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Antioxidant and anti-hyperglycemic potential of pulp extracts of *Irvingia wombolu* fruits

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Abstract
The present study aimed at evaluating the *in vitro* antioxidant and *in vivo* anti-hyperglycemic potential of pulp extracts of *Irvingia wombolu* fruits on streptozotocin-induced diabetic rats. After phytochemical screening, aqueous and hydroethanolic extracts of *I. wombolu* were analysed for polyphenol content. Antioxidant activity was assessed using three methods. The capacity of the extracts to scavenge hydroxyl radical and to chelate metal was also evaluated. Diabetes was induced in 25 rats by intravenous administration of streptozotocin (50mg/kg body weight). The effect of the extracts on blood glucose levels of diabetic rats was monitored at various time intervals for 5h after administration of the extract (first single dose of 400 mg/kg/day). In addition, total cholesterol (TC), HDL cholesterol, triglyceride (TG), MDA, hydroperoxides and plasma antioxidant capacity was determined after a three-week period. The aqueous extract showed the higher polyphenol content while the hydroethanolic extract had the best *in vitro* antioxidant capacity. Glucose levels of treated diabetic rats significantly decreased 3h after administration of extracts ($P<0.05$). There was no significant difference in TC, HDL and LDL cholesterol between diabetic control and treated diabetic rats. In contrast, extracts significantly reduced triglyceride. Treatment with extracts significantly reduced atherogenic risk predictor indices. Plant extracts significantly reduced MDA and hydroperoxide levels. Antioxidant capacity of blood plasma significantly increased after administration of aqueous extract. This study suggests that *I. wombolu* pulp not only could be a good source of antioxidants, but might also have glucose lowering property, with the best *in vivo* activity attributed to aqueous extract.

Keywords: Oxidative stress; polyphenols; diabetes; plant extracts; *I. wombolu*; *Irvingia wombolu*.

Introduction

Chronic amounts of circulating glucose cause toxic effects on the structure and function of organs, including pancreatic islets. There is a considerable evidence that hyperglycemia results in the generation of reactive oxygen species (ROS), ultimately leading to increased oxidative stress in a variety of tissues (Evans *et al.*, 2002). In the absence of an appropriate compensatory response from the endogenous antioxidant network, the system becomes overwhelmed (redox imbalance), leading to the activation of stress-sensitive intracellular signaling pathways (Evans *et al.*, 2002). Multiple biochemical pathways and mechanisms of action of glucose toxicity have been suggested. All of these pathways produce ROS in excess and which over time causes chronic oxidative stress, which in turn causes defective insulin gene expression and insulin secretion as well as increased apoptosis (Robertson, 2004). All these activities can cause or exacerbate diabetes, which is a group of metabolic disorders that result in hyperglycemia. This may happen as a result of decreased insulin production (type-I) or insufficient insulin utilization (type-II) (Marshal and Bangert, 2004). The chronic hyperglycemia of diabetes is associated with long term damage, dysfunction and failure of various organs (Lyra *et al.*, 2006). Some medicinal plants have been reported to be useful in diabetes worldwide and have been used empirically as antidiabetic and antihyperlipidemic remedies (Bhattaram *et al.*, 2002; Hou *et al.*, 2005). Most plants contain glycosides, alkaloids, terpenoids, flavonoids, carotenoids, etc. that have been shown to have antidiabetic effects (Loew and Kaszkin, 2002). Searching for new antidiabetic drugs from natural plants is still attractive because they contain substances which provide safe substitute in the management of diabetes mellitus.

African mango (*I. wombolu*, family Irvingiaceae), a highly valuable and extensively utilised tropical African tree, was identified as a high priority species and a Non-Timber Forest Product (NTFP). It is a local fruit tree with a wide distribution across West and Central Africa. *Irvingia* kernels are used in soup making as they form an important part of the West and Central African diet. Fat extracted from the kernels can be used for food applications, such as in margarine or cooking oil, and is also suitable for soap,

cosmetics and pharmaceuticals (Ejiofor *et al.*, 1987). Flour can be produced from the kernels. Agbor (1994) stated that the roots, leaves and barks of *Irvingia* spp. are used medicinally. Fractions of *Irvingia gabonensis* (a related species) seeds were reported to have hypoglycemic effect (Ngondi *et al.*, 2006). The fruit pulp of *I. wombolu*, however, is bitter and tastes of turpentine, so it is not edible (Ejiofor, 1994). The only part of the *Irvingia* plant that doesn't seem to have other application is it pulp. Hence, the objective of this work was to investigate the antioxidant an anti-hyperglycemic properties of extracts of *I. wombolu*.

