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In vitro and in vivo evaluation of the anti-diabetic, anti-hyperlipidemic and antioxidant properties of aqueous extract of *Millettia laurentii* bark (Fabaceae)

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ABSTRACT

Background & Aim: Hyperglycemia, oxidative stress and dyslipidemia play a major role in the pathophysiology of diabetes and its macro and microvascular complications. Therefore, managing hyperglycemia, oxidative stress and dyslipidemia is an effective way to control diabetes. The present study aimed to evaluate *in vitro* and *in vivo* the anti-diabetic, anti-hyperlipidemic and antioxidant potential of the aqueous extract of *M. laurentii* barks.

Experimental: The *M. laurentii* barks were harvested, treated, dried, ground and an aqueous extraction was carried out (1:10 weight/volume). Subsequently, the anti-hyperglycemic (inhibition of α-amylase and invertase activity) and antioxidant (DPPH radical scavenging, iron III reduction and metal chelating) properties of the aqueous extract was evaluated in vitro. In the in vivo study, 20 male Wistar strain rats with an average weight of 230 to 250 gramme divided into two groups; a negative control group (NC) and a batch of 15 rats. This last batch received an intraperitoneal injection of 45mg/kg BW of streptozotocin then subdivided into 3 groups of 5 rats: positive control group (PC) receiving distilled water, a test group receiving aqueous extract of *M. laurentii* barks (AEML) at the dose of 300 mg/kg BW and a reference group receiving metformin at 20 mg/kg BW. After 21 days of experimentation, the animals were sacrificed, the plasma, serum, hemolysate and liver homogenate were used to evaluate the biomarkers of oxidative stress (catalase, MDA), lipid profile (triglyceride, total cholesterol and HDL-cholesterol) and immunological biomarkers (CRP and NFS).

Results: It emerged that the aqueous extract presented in vitro an anti-hyperglycemic activity (inhibition of invertase and alpha amylase with IC_{50} values 0.015 and 0.38 mg/mL, respectively) and antioxidant activity (DPPH radicals scavenging, reduction of iron III and inhibition of haemolysis). The extract also reduced in vivo, chronic hyperglycemia by -28.44% after 21 days of treatment, improved endogenous antioxidant status, inflammation and lipid profile.

Recommended applications/industries: The findings show that *AEML* has an anti-diabetic, anti-hyperlipidemic and antioxidant properties. Therefore, could be used traditionally in the management of diabetes and its complications in Cameroon. Extending the current control of chronic hyperglycemia is urgently needed in Cameroon to protect human lives.

1. Introduction

Diabetes mellitus is a metabolic disorder characterized by chronic hyperglycemia, resulting from insulin deficiency and/or insulin resistance (Fawziah *et al.,* 2017). The number of people with diabetes continues to grow at a very alarming rate. Indeed, according to the International Diabetes Federation (IDF), more than 463 million people have diabetes worldwide (IDF, 2019). Cameroon does not escape as it has 615,300 people with diabetes (IDF, 2020). The pathophysiology behind this disease involves constitutional (genetic predispositions, age, sex) and environmental (sedentary lifestyle, diet, overweight/obesity) risk factors (IDF, 2019). Both postprandial and fasting hyperglycemia are characteristic of diabetes. Indeed, the excessive consumption of foods rich in saturated fatty acids causes their accumulation in adipose tissue, thus causing excess weight which will be the cause of insulin resistance. Later, this will lead to insulin deficiency, then to chronic hyperglycemia (Pines *et al.,* 2018). In addition, postprandial hyperglycemia results from a regular increase in blood sugar following the consumption of foods with high glycemic indexes; and depends on both the digestibility of carbohydrates and the regulatory capacity of pancreatic cells (Pines *et al.,* 2018). In the long term, chronic hyperglycemia leads to the activation of several cellular pathways, including an overactivation of the electron transporter chain in the mitochondria, thus generating an increased production of ROEs. Free radicals are potential inducers of chronic inflammation through the activation of pro-inflammatory signaling pathways. This inflammation will induce a massive recruitment of leukocytes responsible for the vascular lesions observed in the onset and development of vascular complications of diabetes (Ashok *et al.,* 2019; Archana *et al.,* 2013).

The management of diabetes and its complications involves lifestyle and dietary measures, the use of oral antidiabetics, insulin therapy and anti-inflammatory agents (Fagninou *et al.,* 2019). However, these means of management have not limited the high mortality associated with this pathology; moreover, drugs in combined or non-combined therapies are generally associated with side effects and are expensive. Consequently, research is increasingly turning to the use of natural plants (Wenbi *et al.,* 2019). Indeed, plants constitute an important source of substances with therapeutic potential. They contain considerable amounts of bioactive compounds including polyphenols, flavonoids and alkaloids which have biological properties such as anti-inflammatory, antioxidant, hepato-protective, hypolipidemic and hypoglycemic activities (Djaouida *et al.,* 2019; Mona *et al.,* 2014).

