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Original Research Article

Antihyperglycemic Activity, Total Phenolic, and Total Flavonoid Contents of 96% Ethanol Extract of Pisang Batu (*Musa balbisiana* Colla) Leaves

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ABSTRACT

Diabetes mellitus is a chronic metabolic disorder characterized by elevated blood glucose levels. The leaves of *Musa balbisiana* Colla commonly known as Pisang Batu have been traditionally recognized in Indonesia for their potential to lower blood sugar level. The aim of this study was to determine the antihyperglycemic activity, total phenolic, and total flavonoid contents of the ethanol extract of Pisang Batu leaves. Pisang Batu leaves were extracted by maceration in 96% ethanol. The 96% ethanol extract was subjected to phytochemical screening following standard procedures. The total flavonoid and total phenolic contents of the extract were determined using aluminium chloride colorimetric, and Folin-Ciocalteu methods, respectively. The antihyperglycemic activity of the extract was determined *in vivo* using 5% glucose-induced hyperglycemic mice. Maceration of the dried powdered leaves of Pisang Batu resulted in an extract yield of 12%. Phytochemical screening revealed the presence of flavonoids, tannins, triterpenoids, and saponins. The total flavonoid and total phenolic contents of the extract were found to be 454.87 mg QE/g and 16.14 mg GAE/g, respectively. The extract significantly reduced blood glucose levels in 5% glucose-induced hyperglycemic mice. The percentage reduction in blood glucose by various doses of the extract were 31%, 32%, and 41% at 100 mg/kg, 200 mg/kg, and 300 mg/kg, respectively, and these were comparable to that of the positive control (glibenclamide) with percentage reduction of 45%. In conclusion, the 96% ethanol extract of *M. balbisiana* leaves exhibited promising antihyperglycemic activity, which is likely due to its high phenolic, and flavonoid contents.

Keywords: Musa balbisiana Colla, Total Flavonoid Content, Total Phenolic Content, Antihyperglycemic.

Introduction

Bananas are widely consumed plant due to their comprehensive nutritional content and affordability. Indonesia ranks sixth globally in banana production.[1] Among the various types of bananas, the wild species known as Pisang Batu (Indonesian), Pisang Klutuk (Javanese), or Utti Batu (Bugis) are the most common. This species is known scientifically as *Musa balbisiana* Colla, with synonyms like *Musa bracycarpa* and *Musa sapientum*.[2] Pisang Batu (*Musa balbisiana* Colla) leaves have been used traditionally by Indonesians as eco-friendly food wrappers. These leaves are preferred due to their broad and durable structure, which makes them resistant to tearing during use. Typically, packaging materials come in direct contact with the packaged food. This direct contact can lead to the diffusion of chemical compounds from the packaging material into the food. Therefore, when banana leaves are used as food wrappers, chemical compounds from the leaves may diffuse into the packaged food.

In some regions, the banana plant is used as an alternative traditional preparation for the treatment of fever. In this preparation, a handful of Pisang Batu leaves and a one-inch-long young shoot of Pisang Batu leaves are mixed with water, squeezed, and drained. The young shoots are sliced and mixed into the juice, and a glassful is consumed three times a day.[3] In Bulukumba Regency, South Sulawesi, the young fruit of Pisang Batu is used to treat gastric pain.[4] In East Kalimantan, the sap from the pseudo-stem of the Pisang Batu is utilized in the treatment of diabetes mellitus,[5] and in East Java, the fruit is used as an antidiarrheal agent.[6]