Materials and Methods

Samples extraction: Fruits of *I. wombolu* were harvested in March 2007 from their natural habitat in the Dja region of Cameroon. After drying in an oven at 50^{o}C for 3 days, the pulp was separated from the kernel. The pulp (100g) was ground and extracted by maceration for 48 h with 1 litter of solvent (aqueous or hydroethanolic, 1:1). The resulting supernatant was filtered and evaporated in an oven at 50°C until completely dried. The yield of extracts was 10% and 7.8% respectively for the aqueous and hydroethanolic extracts. Dried extract were ground into powder using an electrical grinder and stored in desiccators.

1. *In vitro* chemical analysis

Phytochemical screening: The quantitative methods described by Trease and Evans (1983) were used to evaluate the presence of tannins, flavonoids, phlobatannins, anthocyanin, quinones, cardiac glycoside and alkaloids.

Folin-antioxidant capacity: Folin reagent diluted 10 times prior to utilization was used to measure the antioxidant capacity via polyphenol content (Singleton *et al.*, 1999). The reagent (1mL) was added to 10 µL of plant extracts. The absorbance at 750 nm was measured after 30 min using a spectrophotometer with catechin as the standard.

DPPH free-radical scavenging assay: The antioxidant activity of the aqueous and hydroethanolic extracts of *Irvingia* was measured in terms of radical scavenging ability, according to the DPPH method (Katalinie *et al.*, 2004). In the procedure, DPPH free radical (violet color) was reduced by antioxidant. The stronger the antioxidant present in the plant extract, the fainter the solution color was. Scavenging activity against the DPPH free radical was studied as follows: 20 µl of extract was introduced into 2 ml of a methanolic solution of DPPH (0.3 mM) and kept in the dark for 30 min. The extract was replaced by methanol for the control and catechin was used as the standard. The absorbance was then spectrophotometrically read at 517 nm. The antioxidant content and inhibition rates of DPPH radical were calculated as milligram of catechin equivalent per gram of sample.

FRAP assay: The antioxidant capacity of each sample was estimated according to the procedure described by Benzie and Strain (1996). Briefly, aqueous or hydroethanolic extracts solution (75µl) was added to 2 ml of FRAP reagent. The free radical scavenging activity was expressed as milligram of catechin equivalent per gram of sample.

ABTS free-radical scavenging assay: The ABTS solution was prepared by mixing 8 mM of ABTS with 3 mM of potassium persulfate in 25 ml of distilled water. The solution was maintained at room temperature in the darkness for 16 hours before use (Re *et al.*, 1999). The $ABTS^{+}$ solution was diluted 10 times with 95% ethanol. Plant extracts (20 µL), was mixed with 1 mL of diluted $ABTS^{+}$ solution and incubated 30 min at room temperature. The absorbance was read at 734 nm after 30 min against ethanol (95%) as a blank.

For each individual antioxidant assay, a catechin (1mM) aliquot was used to develop a standard curve. Results were expressed as milligrams of catechin equivalent per gram of dried extract.

Hydroxyl radical scavenging activity: The hydroxyl radical scavenging activity of the plant samples was determined as described by Halliwell *et al.* (1987). The reaction mixture consisted of $FeCl_3$ (300µM) and aliquots of extracts (2.5mg/ml -10mg/ml) in a final volume of 1 ml. All the reagents were dissolved in potassium phosphate buffer (20 mM, pH 7.4). This was then incubated at 37°C for 1 hour. After incubation, 1 ml of TCA (2.8%) and TBA (1%) were added to the reaction mixture and incubated at 100 °C for 20 minutes. A control tube was prepared similarly except that the extract was replaced by methanol. The absorbance was read spectrophotometrically at 532 nm. The percentage hydroxyl radical scavenging effects of plants extracts were

calculated as follows: % hydroxyl radical scavenging effect = [(Abs1-Abs2)/Abs1] x 100. Where Abs1 is the absorbance of the control and Abs2 is the absorbance of plant extract.

