

Effect of aqueous and ethanol extracts of *Cucurbita maxima* seed on fasting blood glucose in alloxan-induced rats

Muchirah Peninah Njoki^{1, 2}*, Waihenya Rebecca¹, Muya Shadrack¹, Anselimo Makokha³, Laila Abubakar⁴, Simeon Mogaka¹, Hastings Ozwara⁵

¹Department of Zoology, School of Biological Sciences, Jomo Kenyatta University of Agriculture and Technology, Nairobi, Kenya

²Department of Medical Sciences, School of Applied and Health Sciences, Technical University of Mombasa, Mombasa, Kenya

³Department of Food Science, Jomo Kenyatta University of Agriculture and Technology, Nairobi, Kenya

⁴Department of Pure and Applied Sciences, School of Applied and Health Sciences, Technical University of Mombasa, Mombasa, Kenya

⁵Department of Tropical and Infectious Diseases, Institute of Primate Research, Karen, Nairobi, Kenya

*Corresponding author

Muchirah Peninah Njoki
Department of Medical Sciences,
School of Applied and Health
Sciences, Technical University of
Mombasa, Mombasa, Kenya.
Email: muchirahpeninah@tum.ac.ke

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ABSTRACT

Diabetes is a chronic disorder marked by hyperglycemia due to insulin resistance and insufficient insulin action, or both. Synthetic antidiabetic drugs available on the market are associated with severe effects, necessitating the need for alternative antidiabetic drugs. This investigation aimed to determine the antidiabetic potential and phytochemical profiles of aqueous and ethanol seed extracts of *Cucurbita maxima*. The seeds of *C. maxima* were airdried, milled, and extracted using distilled water and ethanol. The antidiabetic effect of the extracts was performed using Wistar rats weighing 160-180 g. Thirty-five rats were assigned to 7 groups: non-diabetic (control), diabetic, glibenclamide-treated, and aqueous or ethanol extract-treated (200 and 400 mg/kg body weight). Alloxan monohydrate was used to induce experimental diabetes. The treatments were orally administered for 28 days. Blood glucose levels and histopathological analysis were performed. The phytochemical screening was conducted utilizing liquid chromatography-mass spectrometry (LC-MS). In diabetic rats, the effects of the two extracts revealed a substantial reduction in levels of fasting blood glucose in a dose-dependent manner, suggesting an antidiabetic effect. The histopathological analysis revealed the restoration of pancreatic β -cells by the extracts. The LC-MS analysis identified phytochemicals in the extracts belonging to classes of flavonoids, phenolic acids, and fatty acids that are associated with antidiabetic effects. Interestingly, the ethanol extract had a better antidiabetic effect compared to the aqueous extract. In conclusion, the aqueous and ethanol extracts of *C. maxima* seed possess phytochemicals that can be used to develop novel antidiabetic agents with less severe effects.

INTRODUCTION

Diabetes is a long-term metabolic disease marked by persistently high blood glucose levels due to insufficient insulin secretion and resistance to insulin action, or both [1]. Diabetes is the fastest growing public health concern globally [2]. It is highly linked to microvascular complications such as neuropathy, nephropathy, and retinopathy, as well as macrovascular complications (such as stroke, cerebrovascular disease, coronary artery disease, and steatohepatitis), and sometimes fatalities [3-5]. According to the International Diabetes Federation, 10.5% (536.6 million) of the adult population in the world suffered from diabetes in 2021 [1, 6]. Most of these individuals lived in low- and middle-income countries. It was also estimated that the number will rise to 643 million and 783 million in 2030 and 2045, respectively [1, 6]. Globally, about 6.7 million fatalities in 2021 were directly associated with diabetes [6, 7].

Type I and type II diabetes mellitus are the most common types of diabetes [1]. Type I diabetes is a T-cell-mediated autoimmune disease characterized by destruction of insulin-producing β cells of Langerhans and inadequate insulin production [8]. Type II



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diabetes results from resistance to insulin action and inadequate insulin secretion by pancreatic β cells [9]. Type II diabetes accounts for approximately 90% of all instances of diabetes, while type I diabetes accounts for about 5 to 10% [6, 10]. Other types of diabetes include monogenic diabetes, gestational diabetes, and secondary diabetes [1]. Diabetes had a significant financial burden amounting to 966 billion USD in 2021 globally [6].

Insulin is primarily responsible for maintaining blood glucose homeostasis. The pancreatic β -cells of the islets of Langerhans are responsible for the synthesis of insulin in the body. After a meal, when blood glucose levels increase, β -cells release insulin into the bloodstream, where it is absorbed by skeletal muscle, adipocytes, and hepatocytes. Insulin reduces or returns blood glucose to normal levels by increasing the absorption of glucose by the adipose tissue and skeletal muscles, as well as inhibiting the liver's endogenous glucose synthesis [11]. In diabetic individuals, the blood sugar levels remain high due to insulin resistance and ineffective action, or both [12].

The management of hyperglycemia in diabetic patients is achieved through lifestyle adjustments and use of synthetic antidiabetic drugs. The lifestyle adjustments include healthy eating and drinking as well as physical exercising [13]. Synthetic antidiabetic drugs are also often prescribed to manage hyperglycemia in individuals. These drugs include injectable medications such as insulin as well as synthetic antidiabetic drugs such as metformin, glibenclamide, and thiazolidinedione, among others [10, 14, 15]. Nevertheless, these medications are associated with severe side effects, including congestive heart failure, gallbladder cancer, gastrointestinal tract toxicity, and hepatotoxicity [9-11]. These data necessitate the need for alternative antidiabetic agents.