Millettia laurentii is a plant belonging to the Fabaceae family commonly called *Wengue*. It is a shrub whose seeds are used by some indigenous populations as a food supplement (Ferdinand *et al.,* 2020). Some studies have reported that different parts of this plant are rich in polyphenols, flavonoids and alkaloids. In Cameroon, the plant is traditionally used as an anti-tumor, antiviral, antiparasitic, antidiabetic and anti-inflammatory (Ferdinand *et al.,* 2020; Clautilde *et al.,* 2013; Adrien *et al.,* 2019). The objective of this study was to evaluate in vitro and in vivo the anti-diabetic, anti-hyperlipidemic and antioxidant properties of the aqueous extract of *M. laurentii* bark.

2. Materials and Methods

2.1. Plant material

The plant material consisted of the bark of *M. laurentii,* harvested in October 2020 in the town of Mbalmayo (Centre Region, Cameroon) at the water and forest school. They were identified at the National Herbarium of Cameroon in comparison with the sample of Letouzey N°10091 collected on March 05, 1970 in the forest of the KOM valley, 25 km east of the confluence NTOM-KOM N°21978 /SRF/Cam. These barks were transported to the Laboratory of Nutrition and Nutritional Biochemistry (LNNB) of the Department of Biochemistry of the University of Yaoundé I, where they were washed, cut into small pieces, dried in the open air until obtaining constant weight, then ground in a blender (Philips Stay Fresh HR3752/00) and aqueous extraction was performed.

2.2. Preparation of the aqueous extract of the barks of M. laurentii (AEML)

Five hundred grammes (500 g) of powder of *M. laurentii* bark was dissolved in 5 L of distilled water (1:10 W/V) and macerated at room temperature for 24 h. The supernatant was collected and then filtered using Whatman No. 3 paper (Whatman International Limited, Ken, England) and the filtrates obtained were dried in

an oven (WGLL-65BE) at 60° C for 72 h to evaporating the solvent which made it possible to obtain the aqueous extract of *M. laurentii* barks**.**

2.3. In vitro study

2.3.1. Evaluation of antihyperglycemic potential

The antihyperglycemic potential was assessed via the inhibition of carbohydrate digestion by α-amylase and invertase tests.

α-amylase inhibition Assay

The α-amylase activity was evaluated according to the protocol described by Foo *et al.* (1998) with modifications and using the Chronolab test kit. α-Amylase (AMS) hydrolyzes 2-chloro-4-nitrophenyl-α-D-maltotrioside (CNPG3) to release 2-chloro-4-nitrophenyl-α-D-maltoside (CNPG2), glucose (G), 2-chloro-4-nitrophenol (CNP) and maltotriose (G3). The initial rate measuring CNP formation was proportional to the catalytic enzyme concentration. Absorbance was read at 405 nm. In the presence of extracts at different concentrations, a reduction in absorbance was interpreted as an inhibition of enzymatic activity. Briefly, a volume of 75 µL of reagent R was diluted in 6 mL of distilled water. Then, 550 mL of diluted reagent R was mixed with 100 µL of extract at increasing concentrations (0.125; 0.25; 0.5; 0.75 and 1 mg/mL). The control was carried out by replacing the extract with water. The whole was pre-incubated at 37°C for 3 min, then 20 µL of α-amylase (0.31 mg/mL) was introduced into the reaction medium. The mixture was incubated for 10 min at 37° C. then brought to a boiling water bath at 100° C. for 5 min to stop the reaction. Another test tube containing no enzyme, but 40 µL of phosphate buffer served as a blank. The tests were carried out in triplicate. The results were expressed as percentage inhibition.

$$\text{Inhibition (\%)} = \frac{(\text{Abs control} - \text{Abs sample})}{\text{Abscontrol}} \times 100$$

Invertase inhibition Assay

Invertase activity was assessed after hydrolysis of sucrose to form glucose.

Briefly, a volume of 290 µL of phosphate buffer was added to 40 µL of enzyme and 250 µL of sucrose, the mixture was pre-incubated at 37 ^{0}C for 10 min. Then, 40 µL of plant extract prepared at different concentrations (0.5; 1; 1.5; 2; 2.5; 5 and 10 mg/mL) were added. The control was carried out by replacing the extract with 40 µL of phosphate buffer. After 15 min of incubation at 37°C, the enzymatic reaction was stopped by heating at 100°C in a water bath for 5 min. The glucose present in the reaction medium was measured using the glucose reagent according to the method of Trinder (1959). The absorbance of the solutions in each tube was read at 505 nm. Another test tube containing no enzyme served as a blank and the tests were carried out in triplicate. The results were expressed as percentage inhibition:

$$\text{Inhibition (\%)} = \frac{(\text{Abs controle} - \text{Abs sample})}{\text{Abscontrol}}$$

2.3.2. Evaluation of antioxidant potential

The in vitro antioxidant potential of the extract was evaluated according to 3 mechanisms: scavenging of the DPPH radical, iron3+ reducing power and metal chelation.