Metal chelating activity: The chelation of ferrous ions by the plants extracts was determined by the method of Denis *et al.* (1994). Briefly described, 0.025 ml of 2mM $FeCl_2$ was added to aliquots of extracts (1-8mg/mL) in 0.5 mL methanol. The reaction was initiated by the addition of 5 mM ferrozine (0.1ml), the mixture was vigorously shaken and left to stand at room temperature for 10 minutes. A control tube contained methanol rather than the extract. The absorbance was then measured spectrophotometrically at 562nm. The metal chelating activity of the plant extracts was calculated as follows:
% metal chelating effect = [(Abs1-Abs2)/Abs1] x 100
where Abs1 is the absorbance of the control and Abs2 is the absorbance of plant extract.

2. *In vivo* experimentation

Animals: Adult male Wistar albino rats weighing 250–300 g were obtained from the animal house of the Department of Biochemistry, University of Yaounde I, Cameroon. The animals were acclimatized in the experimental animal room for 6 days with a 12 h light and 12h dark cycle before the start of experimentation. Standard feed and water was provided *ad libitum* to all experimental animals.

Experimental induction of diabetes: The schedules and procedures were performed in the experimental animal house of the Laboratory of Biochemistry of the University of Yaoundé I, Cameroon. The study was approved by institutional animal ethical committee. Streptozotocin (STZ) was dissolved in 0.1 M cold citrate buffer, pH 4.5, immediately before use. Diabetes was induced by intravenous administration of streptozotocin (50 mg/kg) through the right jugular vein of rats after an overnight fast. Four days after STZ injection, fasting blood glucose was assessed to confirm the diabetic state. Rats with fasting blood glucose values of at least 250 mg/dl were used for the experiment. Fruits extract was suspended in DMSO 10% (v/v) and administered orally through a gavage at a dose of 400 mg/kg body weight/rat/day for 21 days. Tolbutamide was used as reference and was administered at a dose of 80 mg/kg body weight.

Experimental design: In the experiment, a total of 25 rats (20 diabetic surviving rats, 5 normal rats) were used. The rats were divided into five groups of five rats each after the induction of diabetes. One normal control group (only 10% DMSO); one diabetic control group (only 10% DMSO) and three other groups receiving respectively Tolbutamide (80 mg/kg in 10% DMSO), aqueous extract of pulp, hydroethanolic extract of pulp. The body weight and fasting blood glucose levels of all rats were recorded at regular intervals during the experimental period. The Trinder glucose activity test (1969), using glucose oxidase with an alternative oxygen receptor, was used to monitor the blood glucose at 1h 30min, 3 and 5h after first administration of extract using test strip in tail vein blood. To measure lipids and others parameters, oral administration was conducted daily for 21 days.

After 21 days of treatment, the 12-hour-fasted animals were sacrificed by cervical decapitation under anaesthesia. Blood was withdrawn with EDTA tubes and centrifuged at 3000 rpm for 10 min to obtain the plasma which was stored at -20°C for the measurement of biochemical parameters. Tissues (liver, kidney and heart) were collected, washed with saline solution and homogenate prepared in sodium chloride (0.9%). The supernatant was stored at -20°C until analysis. Erythrocyte suspension was made by washing blood residue three times with sodium chloride and followed by lyses with water and stored in the freezer at -20°C for further uses.

Biochemical analysis: Triglycerides were estimated by the GOP-PAP method (Jacobs and Vandemark, 1975), total cholesterol by the CHOD-PAP method (Richmond, 1973) and HDL cholesterol by the CHOD-PAP method (Assmann, 1979). MDA lipid peroxidation marker was estimated by the method of Yagi (1976). Plasma or homogenate (0.1 ml) was added to 0.4 ml of thiobarbituric acid. After boiling at 100°C for 15 min, uncovered tubes were refrigerated in cold water during 15 min. The tubes were later centrifuged at 3000 tr/mn during 5 mn and the absorbance of the supernatant was read at 532 nm. MDA concentration was estimated by using molar extinction coefficient (ε=1.53, $105M^{-1}cm^{-1}$). The content was expressed as µmol/l. Hydroperoxide was determined by the method of Jiang *et al.* (1992). Plasma and homogenate (0.1ml) was added to 0.9 ml of fox reagent. After homogenisation, they were incubated at 37°C for 30 min. The absorbance was measured at 560 nm against a control tube.

The value was expressed as mM hydroperoxide/ 100g of tissue. The antioxidant capacity of plasma was measured using FRAP method.