Medicinal plants offer great promise for treating and managing several diseases due to the presence of phytochemicals that have therapeutic value. The use of herbal medications is growing popularity as alternative therapeutic agents since they have better therapeutic effects with minimal side effects [19, 20]. Some extracts from medicinal plants have revealed the ability to promote regeneration of the pancreas and enhance insulin secretion [21]. The secondary plant metabolites associated with antidiabetic effects include coumarins, flavonoids, alkaloids, carotenoids, phenolic acids, phytosterols, saponins, tannins, and terpenoids [22]. The seeds of *Cucurbita maxima* are used by Kenyan communities to manage diabetes. Nevertheless, there was a paucity of empirical evidence to validate this claim. This investigation therefore aimed to determine the phytochemical composition and antidiabetic effects of aqueous and ethanol extracts of *C. maxima* seed.

MATERIALS AND METHODS

Medicinal sample collection

Fresh parts and fruits of *C. maxima* (pumpkin) were obtained from a commercial pumpkin farm in Embu, Kenya. The seeds and fresh pumpkin samples were taxonomically identified and authenticated by a taxonomist in the Department of Botany at Jomo Kenyatta University of Agriculture and Technology. The specimen voucher was deposited at the department herbarium (Voucher number 4321). The fruits of the pumpkins were cut and opened to yield approximately 2 kg of the seeds. The seeds were washed with distilled water, air-dried, ground into a fine powder, and packed awaiting extraction.

Aqueous extraction of *C. maxima* seed

First, 500 g of the powder was put in 2 L of distilled water, and the mixture was left to stand for 2 h with occasional shaking. The mixture was then incubated for one day at 40 °C. The obtained mixture was decanted, centrifuged at 9000 revolutions per minute (rpm) for 10 minutes, and then filtered via Whatman® GF/C glass microfiber filter paper. The filtrate was lyophilized using a freeze dryer (NANBEI freeze dryer: NBJ-10-1, Zhengzhou, China) and the resulting seed extract was pulverized and stored at 4 °C [23].

Ethanol extraction of *C. maxima* seed

First, 500 g of the seed powder was macerated with 2 L of analytical grade ethanol (Sigma Aldrich, Merck Group, Missouri, United States), shaken for 4 h, and then left to stand for 24 h. The mixture was then filtered using Whatman number 1 filter paper. The extract was concentrated using a vacuum evaporator (Buchi R-200 Rotavapor System, Hampton, United States) at 40 °C. The obtained extract was stored at 4 °C [24].

Animal study

Healthy Wistar rats aged between 8-9 weeks and weighing 160-180 g were utilized in the antidiabetic assay. The rats were acquired and housed at the Institute of Primate Research (IPR), Nairobi. The rats were kept at standard laboratory conditions, fed rodent pellet diet (Unga Group Limited, Kenya), and provided water *ad libitum*. One week before the experiment, the rats were acclimatized. The experiment was conducted based on the animal ethics and guidelines of the Institute of Primate. Ethical approval of the study was sought from the Institute of Primate Research Ethical Review Committee (ISERC/03/2017) before the commencement of the study.

To induce diabetes, alloxan monohydrate (Sigma Aldrich, Merck Group, St. Louis, Missouri, United States) at 180 mg/kg body weight (bw) was injected (single dose) intraperitoneally in overnight fasted rats. The fasting blood glucose was measured after 72 h. The rats with fasting blood glucose exceeding 11.1 mmol/L were regarded as diabetic and employed in this study.

Thirty-five rats were assigned to 7 groups (5 rats/group): a) Non-diabetic rats (control; received vehicle), b) diabetic rats (alloxan monohydrate at 180 mg/kg), c) glibenclamide (reference drug) at a dosage of 5 mg/kg bw treated to diabetic rats, d-e) aqueous extract at doses of d) 200 and e) 400 mg/kg bw were treated to diabetic rats; and f-g) ethanol extract at doses of f) 200 and g) 400 mg/kg bw were treated to diabetic rats. The treatments were administered orally for 28 days [15].

Fasting blood glucose analysis

The fasting blood glucose in each rat was monitored on 0 h, 2 h, 4 h, 6 h, 24 h, 7 day, 14 day, 21 day, and 28 day. Prior to measuring fasting blood glucose levels, the experimental rats were fasted overnight. The blood sample was obtained by first sterilizing the rat tail using 70% surgical spirit, followed by snipping the tip of the tail. A drop of blood was obtained from the tail of each rat and then a OneTouch Select Plus Simple® Glucose Meter (LifeScan IP Holdings, Malvern, Pennsylvania, United States) was utilized to measure the levels of fasting blood glucose.

Histopathological analysis

The experimental rats were euthanized using diethyl ether on day 29, after which they were dissected, and pancreas was detached. Each pancreas was rinsed with normal saline to remove blood and then transferred immediately to 10% formalin. Subsequently, various alcohol-to-water ratios were used to dehydrate sections of pancreas. The tissues were stored in the following progressive alcohol grades for gradual dehydration: 30% and 50% for 5 to 10 h, 70% for 10 to 12 h, 90% for around thirty minutes, and twenty minutes in 100% alcohol. To facilitate sectioning, the dehydrated tissues were embedded in paraffin wax. After adjusting the section's thickness to 6 micrometers (μm), it was stained with hematoxylin and eosin dye and placed in a neutral deparaffinated xylene (DPX) solution to facilitate microscopic studies at magnification of $\times 400$.