M. balbisiana Colla, a member of the Musaceae family, is an herbaceous plant known for its extensive traditional and medicinal applications. This plant is found in various Asian countries, including China, India, Malaysia, Myanmar, Nepal, the Philippines, Sri Lanka, and Thailand.[7] Numerous parts of the plant are used in ethnomedicinal practices to treat common ailments.[8] Phytochemical studies of the ethanol extract of Batu banana leaves have identified flavonoids, tannins,[9] and rutin.[10] GC-MS analysis of the ethyl acetate extract has detected the presence of 2-Methoxy-4-vinylphenol, phytol, vanillin, E-15-heptadecenal, and 1,2-benzenedicarboxylic acid, bis (2-ethylhexyl) ester.[11] The compound 2-Methoxy-4-vinylphenol, also known as Phenol, 4-ethenyl-2-methoxy, falls within the phenolic compound category. In addition, the fruit peel of *M. balbisiana* Colla contains phenolic compounds, tannins, and flavonoids.[12] According to Jeong *et al*. (2011),[13] 2-Methoxy-4-vinylphenol exhibits anti-inflammatory properties. The fruit and fruit peel of this plant demonstrate

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antibacterial, antidotal, anti-inflammatory, diuretic, and laxative effects,[14] as well as antifungal activities.[12]

Research on the antihyperglycemic effect of ethanol extract of *M. balbisiana* Colla flowers, and stems show that the extracts reduce blood glucose, serum cholesterol, and triglycerides levels.[15] Acetone extract of *M. balbisiana* Colla seeds have inhibitory activity on α-amylase and α-glucosidase enzymes,[16] and *M. balbisiana* Colla root extract has been shown to have blood glucose lowering effect through the increase of glucose uptake in cells *in-vivo*.[17]

Hydro-alcoholic extract of *M. balbisiana* Colla flowers have shown significant recovery in animal model of streptozotocin-induced diabetic with significant improvements in parameters such as fasting blood glucose, serum insulin, glycated hemoglobin, antioxidant enzymes, carbohydrate metabolizing enzymes, proapoptotic gene Bax and anti-apoptotic gene Bcl-2, glycemic genes such as Hex-I, and GLUT-4 in mouse liver tissue.[18]

In another study, a basic diet supplemented with *M. balbisiana* Colla fruit and bark extract showed hepatoprotective and hypoglycemic effects with a significant ($P<0.05$) reduction in increasing levels of serum glucose, urea, uric acid, creatinine, triglycerides, total cholesterol, low-density lipoprotein (LDL), very low-density lipoprotein (VLDL), aspartate aminotransferase, alanine aminotransferase, and alkaline phosphatase levels, and significantly increased ($P<0.05$) high-density lipoprotein (HDL) concentration and activity of insulin compared to diabetic control mice.[19] The rhizome extract was found to have strong inhibitory activity against the enzymes α-amylase and α-glucosidase, key enzymes associated with type-2 diabetes.[20] The antihyperglycemic effect, total flavonoid content, and total phenolic content of the 96% ethanol extract of *M. balbisiana* Colla leaves have not been thoroughly studied. Thus, this research seeks to investigate the total flavonoid content, total phenolic content, and antihyperglycemic effects of the 96% ethanol extract of *M. balbisiana* Colla leaves in lowering blood glucose levels in male mice (*Mus musculus*).

Materials and Methods

Materials

UV-Vis Spectrophotometer (UV-Vis AQ8100, Thermo Scientific - USA), analytical balance (Ohaus - USA), rotary evaporator (Heidolph - Germany), drying oven (Memmert - Germany), micropipette (Socorex - Switzerland), hot plate (Thermo Scientific - USA), test strips (Nesco - South Korea), glucometer (Nesco - South Korea). Ethanol (96%) (Smart Lab - Indonesia), gallic acid (Merck - Germany), Folin-Ciocalteu (Merck - Germany), sodium carbonate (Merck - Germany), quercetin (Merck - Germany), aluminium trichloride (Merck - Germany), sodium acetate (Merck - Germany), glucose (Merck).