DPPH radical scavenging

The anti-radical activity of the extract prepared in ethanol at different concentrations (2; 2.5; 3; 3.5 and 4 mg/mL) was evaluated using the DPPH method (Katalinié *et al.,* 2004). For this, a volume of 50 µL of extract at different concentrations was added to 1.950 mL of the freshly prepared methanolic solution of DPPH (0.3 mM). The mixture was incubated in the dark for 30 minutes. The control consisting of DPPH without extract was treated under the same experimental conditions as the tests. Absorbance was read at 515 nm using a spectrophotometer. Catechin was used as a reference.

The trapping percentages were calculated according to the following formula

$$\text{radical scavenging(\%)} = \frac{(\text{Abs control} - \text{Abs sample})}{\text{Abscontrol}} \times 100$$

The scavenging concentration 50 (SC50) parameter was used for the interpretation of the results (Brand-Williams *et al.,* 1996).

Reducing power of iron III

The reducing power of the extract was evaluated via its ability to reduce ferric iron to ferrous iron (Oyaizu *et al.,* 1986). To 1.25 mL of phosphate buffer (pH 6.6; 200 mM), were added 1.25 mL of potassium ferrocyanide (1%) and 0.5 mL of extract prepared at a concentration of 4 mg/mL. The whole was incubated at 50°C for 20 min. Subsequently, 2.5 mL of

trichloroacetic acid (10%) was added and the reaction mixture was centrifuged at 3000g for 10 min. Then 1.25 mL of the supernatant was mixed with 1.25 mL of distilled water and 0.25 mL of iron chloride (0.1%). The absorbance of the final solution was read at 700 nm against the blank prepared in parallel by replacing the extract with distilled water. Catechin was used as a reference.

The results were expressed as percentage reduction according to the formula:

$$\text{reduction of Fe3} + (\%) = \frac{(\text{Abs control} - \text{Abssample})}{\text{Abscontrol}} \times 100$$

Hemolysis inhibition Assay

The method used was that of Arbos *et al.* (2008). Blood from a mal rat (150 g) was collected in a tube containing EDTA and the red blood cells were separated from the supernatant (plasma and white blood cell layer) by centrifugation at 1500 g for 15 minutes. The red blood cells thus obtained were suspended and washed 3 times with NaCl (0.9%). The suspension of red blood cells (hematocrit at 33%) thus obtained was subjected to gentle agitation and then used to evaluate the effect of the extract on the hemolysis induced by Iron3+. A volume of 0.2 mL of extract (5-6.25-7.5-8.75 and 10 mg/mL) prepared in water was mixed with 1 mL of NaCl (0.9%). To the mixture was added 0.1 mL of the red cell suspension and the whole was incubated at room temperature for 30 minutes, then 0.1 mL of CuSO4 (0.1 M) was added to induce hemolysis. After 30 minutes of incubation at room temperature, the absorbance was read at 532 nm against a blank made from 1.3 mL of NaCl and 0.1 mL of the suspension. Total hemolysis (control) was performed by adding 1.2 mL of NaCl (0.9%); 0.1 mL of the suspension for 30 minutes followed by the addition of 0.1 mL of CuSO4 (0.1 M). Catechin was used as a reference.

The results were expressed as a percentage of hemolysis protection according to the formula:

$$\text{inhibition } (\%) = \frac{(\text{Abs control} - \text{Abssample})}{\text{Abscontrol}} \times 100$$

2.4. In-vivo study

2.4.1. Animals

Twenty (20) male rats with average weight of 230 to 250g were provided by the animal facility of the Laboratory of Nutrition and Nutritional Biochemistry (LNNB) of the Department of Biochemistry, Faculty of Sciences of the University of Yaounde I. None of these animals had not been subject to previous experiments and showed no signs of abnormalities. They were placed in standard cages (plastic basins) covered with mesh and received daily a normal diet (ND) of standard composition and tap water *ad libitum*. They were kept at room temperature and subjected to a 12-hour day-night cycle in good ventilation conditions and natural light. The litter was made of sawdust renewed every two days to ensure good hygienic conditions. The experimental protocol and the maintenance of the laboratory animals were carried out in accordance with the standard ethical guidelines for the use of laboratory animals and care as described in the guidelines of the European community and the ethics committee of the Faculty of Sciences of the University of Yaoundé I.

