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Antihyperglycemic and Antioxidant Activity of the Leave Powder Fractions of Psidium guajava L. (Myrtaceae) in High Caloric Sugar Diet Induced Type 2 Diabetic Rats

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ABSTRACT

Psidium guajava is a plant found in many tropical and subtropical regions of the world. The present study was conducted to evaluate the role of Psidium guajava leaf powder fractions in type 2 diabetic rats induced by a high-calorie diet combined with dexamethasone. Powder fractions (≤ 125; 125–200µm) of Psidium guajava leaves were prepared using controlled differential pulverization and sieving, and unsieved powder was obtained by simple grinding. 300 mg/kg of one of the different fractions or the unsieved powder was administered daily, in conjunction with a high-calorie diet, followed by an intraperitoneal injection of dexamethasone for six weeks, to normoglycemic rats.

Six weeks after the induction of diabetes, and regarding antidiuretic activity, the powder fraction ≤ 125 µm maintained fasting blood glucose at the lowest level (91.00 ± 5.31 mg/dL) compared to rats in the diabetic group (138 ± 7.60 mg/dL). Regarding the lipid profile, the total cholesterol level was significantly lower ($P < 0.01$) (50.1 ± 3.2 mg/dL) with the ≤ 125 µm fraction compared to that of rats in the diabetic group (116.3 ± 12.5 mg/dL). Regarding antioxidant activity, the malondialdehyde concentration with the ≤ 125 µm fraction was 6.35 ± 0.192 µM/g versus 14.95 ± 0.77 µM/g observed in rats in the diabetic group.

The leaves of Psidium guajava constitute a powerful antidiabetic agent that could be exploited as an alternative medicine against diabetes and its complications.

Keywords

Antidiabetic, Dexamethasone, Antioxidant, Powder fractions, High-calorie diet, Blood glucose.

Introduction

The Controlled Differential Spraying and Sieving (CDS) process is a dry method for concentrating active ingredients, combining drying, grinding, and sieving operations, patented PTC/W20137379A1 [1,2]. It was developed to replace conventional methods of extracting active plant ingredients (decoction, infusion, hydro distillation, etc.) which can affect the quality and safety of the extracts [1]. This method offers several advantages, including

ease of implementation, a significant reduction in solvent use, application to a wider range of bioactive ingredients with varying sizes and molecular weights, and good preservation of the extracts [1,3]. To validate this tool, which is already gaining popularity, scientific experiments have been conducted on various models. The efficacy of the powder fractions has been validated for the antioxidant [3-7], antiacetylcholinesterase [3], cytoprotective [6], and larvicidal [8] properties of certain plant matrices. The powder fractions have also been explored in the context of metabolic diseases through the evaluation of their antihyperlipidemic properties [9]. However, to the best of our knowledge, the effects of the powder fractions on antidiabetic activities remain unknown.

Globally, the number of people with diabetes was estimated at 463 million in 2018. In Africa, this figure was 19.4 million, with a projected increase to 47.1 million by 2045 [10]. In Cameroon, 6% of the national population was at risk of developing diabetes mellitus and its associated complications (stroke, kidney failure, retinopathy, neuropathy, and leg amputation) [11,12]. Diabetes management can be approached first-line through lifestyle modifications (diet and physical activity) and second-line through insulin injections and oral antidiabetic drugs such as sulfonylureas, glinides, biguanides, glitazones, and alpha-glucosidase inhibitors [13,14]. While these medications do have proven antidiabetic effects, they are not without harmful side effects such as the risk of hypoglycemia and lactic acidosis, and their limited accessibility in rural areas due to their cost [15]. A serious alternative, encouraged by the World Health Organization, is herbal medicine, which offers several advantages: low purchase cost, wide availability, interesting pharmacological activities, and fewer side effects [16]. In this context, our interest focused on the leaves of *Psidium guajava* (Myrtaceae), which are widely used in traditional medicine as an herbal tea to reduce blood sugar. Previously, several scientific studies demonstrated that extracts from the leaves of this plant possess proven antidiabetic properties [17-21]. However, in these studies, the extraction of active ingredients from this plant matrix was carried out using methods involving organic solvents, including ethanolic extraction [17,18], chloroform extraction, hexane extraction and ethyl acetate extraction [21,22]. These methods present several problems, such as the use of large quantities of organic solvents harmful to human health and the constraints related to solvent evaporation, leaving a high level of extraction solvent residues in the extract, which can be toxic [1]. This study aims at demonstrate that the powder fraction extraction process is a much more environmentally friendly and effective method. It thus contributes to the prevention or reduction of diabetes prevalence through the use of antidiabetic plants whose bioactive ingredients have been extracted ecologically.

The general objective of this study is to evaluate the effects of the powder fractions on the antidiabetic activities of *Psidium guajava* leaves in Wistar strain rats.

Materials and Methods

Chemicals and plant material

All chemicals used in this study were of standard analytical purity and were purchased from Sigma Aldrich (St. Louis, USA). The transaminase assay kit was obtained from BIOLABO (Maizy, France). Young leaves of *Psidium guajava* were harvested in May 2024 in the locality of Dang, Ngaoundéré 3rd District (Adamawa Region – Cameroon).