Plant collection and identification

The leaves of Pisang Batu (Figure 1) were collected from the Legok District area, Tangerang Regency, Banten Province, Indonesia, in January 2023. The plant material was identified and authenticated at the Herbarium Jatinangor, Laboratorium Taksonomi Tumbuhan, Jurusan Biologi FMIPA, Universitas Padjadjaran, where it was identified as *Musa paradisiaca* L. with the synonym *Musa balbisiana* Colla. of the family Musaceae, and assigned the voucher number 07/HB/04/2024. The sampling was conducted between 8 AM and 9 AM. After the initial wet sorting to remove impurities, 4 kilograms of *M. balbisiana* Colla leaves were obtained from the initial 8 kilograms of collected leaves. The leaf samples were then dried in an oven at 60°C for 24 hours. The dried herbs were ground using a grinder, weighed, and stored in a well-closed container.

A B

Figure 1: Pisang Batu (*M. balbisiana* Colla); (A). *M. balbisiana* Colla plant and (B). *M. balbisiana* Colla leaves.

Extraction of plant material

The powdered leaves (200 g) of *M. balbisiana* Colla was macerated in 96% ethanol (2 L) for 24 h. The extract was filtered, the solvent was replaced, and the extraction was done five times. The combined extract was concentrated in a rotary evaporator and further dried in an oven at a temperature of 40°C.[21]

Phytochemical screening

The 96% ethanol extract of *M. balbisiana* Colla leaves was subjected to phytochemical screening to detect the presence of secondary metabolites such as flavonoids, phenols, saponins, tannins, alkaloids, steroids, triterpenoids, and quinones following standard procedure.[21]

Determination of total flavonoid content (TFC)

The TFC of ethanol extract of *M. balbisiana* Colla leaves was determined using the colorimetric method as previously described by Chang *et al*., 2002,[22] and modified by Munthe *et al*., 2023.[23]

Briefly; 50 mg of *M. balbisiana* Colla extract was dissolved in 50 mL of 96% ethanol to obtain an extract concentration of 1000 ppm. To 0.33 mL of the extract solution (1000 ppm) was added 0.3 mL of 10% $AlCl_3$, and 0.4 mL of 1 M sodium acetate. The mixture was vortexted and incubated at room temperature for 30 minutes. The absorbance of the reaction mixture was measured at 415 nm using a UV-Vis spectrophotometer. The experiment was done in triplicate. The total

flavonoid content of the extract was expressed as milligram of quercetin equivalent per gram of extract weight (mg QE/g extract).

Preparation of quercetin calibration curve
A stock solution of 100 µg/mL of quercetin was prepared and diluted to concentrations of 20, 40, 60, 80, and 100 µg/mL, with each concentration replicated three times. To 2 mL of each concentration was added 0.1 mL of 10% aluminum trichloride, 0.1 mL of 1M sodium acetate, and 2.8 mL of distilled water, vortexed and incubated at room temperature for 30 minutes. The absorbance of the reaction mixture was measured at 415 nm using a UV-Vis spectrophotometer

Determination of total phenolic content (TPC)
The TPC of ethanol extract of *M. balbisiana* Colla leaves was determined using the colorimetric method as previously described by Szewczyk *et al*., 2021,[24] and modified by Munthe *et al*., 2023.[23]
The ethanol extract of *M. balbisiana* Colla leaves (50 mg) was dissolved in 50 mL of 96% ethanol to obtain a concentration of 1000 µg/mL. To 0.1 mL of the extract solution was added 0.4 mL of Folin-Ciocalteu reagent and 0.5 mL of 7.5% sodium bicarbonate. The reaction mixture was vortexed, and then incubated at room temperature for 30 minutes. Thereafter, the absorbance was measured at 765 nm using a UV-Vis spectrophotometer. The experiment was performed in triplicate. The total phenolic content of the extract was expressed as milligram of gallic acid equivalent per gram of extract weight (mg GAE/g extract).[23]
Preparation of gallic acid calibration curve
From a stock solution of gallic acid (100 µg/mL), dilutions with concentrations of 20, 40, 60, 80, and 100 µg/mL were prepared. To 0.1 mL of each concentration was added 0.4 mL of Folin-Ciocalteu reagent and 0.5 mL of 7.5% sodium bicarbonate. The reaction mixture was vortexed, incubated at room temperature for 30 minutes, and the absorbance was measured at 765 nm using a UV-Vis spectrophotometer.[23]

Evaluation of antihyperglycemic activity
Animals
Male albino mice were used for the study. The mice were housed in a cage and allowed to acclimatize to the laboratory conditions. The mice were fed with standard rodent pellets, and allowed access to drinking water *ad libitum*.