2.4.2. Design of the experimental protocol

The animals were randomized into two groups: a negative control (NC) group consisting of 5 rats and a group of 15 rats subjected to induction of hyperglycemia. The induction was done following the protocol of Al-Shamaony *et al.* (1994) with some modifications. After light ether anesthesia, the batch of 15 rats received intraperitoneally a dose of 45 mg/kgPc of streptozotocin (STZ) dissolved in citrate buffer (100 mM; pH 4.5; 150 mM NaCl). One hour after induction with STZ, the animals received glucose water (20%), in order to avoid hypoglycemic shock following the administration of STZ and 2 hours after food. After 48 hours, the blood glucose levels of the rats were taken by the glucose oxidase method using strips and a Gluco-Plus brand glucometer. Animals with blood glucose levels greater than or equal to 200 mg/dL were diagnosed as diabetic hyperglycemic (Chang, 2000); and left as follows for the rest of the experiment:

- Group I (negative control rats): non- hyperglycemic rats receiving distilled water by daily gavage;
- Group II (positive control): untreated hyperglycemic rats receiving distilled water by daily gavage;
- Group III (trial): hyperglycemic rats receiving *AEML* by daily gavage at a dose of 300mg/kgBW;
- Group IV (reference): hyperglycemic rats receiving metformin by daily gavage at a dose of 20mg/kgBW.

The volume of administration was 5 mL/kgBW. During the experiment which lasted 21 days, the weight of the rats was taken twice a week while the blood

glucose levels were taken for each group at regular time intervals of seven days until twenty- first (21st) days. The calculation of the percentage changes in blood glucose and weight was performed as follows:

Glycemic (%) $= \frac{Glycemic\,(GX) - Glycemic\,(G0)}{Glycemic\,(G0)} \times 100$;

G0: Blood glucose at day = 0, Gx: Blood glucose after each 7 days

Weight variation (%)

$$= \frac{(Finalweight - Initialweight)}{Finalweight\,(g)} \times 100$$

2.4.3. Euthanasia of animals and preparation of biological samples

At the end of the treatment, the animals were fasted for 12 hours and after light anesthesia with ether, the rats were euthanized by cardiac puncture. Blood was collected in dry tubes for serum preparation and EDTA tubes for plasma and hemolysate preparation. Blood collected in EDTA tubes was centrifuged (10 min, 900g, 25°C) and plasma was collected, aliquoted and stored in Eppendorf tubes at -20°C for biochemical assays. Subsequently, a volume of 100 µL of pellet was introduced into conical tubes then washed in 2 mL of an NaCl solution (0.9%) and centrifuged (10 min, 900 g, 25°C). This process was repeated twice. Hemolysis of erythrocytes is carried out by adding 2 mL of distilled water followed by centrifugation at 900 g for 10 min at 25°C. The supernatant (hemolysate) was collected, aliquoted and stored at -20°C. The liver was removed by dissection, it was washed in the NaCl solution (0.9%), drained, weighed and ground separately in a mortar; then the ground material was homogenized in 10% (weight/volume) of 0.1 M phosphate buffer, pH 7.4. The homogenates obtained were stored at -20°C.

2.4.4. Effect on oxidative stress

The markers evaluated were erythrocyte catalase activity according to the method described by Sinha (1972) and hepatic malondialdehyde (MDA), a marker of lipid peroxidation according to the method described by Yagi (1976).

2.4.5. Effect on immunological parameters

Hematological parameters were analyzed using a Beckman Coulter Medonic. The parameters analyzed were: White Blood Cells, Neutrophils, Monocytes and Lymphocytes. The C-reactive protein (CRP) assay was performed according to the protocol of Ritchie *et al.* (1971), based here on turbidimetric measurements.

2.4.6. Effect on plasma lipid profile and atherogenic risks

Total cholesterol (TC), triglycerides (TG) and high-density lipoprotein cholesterol (HDL-c) were evaluated in plasma using the chronolab kits. Low density lipoprotein cholesterol (LDL-c) and very low-density lipoprotein cholesterol (VLDL-c) were determined from the formula of Friedewald, Levy and Friedrickson (1972).

$$LDL - c\ (mg/dL) = TC - (HDL - c + TG/5)$$

$$VLDL - c\ (mg/dL) = TG/5$$

The lipid profile parameters were used to evaluate the Atherogenic Index of Plasma (AIP) (Althunibat *et al.,* 2019), Atherogenic Coefficient (AC) (Ikewuchi and Ikewuchi, 2009), Cardiac Risk Ratio (CRR) (Ikewuchi and Ikewuchi, 2009), and Cardioprotective Index (CPI) (Oršoli´*et al.,* 2014) according to the following formulas:

$CRR1 = \frac{TG}{HDL-C}$; $CRR2 = \frac{LDL-c}{HDL-C}$

$AC = \frac{TG-HDL-C}{HDL-C}$;

$AIP = \log\frac{TG}{HDL-C}$

$CPI = \frac{HDL-C}{LDL-C}$

2.5. Statistical analysis

Results were expressed as the mean ± standard error of the mean and percent change. Statistical analysis was performed using SPSS (Statistical Package for Social Science) version 20.0 for Windows. The one-way ANOVA (Analysis Of Variance) test followed by a Tukey post-hoc test was used for the comparison between the groups. Results with $P<0.05$ were considered significantly different. The Excel software made it possible to process the results and draw the graphs.

