irvingia gabonensis* were also harvested in the Nyabibete village of Zoetele and were then identified at the National Herbarium of Cameroon described as follows: From the Irvingiaceae family. In comparison with the material of D.W. Thomas Martin No. 7163 from Herbarium collection specimen No. 55926/HNC.

The harvested plant parts were freed of impurities. Dried in the shade and then ground using a mill until fine powders were obtained. These powders were subjected to sequential extractions in distilled water and in a hydro-ethanolic solvent according to the procedures below:

Preparation of aqueous extracts

Two hundred and fifty grams (250 g) of dried fruit powder of *Tetrapleura tetraptera* and 250 g of *Irvingia gabonensis* bark separately were boiled for 10 min in 2 L of distilled water then left to macerate for 24 hours and then filtered using Wattman paper. The filtrates were collected. 1 L of water was added to the residues and the macerations continued every 24 hours until the residues were completely exhausted. Then proceeded to a mixture of all the respective filtrates which were finally freeze-dried to obtain extracts which were used for subsequent analyzes and tests.

Preparation of hydro-ethanolic extract

Two hundred and fifty grams (250 g) of dried powder of *Tetrapleura tetraptera* fruits and 250 g of *Irvingia gabonensis* bark separately were macerated in 2 L of a 70/30 (v/v) hydro-ethanolic solvent for 72 hours. The residues obtained were subjected to continuous macerations until obtaining residues with little color. The filtrates obtained were freeze-dried.

Phytochemical analyzes of freeze-dried extracts

Phytochemical screening of extracts

The extracts were screened for detection of different chemical families according to the methods previous described by Odebiyiand Safowara(1978). In brief. phenolic compounds were detected using the ferrocyanide reaction; triterpenes and sterols were revealed by their reactivity with anhydrous acetate and sulphuric acid. Alkaloids were detected using Mayer reagent, whereas the presence of saponins revealed based on their foaming property. Tanins and flavonoids were revealed using ferric chloride and hydrochloric acid. Respectively. Anthraquinones were detected in extracts by the

chloroform/petroleum system. while the presence of lipids was assessed on filter paper.
Quantitative analyses of tannin, saponin, alkaloid and phenolic compounds were carried out as previously described by Teugwa et al. (2013).

Experimental animals
The animals (Wistar albino rats used for antidiabetic activity testing and the Balb/c mice used in toxicity studies were both provided by the animal facility of the Medical Research and Applied Biochemistry Laboratory, Faculty of Health Sciences, University of Buea.

Evaluation of the antidiabetic potential of plant extracts
Hypoglycemic and anti-hyperglycemic activity of extracts
This test was done using the Oral Glucose Tolerance Test. as previously described by Zofou et al. (2017) with slight modifications. In brief, a total of 30 rats were divided into 6 groups having a mass between 150-200 g. The different treatments were administered orally as shown in Table 1. During the treatment period, water and feed were available to the mice ad libitum.

Table 1: Treatment in the different experimental groups for the oral glucose tolerance test (OGTT)

Group 1:	2g/kg glucose + Distilled water (5ml/kg)
Group 2:	2g/kg glucose + 10 mg/kg Metformin (positive control)
Group 3:	2g/kg glucose + 1000 mg/kg Aqueous extract of the fruits of *Tetrapleuratretraptera*
Group 4:	2g/kg glucose + 1000 mg/kg Hydroethanolic extract of *Tetrapleura tetraptera* fruits
Group 5:	2g/kg glucose + 1000 mg/kg Aqueous extract of *Irvingia gabonensis* bark
Group 6:	2g/kg glucose + 1000 mg/kg Hydroethanolic extract of *Irvingia gabonensis* bark

The tail of each animal was gently massaged to increase blood flow and pricked using a lancet at the tip of the tail to collect a drop of blood. The blood sample collected migrated by capillary action when applied on a glucose strip already inserted in a glucometer (CodeFree®. SD Biosensor. South Korea). The fasting blood sugar (FBS) level was measured and the time recorded as $t_{-30mins}$. Immediately after FBS measurement. animals were orally administered corresponding products (tea extract for the test groups. glibenclamide for the positive control and distilled water for the negative control group). After 30 mins, fasting blood sugar levels of the animals were again measured (time recorded as t_{0mins}) and all the animals were then given an oral dose of glucose (2 g/kg body weight). The fasting blood sugar levels of the animals were then further determined after 30 mins, 60 mins, 90 mins, 120 mins and 240 mins (referred to as $t_{+30\ min}$. $t_{+60\ min}$. $t_{+90\ min}$. $t_{+120\ min}$ and $t_{+240\ min}$).