Production of powder fractions from Psidium guajava leaves by the controlled differential pulverization and sieving method

The harvested young leaves of *Psidium guajava* were washed and then shade-dried for 7 days before being pounded in a mortar to reduce the particle size and facilitate their passage through the electric grinder. The pounded leaves were fed into a Biobase Disintegrator (Model MPD-102) electric mill to produce fine powder fractions. The resulting powder was divided into two equal parts. The first part was passed through a sieve column to obtain the particle size fractions using an Endecotts (Model Minor 1332-06) sieve shaker, following the method described by Deli et al. [2]. This first portion of powder was deposited on top of a stack of five sieves with decreasing mesh sizes: >355 µm; 355-250 µm; 250-200 µm; 200-125 µm and ≤ 125 µm particles were placed on a platform directly connected to the sifter motor shaft. Five particle size fractions of powder were obtained, of which the ≤ 125 µm and 125-200 µm fractions were the finest and were selected for further study simply because these finer fractions are rich in antidiabetic bioactive compounds [1]. The remaining unsieved powder was used for the second portion of the sample.

Qualitative Phytochemical Analysis

Powder fractions from *Psidium guajava* leaves were screened for the detection of different families of chemical compounds according to the standard method described by Irayya Gurayya et al. [22].

Evaluation of the hypoglycemic and antidiabetic activity of powder fractions from *Psidium guajava* leaves Animal material

The animal material for this study consisted of adult male Wistar rats (Rattus norvegicus) aged 8 to 10 weeks, with a body mass between 150 and 170 g. These rats were reared at the Animal Facility of the Biophysics Laboratory, Food Biochemistry and Nutrition (LABBAN) of the National School of Agro-Industrial Sciences (ENSAI) at the University of Ngaoundere. They were kept at room temperature with alternating 12-hour light/12-hour dark cycles and had free access to standard feed and tap water. After rearing, the animals were adapted to these conditions for one week before the start of the experiments. The animals were treated in accordance with the guidelines of the European Union Directive on the Ethical Evaluation of Animal Experiments (EEC Directive 2010/63/EEC).

Evaluation of hypoglycemic activity

The hypoglycemic activity was evaluated according to the protocol described by Dzeufiet et al. [23]. This protocol involved force-feeding normal glycemic rats, which had been fasted for 15 hours, with only the ≤ 125 µm, 125–200 µm, and unsieved powder of *Psidium guajava* leaves for the test groups, while the normal control group was force-fed with distilled water. The plant leaf

powder fractions were administered using a feeding tube. Blood glucose levels were measured at 0 hours before treatment and then successively at 30 minutes, 1 hour, 2 hours, 3 hours, 5 hours, and 7 hours after treatment administration. To do this, a drop of blood was taken from a notch made on the distal end of each animal's tail onto the reactive area of a test strip mounted on a "One Touch Ultra" type glucometer for automatic blood glucose reading.

Evaluation of Antidiabetic Activities

Induction du diabète de type 2

The type 2 diabetes was induced using hypercaloric sugar diet (HSD). As shown in Table 1 below, following the MACAPOS1 model (Maize Cassava Palm Oil and Sucrose) [24] modified by the additional intraperitoneal administration of dexamethasone (NDC 57319–519-05, Phoenix) at 0.2 mg/kg body weight [25], once daily for six weeks. The treatment consisted of administering metformin, powder fractions ≤ 125 µm, 125–200 µm, or unsieved powder.

Table 1: Composition of the sugary high-calorie diet and the normal diet [24].

Ingredients	Normal Diet (g/kg)	HSD (g/kg)
Corn	250	300
Wheat	100	250
Soy	100	100
Fish powder	120	30
Sucrose	-	80
Palm oil	-	100
Farine d'os	25	25
Palm kernel meals	50	-
Cassava	-	30
Salt	05	05
Vitamin mix	10	10
Energy kcal/kg	2530	4060

Abbreviation: HSD, high-caloric- sucrose Diet.

Thirty (30) male rats with a body mass between 140 and 160 g were divided into six (6) groups of five (5) rats each and treated daily for 6 weeks as follows:

- Group 1: Normal control (NC) rats received a normal diet and distilled water (10 mL/kg/day);
- Group 2: Diabetic control (DC) rats received a high-caloric diet, 10 mL/kg/day of distilled water and sucrose (4 g/kg bw);
- Group 3: Metformin-treated (MET) rats received a high-caloric diet, Metformin Hydrochloride (NDC 0378–6001-91, Mylan Pharmaceuticals Inc.) (20 mg/kg bw) and sucrose (4 g/kg bw);
- Group 4: fractions-treated, rats received a high-caloric diet, 300 mg/kg of the fraction (≤ 125 µm) of *M. indica* leaves per os and sucrose (4 g/kg bw) per os;
- Group 5: fractions-treated rats received a high-caloric diet, 300 mg/kg of the fraction (120–200 µm) of *M. indica* leaves per os and sucrose (4 g/kg bw);
- Group 6: Unsieved powder-treated rats received a high-caloric sucrose diet, 300 mg/kg of unsieved powdered *M.indica* leaves per os, and 4 g/kg sucrose [26].