Ethical approval
Ethical approval for the study was obtained from the Ethics Committee of the Faculty of Health Sciences, Universitas Pelita Harapan, with the ethical license number 0013/PE.KEPK-FIKes-UPH/IV/2023.

Animal grouping and administration of extract
Fifteen (15) male albino mice were divided into five groups, a negative control group, which received 0.5% sodium carboxymethylcellulose (Na-CMC), a positive control group, which received 5 mg/kg of glibenclamide, and three treatment groups, which received 96% ethanol extract of *M. balbisiana* Colla leaves at doses of 100 mg/kgBW, 200 mg/kgBW, and 300 mg/kgBW. Prior to the treatment, the mice were fasted for approximately 8 hours. Hyperglycaemia was induced by administration of 5% glucose, and after thirty (30) minutes, the mice were administered the test samples according to the groupings highlighted above. With the aid of a glucometer, blood glucose levels were measured at 30, 60, and 120 minutes after administration of the test samples.[25]

Statistical analysis
Data were subjected to One-Way analysis of variance (ANOVA) using the Statistical Package for the Social Sciences (SPSS) edition 19. Comparison between mean values were done using the Tukey HSD Post Hoc test. Significant difference was established at P-value < 0.05.

Results and Discussion

Extraction Yield
The percentage yield of *M. balbisiana* Colla leaves extract following maceration in 96% ethanol is presented in Table 1. The extract yield was 12% compared to the weight of the dried powdered plant material. The value obtained indicate a relatively high extraction efficiency and the effectiveness of the extraction method and solvent in extracting the secondary metabolites from the plant.

Table 1: Yield of *M. balbisiana* Colla Leaf Extract

Weight of dried powdered leaves (g)	Extract Weight (g)	Yield (%)
100	12	12%

Phytochemical constituents of M. balbisiana Colla leaves
Phytochemical screening of *M. balbisiana* Colla leaves revealed the presence of flavonoids, phenols, saponins, tannins, alkaloids, and steroids (Table 2). Previous studies have shown that *M. balbisiana* Colla leaves contain compounds such as flavonoids, polyphenols, sesquiterpenoids, triterpenoids, monoterpenoids, and saponins.[26] The identification of flavonoids and saponins in the *M. balbisiana* Colla leaf extract corroborates the findings of Pane (2013),[27] which reported the presence of these compounds in the stem of *M. balbisiana* Colla.

Table 2: Phytochemical constituents of *M. balbisiana* Colla ethanol leaf extract

Phytochemical Compounds	Test Method	Observation	Inference
Flavonoids	Willstatter	Reddish-brown colour	+
Saponins	Forth	Persistent foaming	+
Phenol	$FeCl_3$	Blue colour	+
Tannins	$FeCl_3$ 1%	Blue colour	+
Alkaloids	Dragendorff	Red Precipitate	-
Steroids	Lieberman-Burchard	Blue, Green colour	+
Triterpenoids	Lieberman-Burchard	Red, Purple	-
Quinone	NaOH	Red colour	-

(+): indicate presence of phytocompound, (-): indicate absence of phytocompound

Total flavonoid and total phenolic contents of M. balbisiana Colla leaves extract

The total flavonoid content of the extract was determined using aluminium chloride colorimetric method. The aluminum chloride method is the most commonly used method for determining total flavonoid content. This method is based on a complexation reaction between aluminum chlorides and the carbonyl group at C4 and hydroxyl group at C3 of the flavonoid compound. The complex produced is stable and can easily be detected in the presence of sodium acetate. The total flavonoid content of the extract of *M. balbisiana* Colla leaves was estimated from a linear regression equation of quercetin (QE) calibration curve (y = 0.003x - 0.0394) with R^2 value of 0.979 (Figure 2). The total flavonoid content of the extract was found to be 454.76 ± 0.057 mg QE/g extract (Table 3).