Percentage of suppression of the postprandial glycemic peak: This parameter allows the evaluation of the percentage with which the different treatments canceled the glycemic spike observed after the administration of 2 g/kg of glucose. It is materialized by the formula:
%PS: percent glycemic peak suppression = $[Glu+30_{control} - Glu+30_{test}*100]/Glu+30_{control}$.
$Glu+30_{control}$: Blood glucose at $t_{30}$of the negative control (distilled water); $Glu+30_{test}$: Blood glucose at t_{30} of the test group being considered (extract or reference drug).
Blood glucose restoration time (t_R)
This parameter corresponds to the time following glucose administration. Where the FBS recorded were for the first time similar to the initial value.
Subacute antidiabetic activity of extracts
Only extract(s) with the best results in the Acute hypoglycemic/anti-hyperglycemic activity were considered for subsequent antidiabetic activity testing (subacute antidiabetic activity assessment).
Induction of type 2 diabetes
The Streptozotocin-Wistar rat model was used starting with a total of 25 animals, weighing 150 g and above. The selected rats were fed for 21 days on a High-fat diet to render them obese. The animals were then weighed and their FBS taken to confirm their "pre-diabetic" status (Body weight of 200g and above, FBS >120). Those fulfilling these criteria were submitted to streptozotocin type 2 diabetes induction at a dose of 40mg/Kg. The animals were observed for 2 days to appreciate water and food consumption, and volume of urinary excretion. Once substantial changes were observed. the fasting blood sugar measurement was measured to confirm the diabetic status (FBS of 200mg/dL and above) and considered for the test.

Treatment of rats during subacute antidiabetic activity
A total of 20 diabetic rats were divided into 4 groups of 5 rats each and treated with 5mL/Kg distilled water (Negative control). 10mg/Kg Metformin (Positive control), 250mg/Kg and 500mg/Kg extract of *I. gabonensis* stem bark [IGHE (250) and IGHE (500), respectively]. The different treatments were administered for 21 consecutive days by oral administration using an esophageal probe.
The antidiabetic activity was determined based on both clinical markers (Change in food and water intake. urine elimination and body weight) and the main biochemical indicator (fasting blood sugar levels). Volume of urine eliminated, food and water consumed were measured daily, except for Days 5, 10, 15 and 21 where animals were put to fasting.The animals were also monitored for any behavioural changes, and their body weight and FBS

measured on day 0 (before drug administration), day 5, day 10, day 15 and day 21 (end of the treatment period).
Further, the effect of the different treatments was assessed on some key functions which are known to be affected in diabetes. These include the liver (ALT, AST) and the kidney (creatinine and urea levels), and lipid metabolism (total cholesterol. triglycerides. HDL, LDL and VLDL). At the end of the treatment period. the rats were sacrificed and blood samples were collected through cardiac puncture into tubes.

Evaluation of the acute toxicity of the extracts

The acute toxicity test was conducted at a dose of 2000 mg/kg. In brief, animals were divided into five groups consisting of five male mice each having a weight between 15-30g: Control group which received 10 ml/kg of distilled water and the 4 other groups which received respectively 2000 mg/kg of the various extracts using a gastro-esophageal probe. The animals were observed individually for the next four hours post administration, and thereafter, monitored daily for a 14-day period. Signs of toxicity including hair/skin change, motility, tremors, weight, grooming, respiratory stress, sensitivity to noise after metal shock, stool appearance and death were considered. The acute toxicity test was repeated female animals. for the exacts that showed the best antidiabetic activity to ascertain their safety.

Ethical considerations.

The proposal including the different procedures deployed in the present work were reviewed and approved by the Joint Institutional Review Board for Animal & Human Bioethics (JIRB) of the University of Yaounde 1, and ethical clearance was obtained from with reference number BTC-JIRB2022-028.