From the first to the sixth week of treatment, all animals received intraperitoneal dexamethasone (0.2 mg/kg body weight), except for the normal control group, which received intraperitoneal saline (5 ml/kg). At the end of the sixth week of treatment, when the fasting blood glucose of the diabetic control group animals was ≥ 126 mg/dL, an oral glucose tolerance test was performed to confirm the onset of type 2 diabetes (postprandial blood glucose ≥ 126 mg/dL). This test consisted of administering 4 g/kg body weight of D-glucose to the animals in each group. The blood glucose of the animals in each group was then measured just before administration of the treatment (distilled water, powder fraction, or unsieved powder), followed by the D-glucose treatment. Blood glucose was measured again every 30 minutes for 2 hours. Blood glucose levels were measured using a ONE TOUCH Ultra glucometer [27]. At the end of treatment, all rats were fasted for 15 hours and then anesthetized by intraperitoneal injection of a mixture of ketamine (50 mg/kg) and diazepam (10 mg/kg) [28]. After anesthesia, all rats were sacrificed by incision of the jugular vein, and blood was collected in dry tubes for measurement of lipid profile parameters and transaminases. The liver and kidneys of each animal were removed immediately after blood collection, rinsed in Ringer's lactate (RL) solution, drained, and 0.2 g of each organ was ground and homogenized in a buffer. The homogenate was then centrifuged at 3,000 rpm for 15 min, and the supernatant was collected and stored at −20 °C for the evaluation of oxidative stress parameters.

Biochemical Analysis

Fortress Diagnostics kits (BXC0317A, UK) were used to measure triglyceride (TG), total cholesterol (TC), and high density lipoprotein (HDL) cholesterol levels, according to the manufacturer's protocols. Low density lipoprotein (LDL) cholesterol concentrations were calculated from total cholesterol (TC), triglyceride (TG), and HDL cholesterol values using the following formula: [29]

$$[LDL - Cholesterol] = [TC] - [HDL - Cholesterol] - \frac{TC}{5} \text{ ---------- (1)}$$

Alanine aminotransferase (ALAT) and aspartate aminotransferase (ASAT) activities were assessed using Fortress diagnostic kits (BXC0212A and BXC0202A, respectively) following the manufacturer's protocols.

Serum creatinine was assessed using the kinetic method [30]. In this case, 100 µL of standard creatinine, 100 µL of distilled water, and 100 µL of sample were added to the standard tube, control tube, and test tube, each containing 500 µL of reaction medium. The entire mixture was homogenized, and the absorbances of the standard and test tubes were read spectrophotometrically at 500 nm against the blank at 30 and 90 s. The creatinine concentration was calculated as follows:

$$[Creat] = \frac{A_{sample}}{A_{Std}} \times C_{Std} \text{ ---------- (2)}$$

Where [Crea]: creatinine concentration (mg/dL); A_{sample}, absorbance of the sample; A_{std}, absorbance of the standard; C_{std}, standard concentration (200 mg/dL). For malondialdehyde (MDA)

determination, 125 µL of trichloroacetic acid (TCA, 20%) and 250 µL of thiobarbituric acid (TBA, 0.67%) were added to test tubes containing 250 µL of Tris–HCl buffer (50 mM, pH 7.4) or homogenate. The tubes were subsequently capped with glass beads, heated at 90 °C in a water bath for 10 minutes, cooled with tap water, and centrifuged at 3000 rpm for 15 minutes at room temperature using an ANKE LD-6000B centrifuge. The supernatant was pipetted and the absorbance was read at 530 nm against the blank [31]. The MDA concentration of each sample was obtained from the following formula:

$$[MDA] = \frac{\Delta DO}{\varepsilon * L * m} \quad \text{----------(3)}$$

Where [MDA]: MDA concentration (mol/g of organs); ΔDO, optical density of the assay-optical density of the blank; L, optical path length (1 cm); ε, molar extinction coefficient (13,600 $mol^{-1}.cm^{-1}$); m, organ mass (g).

For the superoxide dismutase (SOD) assay, 200 µL of adrenaline (0.3 mM) and 1666 µL of carbonate buffer (0.05 M, pH 10.2) were added to the test tubes containing 134 µL of sample. In the blank tubes, 200 µL of adrenaline (0.3 mM) was added to 1800 µL of carbonate buffer (0.05 M, pH 10.2). After homogenization of all the different tubes, the absorbances of the test tubes were read against the blank at 480 nm at 20 and 80 s [31] SOD activity was determined according to the formula:

$$\%\boldsymbol{Inhibition} = \mathbf{100} - \frac{(A_{20s} - A_{80s})test}{(A_{20s} - A_{80s})blank} \times \mathbf{100} \quad \text{---------- (4)}$$

Where A_{20s}: Absorbance measured at 20 s; A_{80s}, Absorbance measured at 80 s.

For catalase (CAT) assay, 187.5 µL of phosphate buffer (0.1 mM; pH 7.5) was added to a test tube containing 12.5 µL of homogenates and 12.5 µL of distilled water for the control tube. 50 µL of hydrogen peroxide (50 mM) was added to each tube and incubated for 1 minute at room temperature. 500 µL of potassium dichromate/glacial acetic acid (5%) was then added to all tubes and the mixture was heated to 100 °C for 10 minutes. After cooling the tubes, the absorbance of the tubes was read at 570 nm relative to the control tube [32]. The specific activity of catalase was determined according to the formula:

$$\boldsymbol{Act\ CAT} = \frac{A_{test} - A_{blank}}{a \times t \times m} \quad \text{---------- (5)}$$

where Act CAT: catalase activity (mM H_2O_2/min/g organs); A.test, absorbance of the test tubes; A_{blank},: absorbance of the blank tube; **a**, slope of the calibration curve; **t,** reaction time (1 min); **m**, mass of test organ (g).

Analyses statistiques

The results obtained were expressed as mean ± standard error of mean (SEM). Data processing was carried out using Microsoft Excel 2021 software and their exploration was done using Graph Pad Prism software version 5.03. The analysis of variance test "Two-way ANOVA" followed by Turkey's multiple comparison test was carried out using GraphPad prism 10.1.0 software.