3. Results and discussion

3.1. Effect of extract on inhibition of α-amylase and invertase activity

The Ic_{50} of the aqueous extract of *M. laurentii* bark are shown in Table 1. The extract inhibited invertase and alpha amylase with Ic_{50} of 0.015 and 0.38 mg/mL respectively. Compared to the reference drug (acarbose), the extract showed greater inhibitory activity on alpha amylase. This effect could be attributed to the presence of flavonoids such as flavonols or isoflavones which are potential inhibitors of α-amylase and α-glucosidase (Mojica *et al.,* 2015). Indeed, the work of Gaikwad *et al.* (2014) showed that compounds such as berberines and epicatechins (flavonoids) inhibit α-glucosidase by preventing the binding of the enzyme to its substrate which is sucrose.

Table 1. Inhibition concentration (IC_{50}) of the extract.

Enzyms	IC_{50} (mg/mL)	
	AEML	Acarbose
Alpha-amylase	0.38	2.73
Invertase	0.015	/

3.2. Antioxidant potential of the extract

The IC_{50} of the aqueous extract of *M. laurentii* bark are shown in Table 2. The extract showed DPPH• radical scavenging activity and protection of the erythrocyte membrane against the effects of Cu^{2+} pro-oxidants by chelation him. The IC_{50} were 333.60 and 2.354.28 mg/mL respectively for DPPH• trapping and hemolysis inhibition. The extract showed lower antiradical and antihemolytic activity than catechin. Similarly, the extract reduced ferric ions in a concentration-dependent manner. The percentages of reduction ranged from 61.04 to 69.63% respectively. The extract showed a reduction capacity of Fe^{3+} lower than that of catechin. This anti-radical activity would result from the content of polyphenols and in particular flavonoids in the extract, which, by the redox potential of their OH groups, would be capable of yielding a proton and/or an electron, thus trapping the DPPH radical. Indeed, the low potential redox of flavonoids (FLOH) makes them thermodynamically capable of reducing free radicals (R•) by transferring hydrogen atoms from hydroxyl groups. This reaction gives rise to the flavonoxyl radical (FLO•) and to the stable radical molecule (RH), the FLO• will subsequently undergo a structural rearrangement allowing the redistribution of the single electron on the aromatic ring and the stabilization of the aroxyl radical (Prochazkova *et al.,* 2011). The electron-donating capacity of these phenolic compounds was substantiated via the ability of the extracts to reduce ferric iron to ferrous iron (Table 3). Finally, the extract chelated the Cu^2+ ions, chelation materialized by the inhibition of hemolysis (Table 2). This activity would be due to the presence of phenolic compounds which would prevent hemolytic effects induced by Cu^{2+} ions and would protect against the stiffening of the erythrocyte membrane.

Table 2. Antiradical activity and chelating power of the extract.

Ic_{50}	IC_{50} DPPH (mg/mL)	IC_{50} hémolyse (mg/mL)
Extract	333.60	2.35
Catechin	1.45	2.30

Table 3. Antiradical activity and chelating power of the extract

Concentrations	2 mg/mL	2.5 mg/mL	3 mg/mL	3.5 mg/mL	4 mg/mL
% Reduction Fe^{3+}	61.04± 0.11	62.25±0.04	65.40±0.09	67.81±0.04	69.63±0.05
Catechin					51.09±2.2

Values are expressed as mean ± standard error.

3.4. Influence of AEML on the evolution of body weight

The variation in body weight observed during the 21 days of experimentation is shown in Figure 1. A significant decrease in weight (P<0.05) is revealed throughout the untreated hyperglycemic rats compared to the normoglycemic control group (NC). However, the groups treated with the extract and the reference drug (metformin) significantly gained weight (P<0.05) from the 14th week. These beneficial effects of the extract could be due to its high in polyphenols. Indeed, although it is established that phenolic compounds have the ability to reduce overweight by inhibiting the expression of genes involved in lipogenesis, digestion and absorption of lipids (Keunae and Hwang, 2011). The limitation of weight loss observed in the group treated with extract can be explained by the fact that; phenolic compounds in addition to their role in activating lipolysis; also play a role in regulation of lipid metabolism. Like the extract, the reference drug (metformin) also limited this weight loss from the 14th day, which suggests that this antidiabetic drug could also play a role in regulation of lipid metabolism.

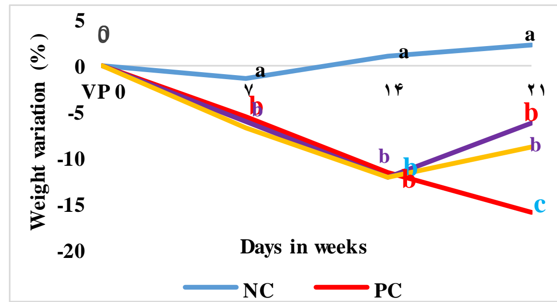

Figure 1: Effect of AEML on the weight variation of experimental rats. Values are expressed as mean ± standard error. NC: Negative control; PC: Positive control; AEML: rats treated with 300 mg/kg of *M. laurentti* bark extract; REF: Reference (diabetic rats + 20mg/Kg of metformin. The points assigned different letters (a, b, c, d) are significantly different (P<0.05).