Statistical analysis

The Statistical Package for Social Science (SPSS) software version 20.0 for Windows was used for the statistical analysis of the results. The one-factor ANOVA (Analysis of Variance) test was employed to compare the means of the different groups. The results were expressed as the mean ± standard deviation at the 5% significance level. At the end, the graphical representations of the different results were built in Microsoft Excel 2016.

Results

Extraction yields and phytochemical characteristics of the extracts: The yields in hydro-ethanolic extraction were higher **(**22.9% and 6.8% for *Tetrapleura tetraptera* and *Irvingia gabonensis*, respectively), as compared to water-extraction. Table 2 presents the main secondary metabolites of interest revealed in the different extracts of *I. gabonensis*stem bark and *T. tetraptera* fruits, using semi-quantitative methods.

Table 2: Phytochemical constitution of extracts (semi-quantitative analysis)

	TTAq	TTHE	IGAq	IGHE
Alkaloids	+++	+++	-	++
Flavonoïdes	++	++	++	+
Tannins	+	+	+++	+++
Saponines	+++	+++	+	++
Anthocyanines	-	-	+	+
Phénols	+	-	+	+
Triterpènes	+	-	-	++

+++: strongly positive; ++: moderately positive; +: weakly positive; -: negative. TTAq: aqueous extract of *T. tetraptera*; TTHE: hydroethanolic extract of *T. tetraptera;*IGAq: aqueous extract of *I. gabonensis* and IGHE: hydroethanolic extract of *I. gabonensis.*

The results show that TTAq and TTHE were highly concentrated in alkaloids and saponins, unlike IGAq and IGHE which are highly concentrated in tannins. All extractions showed low amounts of anthocyanins, flavonoids, phenols and triterpenes.Table 3further presents the amount of selected secondary metabolites in different extracts.TTAq had the greatest quantity of phenolic compounds,saponins and alkaloids as compared to other extracts. However,the concentration of tannins in IGHE(2446.84 mg/100g) was the highest.

Table 3: Quantification of metabolites of interest

	TTAq	**TTHE**	**IGAq**	**IGHE**
Polyphenols (mgEAG/g)	414.28 ±025	398.97± 0.00^{b}	331.15± 0.43^{d}	389.38± 0.25^{c}
Alkaloids (µgEQui/mg)	6379.58±7.21^{a}	3354.58 ±7.21^{b}	2917.08 ±7.21^{c}	4679.58 ±7.21^{d}
Tannins (mg/100g)	1886.07±1.69^{a}	2246.84 ±2.35^{b}	103.30± 1.88^{d}	2446.84 ±2.60^{c}
Saponins (mg/100g)	613.76 ±9.98^{a}	320±9.98^{b}	95.36±9.98^{d}	256.64± 9.98^{c}

TTAq: aqueous extract of *T. tetraptera*; TTHE: hydroethanolic extract of *T. tetraptera;*IGAq: aqueous extract of *I. gabonensis* and IGHE: hydroethanolic extract of *I. gabonensis.*

One same row, the values with similar letters are statistically comparable with each other, unlike those carrying different letters

Hypoglycemic and anti-hyperglycemic activities of the different extracts

The effects of the different extracts on the rat's tolerance to glucose (Oral Glucose Tolerance Test) is summarized in Figure 1, which shows the variations in glycemia before and after administration of the treatments and oral glucose.

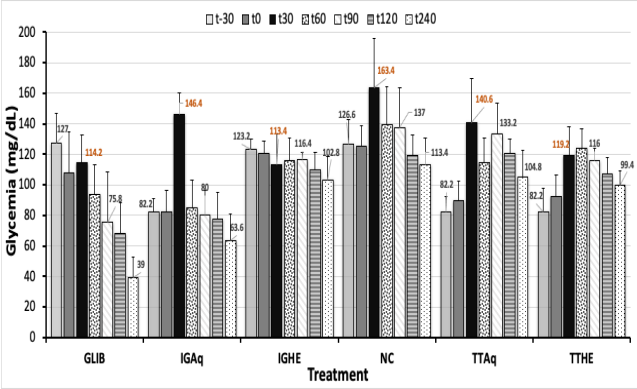

Figure 1: Evolution of Fasting Blood Sugar in the different experimental groups.