Results

Qualitative phytochemical composition of powder fractions of Psidium guajava leaves Qualitative phytochemical analysis of PTC powder fractions and unsieved powder of Psidium guajava leaves revealed the presence of polyphenols, flavonoids, triterpenes, tannins and saponins in all extracts and alkaloids only in the unsieved powder (Table 2).

Table 2: Qualitative phytochemical characteristics of powder fractions and unsieved powder of Psidium guajava.

Composés	Fraction ≤ 125 µm	Fraction 125 – 200 µm	Poudre non tamisée
polyphénols	+++	+++	++
Alcaloïde	-	-	+
Flavonoïdes	+++	+++	++
Triterpènes	+++	+++	++
Tanins	+	++	++
Saponines	++	++	++

-: Absence of the compound; **++:** average presence of the compound; **+++:** high presence of the compound.

Hypoglycemic effect of powder fractions and unsieved powder of Psidium guajava leaves on normal glycemic rats

At 0 hours before administration, the fasting blood glucose levels of normal animals were statistically similar (P ≥ 0.05), with a mean value of 75.93 ± 2.81 mg/dL.

From 0 to 7 hours after administration of the plant extracts, a continuous decrease in blood glucose levels was observed in rats receiving the ≤ 125 µm and 125–200 µm fractions of *Psidium guajava* leaves, decreasing from 78.3 ± 7.71 to 43.23 ± 3.30 mg/dL and from 81.0 ± 5.5 mg/dL to 43.5 ± 4.8 mg/dL, respectively. This decrease was highly significant ($p < 0.01$) compared to the normal control from 5 to 7 hours. Unsieved *Psidium guajava* leaf powder resulted in a decrease in blood glucose levels from 71.03 ± 5.15 mg/dL to 54.00 ± 1.32 mg/dL, respectively, from time 0 to time 7 hours, but this decrease was only statistically significant ($p < 0.05$) at 7 hours post-administration.

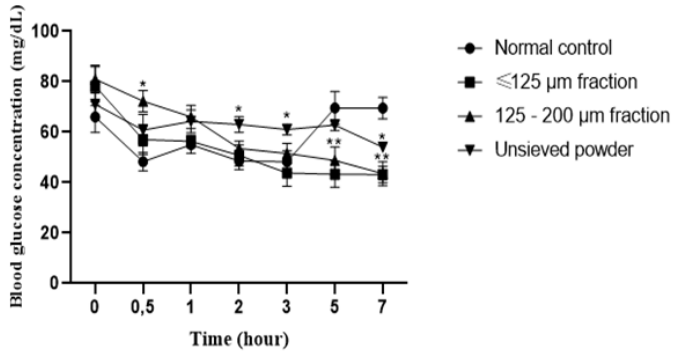
Comparatively, the ≤ 125 µm fraction exhibited the greatest hypoglycemic effect, followed by the 125–200 µm fraction, and finally the unsieved powder, with respective blood glucose differences of 37.50, 35.07, and 17.00 mg/dL (Figure 1).

Antidiabetic effect of powder fractions and unsieved powder of *Psidium guajava* leaves on normal glycemic rats

On day 0 before treatment, blood glucose levels in the different animal groups were not significantly different (P ≥ 0.05) and averaged 72.33 ± 3.01 mg/dL. From 0 to 3 weeks, blood glucose levels in all animal groups evolved similarly without significant differences (P ≥ 0.05). From the 3rd to the 6th week, blood glucose levels in the different treated animal groups began to increase significantly ($p < 0.05$) compared to the normal control group, whose blood glucose level was 61.0 ± 3.8 mg/dL. After 6 weeks, the 125-200 µm fraction significantly reduced ($p < 0.05$) blood glucose levels in the rats of this test group (91.0 ± 13.00 mg/dL)

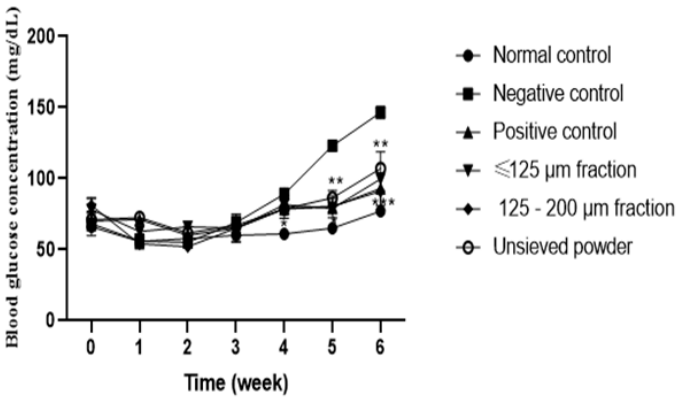
compared to the negative control (146.50 ± 4.12 mg/dL). This reduction was not significantly different from that of the group receiving the ≤ 125 µm fraction, whose blood glucose level was 93.0 ± 1.00 mg/dL. Similarly, compared to the reference drug (Metformin), no significant difference ($p \geq 0.05$) was observed. The unsieved powder caused the greatest reduction in blood glucose compared to the PTC powder fractions. The blood glucose values of the groups that received the PTC powder fractions are close to those of the normal control (77.0 ± 3.7 mg/dL) (Figure 2).