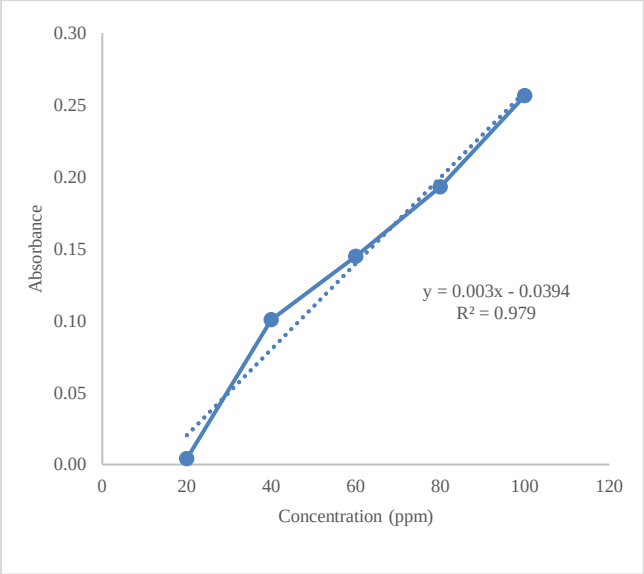

Figure 2: Quercetin calibration curve

On the other hand, the total phenolic content of the extract was determined using the Folin-Ciocalteu reagent. Folin-Ciocalteu (F-C) (Phosphomolybdotungstic reagent, $3H_2O•P_2O_5•13WO_3•5MoO_3•10H_2O$) reacts with a phenolic compound producing a blue colour [$(PMoW11O_4)4-$] that can be measured using a spectrophotometer at a wavelength of 760 nm. The blue colour of the solution is due to the molybdenum metal (Mo(VI)) in the complex being reduced to Mo(V) in the presence of electron donor such as antioxidant. Gallic acid is used as a standard for the determination of total phenolic content.[28]

The total phenolic content of *M. balbisiana* Colla leaf extract was estimated from the linear regression equation of the gallic acid calibration curve (y = 0.0111x - 0.1252) with an R^2 value of 0.9973 (Figure 3). The total phenolic content of the extract was estimated as 16.14 ± 0.013 mg GAE/g (Table 3). Flavonoids are polyphenolic compounds characterized by two phenyl rings linked by a propane bridge, resulting in a 15-carbon flavan structure (C6-C3-C6).[29] They are a class of low molecular weight phenolic compounds widely found in plants. In higher plants, flavonoids are among the most prevalent compounds. Within most angiosperm families, flavonoids are commonly identified as flower pigments. Major flavonoid groups include anthocyanins, flavonols, flavanones, isoflavones, and flavonoids.[30]

Previous studies by Basumatary and Nath (2018)[31] found that the inflorescence extract of *M. balbisiana* Colla contains 5.209 ± 0.428 mg QE/g extract of total flavonoids and 16.318 ± 0.544 mg GAE/g extract of total phenolics. In contrast, research by Revadigar *et al.* (2017)[32] reported that the ethanol extract of the same part of the plant contained 4.85 ± 0.05 mg QE/g extract of total flavonoids and 92.02 ± 0.40 mg GAE/g extract of total phenolics. Additionally, Trieu *et al.* (2020)[33] found that the fruit of *M. balbisiana* Colla contains 162.64 ± 3.39 mg GAE/g extract of total phenolics and 1.222 ± 0.007 mg QE/g extract of total flavonoids. Based on the findings from the present study, the 96% ethanol extract of *M. balbisiana* Colla leaves has a high total flavonoid content and a relatively high total phenolic content, indicating its potential as a powerful natural antioxidant capable of reducing free radicals effectively.