TTAq

aqueous extract of *T. tetraptera*; TTHE: hydroethanolic extract of *T. tetraptera;*IGAq: aqueous extract of *I. gabonensis* and IGHE: hydroethanolic extract of *I. gabonensis.* GLIB: Glibenclamide (5mg/Kg); NC: Negative Control (Distilled water, 5mL/Kg).

NB: The effect of the different treatments was assessed by comparing the glycemia values at t-30 against t_0, while Postprandial peak was measured at t_{30}. To this end, it is found that all both extracts of *T.teraptera*caused an increase in glycemia(with delayed postprandial peak in hydroethanol extract). However, the groups treated with the extracts of *I. gabonensis*witnessed adecline in their fasting blood sugar, and their glycemia restored completely after postprandial peak similarly to the positive control group (Glibenclamide). Table 4 below present more information on the postprandial peak suppression rates and glycemia restoration time of the different treatments.

Table 4: Postprandial Peak Suppression Rates and Glycemia Restoration Time of the different treatments

Treatment	Postprandial glycemic peak (%)	Percent post prandial peak suppression (%)	Glycemia restoration Time (min)
TTAq	75.65±56.78 ^{a}	-157.61±193.38 ^{a}	>240
THE	51.05±45.80 ^{ab}	-73.84±155.99 ^{ab}	>240
IGAq	79.16±19.73 ^{a}	-169.58±67.20 ^{a}	60
IGHE	-7.87±16.30^{bc}	126.83±55.52 ^{c}	*
GLIB	-9.57±12.87^{bc}	132.60±43.84 ^{c}	*
Negative control	29.37±22.27 ^{b}	0.00±75.82^{bc}	120

TTAq: aqueous extract of *T. tetraptera*; TTHE: hydroethanolic extract of *T. tetraptera;*IGAq: aqueous extract of *I. gabonensis* and IGHE: hydroethanolic extract of *I. gabonensis.* GLIB: Glibenclamide (5mg/Kg); NC: Negative Control (Distilled water, 5mL/Kg).

From the above. only EHIG and glibenclamide were able to prevent the development of the glycemic peak after ingestion of 2g/kg of glucose, reflected in a decrease of glycemia (-7.87% and -9.57% for IGHE and GLIB, respectively).Glycemia returned to the initial in animals receiving IGHE, IGAq, GLIB and Negative Control groupafter 30, 60, 90 and 120 minutes, respectively. However, the groups receiving the fruit extracts of *T. tetraptera* saw their blood sugar levels significantly above the normal (initial) values throughout the assay duration.

In view of the findings from the acute hypoglycemic and anti-hyperglycemic activity, only the hydro-ethanolic extract of *I. gabonensis* (IGHE) was retained for further investigation (subacute antidiabetic and safety evaluation).

Subacute antidiabetic activity of hydro-ethanolic extract of *I. gabonensis* stem bark (IGHE)Effect of the different doses of IGHE on clinical markers of DM in Wistar rats: Figure 2 summarizes the effect of the different treatments on the clinical marker of DM in STZ-induced rats.

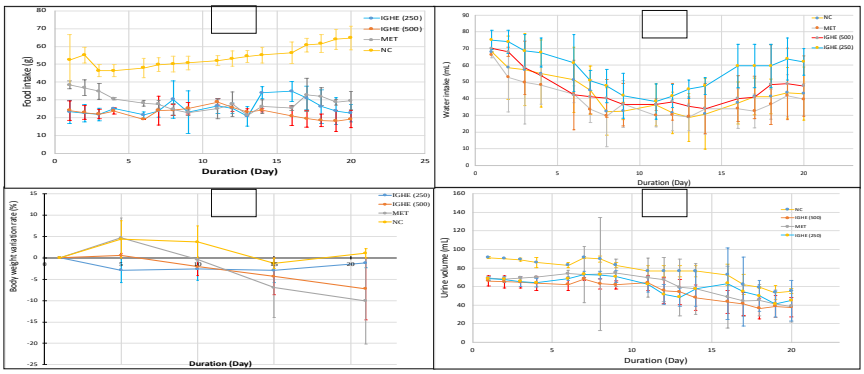

Figure 2: Effect of the different treatments on clinical markers of DM in Wistar STZ-induced rats.