Liquid chromatography-mass spectrometry analysis

One gram of each extract was weighed, dissolved in 1 mL in double distilled water/acetonitrile, 95/5 (v/v) with 0.1 % formic acid (Sigma Aldrich, Merck Group, St. Louis, Missouri, United States), centrifuged at 15,000 rpm for 2 minutes, and then transferred into clean labeled vials. From each extract, a stock solution of 1 mg/1 mL was prepared and 20 μL of the extract was injected into a liquid chromatography-mass spectrometry (LC-MS)-electrospray ionization (ESI) vial. The operating conditions were as follows: a quaternary LC pump (Model 1200, Agilent Technology, Palo Alto, California, United States) coupled to Agilent MSD 6120-Triple quadruple MS with an electrospray source (Agilent Technology, Palo Alto, California, United States) was utilized. ChemStation software (Hewlett-Packard) was used to control the system. Reversed-phase liquid chromatography was conducted on an Agilent Technology 1200 infinite series, Zorbax SB C 18 column, 2.1 \times 50 mm, 1.8 μm (Phenomenex, Torrance, California, United States) using the following gradient program: 0 minute, 5 % B; 0-5 minutes, 5-50 % B; 5-10 minutes, 50-80 % B; 10-15 minutes, 80-100 % B; 15-25 minutes, 100 % B; 25-30 minutes, 5 % B; 30-35 minutes, 5 % B (Water; Acetonitrile). The flow rate was maintained at 1 mL/min.

A scan range of 100 to 1500 m/z was used to collect data in both positive and negative ion modes. Every ion had a dwell period of 50 milliseconds. The mass spectrometer had the following additional settings: capillary voltage, 3.0 kV; cone voltage, 70 V; extract voltage, 5 V; RF voltage, 0.5 V; source temperature, 110 $^{\circ}\text{C}$; nitrogen gas temperature for desolvation, 380 $^{\circ}\text{C}$; and 400 L/nitrogen gas flow. Serial dilutions (1-100 ng/ μL) of rutin hydrate standard were analyzed using LC-MS-ESI in full scan mode to generate the following linear calibration curve: $[y = 5578.4x - 39094 \text{ (} R^2 = 0.9990\text{)}]$. The equation served as a basis for external quantification. Automated quantification of the compounds was acquired in reference to the calibration curve and instrumentally tabulated against the exact mass ions.

Statistical analysis

Raw data was analyzed using GraphPad Prism version 9. The mean \pm standard error of the mean (SEM) was used to express descriptive statistics. An inferential statistic, one-way ANOVA (analysis of variance), was employed to test for statistical differences among different treatment groups. In case of statistical differences, the data was subjected to Tukey's multiple comparisons. To compare the effects of the two extracts, an independent t-test was computed. The level of significance was set at p less than 0.05.

RESULTS

Effects of aqueous and ethanol seed extracts on levels of fasting blood glucose in rats

The aqueous seed extract of *C. maxima* at 200 and 400 mg/kg bw demonstrated an antidiabetic effect following alloxan-induced diabetes in rats (Table 1). The diabetic control rats had higher levels of fasting blood glucose from 2 h of the experiment. Treatment with aqueous seed extract at 200 and 400 mg/kg bw substantially reduced the fasting blood glucose levels relative to the levels ($p < 0.05$) in diabetic control rats. The levels of fasting blood glucose in normal control rats remained remarkably low throughout the experiment ($p < 0.05$; Table 1). The baseline of all studied groups did not vary significantly ($p > 0.05$) before the induction of diabetes (Table 1).

The antidiabetic effect of aqueous seed extract at the two doses did not differ significantly ($p > 0.05$) in 2 h. Nevertheless, in 4, 6, and 24 h, the extract dose of 400 mg/kg bw significantly lowered the blood glucose levels compared to the levels noted ($p < 0.05$) at 200 mg/kg bw (Table 1). In 2, 4, and 6 h, the fasting blood glucose levels in glibenclamide-treated rats were substantially lower relative to levels noted ($p < 0.05$) in extract-treated rats at the two studied doses, although in 24 h, the blood glucose levels were insignificant compared to levels of the higher dose of the extract ($p > 0.05$). The blood glucose levels in diabetic control rats were significantly higher relative to levels observed in other treatment groups in 4, 6, and 12 h ($p < 0.05$). The non-diabetic control rats had considerably lower fasting blood glucose levels than those recorded in other treatment groups in 0, 2, 4, 6, and 24 h ($p < 0.05$; Table 1).

On days 7 and 21, the levels of fasting blood glucose in aqueous seed extract of *C. maxima* at 400 mg/kg bw were substantially lower than those at 200 mg/kg bw ($p < 0.05$), although the levels at the two doses were insignificant ($p > 0.05$) on days 14 and 28 (Table 1). The levels of fasting blood glucose in glibenclamide-treated rats were significantly lower on days 7, 21, and 28 ($p < 0.05$). On days 7, 14, 21, and 28, the fasting blood glucose levels in diabetic control rats were considerably greater compared to those seen in the other treatment groups ($p < 0.05$). Nevertheless, in the same period, the fasting blood glucose levels were substantially lower in non-diabetic control rats ($p < 0.05$; Table 1).

Table 1. Effect of *C. maxima* aqueous seed extract on levels of fasting blood glucose on alloxan-induced diabetes in rats.

Treatment	Blood glucose levels (mmol/L)									
	Baseline	0 h	2 h	4 h	6 h	24 h	Day 7	Day 14	Day 21	Day 28
Non-diabetic	5.12±0.20 ^a	4.88±0.34 ^b	4.94±0.27 ^c	4.68±0.32 ^d	5.04±0.28 ^e	5.08±0.18 ^d	5.06±0.29 ^e	5.28±0.19 ^d	5.20±0.19 ^e	5.04±0.22 ^d
Diabetic	5.20±0.21 ^a	28.90±0.58 ^a	29.16±0.74 ^a	29.78±0.86 ^a	29.48±0.43 ^a	30.58±0.68 ^a	30.08±0.71 ^a	31.54±1.97 ^a	29.42±0.75 ^a	29.62±0.50 ^a
Glibenclamide	5.20±0.23 ^a	30.46±0.84 ^a	25.14±0.35 ^b	22.04±0.56 ^c	19.92±0.34 ^d	19.78±0.38 ^c	13.28±0.54 ^d	13.06±0.55 ^c	12.16±0.44 ^d	11.89±0.30 ^c
200 mg/kg bw AqE	5.32±0.28 ^a	29.64±0.36 ^a	28.80±0.44 ^a	27.94±0.38 ^a	26.56±0.33 ^b	25.00±0.26 ^b	21.84±0.37 ^b	19.06±0.35 ^b	18.44±0.44 ^b	15.14±0.34 ^b
400 mg/kg bw AqE	5.24±0.25 ^a	29.82±0.31 ^a	27.78±0.23 ^a	25.10±0.41 ^b	23.12±0.28 ^c	19.74±0.35 ^c	17.22±0.28 ^c	15.30±0.33 ^{bc}	14.96±0.20 ^c	13.90±0.21 ^b