Statistical analysis: *In vitro* measurements were carried out in triplicate. All data were expressed as mean values ± standard deviation. Significant differences among the groups were determined by the test of Kruskal-Wallis following by the post hoc of Bonferonni using the SPSS statistical analysis program. Statistical significance was considered at $P<0.05$.

Results

The result of phytochemical screening showed that the African mango pulp is rich in compounds known to have antioxidant activity like tannins, phlobatannins, flavonoids, anthocyanin, cardiac glycosides and alkaloids (Table 1). Table 2 depicts the results of Folin, DPPH, ABTS and FRAP antioxidant activities of the different pulp extracts. Folin test result showed that, compared to the hydroethanolic extract the aqueous extract had higher antioxidant capacity, indicating greater polyphenol content. However, with the other methods, the hydroethanolic extract depicted the higher antioxidant capacity. Results of the hydroxyl radical scavenging activity and metal chelating activity of plant extracts versus concentrations are shown in Table 3. These extracts had the ability to scavenge radicals produced in the body during metabolic reactions.

Table 1: Phytochemical screening of plant extract.

	A	S	T	F	GC	P	SR	AN	Q
Aqueous extract	+	-	+	+	+	+	+	+	+
Hydroethanolic extract	+	-	+	+	+	+	+	+	-

A= Alkaloid, S= Saponine, T= Tannin, F= Flavonoid, GC= Cardiac Glycoside, P= Phlobatannin, SR= reducing sugar, AN= Anthocyanin, Q= Quinone.

Table 2: The antioxidant activity of *I. wombolu* pulp.

Fractions	Polyphenol content (mg catechin equivalent/g)	DPPH (% of inhibition by extract)	ABTS (% of inhibition by extract)	DPPH (mg catechin equivalent/g)	ABTS (mg catechin equivalent/g)	FRAP (mg catechin equivalent/g)
Aqueous extract	119.81±39.62	37.86±0.03	55.53±0.33	42.016±0.06	11.28±0.13	15.55±0.11
Hydroethanolic extract	61.83±26.14	66.38±3.17	97.39±0.10	415.09±27.09	27.94±0.03	19.25±1.11

Values are given as mean ± SD and expressed as % of inhibition and mg equivalent catechin/g of dry weight. Catechin was used as standard.

Table 3: Hydroxyl radical scavenging and metal chelating activities of plant extracts at different concentrations.

Concentrations (mg/ml)	Hydroxyl radical scavenging activity (% inhibition by extract)		Metal chelating activity (% inhibition by extract)	
	Aqueous extract	Hydroethanolic extract	Aqueous extract	Hydroethanolic extract
0.25	20.62	35.13	32.67	60.97
0.50	24.43	25.66	33.20	34.57
0.75	26.38	24.27	50.59	36.22
1	31.63	33.69	30.90	34.01

Values are given as mean ± SD. There was no a significant difference between plant extracts independently of the concentration.

Table 4: Blood glucose (mg/dl) after hypoglycemic test of control and experimental groups of rats.

Groups	0	90 min	180 min	300 min
Control	76.5±7.22	75.75±4.91	69.25±3.56	69.75±2.58
Diabetic control	321.25±3.66 [a]	322.25±3.25 [a]	321.25±5.66 [a]	321.5±3.03 [a]
Diabetic + tolbutamide	329.75±3.35	298.25±4.13*	213.25±3.99*	109±3.71*
Diabetic + aqueous extract	361.5±39.91	355±23.61*	295.25±33.65*	152.75±3.71*
Diabetic + hydroethanolic extract	344±45.61	296±40.91*	234.66±29.9*	202±33.18*

Values are expressed as mean ± SD (n=5); [a] Values are statistically significant at $p<0.05$ compared to normal control rats;
* Values are statistically significant at $p<0.05$ compared to diabetic control rats.

Table 5: Effect of plant's extract on the glucose levels (mg/dl) after 21 days of treatment.

Groups	0	14	21
Control	74.5±2.9	72.25±2	73.35±1.9
Diabetic control	347.2±43.9 [a]	288±22 [a]	205.56±45.1 [a]
Diabetic + tolbutamide	278±29.4*	131±2.9*	62.4±6.3*
Diabetic + aqueous extract	361.5±34.9*	79.33±7.07	84.33±1.4*
Diabetic + hydroethanolic extract	366.25±50.74*	195.5±55.6*	134±7.75*

Values are expressed as mean ± SD (n=5); [a] Values are statistically significant at p<0.05 compared to normal control rats;
* Values are statistically significant at p<0.05 compared to diabetic control rats.