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A: Effect on food intake; **B:** Effect on body weight; **C:** Effect on Water intake; **D:** Effect on Urine excretion volume. IGHE (500): hydroethanolic extract of *I. gabonensis at 500mg/Kg;* IGHE (250): hydroethanolic extract of *I. gabonensis at 250mg/Kg; MET: Metformin (10mg/Kg); NC: Negative Control (Distilled water, 5mL/Kg).*

The groups treated with the hydroethanolic extracts of *I. gabonensis*and those with Metformin at witnessed agradual reduction in the amount of food consumed from Day 1 through Day20 of the treatment, which was more pronounced in IGHE (500mg/Kg) at the end of the assay. The control group receiving distilled water instead showed a consistently high and increasing food intake. Regarding body weight, there was not statistically significant difference among experimental groups, despite a slight increase noticed in the negative control. Likewise, water intake was statistically similar across experimental groups. Concerning urine excretion, urine volume remained higher in the negative control as compared to the groups receiving either the extracts or the reference drug metformin, with the IGHE (500mg/Kg) animals showing the lowest urine volumes towards Day 20 of the assay.

Effect on Glycemic control (Variation in Fasting Blood Sugar in the different doses of IGHE) The evolution of Fasting Blood Sugar in the different experimental groups is presented in Figure 3.

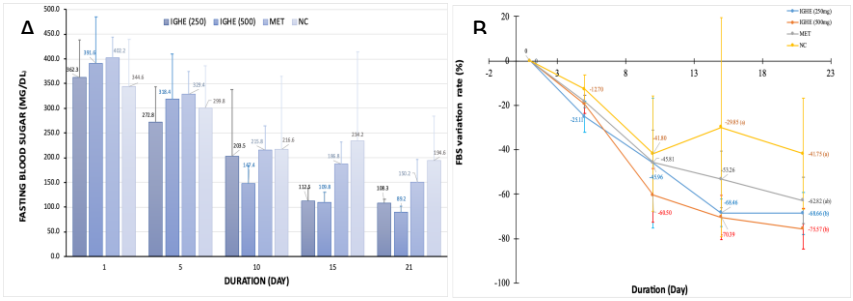

Figure 3: Effect of the different treatments on Fasting Blood Sugar (FBS) of diabetic rats

A: Effect on FBS; **B:** FBS Variation rate in the different experimental groups

IGHE (500): hydroethanolic extract of *I. gabonensis at 500mg/Kg;* IGHE (250): hydroethanolic extract of *I. gabonensis at 250mg/Kg; MET: Metformin (10mg/Kg); NC: Negative Control (Distilled water, 5mL/Kg).*

The group treated with Metformin showed a reduction in FBS from Day 1 to Day 21, (62.82%)compared to the 41.74% reduction recorded in the negative control group, though the FBS value was not statistically different from that of the negative control group on Day 21 (P=0.092).Administration of the hydroethanolic extracts of *I. gabonensis* at 250mg/Kg and 500mg/Kg led to more pronounced drops in FBS in diabetic rats (68.65% and 75.57%, respectively) with the FBS on Day 21 significantly lower than that of the negative control group (P<0.05).

Recovery rates from STZ-induced diabetes

The recovery rate in the different experimental groups is summarised in Figure 4. From these results, only one death was recorded in the group which received 250mg/Kg of *I. gabonensis* extract on Day 3 and this animal was identified with high glycemia (565mg/dL). IGHE (500) scored the highest recovery rate (100%), followed by the reference drug metformin (80%), and IGHE (250) while 3 out of the 5 animals of the negative control group remained diabetic after the 21-day period of the study.