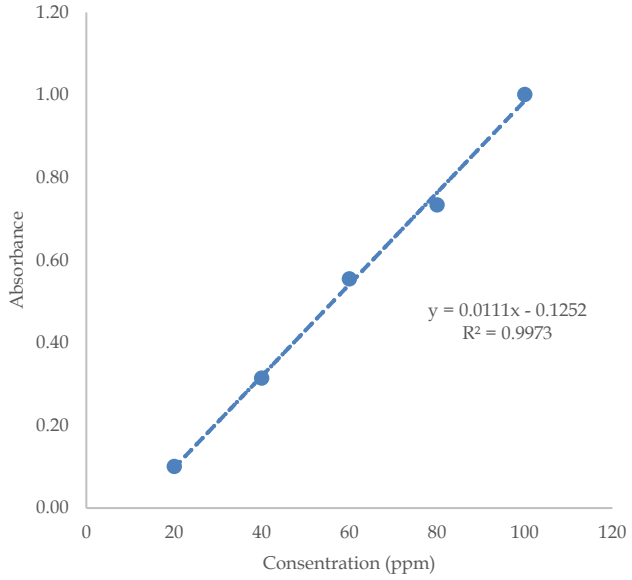

Figure 3: Gallic acid calibration curve

Antihyperglycemic effect of ethanol extract of M. balbisiana Colla leaves

The antihyperglycemic effect of a 96% ethanol extract of *M. balbisiana* Colla leaves was evaluated in glucose-induced hyperglycemic mice. From the result of the study, the mean initial blood glucose level (T0) before glycemia induction in the different groups were as follows: 114.66 mg/dL in the negative control group (Na CMC), 138.33 mg/dL in the positive control group (Glibenclamide 5 mg/kg), 126 mg/dL in the 100 mg/kg group, 129.67 mg/dL in the 200 mg/kg group, and 160.67 mg/dL in the 300 mg/kg group. These results indicate that the blood glucose levels of the mice were within the normal range.

The mean blood glucose levels immediately after glucose induction (T1) were as follows: 137.67 mg/dL in the negative control group (Na CMC), 163.67 mg/dL in the positive control group (Glibenclamide 5 mg/kg), 140.67 mg/dL in the 100 mg/kg group, 149.33 mg/dL in the 200 mg/kg group, and 187.67 mg/dL in the 300 mg/kg group. These results indicate that 5% glucose administration effectively increased blood glucose levels in mice, as reflected by the increase in blood glucose levels in all the groups immediately after 5% glucose administration (T1) (Figure 4).

Measurement of the average decrease in blood glucose levels after administration of the test preparations, showed that the negative control group continued to experience an increase in blood glucose levels up to 90 minutes, with an increase of 31%. In contrast, the positive control group (Glibenclamide 5 mg/kg) and the groups that received *M. balbisiana* Colla leaves extract at doses of 100 mg/kg, 200 mg/kg, and 300 mg/kg experienced decreases in blood glucose levels by 45%, 31%, 32%, and 41%, respectively after 90 minutes (Figure 5).

Comparison of the percentage reduction in blood glucose in the positive control group (Glibenclamide 5 mg/kg), and each of the extract treated groups (100 mg/kg, 200 mg/kg, and 300 mg/kg) showed that there was no significant difference in the percentage reduction of blood glucose levels between the positive control group and the extract treated groups ($p = 0.834$) for 100 mg/kg group, ($p = 0.846$) for 200 mg/kg group, and ($p = 0.999$) for 300 mg/kg group. Based on these results, it could be inferred that 96% ethanol extract of *M. balbisiana* Colla leaves at a dose of 100 mg/kgBW is an effective dose as an antihyperglycemic agent.