Table 6: Effect of plant extracts on lipid profile.

Groups	TC (mg/dl)	LDL-C (mg/dl)	HDL-C (mg/dl)	TG (mg/dl)
Control	74.05±5.58	24.54±9.80	23.84±9.39	46±2.68
Diabetic control	85.07±6.88	55.84±9.51 [a]	1.21±0.13 [a]	160.70±11.96 [a]
Diabetic + tolbutamide	32.85±4.84	11.38±3.12*	8.44±5.64	27.73±17.09*
Diabetic + aqueous extract	77.33±9.71	52.91±6.69	8.69±2.73	44.80±16.66*
Diabetic + hydroethanolic extract	96.20±1.10	80.47±2.31*	9.95±1.00	24.95±12.62*

Values are expressed as mean ± SD (n=5); [a] Values are statistically significant at p<0.05 compared to normal control rats;
* Values are statistically significant at p<0.05 compared to diabetic control rats.

Table 7: Effect of plant extract on the atherogenic risk indices.

Groups	TC/HDL-C	LDL-C/HDL-C
Control	1.56±0.19	0.44±0.17
Diabetic control	71.59±12 [a]	47.20±10.75 [a]
Diabetic + tolbutamide	5.06±2.05*	1.98±0.96*
Diabetic + aqueous extract	9.84±3.10*	8.17±2.02*
Diabetic + hydroethanolic extract	9.75±0.88*	8.17±0.92*

Values are expressed as mean ± SD (n=5); [a] Values are statistically significant at *p*<0.05 compared to normal control rats;
* Values are statistically significant at p<0.05 compared to diabetic control rats.

Table 8: Effect of plant extract on the body weight (g) management of control and experimental groups of rats.

Groups	0	7	14	21
Control	254.33±7.23	278±8.18	282±6.65	284±7.63
Diabetic control	214.13±22.09	222.33±22.57	210.32±15.31	200.76±23.09
Diabetic + tolbutamide	235.82±16.20	259.67±26.23	247.4±21.82	244.77±17.94
Diabetic + aqueous extract	218.7±37.64	247.15±43.57	243.73±23.78	248.17±41.22
Diabetic + hydroethanolic extract	209.07±7.39	228.19±11.74	223.09±20.38	217.62±22.49

Values are expressed as mean ± SD (n=5); [a] Values are statistically significant at p<0.05 compared to normal control rats;
* Values are statistically significant at p<0.05 compared to diabetic control rats.

Table 9: Effect of plant extract on the level of MDA in plasma, liver and kidney.

Groups	Plasma (µM)	Liver (µM/g de tissue)	Kidney (µM/g de tissue)
Control	1.55±0,24	3.52±0,22	4.02±0,46
Diabetic control	2.29±1.14	6.72±0,24 [a]	5.56±0,16 [a]
Diabetic + tolbutamide	1.67±0.61	2.98±0.72*	1.83±0.36*
Diabetic + aqueous extract	1.43±0.43	2.36±0.36*	1.99±0.18*
Diabetic + hydroethanolic extract	1.70±0.31	3.13±0.52*	2.70±0.1*

Values are expressed as mean ± SD (n=5); [a] Values are statistically significant at p<0.05 compared to normal control rats;
* Values are statistically significant at p<0.05 compared to diabetic control rats.

Table 10: Effect of plant extract on the level of hydroperoxide (mM/100g of tissue) in liver and kidney.

	Liver	Kidney
Control	2.05±0.03	1.68±0.04
Diabetic control	1.98±0.13	2.86±0.15 [a]
Diabetic + tolbutamide	1.69±0.24	2.32±0.32
Diabetic + aqueous extract	1.21±0.09*	1.20±0.14*
Diabetic + hydroethanolic	1.51±0.11*	1.30±0.045*

Values are expressed as mean ± SD (n=5); [a] Values are statistically significant at p<0.05 compared to normal control rats;
* Values are statistically significant at p<0.05 compared to diabetic control rats.

Table 11: Effect of plant extract on the antioxidant capacity of blood plasma.