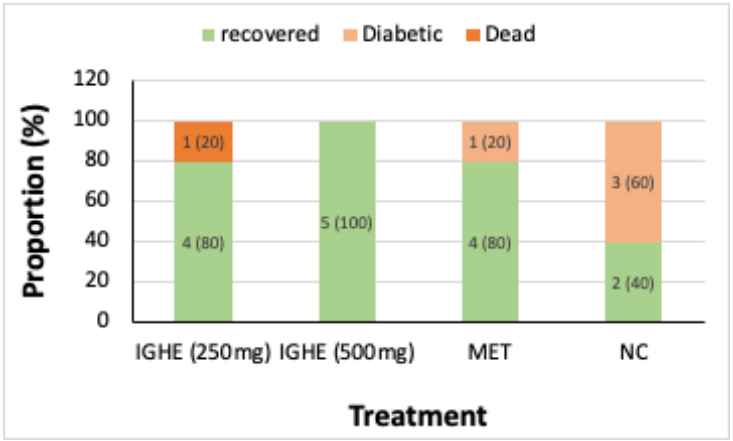

Figure 4: Recovery rate of diabetic rats based on survival and glycaemic control

IGHE (500): hydroethanolic extract of *I. gabonensis at 500mg/Kg;* IGHE (250): hydroethanolic extract of *I. gabonensis at 250mg/Kg; MET: Metformin (10mg/Kg); NC: Negative Control (Distilled water, 5mL/Kg).*

Effect of different treatments on selected key vital functions

Effects of EHIG on lipid metabolism: The lipid profile of the different experimental groups are presented in Table 5.

Table 5: Effect of the different treatment of markers of dyslipidemia

Treatment	Total Cholesterol (mg/dl)	Triglycerides (mg/dl)	HDL (mg/dl)	LDL (mg/dl)	VLDL (mg/dl)
IGHE (250m)	81.50±23.75^{a}	97.39±36.77^{ab}	19.89±31.82^{a}	42.13±21.74^{ab}	19.4776±7.35^{ab}
IGHE (500)	64.85±22.82^{a}	42.09±41.77^{a}	39.46±37.12^{a}	30.99±25.75^{a}	8.4179 ±8.35^{a}
MET	70.54±19.85^{a}	67.5373±48.48^{a}	35.31±27.75^{a}	51.54±2.11^{ab}	13.5075±9.70^{a}
NC	108.12±39.43^{a}	147.4627±48.62^{b}	49.55±19.05^{a}	74.69±33.55^{b}	29.4925±9.72^{b}

IGHE (500): hydroethanolic extract of *I. gabonensis at 500mg/Kg;* IGHE (250): hydroethanolic extract of *I. gabonensis at 250mg/Kg; MET: Metformin (10mg/Kg); NC: Negative Control (Distilled water, 5mL/Kg).*

The concentration of triglycerides, LDL cholesterol and VLDL cholesterol were the lowest in animals received the *I. gabonensis* at 500mg/Kg, and values for these markers were significantly lower than those obtained in the negative control group. In general, IGHE (500) displayed greater effects on lipids profile compared to IGHE (250) and MET. However, there was no statistically difference among the different treatment for HDL and total cholesterol.

Effects of EHIG on liver and kidney functions

The effect of the different treatments on selected markers of liver (ALT and AST levels)and kidney (Creatinine, Urea) functions are presented in Table 6 below.

Table 6: Effects of the different treatments on selected markers of the liver and kidney functions

Traitement	ALAT (U/L)	ASAT (U/L)	Urea (mg/dL)	Creatinine (mg/dL)
EHIG (250)	71.90±9.05^{ab}	60.52±7.03^{ab}	26.11±7.01^{ab}	3.20±4.50^{ab}
EHIG (500)	32.90±5.11^{a}	34.42±6.32^{a}	16.73±2.22^{a}	1.36±4.07^{a}
MET	47.98±0.01^{a}	42.29±2.51^{a}	32.05±2.01^{ab}	7.70±5.16^{b}
NC	127.05±3.21^{b}	67.32±7.10^{ab}	58.70±11.13^{b}	8.24±14.03^{b}

IGHE (500): hydroethanolic extract of *I. gabonensis at 500mg/Kg;* IGHE (250): hydroethanolic extract of *I. gabonensis at 250mg/Kg; MET: Metformin (10mg/Kg); NC: Negative Control (Distilled water, 5mL/Kg).*

Administration of 500mg/Kg of IGHE as well as Metformin at 10mg/Kg reduced significantly the serum levels of ALT, compared to the group treated only with water at 5mL/Kg. There was also a slight decrease in AST levels in these groups, though not statistically significant (p = 0.09).

The level of urea and creatinine in the groups treated with *I. gabonensis* extract were the lowest compared to MET and the control groups.