Using one-way ANOVA and Tukey's multiple comparisons, the means with mean±standard error that do not share a lowercase superscript letter along the column vary significantly ($p < 0.05$). Non-diabetic = normal control; diabetic = alloxan treated-negative control; glibenclamide = reference drug treated to diabetic rats; AqE = aqueous extract treated to diabetic rats.

The rats that were administered with ethanol seed extract of *C. maxima* at 200 and 400 mg/kg bw noted a reduction in the levels of fasting blood glucose on alloxan-induced diabetes in rats (Table 2). The levels of fasting blood glucose at the baseline did not differ considerably in all the studied groups ($p > 0.05$). From 2 h of the experiment, the diabetic control rats had considerably higher levels of fasting blood glucose ($p < 0.05$).

The non-diabetic rats noted unchanged fasting blood glucose levels in the entire experiment ($p>0.05$; Table 1).

In 2 and 4 h of the study, the fasting blood glucose levels of two doses of ethanol seed extract were nonsignificant in diabetic rats ($p>0.05$). Nonetheless, in 6 and 24 h, the levels of blood glucose in rats that were administered ethanol seed extract at 400 mg/kg bw were significantly lower than those noted ($p<0.05$) at 200 mg/kg bw (Table 2). The levels of fasting blood glucose in glibenclamide-treated rats statistically matched those of rats that received ethanol seed extract at the two doses in 2 and 4 h ($p>0.05$). Similarly, in 6 and 24 h, the levels of fasting blood glucose in animals treated with glibenclamide were statistically similar to the levels noted in animals treated with ethanol seed extract ($p>0.05$) at 400 mg/kg bw. The levels of fasting blood glucose in diabetes control rats remained substantially higher compared to other treatment groups in 2, 4, 6, and 24 h ($p<0.05$). Contrarily, the non-diabetic control rats had substantially lower levels of fasting blood glucose relative to the levels ($p<0.05$) of the other treatment groups in all the studied times (Table 2).

On days 14, 21, and 28, the levels of blood glucose in animals treated with ethanol seed extract were statistically insignificant ($p>0.05$) at the two studied doses (Table 2). On days 7 and 14, the effect of glibenclamide on the fasting blood glucose levels did not differ significantly relative to the levels seen in extract-treated rats at the two doses ($p>0.05$). The fasting blood glucose levels were considerably greater in diabetes control rats compared to levels noted in ($p<0.05$) other treatment groups on days 7, 14, 21, and 28. In the same period, the non-diabetic control rats revealed substantially lower fasting blood glucose levels relative to levels observed in the other studied groups ($p<0.05$; Table 2).

Table 2. Effect of *C. maxima* ethanol seed extracts on levels of fasting blood glucose on alloxan-induced diabetes in rats.

Treatment	Blood glucose levels (mmol/L)									
	Baseline	0 h	2 h	4 h	6 h	24 h	Day 7	Day 14	Day 21	Day 28
Non-diabetic	5.12±0.20 ^a	4.88±0.34 ^b	4.94±0.27 ^c	4.68±0.32 ^c	5.04±0.28 ^d	5.08±0.18 ^d	5.06±0.29 ^d	5.28±0.19 ^c	5.20±0.19 ^d	5.04±0.22 ^d
Diabetic	5.20±0.21 ^a	28.90±0.58 ^a	29.16±0.74 ^a	29.78±0.86 ^a	29.48±0.43 ^a	30.58±0.68 ^a	30.08±0.71 ^a	31.54±1.97 ^a	29.42±0.75 ^a	29.62±0.50 ^a
Glibenclamide 200mg/kg bw	5.20±0.23 ^a	30.46±0.84 ^a	25.14±0.35 ^b	22.04±0.56 ^b	19.92±0.34 ^c	19.78±0.38 ^c	13.28±0.54 ^{bc}	13.06±0.55 ^b	12.16±0.44 ^b	11.89±0.30 ^b
EtE 400mg/kg bw	5.24±0.21 ^a	29.04±0.39 ^a	26.00±0.48 ^b	23.54±0.38 ^b	22.64±0.40 ^b	22.02±0.30 ^b	14.86±0.28 ^b	12.68±0.20 ^b	11.38±0.26 ^{bc}	10.72±0.14 ^{bc}
EtE	5.20±0.23 ^a	29.56±0.67 ^a	26.52±0.52 ^b	22.64±0.33 ^b	21.00±0.45 ^c	18.34±0.32 ^c	11.58±0.32 ^c	10.16±0.25 ^b	10.30±0.32 ^c	10.14±0.25 ^c

Using one-way ANOVA and Tukey's multiple comparisons, the means with mean±standard error that do not share a lowercase superscript letter along the column vary significantly ($p<0.05$). Non-diabetic = normal control; diabetic = alloxan treated-negative control; glibenclamide = reference drug treated to diabetic rats; EtE = ethanol extract treated to diabetic rats.

In comparison, the effects of aqueous and ethanol seed extracts of *C. maxima* at 200 mg/kg bw on fasting blood glucose levels did not differ significantly in 0 h ($p>0.05$). Nevertheless, at the same dose, the levels of fasting blood glucose were considerably lower in rats that received ethanol extract relative to the levels noted in rats that received aqueous extract in 2, 4, 6, and 24 h ($p<0.05$; Figure 1). At 400 mg/kg bw, the effect of the two extracts did not differ significantly on fasting blood glucose levels in 0 and 2 h ($p>0.05$). Nonetheless, the fasting blood glucose levels in rats that were administered ethanol seed extract at 400 mg/kg bw were substantially lower relative to levels observed in rats that received aqueous seed extract in 4, 6, and 24 h ($p<0.05$; Figure 1).