3.5. Effect of the extract on fasting hyperglycemia

Table 4 shows the result of the change in glycemia throughout the treatment, expressed as mean glycaemia and as a percentage change. The injection of streptozotocin caused a significant increase in blood sugar in all groups of animals (PC, *AEML* and REF) compared to the normoglycemic control group(440.6 mg/dL versus 70 mg/dL). After 21 days of treatment, the extract caused a significant decrease in blood sugar from the 14th day (-9.99%), which increased on the 21st (-28.44%). The extract was less effective than metformin which resulted in a reduction of -32.75% on the 21st day. Indeed, streptozotocin is the most widely used chemical in the induction of diabetes (Manik *et al.,* 2017). It's a nitrosated glucosamine that causes a selective cytotoxic effect on β-cells of islets of Langerhans (Manik *et al.,* 2017). Thus, its administration caused a sudden increase in glycemia in all groups of animals (PC, *AEML* and REF) compared to the normoglycemic control group. The administration of the extract significantly reduced blood sugar from the 7th day of treatment. These results could be explained by the presence of alkaloids in the extract which have the ability to stimulate insulin secretion by residual beta cells and to inhibit glucose-6-phospatase and phosphoenolpyruvate carboxykinase; keys enzymes of gluconeogenesis (Djaouida *et al.,* 2019). Moreover, postprandial hyperglycemia has being strongly involved in the occurrence of chronic hyperglycemia; the ability of the extract to lower blood sugar could also partly due to the ability of the alkaloids and flavonoids found in the extract to inhibit carbohydrate digestion enzymes (β-galactosidase, α-glucosidase, α-amylase and invertase) (Znifeche., 2019).

Table 4: Change in fasting glycaemia after administration of STZ in rats

Groupes		t= 0 day	t= 7 days	t= 14 days	t= 21 days
Negative control (NC)	Blood glucose (mg/dL)	70.0±2.4	75.0±3.27	85.0±8.28	65.5±3.32
	Variation (%)	(0)	(7.21) ^{a}	(21.73) ^{a}	(-6.41) ^{a}
Positive control (PC)	Blood glucose (mg/dL)	440.6±20.22	451.4±63.99	490.4±47.56	493.4±30.05
	Variation (%)	(0)	(2.19) ^{b}	(11.31) ^{b}	(12.27) ^{b}
AEML (300 mg/kg .BW)	Blood glucose (mg/dL)	489.4±59.13	453±7.45	433.8±23.65	346.2±39.07
	Variation (%)	(0)	(-6.45) ^{b}	(-9.99) ^{c}	(-28.44) ^{c}
REF (20 mg/kg.BW)	Blood glucose (mg/dL)	494.2±42.66	470.0±45.68	375.2±49.87	332.33±26
	Variation (%)	(0)	(-4.64) ^{c}	(-23.15) ^{d}	(-32.75) ^{b}

Values are expressed as mean ± standard deviation, NC: Negative control; PC: Positive control; AEML: group treated with 300mg/Kg BW of aqueous extract of *M. laurentti* barks; REF: diabetic rats + 20mg/Kg BW of metformin values in parentheses represent percentage changes in blood glucose levels; values assigned different letters (a, b, c, d) are significantly different between groups at the same time (P<0.05).

3.6. Effect of the extract on endogenous oxidative status

Figure 2 shows the results of the effect of the extract on catalase activity and plasma malondialdehyde (MDA) concentration. There is a significant decrease (P<0.05) in erythrocyte catalase activity (Figure 2A), as well as an increase in plasma MDA levels (Figure 2B) in rats of the untreated hyperglycemic group (PC) compared to the normoglycemic group (NC). However, administration of the extract resulted in a significant increase in catalase activity and a significant reduction in plasma MDA levels. Compared to the reference group, the extract showed significantly higher

activities. Oxidative stress is a factor involved in the development of vascular lesions leading to the complications associated with diabetes. It is involved in the abnormal activation of pro-inflammatory signaling pathways leading to increased secretion of pro-inflammatory cytokines such as IL-6, TNF-α which induce the production of CRP in the liver (Saeed *et al.,* 2017). In contrast, inflammation accelerates the production of reactive oxygen species and decreases the body's antioxidant defense capacity. The extract improved endogenous antioxidant status by increasing erythrocyte catalase activity while reducing hepatic MDA levels. This could be explained by the ability of the flavonoids in the extract to inhibit the expression of the KEAP1 gene. Indeed, the KEAP1 protein is known to bind to factor 2 linked to Nrf2 and promote its degradation. Nrf2 is known to activate several antioxidant enzymes such as Catalase (Mallique *et al.,* 2020).