Figure 1: Variation in blood glucose levels after single administration of powder fractions and unsieved powder of *Psidium guajava* leaves in normal glycemic rats.



Values were expressed as means ± standard deviations (n = 5). Results with different symbols at each point indicate significant differences. *P < 0.05; **P < 0.01 compared to the normal control.

Figure 2: Fasting blood glucose after repeated administration of powder fractions and unsieved powder of *Psidium guajava* leaves to rats.

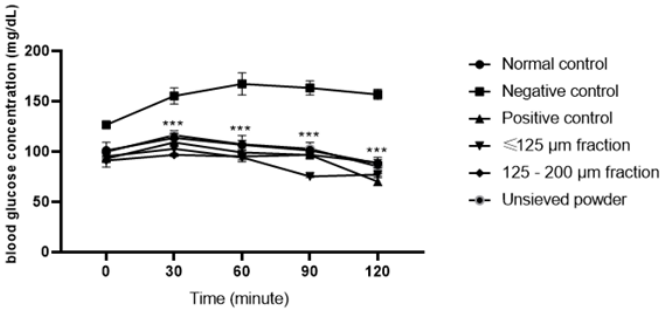


Values were expressed as means ± standard deviations (n = 5). Results with different symbols at each point indicate significant differences. *P < 0.05; **P < 0.01; P < 0.001 compared to the negative control

Effect of powder fractions and unsieved powder of *Psidium gaujava* leaves on oral glucose tolerance

At 0 minutes before D-glucose administration, blood glucose levels in all rat groups were normal (below 126 mg/dL) except for the negative control, which had a blood glucose level of 146.50 ± 4.10 mg/dL. From 0 to 30 minutes after administration, an increase in blood glucose was observed in all rat groups. The glycemic responses of the PTC powder fractions and the normal control were significantly similar ($P \geq 0.05$) at 30 minutes. From 30 to 120 minutes after glucose loading, blood glucose levels in all rat groups decreased significantly compared to the negative control, with the value decreasing from 155 ± 19.63 mg/dL to 156.73 ± 13.26 mg/dL. Comparatively, a significant decrease ($p < 0.05$) in blood glucose was observed in the group receiving the ≤ 125 µm fraction compared to those receiving the 125 – 200 µm fraction and the unsifted powder of *Psidium gaujava* leaves (Figure 3).

Figure 3: Variation in blood glucose levels after an oral glucose load.

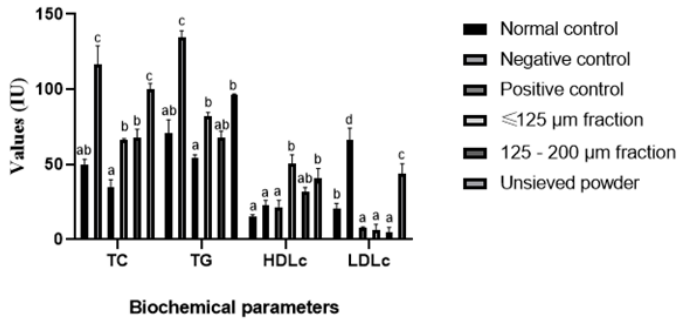


Values were expressed as means ± standard deviations (n = 5). Results with different symbols at each point indicate significant differences. **P < 0.01; ***P < 0.001 compared to the negative control

Effect of powder fractions and unsieved powder of *Psidium gaujava* leaves on the lipid profile

The ≤ 125 µm fraction significantly decreased total cholesterol levels compared to the negative control (116.36 ± 21.73 IU) and compared to the unsieved powder (99.87 ± 19.24 IU). Furthermore, these fractions reduced total cholesterol levels to a value that was not statistically different ($P \geq 0.05$) from the normal control (50.00 ± 11.17 IU). In contrast, metformin resulted in a significantly greater decrease in total cholesterol levels ($P < 0.05$) than the PTC fractions (Figure 4). Furthermore, the ≤ 125 µm, 125–200 µm, and unsieved powder fractions reduced triglyceride levels to a value that was not significantly different ($P \geq 0.05$) from the normal control but was significantly ($P < 0.05$) lower than that of the negative control. The positive control resulted in the greatest reduction in triglyceride levels. The ≤ 125 µm fraction resulted in a significant increase in HDL cholesterol levels compared to metformin. This increase was not significantly different from that of the unsieved Psidium guajava leaf powder. Rats receiving the 125–200 µm fraction of Psidium guajava leaves had HDL cholesterol levels that were statistically similar to those of the normal and negative controls. PTC powder fractions and metformin significantly reduced LDL cholesterol levels compared to unsieved powder and the negative control (66.25 ± 12.91). This reduction was significantly greater than that of the normal control.

Figure 4: Effect of powder fractions and unsieved powder of *P. guajava* leaves on lipid profile.

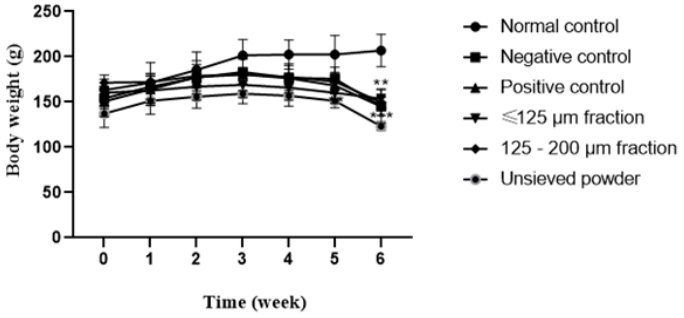


The bars represent means ± standard deviations (n = 3). Those with different letters above them indicate significant differences ($p < 0.05$). Abbreviations: TC: Total cholesterol; TG: Triglyceride; HDL: High-density lipoprotein; LDL: Low-density lipoprotein.