	FRAP
Control	173.6 ± 4.8
Diabetic control	140 ±10.42
Diabetic + tolbutamide	184 ± 19.9
Diabetic + aqueous extract	216 ± 7*
Diabetic + hydroethanolic extract	161 ± 27.71

Values are expressed as mean ± SD (n=5); [a] Values are statistically significant at p<0.05 compared to normal control rats;
* Values are statistically significant at p<0.05 compared to diabetic control rats.

Tables 4 to 11 present the results of *in vivo* experimentation. The effect of aqueous and hydroethanolic extracts of *I. wombolu* on the blood glucose levels of experimental animals was determined at various time intervals for 5 h after oral administration at 400 mg dose kg^{-1} body weight (Table 4). There was a significant reduction (P<0.05) of blood glucose levels 3 h after treatment of diabetic rats compared to the diabetic control. Aqueous extracts showed the best reduction after 5 h of treatment. Table 5 presents the change in blood glucose levels in control and experimental groups after 21 days of treatment. The blood glucose level in the control rats did not change much during the treatment. In diabetic control rats, the blood glucose concentration remained elevated during the experimentation. The diabetic rats treated with aqueous extract of *I. wombolu* showed a significant decrease (P<0.05) in blood glucose compared to the normal control after 2 weeks of treatment.

As shown in Table 6, there was a significant difference (P<0.05) between total cholesterol content in diabetic control compared to normal group. It was observed that LDL concentration of diabetic control rats was significantly elevated (p<0.01) as compared to normal group. The aqueous and hydroethanolic extracts did not change the TC, LDL cholesterol and HDL cholesterol levels. Triglycerides level was significantly increased (P<0.01) in diabetic control comparing to normal control. There was a significant difference in TG level between the diabetic control and treated diabetic rats, with aqueous and hydroethanolic extracts of *I. wombolu*. The percentage of reduction of each extract was 72.12% and 84.47% respectively. In particular, the value of aqueous extract of *I. wombolu* treated diabetic rats was less than those of tolbutamide treated diabetic rats. The effect of aqueous and hydroethanolic extracts on atherogenic indices of experimental animals was determined using the ratios CT/HDL and LDL/HDL (Table 7). There was a significant (p<0.05) elevation of atherogenic risk in diabetic control compared to normal control and treated diabetic rats. So the aqueous and hydroethanolic extracts of plant caused the reduction in atherogenic risk as same extent as the reference tolbutamide.

There was a significant decrease in the body weight of diabetic rats compared to the normal rats. Upon treatment with plant extracts and tolbutamide, the weight gain was improved (Table 8). The results of the effect of extracts on the level of MDA in plasma, liver and kidney are presented in Table 9. There was no significant difference between plasma MDA in all groups. The level of liver and kidney MDA was significantly elevated in diabetic control compared to treated diabetic and normal groups. The level of hydroperoxide in liver and kidney are significantly lower in diabetic rats receiving aqueous and hydroethanolic extracts compared to diabetic control rats (Table 10). The administration of aqueous and hydroethanolic extracts significantly (p<0.05) brought down hydroperoxide values in streptozotocin induced diabetic rats to 38.8 and 31.12% respectively in liver and 58.04 and 54.5%

respectively in kidney. These extract showed a significant reduction compared to diabetic rats treated with tolbutamide and normal control. The best reduction was obtained with aqueous extract both in liver and kidney. Administration of plant aqueous extract induced a significant elevation of plasma antioxidant capacity. There was no significant difference between diabetic control and normal control rats (Table 11).

Discussion

In vitro analysis

The phytochemical study of pulp extracts revealed the presence of polyphenol-rich compounds. Polyphenols have been suggested to decrease the oxidative stress in human especially through inhibition of the LDL-cholesterol oxidation (Fuhrman and Aviram, 2001). Flavonoids found in the pulp extract may inhibit the oxidative stress by: 1) scavenging free radicals by acting as reducing agent, hydrogen atom donating molecules or singlet oxygen quenchers; 2) chelating metal ions; 3) sparing other antioxidants (e.g. carotene, vitamin C and E); and 4) preserving HDL associated serum paraoxonase activity (Fuhrman and Aviram, 2001). Antioxidant properties of polyphenols are related to their chemical structure and depend on the number and arrangement of their phenolic hydroxyl groups (Bravo, 1998; Heim, 2002; Rice-Evans, 2001). The amount of phenolics varies considerably in the different pulp extracts. In fact, the polyphenol content in the aqueous extract is 1.94 times higher than the polyphenol content of hydroethanolic extract. This result showed the influence of the type of solvent used on the polyphenol content. Oviasogie *et al.* (2009) also found that methanolic pulp extract of *I. wombolu* contained a high level of total phenolic compound.