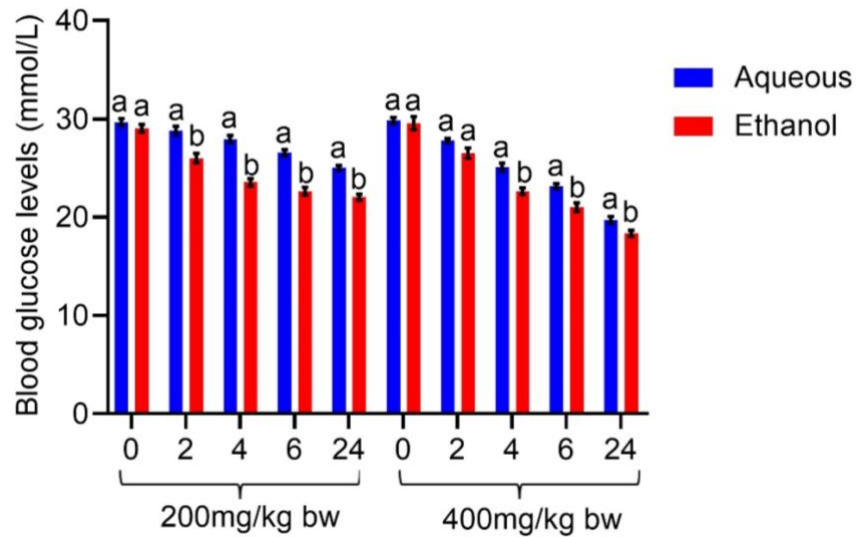


Figure 1. Hourly comparison of the effects of aqueous and ethanol seed extracts of *C. maxima* on levels of fasting blood glucose on alloxan-induced diabetes in rats. Aqueous and ethanol extract was treated to diabetic rats. Using an independent t-test, bars with distinct lowercase letters within each time differ significantly ($p < 0.05$).

The fasting blood glucose levels were also compared on the treatment days. The diabetic rats that were administered ethanol seed extract at 200 mg/kg bw revealed a substantial decline in their fasting blood glucose levels compared to the levels seen in rats that received aqueous seed extract on days 7, 14, 21, and 28 of the experiment ($p < 0.05$; Figure 2). At 400 mg/kg bw, treatment with the ethanol seed extract of *C. maxima* substantially lowered levels of fasting blood glucose levels compared to the levels noted in diabetic rats that received aqueous seed extract on days 7, 14, 21, and 28 of the experiment ($p < 0.05$; Figure 2).

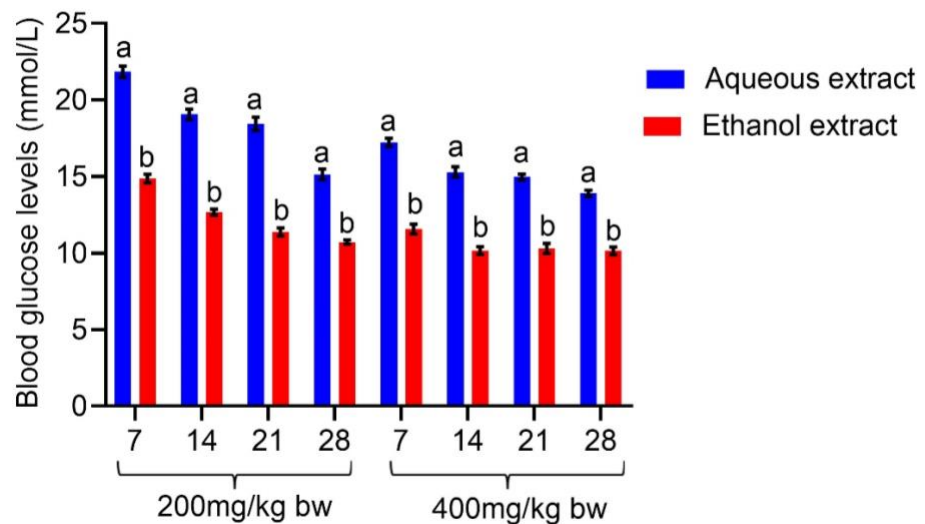


Figure 2. Day wise comparison of the effects of aqueous and ethanol seed extracts of *C. maxima* on levels of fasting blood glucose on alloxan-induced diabetes in rats. Aqueous and ethanol extract was treated to diabetic rats. Using an independent t-test, bars with distinct lowercase letters within each time differ significantly ($p < 0.05$).

Effect of the extracts on histopathology of the pancreas in diabetic rats

The histopathology of the pancreas in normal control rats had normal β -cells of islets of Langerhans (Figure 3). The induction of diabetes in rats resulted in degeneration of pancreatic β -cells, pancreatic inter-lobular fibrosis, and massive infiltration of neutrophils enclosed within an eosinophilic fibrous capsule. However, treatment with the reference drug, glibenclamide and the two extracts revealed restoration of the pancreatic β -cells. At 200 mg/kg bw of the two extracts, the histopathology of the pancreas showed partly mild regeneration of pancreatic β -cells. At 400 mg/kg bw, the effects of the two extracts revealed a moderate regeneration of the β -cells (Figure 3).