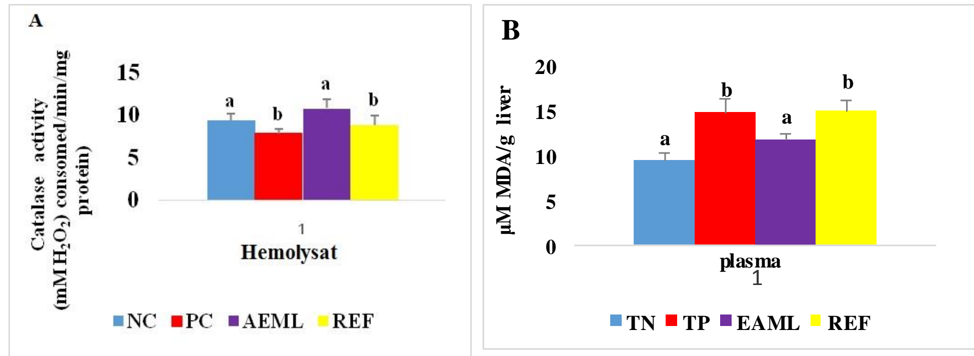

Figure 2: Effect of EAML on endogenous oxidative status. (**A**) Effect of AEML on catalase activity, (**B**) Effect of AEML on lipid peroxidation. The values are expressed as the mean ± standard deviation, the values in parentheses represent the variations in blood glucose levels as a percentage; Values assigned different letters (a, b, c, d) are significantly different (P<0.05).

3.7. Effect of AEML on some inflammatory parameters

Table 5 shows the effect of AEML on serum C-reactive protein concentration. There is a significant increase (P<0.05) in the serum concentration of C-reactive protein in the rats of the PC group compared to the NC group (17.06 versus 8.99 µg/dL). Administration of the extract led to a significant drop in this concentration compared to the PC group (9.5 versus 17.06 µg/dL). The effect of the extract was comparable to that obtained with the reference drug (metformin). Table 6 shows that a significant increase (P<0.05) in white blood cells, lymphocytes and monocytes was observed in the rats of the untreated hyperglycemic group compared to the normoglycemic control group. The administration of the extract led to a significant drop in these hematology parameters. An inflammatory state is associated with a high production of inflammatory cells (Qingliang *et al.,* 2019); this is why the determination of inflammatory cells (white blood cells, lymphocytes, Neutrophils) in this study; revealed a significant increase in those of white blood cells, including lymphocytes and Neutrophils; in the untreated hyperglycemia group compared to the normoglycemic control group. The increase in those of white blood cells in untreated hyperglycemic rats could be the result of anemia (Rao *et al.,* 2003). Indeed, a variation in hematological parameters in a subject is synonymous with an anomaly in the functioning, morphology or metabolism of erythrocytes, leukocytes and platelets (Effozougba *et al.,* 2019). The non-variation of the hematological parameters in the treated rats compared to the normoglycemic control rats could mean that our extract would not affect the hematological parameters of the treated hyperglycemic rats, but would rather act as a regulator of these parameters.

Table 5: Effects of the extract on serum C-reactive protein concentration

Groups	NC	PC	AEML	REF
Concentration (µg/dL)	8.99±0.36 [a]	17.06±1 [b]	9.5±0.58 [a]	9.53±0.09 [a]

Values are expressed as mean ± standard error. NC: Negative control; PC: Positive control; AEML: rats treated with 300 mg/kg BW of *M. laurentti* bark; REF: Reference (diabetic rats + 20mg/Kg BW of metformin. The points assigned different letters (a, b, c, d) are significantly different (P<0.05).

Table 6: Effect of extract on hematological parameters.

Parameters	NC	PC	AEML	REF
White blood cells (10^3/µL)	10.88 ± 0.47[a]	16.04 ± 1.00[b]	12.23 ± 1. 43[a]	10.31±0.88[a]
Neutrophils (%)	14.8 ± 3.92[a]	22.1 ± 10.6[b]	16.17 ± 3.05[a]	31.35±0.75[b]
Lymphocyts (%)	72.87 ± 3.05[a]	82.53 ± 5.1[b]	73.04 ± 2.1[a]	58.8 ± 0.2[c]

Values are expressed as mean ± standard error. NC: Negative control; PC: Positive control; AEML: rats treated with 300 mg/kg BW of *M. laurentti* bark; REF: Reference: diabetic rats+ 20mg/Kg BW of metformin. The points assigned different letters (a, b, c, d) are significantly different (P<0.05).