In order to neutralize and fight against the deleterious effects of ROS, various antioxidant strategies have evolved either by increasing the endogenous antioxidant enzyme defences or by enhancing the non-enzymatic defences through dietary or pharmacological means. The antiradical activities of various antioxidants were determined using the free radical: 2,2-Diphenyl-1-picrylhydraxyl ($DPPH^{\bullet}$) and 2,2'-azinobis (3-ethylbenzothiazoline-6-sulfonic acid) ($ABTS^{\bullet+}$). The ABTS assay measures the relative ability of antioxidant to scavenge the $ABTS^{\bullet+}$ generated in aqueous and organic solvent systems. $DPPH^{\bullet}$ is a stable radical which loses its activity at 515 nm when reduced by an antioxidant or a free radical species. It is widely used to determine antiradicals/antioxidant activity of purified phenolic compounds as well as natural plants extracts (Brand-Williams, 1995; Mahinda and Shahidi, 2000). In the present research, the hydroethanolic extract of pulp has shown the best antiradical properties independently of the methods. This could be explained by the structure, number and arrangement of their phenolic hydroxyl group responsible for these activities. FRAP measures the ferric reducing ability of the antioxidant molecule. It is known that the antioxidant properties of many compounds are directly related to their reducing power. The analyses have showed that reducing power varied from 15.35±0.11 mg catechin equivalent/g of pulp extracts to 19.25±1.11 mg catechin equivalent/g of pulp extract for aqueous and hydroethanolic extracts respectively.

The hydroxyl radical is formed *in vivo* by high energy irradiation leading to homolytic cleavage of water or from H_2O_2 in a metal catalysed process (Cheeserman and Slater, 1993). Hydroxyl radical can attract hydrogen atoms from biological molecules, including thiols leading to the formation of sulphur radicals capable of combining with oxygen to regenerate oxysulphur radicals which also damage biological molecules (Halliwell, 1991). Endogenously, a free metal ion will react with H_2O_2 to produce the deadly free radical (OH^{-}). Hence, scavenging of H_2O_2 and metal chelating processes are important for the cell (Gülçin *et al.*, 2003). The pulp extracts showed moderated hydroxyl radical scavenging activity with hydroethanolic extracts being the strongest scavenger at 0.25 mg/ml. These extracts also showed moderated scavenging activity against metal. Thus these extracts can be use in reducing the effect of hydroxyl radical and metal activity against biological molecules.

Effect of extracts on plasma glucose level

Streptozotocin-induced diabetes provides a condition of insulinopenia and has been described as a useful experimental model to evaluate the activity of hypoglycemic agents (Bailey and Flatt, 1986). In the present study, a single administration of the aqueous and hydroethanolic extracts of *I. wombolu* effectively lowered the blood glucose level in streptozotocin-diabetic rats compared to their control (Figure 5). Streptozotocin selectively damaged the pancreatic insulin secreting β-cells, leaving less active cells and resulting in a diabetic state (Junod *et al.*, 1969). Both experimental and clinical studies suggested

that oxidative stress plays a major role in the development and progression of both types of diabetes mellitus. Free radicals are formed disproportionately in diabetes by glucose oxidation, non enzymatic glycation of proteins and subsequent oxidative degradation of glycation proteins. Diabetes is usually accompanied by impaired antioxidant capacity. In diabetic rats tolbutamide was used as positive control of known drug because this hypoglycemic agent requires the presence of functioning β-cells of islets of Langerhans. The administration of aqueous and hydroethanolic extracts in diabetic rats may act by a direct stimulation of insulin secretion in remaining β-cells. This effect could be attributed to compounds like glycosides, alkaloids, flavonoids, anthocyanin, tannins found in pulp extracts (Table 1) that are frequently reported to have antidiabetic effect (Loew and Kaszkin, 2002). Action of the extracts may involve insulin-like extrapancreatic mechanisms such as the stimulation of glucose utilisation and the reduction of hepatic gluconeogenesis. The best hypoglycemic activity was attributed to aqueous extract of *I. wombolu*. This result may be due to its higher level of polyphenolic compounds shown *in vitro*.