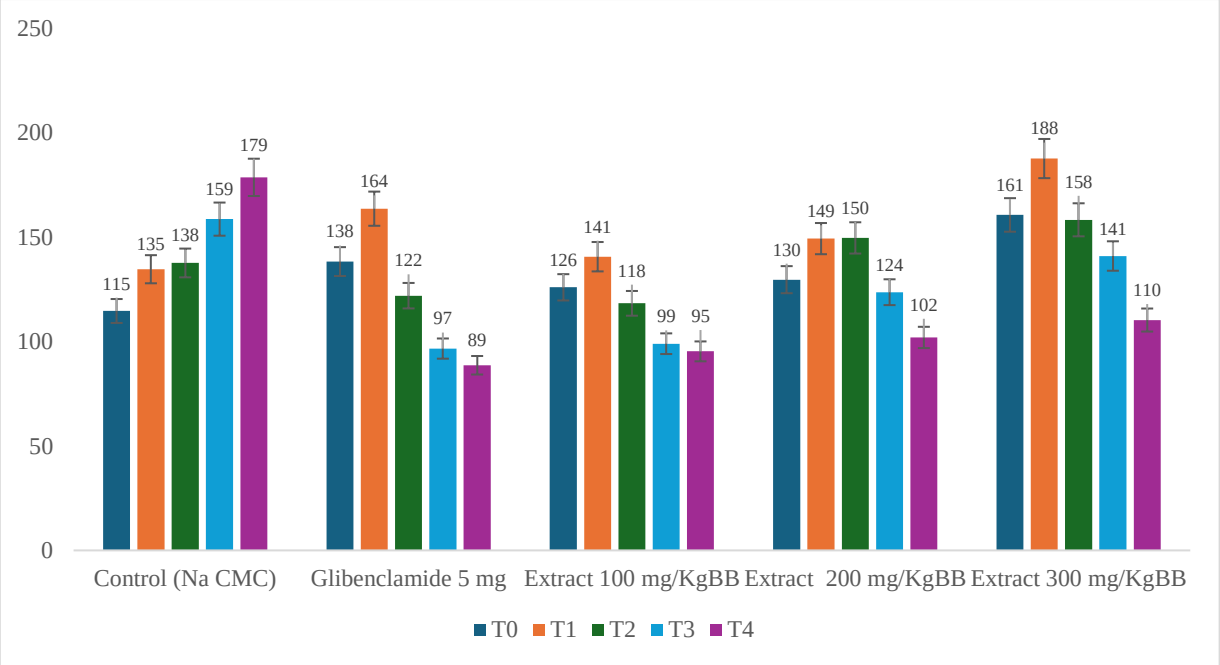

Figure 4: Blood glucose level of mice at various time intervals after administration of *M. balbisiana* Colla leaf ethanol extract. T0: Before glycemia induction, T1: 0 min (immediately after glycemia induction), T2: 30 min, T3: 60 min, T4: 90 min

Table 3: Total Flavonoid and Total Phenolic Contents of ethanol extract of *M. balbisiana* Colla leaves

Total Flavonoid Content (mg QE/g Extract)	Total Phenolic Content (mg GAE/g Extract)
454.76 ± 0.057	16.14 ± 0.013

Data represent mean ± Standard deviation (SD) of triplicate determination

The reduction in blood glucose levels in mice after administration of *M. balbisiana* Colla leaves extract may be influenced by the high content of total flavonoids and total phenolics in the extract.