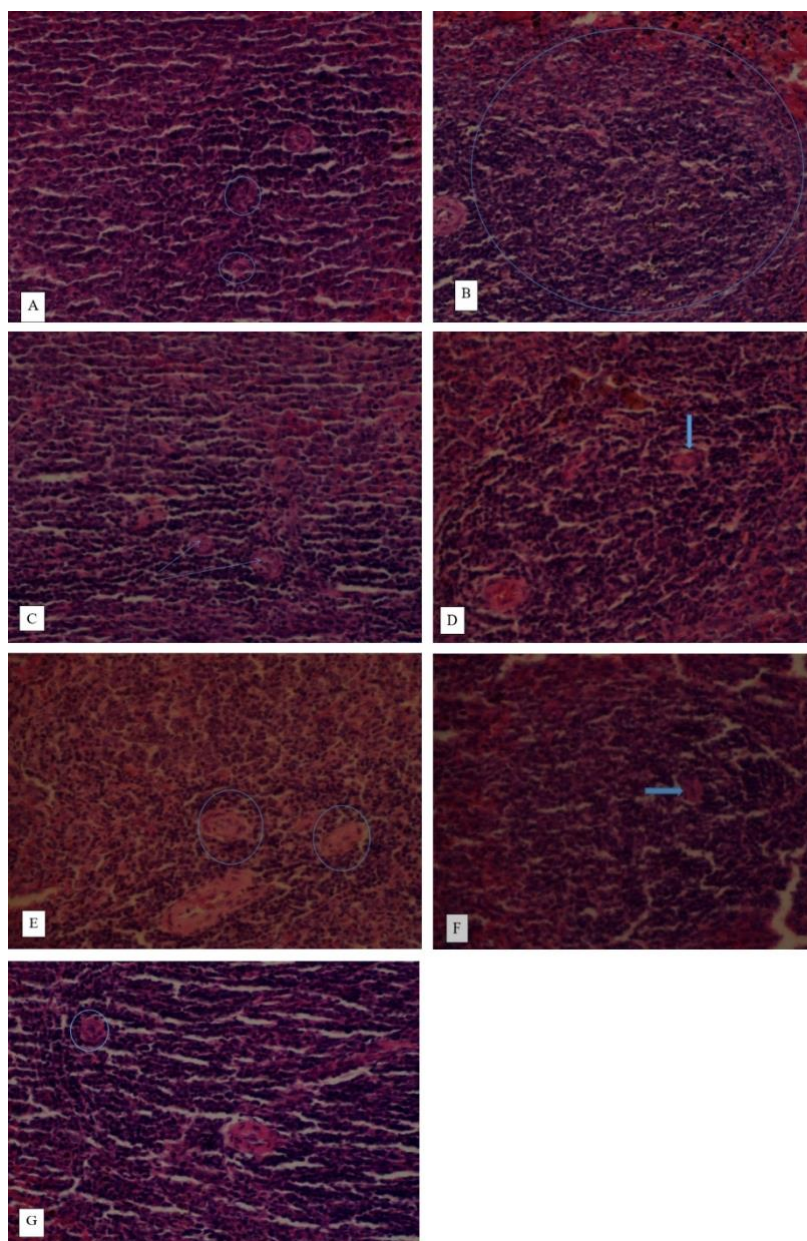


Figure 3. Effects of aqueous and ethanol seed extracts of *C. maxima* on histopathology of pancreatic tissue on alloxan-induced diabetes in rats. A) Non-diabetic rats (control); normal pancreatic β -cells of the islets of Langerhans; B) Diabetic rats; degenerated pancreatic β -cells; C) glibenclamide was treated to diabetic rats (regenerated pancreatic β -cells); D) Ethanol seed extract at 200 mg/kg bw-treated diabetic rats (partially regenerated pancreatic β -cells), E) Ethanol seed extract at 400 mg/kg bw-treated diabetic rats (regenerated pancreatic β -cells), F) aqueous seed extract at 200 mg/kg bw-treated diabetic rats (partly regenerated pancreatic β -cells), and G) aqueous seed extract at 400 mg/kg bw-treated diabetic rats (regenerated pancreatic β -cells); magnification = $\times 400$.

Phytochemical composition of seed extracts of *C. maxima*

The LC-MS analysis of the two extracts identified phytochemicals that belonged to classes of phenolic acids, flavonoids, and fatty acids (Figures 4 and 5). The aqueous extract detected phenolic acids such as 4-hydroxybenzoic acid (retention time in minutes (RT) = 15.77), concentration in $\mu\text{g}/\text{mg}$ (Conc.) = 1.67), protocatechuic acid (RT of 15.36, Conc. = 0.29), 4-hydroxycinnamic acid (RT of 2.98, Conc. = 68.67), vanillic acid (RT of 14.89, Conc. = 0.03), gallic acid (RT = 2.96, Conc. = 68.67), quinic acid (RT = 10.52, Conc. = 0.08), ferulic acid (RT = 14.82), rosmarinic acid (RT = 13.8), 3-feruloylquinic acid (RT = 13.63) and chicoric acid (RT = 13.7; Conc. = 0.03) (Figure 4).

Flavonoids such as biochanin B (RT = 10.52, Conc. = 0.60), calconaringenin (RT = 14.8, Conc. = 0.64), eriodictyol (RT = 14.9, Conc. = 0.64), taxifolin (RT = 14.33, Conc. = 0.76), epigallocatechin (RT = 12.82, Conc. = 0.02), cirsiolol (RT = 14.62, Conc. = 0.21), genistin (RT = 12.01, Conc. = 0.54), Kaempferol-3-glucorhamnoside (RT = 13.8; Conc. = 0.03), diosimin (RT = 12.45, Conc. = 0.02) and myricetin (RT = 10.89, Conc. = 0.33, negative mode) were detected in the aqueous extract (Figure 4). Fatty acids, including palmitoleic acid (RT = 10.42, Conc. = 0.42), linoleic acid (RT = 14.3, Conc. = 0.17, negative mode) and oleic acid (RT = 11.64, Conc. = 2.41, negative mode) were also detected in LC-MS analysis of the aqueous extract (Figure 4).