Effect of powder fractions and unsieved powder of *Psidium gaujava* leaves on body weight

The body mass of animals in the diabetic control group, the positive control group, and the groups treated with powder fractions ≤ 125 µm and 125–200 µm was significantly ($p < 0.01$) lower than that of animals in the normal control group at the end of the sixth week of treatment. Similarly, the body mass of animals in the group treated with unsieved powder was even more significantly lower ($p < 0.001$) than that of animals in the normal control group at the end of the sixth week (Figure 5).

Figure 5: Change in body weight of rats after 6 weeks of treatment.



Values were expressed as means ± standard deviations (n = 5). Results with different symbols at each point indicate significant differences. *P < 0.05; **P < 0.01; ***P < 0.001 compared to the negative control; ≠P < 0.05; ≠P < 0.01; ≠P < 0.001 PTC fractions compared to unsieved powder

Effect of powder fractions and unsieved powder of *Psidium gaujava* leaves on oxidative stress parameters

The alanine aminotransferase activity of the negative control was significantly higher (48.10 ± 5.13 IU/L) than that of the other rat groups. The normal control showed the lowest value (13.4 ± 1.87 IU/L). Furthermore, no statistically significant difference ($P \geq 0.05$) was observed between the ≥ 125 µm fraction (31.12 ± 0.22 IU/L), the 125–200 µm fraction (28.7 ± 3.34 IU/L), and the unsieved powder (27.1 ± 1.92 IU/L). Regarding aspartate aminotransferase activity, it was significantly higher in the negative control (93.3 ± 8.59 IU/L), while the lowest value (38.17 ± 4.54 IU/L) was observed in the 125–200 µm fraction. However, no significant difference ($P \geq 0.05$) was observed between the 125–200 µm fraction, the ≥ 125 µm fraction, and the unsieved powder. Similarly, no statistically significant difference ($P \geq 0.05$) was observed between the normal and positive controls. Finally, no significant difference ($P \geq 0.05$) was noted between the serum creatinine levels of the different groups of rats, although a slight increase in serum creatinine was observed in the negative control (Table 3).

Effect of powder fractions and unsifted powder on transaminase activity and creatinine levels

After 6 weeks of treatment, the liver levels of malondialdehyde (MDA) were 6.41±0.51, 14.95±0.77, 6.47±0.70, 6.35±0.92, and 7.24±1.35 µM/g in normal control rats, diabetic control rats, positive control rats, animals treated with the powder fraction ≥ 125 µm, and those treated with unsieved *Psidium guajava* leaf powder, respectively. The superoxide dismutase (SOD) levels during this time were 19.9±2.98, 9.9±4.95, and 32.3±2.44. 27.9±2.98 and 20.9±2.98 IU/mL respectively in normal control rats, diabetic control, positive control, animals treated with the powder fraction ≥ 125 µm and those treated with unsieved powder of *Psidium guajava* leaves (Table 5).

Effect of powder fractions and unsieved powder of *Psidium gaujava* leaves on the elimination of reducing sugars in feces

The total sugar content in rat feces was measured using the ≤ 125 µm powder fraction, which proved to be the most effective during the study (Figure 7).

At week 1, the reducing sugar content of the different rat groups in this study was statistically similar ($P \geq 0.05$), with a mean value of 1.55 ± 0.01 mg EG/g DW. At week 2, a significant decrease ($p < 0.05$) in reducing sugar content was observed in the feces of the negative control rats (0.43 ± 0.046 mg EG/g DW) compared to the positive control (2.174 ± 0.012 mg EG/g DW), and the ≤

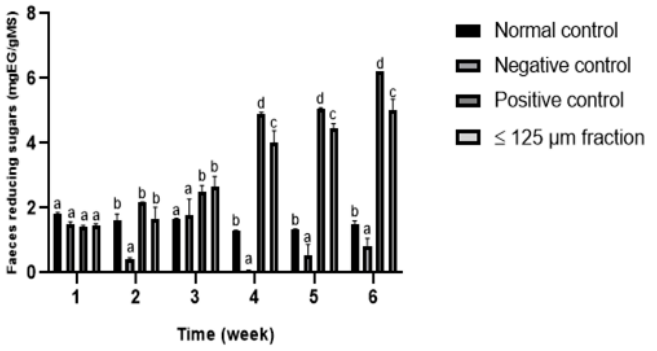
Table 3: Effect of PTC powder fractions and unsieved *Psidium guajava* leaf powder on some biochemical parameters.

Parameters	Normal Control	Negatif Control	Positif Control	≥ 125 µm Fraction	125 – 200 µm Fraction	Unsieved powder
ALAT (UI/L)	13.4±1.87^{a}	48.1±5.13^{bc}	31.12±0.22^{ab}	21.7±4.06^{ab}	28.7±3.34^{ab}	27.1±1.92^{ab}
ASAT (UI/L)	68.2±5.79^{b}	93.3±8.59^{c}	67.2±2.98^{b}	41.13±5.60^{a}	38.17±4.54^{a}	41.7±9.24^{a}
Créatinine (mg/dl)	0.81±0.03^{a}	1.87±0.21^{a}	1.05±0.10^{a}	1.00±0.17^{a}	0.98±0.17^{a}	1.1±0.25^{a}

Values represent means ± standard deviations (n = 3). Results with different superscript letters in the same row indicate significant differences ($p < 0.05$); Abbreviations: ALAT: Alanine aminotransférase; ASAT: Aspartate aminotransférase.