Flavonoids such as quercetin have been shown to possess antihyperglycemic activity by regulating glucose homeostasis, sensitizing the secretion of insulin, enhancing glucose utilization in peripheral tissues, and inhibition of intestinal glucose absorption.[34] The mechanisms by which quercetin reduces blood glucose levels include inhibiting glucose transporter activity,[34] increasing glucose uptake through activation of the AMPK signalling pathway in skeletal muscle cells,[35, 36] reducing hepatic glucose production,[37] inhibiting α-glucosidase,[38] protecting against pancreatic islet beta cell damage, and promoting beta cell regeneration.[39]

Similarly, phenolic compounds like gallic acid exhibits antihyperglycemic and anti-hypertriglyceridemic effects, and provides protection to pancreatic β cells. The mechanism by which gallic acid reduces blood glucose levels involves the upregulation of the transcription factor peroxisome proliferator-activated receptor-γ (PPAR-γ) in the nucleus. This upregulation enhances differentiation and insulin sensitivity in adipocytes. Additionally, gallic acid boosts cellular glucose uptake by stimulating the phosphatidylinositol 3-kinase (PI3K)/p-Akt signalling pathway and facilitating the translocation of GLUT1, GLUT2, and GLUT4, which are insulin-responsive glucose transporters.[40] Furthermore, gallic acid significantly enhances insulin secretion in pancreatic β cells and decreases apoptosis of these cells.[41]

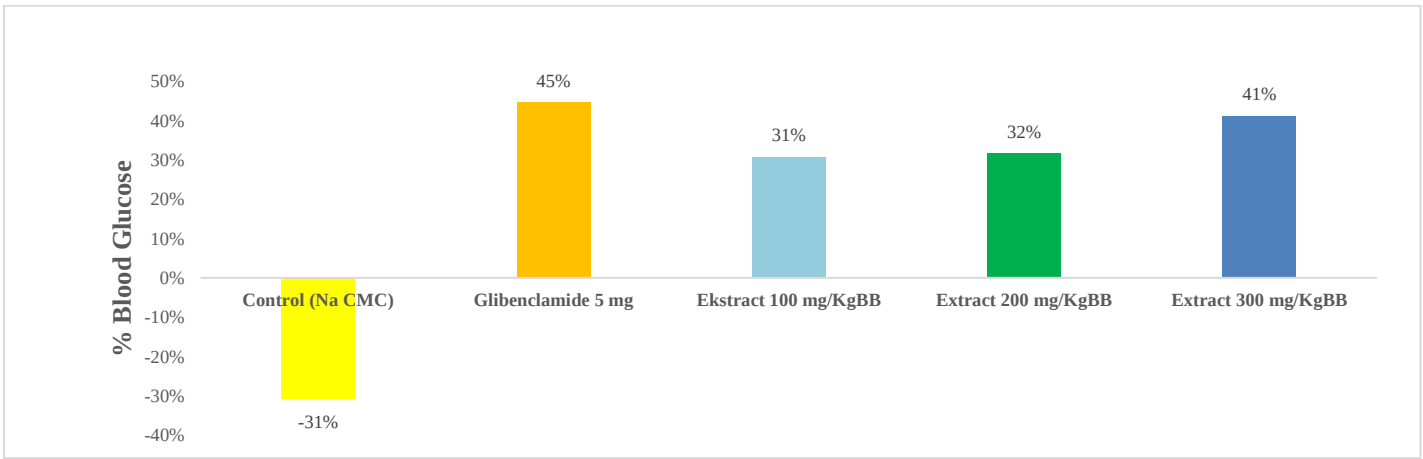

Figure 5: Percentage decrease in blood glucose levels in mice after administration of *M. balbisiana* Colla leaf ethanol extract

Conclusion

From the results of the study, the 96% ethanol extract of *Musa balbisiana* Colla leaves have been found to contain flavonoids, tannins, triterpenoid steroids, quinones, and saponins. It has a total flavonoid content of 454.87 mg QE/g extract and a total phenolic content of 16.14 mg QE/g extract. Findings from the present study have also demonstrated that 100 mg/kg BW dose of the 96% ethanol extract of *M. balbisiana* Colla leaves is effective as an antihyperglycemic agent.

Conflict of Interest

The authors declare no conflicts of interest.

Authors' Declaration

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

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