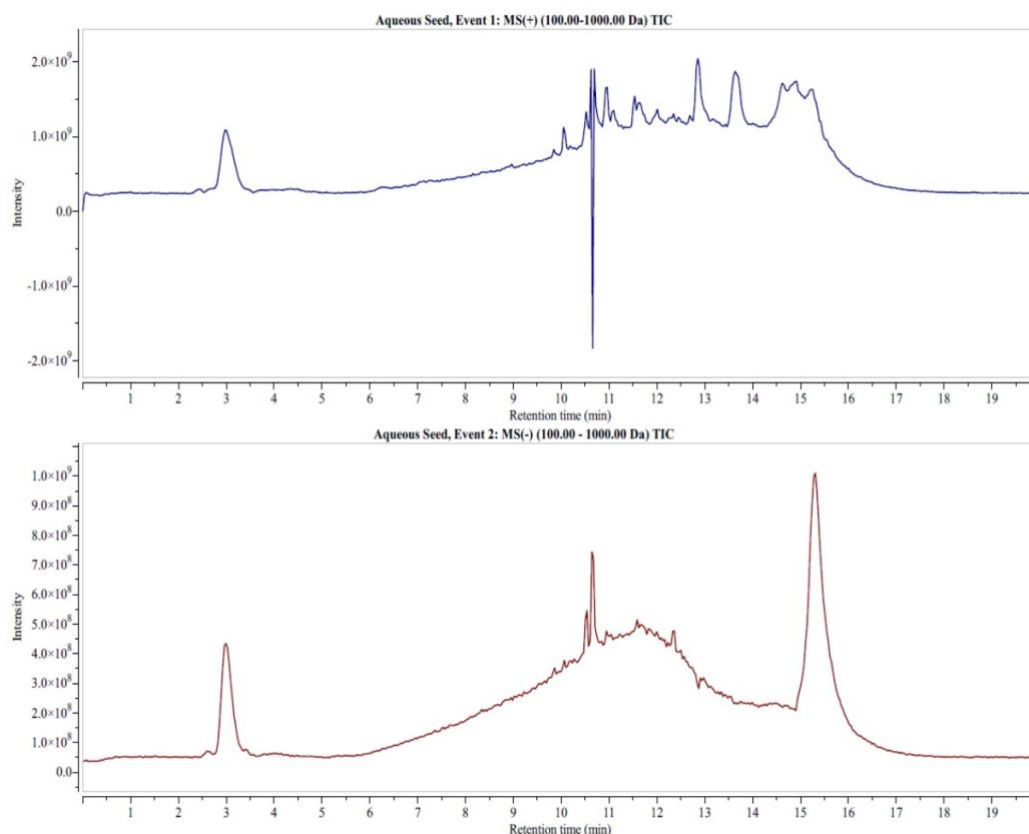


Figure 4. LC-MS chromatogram of aqueous seed extract of *C. maxima* (upper chromatogram = positive mode (MS +), lower chromatogram = negative mode (MS -)).

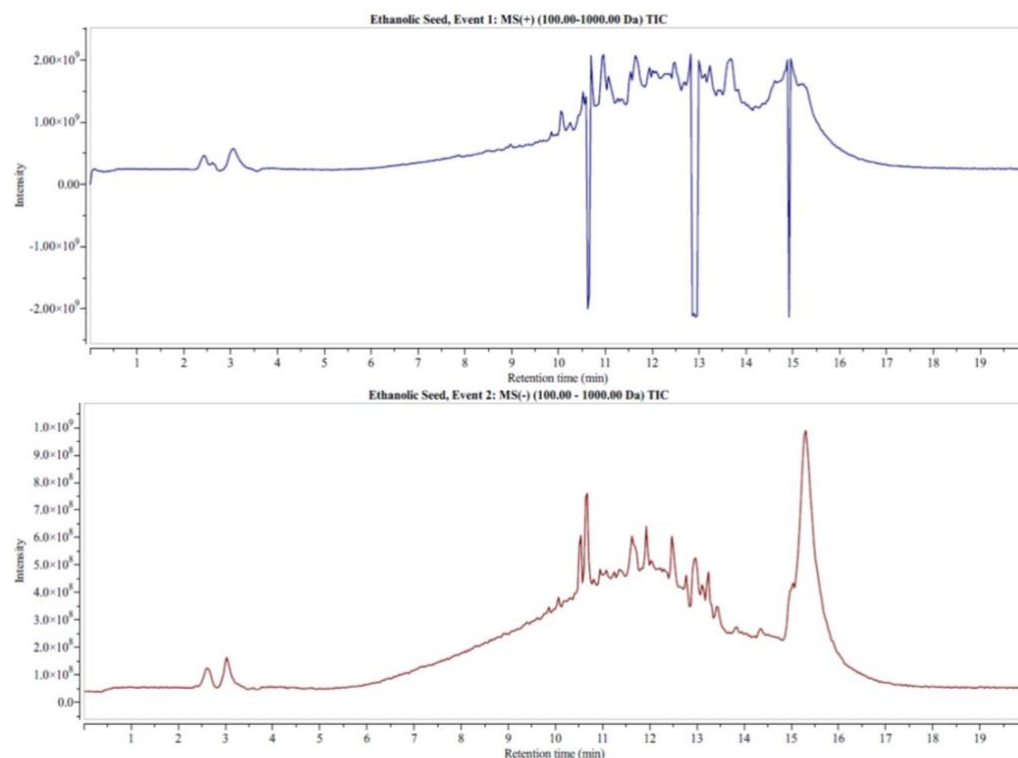


Figure 5. LC-MS chromatogram of ethanol seed extract of *C. maxima* (upper chromatogram = positive mode (MS +), lower chromatogram = negative mode (MS -)).