Acute toxicity profile of the different extracts

Effect of the extract on bodyweight in mice: Findings from the acute toxicity study showed that there was a slight increase in body weight in males for both the control group and the test groups (MI, AC+MI+PA) from day 0 to day 7, where the increase was highest in the group which received AC+MI+PA. Body weight decreased in all the groups from day 7 to day 14 especially in the control group. However, there was no significant difference between the test groups and the control group on days 7 and 14 with p value≥0.05.

In females, a similar trend was observed from day 0 to day 7, as there was a general increase in body weight both in the test groups (MI and AC+MI+PA) and the control group. From day 7 to day 14, there was an increase in body weight in the control group and in the test group which received AC+MI+PA while the body weight remained constant in animals which received MI. However, there was no significant difference in body weight between the control group and the test groups with p value of 0.342 and 0.366 on day 7 and 14 respectively.

Effects of teas on water and food consumption in mice: Water and food intake increased in all the groups. However, no significant difference was observed between the test groups.

Physical appearance and mortality: No loss of fur was observed in any of the groups and none of the groups had a change in fur and eye color. No abnormal secretions from the eyes, nose and genitals were observed. Movements andreactivity to stimuli was assessed by agility. Animals in control and test groups moved with

usual ease and all the animals involved survived through the experiment.

Effect of extracts on mouse weight

During the 14-day monitoring of mice upon administration of the different extracts, there was an increase in body weight in general in all the groups in both males and females, but there was no significant difference (P= 0.68) in weight between the groups that took IGHE compared to the control group during the two weeks of the study.

DISCUSSION

The management of type 2 diabetes is a serious thorn for people living with it. Not only economically but also by the side effects caused by these palliatives like drugs. In the quest for new drugs, particularly from locally available and affordable sources, the present work was prompted focusing on two medicinal plants commonly used in folk medicine in the rain forest regions of Cameroon, namely *Terapleura terapleura* fruits and *Irvingia gabonensis*. The choice of the plant parts and the methods of extraction were inspired by the traditional use of these plants, while a two-step approach was employed. From a first step consisting of evaluating the acute hypoglycemic and anti-hyperglyemic activity through OGTT, the hydroethanolic extract of *Irvingia* gabonensise merged as the most promising candidate with both a significant suppression of postprandial peak and fast recovery in FBS (30 minutes after administration of 2g/kg of glucose) in healthy Wistar rats. *I. Gabonensis* also known as African Bush Mango,is a West African culinary fruit spice and the mucilage from this fruit seed is used to make traditional soups and sauces (Sun and Chen, 2012). The plant has been reported to possess a wide range of applications in Cameroonian folk medicine, including treatment of gonorrhea, gastrointestinal and hepatic disorders, wounds infection, diabetes, analgesis (Ngondi et al., 2005; Kuete and Efferth, 2010). Otitolaiye et al. (2023) recently reported that the alcohol extract had higher Total Antioxidant Power (AP) and significantly higher concentrations of all the phytochemicals measured as well as the *in vitro* antioxidant capacity, in comparison to the aqueous extracts. Findings from the present study also revealed higher contents of hydroethanolic extract in Polyphenols, Alkaloids, Tannins, Saponins and Triterpenes, compared with the aqueous extract, thus corroborating the previous work.

It has been extensively reported that the passage of glucose from the blood to the peripheral tissues, in this case the hepatic tissues, is likely to be facilitated by various effects of phenolic substancesas well (Subash-Babu et al. 2008). However, the low activity observed in the fruit extracts of *Tetrapleura tetraptera* may also be due to the presence of simple sugars that it contains, thus contributing to the increase in blood sugar. In this regards, Dosunmu (1997) demonstrated that the fruits of *Tetrapleura tetraptera* contain simple carbohydrates like glucose, fructose and sucrose.