Effect of extracts on plasma lipid content

In the diabetes mellitus abnormal increased levels of lipid, lipoprotein and lipid peroxides in plasma may be due to the abnormal lipid metabolism (Suckling *et al.*, 1993). Although abnormalities in cell cholesterol metabolism could be partly responsible for the changes in the plasma cholesterol levels in diabetes, the precise mechanisms underlying these enzymatic changes have yet to be elucidated (Retnam *et al.*, 1983; Bopanna *et al.*, 1997).

It was found that total cholesterol of rats treated with aqueous extract was lower although not significantly different compared to control diabetic. The significant increased of LDL cholesterol level in diabetic control compared to the normal control could be explained by the fact that insulin could increase the number of LDL receptors, so chronic insulin deficiency might be associated with a diminished level of LDL receptors. This causes the increase in LDL particles and result in the increase in LDL-cholesterol value. It has been suggested that the increase in triglyceride may be due to insulin deficiency which results in faulty glucose utilization, causes hyperglycemia and mobilization of fatty acids from adipose tissue. In diabetes blood glucose is not utilized by tissue and that condition lead to hyperglycemia. The fatty acids from adipose tissue are mobilized for energy purpose and excess fatty acids are accumulated in the liver, which are converted to triglyceride (Shih *et al.*, 1997). All treated diabetic rats have shown a significant ($P<0.05$) reduction of plasma triglyceride. So, aqueous and hydroethanolic extracts has the ability to reduce triglyceride level in diabetic probably by enhancing the stimulation of insulin secretion or acting through regeneration of beta cells.

Effect of extracts on lipid peroxidation

Free radicals are formed disproportionately in diabetes mellitus by glucose degradation, non-enzymatic glycation of proteins, and the subsequent oxidative degradation, which may play an important role in the development of complications in diabetic patients. The generation of free radicals may lead to lipid peroxidation and several damages in diabetes mellitus (Mahboob *et al.*, 2005).

In the present study, the degree of lipid peroxidation was measured in terms of malondialdehyde (MDA) concentration and hydroperoxide level. We have observed that MDA levels, a lipid peroxidation product as markers of oxidative stress, were significantly elevated in liver and kidney of diabetic control rats compared to control rats. This finding clearly showed that diabetic rats were exposed to an increased oxidative stress via lipid peroxidation. Administration of plant extracts significantly reduced MDA content in liver and kidney probably due to their polyphenol content. The higher reduction was obtained with aqueous extract. Lipid peroxidation of membrane, associated with increased membrane rigidity and reduced cell survival has been implicated in diabetes mellitus (Selvam and Anuradha, 1988). The observed increase in hydroperoxide level in diabetic control rats could be attributed to the increase in peroxidative damage of lipids, thereby contributing to alterations in lipids and antioxidant status. Polyphenol-rich extracts of *I. wombolu* have significantly reduced the level of hydroperoxide compared to normal and diabetic controls and tolbutamide treated diabetic rats.

Effect of plant extracts on the antioxidant capacity of plasma

In this study, depletion of total antioxidant capacity estimated by FRAP method in diabetic and control rats were done. Compared to the normal control group the total antioxidant capacity was low in diabetic control. This severe depletion may be due to poorer glycemic regulation in diabetic untreated rats. However, the supplementation with aqueous and hydroethanolic extract of *I.*

wombolu was very helpful in the management of hyperglycemia and enhanced the plasma total antioxidant capacity. The aqueous extract had the best improvement, probably due to its high capacity to control blood sugar.

Conclusion

Based on the aforementioned results, we concluded that aqueous and hydroethanolic extracts of *I. wombolu* pulp have potential antioxidant activity *in vitro* and hypoglycemic effect in streptozotocin-induced diabetic rats. The effect was found to be significantly more effective with aqueous extract compared to tolbutamide. These extracts have also shown an effect on atherogenic risk, triglyceride and lipid peroxidation levels. Therefore, these extracts could be used as good source of antioxidants as well as effective and alternative treatment of diabetes.

Ethical Approval

The study was approved by the Animal Ethics Committee of the Faculty of Sciences, University of Yaounde I, Cameroon.

Conflict of Interests

Authors have no conflicting interests.

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