3.8. Effect of the extract dyslipidemia and Atherogenic **risks**

It appears from Table 7 that hyperglycemia led to an alteration of lipid metabolism in animals, characterized by a significant increase (P<0.05) in the content of triglyceride, total cholesterol, VLDL-cholesterol and LDL-cholesterol as well a decrease in HDL-cholesterol in the untreated hyperglycemic group (PC) compared to the normoglycemic group (NC). However, the administration of the extract led to a positive improvement in these parameters, in particular the reduction in triglycerides, VLDL-cholesterol and LDL-cholesterol as well as an increase in HDL-cholesterol, just like the reference molecule. Table 8 represents the effect of the extract on the CRR, AC, AIP and CPI. It appears that the CRR, AC and AIP were higher in the rats of the positive control group compared to the normal group while ICP was lower in the untreated group compared to the normal group (P<0.05). The administration of the extract significantly reduced (P<0.05) the CRR, AC and AIP while increasing CPI. The effect of the extract was comparable to that of the reference medicine. The alteration of the action of insulin and the insulin deficiency observed during diabetes also lead to disorders in lipid metabolism characterized by hypertriglyceridemia, an increase in LDL cholesterol, and a decrease in HDL cholesterol (Kumar *et al.,* 2012). The results obtained in the present study show a significant increase in triglyceridemia, LDL cholesterol, VLDL cholesterol and a decrease in that of HDL cholesterol in rats of the untreated hyperglycemic group compared to the normoglycemic group (Table 7). These high levels of triglyceride, VLDL-cholesterol and LDL-cholesterol reflect the increase in CRR, AC, AIP and the decrease in CPI in these groups of animals (Table 8). Administration of the extract resulted in a decrease in triglycerides, LDL-C, VLDL-C and an increase in HDL-C. These hypotriglyceridemic and hypocholesterolemic properties of the extract are reflected by the drop in CRR, AC, AIP and by the increase in CPI in these groups of animals. This effect of the extract could further be attributed to their polyphenol content, which are capable of stimulating lipoprotein lipase and inhibiting HMG-CoA reductase, which are key enzymes involved respectively in triglyceride catabolism and cholesterol synthesis (Abdulazeez, 2011).

Table 7: Effect of the extract on dyslipidemia induced by hyperglycemia.

	NC	PC	AEML	Reference group
TG (mg/dL)	74.61±0.9[a]	122.71±1.0[b]	45.69±0.60[c]	61.98±2.52[a]
TC (mg/dL)	119.26±1.82[a]	170.58±3.72[a]	90.31±2.56[b]	177.04±8.64[a]
VLDL-C(mg/dL)	14.92±0.44[a]	24.54±0.80[b]	9.31±1.44[c]	11.59±4.54[a]
LDL-C(mg/dL)	94.27±1.18[a]	163.35±0 .38[b]	48.68±0.64[c]	31.76±1.82[d]
HDL-C(mg/dL)	40.90±1.29[a]	31.76±3.72[b]	50.77±1.05[c]	41.66±1.74[a]

Values are expressed as mean ± standard error. NC: Negative control; PC: Positive control; AEML: rats treated with 300 mg/kgBW of *M. laurentti* bark; Reference: diabetic rats + 20mg/Kg BW of metformin. TG: Triglycerides, TC: Total cholesterol, VLDL-C: Very low-density lipoprotein cholesterol, LDL-C: Low density lipoprotein cholesterol, HDL-C: High density lipoprotein cholesterol. The values assigned different letters (a, b, c, d) are significantly different (P<0.05).

Table 8: Effect of the extract on Atherogenic risks.

	NC (negative control)	PC (positive control)	AEML (treated group)	Reference group
CRR1	1.86±0.16[a]	3.86±1.04[b]	0.90±0.85[c]	1.39±1.01[a]
CRR2	2.36±0.05[a]	5.14±0.85[b]	0.95±0.06[c]	0.76±0.55[c]
AC	73.61±0.45[a]	121.71±2.66[b]	44.69±0.77[c]	56.98±1.24[a]
AIP	0,27±0,001[a]	0.58±0.05[b]	0.04±0.001[c]	0.14±0.001[d]
CPI	0.42±0.02[a]	0.19±0.01[b]	1.04±0.03[c]	1.31±0.07[c]

Values are expressed as mean ± standard error. NC: Negative control; PC: Positive control; AEML: rats treated with 300 mg/kg of *M. laurentti* bark; Reference: diabetic rats + 20mg/Kg of metformin. CCR: Cardiac Risk Ratio, AC: Atherogenic Coefficient, IAP: Atherogenic Plasma Index, ICP: Cardio-Protective Index. Values assigned different letters (a, b, c, d) are significantly different (P<0.05).

4. Conclusion

The results of this study reveal that the aqueous extract of *M. laurentii* bark has anti-diabetic, anti-hyperlipidemic and antioxidant properties; therefore, could be used in the management of diabetes and its complications. This work highlights the beneficial effects of *M. laurentii* traditionally used in the management of diabetes. In order to refine our work, we propose to evaluate the effect of the extract on the regulatory capacity of pancreatic beta cells in culture, on the activity of HMG-CoA reductase as well as to characterize the bioactives compounds present in our extract that may be responsible for its biological effects.

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