125 µm fraction (1.67 ± 0.55 mg EG/g DW) was also significantly reduced. Furthermore, no significant difference ($P \geq 0.05$) was observed between the normal control, the positive control, and the ≤ 125 µm fraction. At week 3, a slight increase in sugar content was observed in the feces of all rat groups. However, this increase was significantly ($p < 0.05$) greater in the positive control and the group receiving the ≤ 125 µm fraction. The same trends were observed from week 4 to week 6, with reducing sugar levels in the feces reaching their maximum values in the positive control and the group receiving the ≤ 125 µm fraction.

Figure 7: Effect of powder fractions and unsieved powder of *Psidium guajava* leaves on the reducing sugar content in rat feces



The bars represent the means ± standard deviations (n = 3). Those with different letters above them indicate the presence of significant differences ($p < 0.05$).

Discussion

The overall objective of this study was to evaluate the effects of powder fractions on the antidiabetic activities of *Psidium guajava* leaves in Wistar strain rats.

In the hypoglycemic process, it has been shown that the powder fractions and unsieved powder of *Psidium guajava* leaves have the ability to lower blood glucose levels in fasting rats with normal glucose levels. However, this decrease varies depending on the extract. Thus, the ≤ 125 µm fraction exhibited the greatest hypoglycemic activity, which, nevertheless, was not significantly greater than that of the 125–200 µm fraction. This decrease in blood glucose is thought to be due to the fact that certain bioactive compounds in *Psidium guajava* stimulate insulin secretion by the β cells of the pancreatic islets of Langerhans and/or increase insulin sensitivity. The hypoglycemic effect of the plants is attributed to the presence of certain molecules such as flavonoids, alkaloids, saponins, tannins, and triterpenes [33]. Authors such as Keun et al. [20] showed that guava leaves inhibit protein phosphatase 1B and lead to a significant decrease in blood glucose levels in induced diabetic mice. This suggests that these fractions have an interesting pharmacological profile. The greater hypoglycemic effect observed in the groups receiving the powder fractions compared to the group receiving the unsieved powder could be explained by the phenolic compound content of these extracts. Hypoglycemic effects resulting from treatment with plants are generally attributed to their ability to improve pancreatic tissue performance, which implies either an increase in insulin secretion or a reduction in intestinal glucose absorption [34].

It should be noted that type 2 diabetes was successfully induced through a high-calorie, sugary diet combined with dexamethasone. In our study, this diabetes was characterized by fasting hyperglycemia exceeding 126 mg/dL, indicating underlying insulin resistance. The insulin resistance responsible for the chronic hyperglycemia is thought to be due to the action of dexamethasone. This is an exogenous glucocorticoid used in the treatment of numerous conditions, such as inflammation and autoimmune diseases. Long-term corticosteroid therapy has adverse effects, including insulin resistance and dyslipidemia. Dexamethasone increases the production of glucose by the liver from amino acids and glycerol (gluconeogenesis) [35]. It also promotes the inhibition of adiponectin gene expression and secretion. Indeed, adiponectin is an important hormone in the regulation of insulin resistance. It is secreted in response to an inflammatory reaction due to excessive fat deposition in adipose tissue, and its inhibition would therefore lead to a loss of insulin receptor sensitivity [36]. Furthermore, dexamethasone promotes the redistribution and/or differentiation of adipocytes by stimulating lipolysis, thus allowing a considerable increase in the plasma level of non-esterified fatty acids. These fatty acids are capable of inducing insulin resistance by masking insulin receptors [35]. In addition to chronic hyperglycemia, an increase in body weight is observed in diabetic rats. This increase is thought to be due to the accumulation, in the form of fat, of the excess energy provided by the high-calorie, high-sugar diet. Mvongo et al. [27] showed an increase in fats in diabetic-induced

Table 5: Effect of PTC powder fractions and unsieved *Psidium guajava* leaf powder on some parameters of oxidative stress in the rat liver after 6 weeks of treatment.

	NC	NeC	CP+	≥ 125 µm	125 – 200 µm	Uncieced Unsieved powder
MDA (µM/g)	6.41±0.51	14.95±0.77**	6.47±0.70##	6.35±0.92##	6.67±0.13##	7.24±1.35##
NO (mM)	0.190±0.03	0.3±0.00**	0.23±0.04	0.31±0.00	0.16±0.00	0.24±0.00
GSH (mM/g)	0.14±0.02	0.19±0.03	0.14±0.00	0.16±0.00	0.13±0.02	0.13±0.02
SOD (UI/mL)	19.9±2.98	9.9±4.95**	32.3±2.44##	27.9±2.98##	21.4±2.49##	20.9±2.98##

Values represent means ± standard deviations (n = 5). Significant differences **p < 0.05 compared to normal control (NCo) and 0.5p compared to diabetic control (DC).

Abbreviations: GSH, reduced glutathione; MDA, malondialdehyde; NO, nitric oxide; SOD, superoxide dismutase.

rats by the sugary hypercaloric diet.