The next steps in this work therefore focused based on the evaluation of the subacute antidiabetic activity of the most active extract, namely IGHE. For this purpose, two doses were considered, 250mg/Kg and 500mg/Kg for 21 days. From this assay, reduction in blood sugar of up to 75.57% was scored for 500mg/Kg dose, compared to the68.66% for 250mg/Kg, which was also significantly greater than the negative control. Overall, diabetic rats treated with IGHE at 500mg/Kg completely recovered from the diseases while a recovery rate of 80% was recorded for 250mg/Kg dose. The results obtained are in line with those of Omonkhua et al. (2014) who worked on samples from Nigeria and could demonstrate the antidiabetic properties of aqueous extract of *I.gabonensis* bark in type 1 diabetes model. Atanu et al. (2022) also reported the antioxidant activity of significant in vitro inhibitory effect of the non-polar (n-hexane and chloroform) extracts of *I. gabonensis* on alpha-amylase and alpha-glucosidase. extracts of *Irvingia gabonensis* leaves. Heliyon.The antidiabetic activity observed could be partly attributed to the presence of saponins among other potential active ingredients. In fact, *I. gabonensis* total saponin fractions (ITSF) have been demonstrated to exert antihyperlipidaemic and antioxidant and antidiabetic effects (Omonkhua et al., 2014; Onoagbe and Omonkhua, 2013). furthermore, Terminalin, a tannin isolated from African Mango this plant was found to stimulate glucose uptake through inhibition of Protein tyrosine phosphatases (Yoon et al., 2022).

Further, the hydroethanolic extract of *I gabonensis* significantly improved the lipid profile of diabetic animals, reflected in a decrease in TG, LDL and VLDL. However, there was no significant difference in HDL levels across groups.The effect on lipid profile could be due to the presence of saponins which possess hypolipidemic effects (Francis et al. 2002; Gupta et al. 2009). Similarly, there was an improvement in markers of liver (ALT and AST) and kidney (urea and creatinine) functions, confirming the hepatoprotective and nephroprotective effects of *I gabonensis*stem bark, particularly the hydroethanolic extract at 500mg/Kg.

The hepatoprotective and nephroprotective effects of *I. gabonensis* was previously reportedby Oluwafemi et al. (2014b) with the aqueous extracts of the bark. The antioxidant potential of the ethanolic extract of *I. gabonensis* bark was also reported, with an increase in glutathione, glutathione peroxidase and superoxide dismutase (Oluwafemi et al., 2014b; Otitolaiye et al., 2023).

More interestingly, no significant sign of acute toxicity was observed with the *I. gabonensis*. This corroborates with findings from several authors on other parts or extracts of the plant. Kothari et al. (2012) investigated the extract from the kerneland the results of subchronic toxicity study

suggest the no-observed-adverse-effect level (NOAEL) as ⩾2500 mg/kg bw/day, the highest dose tested.

Other findings indicate that ethanol extract of the root barks was found to be practically safe after acute administration, but reported some histomorphological alterations in the liver and kidney after prolonged administration in the sub-acute dosages (Nuhu et al., 2020). Further investigations are therefore necessary to ascertain the safety of the hydroethanolic extract of *I. gabonensis*stem bark. Formulating Improved Traditional Medicines from this extract is equally envisaged as future direction.

Conclusion

Out of the four extracts prepared from *T. tetraptera* and *I. gabonensis*, and tested for their antidiabetic potential, only the hydroethanol extract of the stem bark of *I. gabonensis* showed promising hypoglycemic and anti-hyperglycemic activity from preliminary screening. Of the two doses of this extract considered for the subacute antidiabetic assessment, the dose of 500mg/Kg demonstrated the highest activity reflected in significant improvement in both clinical and biochemical markers with a 100% recovery rate after 21 days treatment of the STZ-induced diabetic Wistar rats. At this dose, the *I. gabonensis* extract was shown to also improve liver and kidney functions as well as mitigating dyslipidemia in diabetic rats. Overall, its pharmacological properties were above those of the reference drug metformin at 10mg/Kg dose. More interestingly, the acute toxicity study revealed a relative safety of this extract in mice. Further investigations are therefore envisaged to ascertain the suitability of *I. gabonensis* extract to serve as pharmaceutical active principle or source of new drug for diabetes management. These could include advanced toxicity studies, and formulation into Improved traditional Medicines of categories 3 or 4.

Declaration

The authors declare that there is no conflict of interest.

Authors' Contributions

DZ and CTM conceived the work, oversaw the laboratory work and drafted the manuscript; GLS and EEM carried out the bench work and took part in drafting the manuscript. All the authors approved the last version of the work and its submission to the journal.

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