In the antidiabetic process, defined by the ability of the fractions to prevent the onset of diabetes, it is noted that, like metformin, powder fractions and unsieved powder have demonstrated proven antidiabetic properties. The efficacy of metformin is explained by its mechanisms of action. Metformin's effect on blood glucose levels results primarily from a reduction in hepatic glucose production through inhibition of gluconeogenesis [37]. It potentiates the effect of insulin on muscle glucose uptake, increasing its storage as glycogen without affecting its oxidative metabolism [38]. Furthermore, due to their similar efficacy, the antidiabetic action of powder fractions and unsieved powder is thought to be due to an insulin-sensitizing effect of the latter. They would thus promote the peripheral sensitivity of target tissues (liver, muscles, adipose tissue) to insulin, thereby allowing the storage of glucose as glycogen in muscles and fatty acids as triglycerides in adipose tissue, while decreasing their differentiation. These powder fractions of Psidium guajava leaves would thus correct insulin resistance, leading to a normalization of blood glucose levels. Similar results were observed in the work of Deutchoua et al. [39] on the aqueous extract of Clerodendrum thomsoniae leaves. The comparative study between the antidiabetic effects of powder fractions and unsieved *Psidium guajava* leaf powder showed a superior therapeutic effect of the powder fractions compared to that of the unsieved powder. A plausible explanation is that fractions have high concentrations of total polyphenols in the finest cells of the study, and these polyphenols may have stimulated glucose uptake by peripheral cells. This, in turn, decreased glucose absorption, leading to a sustained drop in blood glucose levels.

Furthermore, analysis of fecal sugar content revealed consistently high concentrations in both the positive control rats (receiving metformin) and the group receiving the 125 µm fraction of Psidium guajava leaves. This result suggests that the powder fraction slows intestinal carbohydrate absorption and decreases postprandial plasma glucose concentration. These fractions would thus act similarly to metformin, which, in this study, serves as the reference drug. By slowing intestinal absorption of reducing sugars, these sugars can reach the colon and subsequently be eliminated in the feces.

Given that diabetes is positively associated with dyslipidemia, the lipid profile of different rat groups was evaluated. This dyslipidemia, known as diabetic dyslipidemia, is generally characterized by elevated levels of total cholesterol, low density lipoproteins (LDL), very low density lipoproteins (VLDL), triglycerides, and an atherogenic index, as well as increased body weight and decreased HDL cholesterol. In this study, the negative control group exhibited the aforementioned characteristics, suggesting that it suffered from dyslipidemia resulting from a high-calorie, high-sugar diet (MACAPOS1). Indeed, rats fed a high-calorie (energy-rich) diet store a large portion of the food as visceral and testicular fat [27,39]. This will therefore lead to an increase in the hepatic concentration of triglycerides and free fatty acids, which itself results from the mobilization of these lipids from adipose tissue to the liver [40]. The results of this study showed that the powder fractions and the unsieved powder have an effect against dyslipidemia and weight gain. The decrease in triglycerides and cholesterol could result, on the one hand, from the inhibition of 3-hydroxy-3-methylglutaryl CoA reductase (a key enzyme in endogenous cholesterol synthesis) and, on the other hand, from the stimulation of LDL-R receptors, promoting the uptake and clearance of VLDL and LDL [41,42]. This role can be attributed to phenolic compounds and saponins. Phytochemical screening of guava leaves revealed the presence of saponins [43]. These substances are known to have antihyperlipidemic and antihypercholesterolemic properties [44]. In this study, an increase in HDL levels was also observed. HDL also helps lower LDL and VLDL levels by removing excess cholesterol peripherally to the liver, which is the main organ of cholesterol metabolism [45].

The activity of transaminases, particularly alanine and aspartate aminotransferase, was assessed to detect potential liver dysfunction. Transaminases are enzymes that catalyze the transfer of the amine group from amino acids to a carbonyl group when a cell has sustained damage [46]. They are biomarkers of organ damage affecting the liver, heart, muscles, or blood. In this study, the negative control showed a significant increase in ALT levels compared to the normal control, indicating hepatocellular injury or necrosis. ALT is primarily synthesized by hepatocytes and released into the bloodstream; it is specific to hepatocellular injury, making it an indicator of hepatotoxicity [47]. Although the ALT concentrations of the positive control, lots receiving the 125µm, 125-200µm fractions and unsieved powder were slightly higher than the normal control, no significant difference was observed. This result indicates the absence of liver damage or the presence of minimal damage. Furthermore, AST is an enzyme found primarily in the liver, heart, muscles, and red blood cells [48,49]. Its presence in large quantities would suggest potential dysfunction of these organs. The AST concentration in rats receiving the 125 µm, 125–200 µm fractions and the unsieved powder was significantly decreased compared to the normal control, while it was significantly increased in the negative control. This result suggests that guava leaves improve liver, heart, and muscle function by preventing potential damage. Serum creatinine allows for the assessment of kidney function. Indeed, creatinine is a product of protein metabolism with a normally constant value [50]. In this study, no significant variation was observed, suggesting that the powder fractions and the unsieved powder of Psidium guajava leaves have no toxic effects on the kidneys.

Conclusion

The PTC fractions (125 µm and 125–200 µm) of Psidium guajava leaves exhibited a superior phenolic compound composition and good antioxidant activity in vivo. Furthermore, these fractions demonstrated a good hypoglycemic effect and a significant antidiabetic effect, with good maintenance of the lipid profile. Moreover, they showed no signs of toxicity, and once these mechanisms of action are studied, powders of Psidium guajava leaf fractions could be used as a food additive for people with diabetes.

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