DISCUSSION

In this study, the effects of aqueous and ethanol seed extracts at doses of 200 and 400 mg/kg bw of *C. maxima* attenuated elevated fasting blood glucose levels, including restoration of damaged pancreatic β -cells following alloxan-induced diabetes in rats. The LC-MS analysis of the two extracts detected phytochemicals that belonged to the classes of phenolic acids, flavonoids, and fatty acids. These plant secondary metabolites have been documented to possess antidiabetic effects [26].

After diabetic rats were administered the two extracts for 28 days, the blood glucose levels gradually lowered throughout the treatment period, suggesting that a higher dosage had a more potent antidiabetic effect. Various research studies have also reported the antidiabetic effects of medicinal plant extracts following alloxan-induced diabetes in animal models. A study by Sharma *et al.* [27] found that *C. maxima* ethanol seed extract had an antidiabetic effect on streptozotocin-induced diabetes in rats. Kushawaha *et al.* [28] revealed that *C. maxima* aqueous seed extract had an antidiabetic effect on streptozotocin-induced diabetes in rats. Atta *et al.* [29] demonstrated that *C. maxima* fruit methanol extract attenuated streptozotocin-induced diabetes in rats. Suwannapong *et al.* [30] reported that pulp extract of *C. maxima* had an antidiabetic effect on streptozotocin-induced diabetes in rats.

Alloxan, an unstable organic compound, is commonly utilized as a diabetogenic agent in experimental research [25]. Alloxan is widely recognized for its ability to damage pancreatic β -cells of the islets of Langerhans, which consequently impairs insulin secretion, thereby causing hyperglycemia [31]. Alloxan-induced diabetes is caused by two different mechanisms: first, it selectively inhibits glucose-induced insulin secretion by specifically inhibiting glucokinase, the β -cell's glucose sensor; second, it produces reactive oxygen species (ROS) that destroy insulin-producing pancreatic β -cells in the

pancreas, leading to reduced synthesis and release of insulin, thereby causing hyperglycemia [32].

Glibenclamide is highly prescribed to manage diabetes mellitus [33], hence the choice of this study. Glibenclamide is classified as part of the second-generation of sulfonylurea. Its mechanism of action involves inhibiting adenosine triphosphate-sensitive K^+ channels of pancreatic β -cells, which increases insulin secretion and sensitivity of insulin-dependent organs, such as the muscles, adipose tissues, and liver, leading to the utilization of glucose [34].

Antidiabetic effects of medicinal plants have been linked to a number of mechanisms, including stimulation of insulin secretion by the pancreas, suppression of enzymes that break down carbohydrates, increased expression of glycogen-regulatory enzymes in the liver, and enhanced uptake of glucose by skeletal muscles and adipocytes [35]. The effect of two extracts in this study could have occurred through a variety of mechanisms, including slowing down the gut's absorption of carbohydrates, boosting the production of insulin by potentially regenerated β -cells, reducing the endogenous synthesis of glucose by the liver, or stimulating the absorption of glucose by skeletal muscles and adipocytes [19, 22]. Also, the restoration in the integrity of the pancreas tissue as shown in this study could have possibly enhanced the function of regenerated pancreatic β -cells, thus increasing the secretion of insulin [31].

In this study, phytochemicals associated with antidiabetic effects, such as phenolic acids, flavonoids, and fatty acids, were detected using LC-MS analysis. The flavonoids included biochanin B, calconaringenin, eriodictyol, taxifolin, epigallocatechin, cirsiol, genistin, kaempferol-3-glucorhamnoside, diosimin, myricetin, leutolin, and apigenin. Biochanin B exerts its antidiabetic effect through stimulation of β -cells to secrete insulin, as well as reducing the activity of gluconeogenesis enzymes and enhancing glycolytic enzymes [36]. Chalconaringenin (isosalipurpol or naringenin chalcone) inhibits the absorption of glucose in the intestines as well as renal glucose reabsorption, thus causing an antidiabetic effect [37]. Eriodictyol has demonstrated its antihyperglycemic effect through inhibition of lipid peroxidation caused by ROS [38].

Taxifolin (dihydroquercetin) exerts its antidiabetic effect by promoting the translocation of glucose transporter 4 (GLUT4), which is involved in insulin-regulated glucose absorption in fat and muscle cells [39]. Genistein has shown its antidiabetic effect through enhanced β -cell proliferation, suppression of production of hepatic glucose, and reduction in β -cell apoptosis [40]. Myricetin inhibits α -glucosidase and α -amylase, prevents digestion of carbohydrates, and enhances expression of GLUT4 to exert its antidiabetic effect [41]. Luteolin has been shown to reverse hyperlipidemia and oxidative stress, thus exerting its hyperglycemia effect [29, 30]. Apigenin enhances the release of insulin from the pancreas and stimulates the metabolism of glucose and its delivery to peripheral tissues [44].

The phenolic acids that were detected using LC-MS included 4-hydroxybenzoic acid, vanillic acid, gallic acid, quinic acid, protocatechuic acid, 4-hydroxycinnamic acid, ferulic acid, rosmarinic acid, 3-feruloylquinic acid, chicoric acid, and syringic acid. 4-Hydroxybenzoic acid has been shown to exert its antidiabetic effect by enhancing peripheral glucose consumption [45]. Gallic acid exerts its antidiabetic effect by increasing glucose consumption, including the reduction in the synthesis of reactive oxygen species and enhanced glucose uptake [33, 34]. 4-Hydroxybenzoic acid also exerts its antihyperglycemic effect through enhanced insulin secretion and expression of GLUT4 [48]. Chicoric acid increases insulin secretion and enhances glucose uptake in muscle cells to exert its antidiabetic effect [49]. 4-Hydroxycinnamic acid stimulates

insulin secretion in the pancreas, inhibits hepatic gluconeogenesis, enhances glucose uptake, improves pancreatic β -cell functionality, and delays the digestion of carbohydrates and absorption of glucose [37, 38].

Ferulic acid restores pancreatic architecture and modulates important diabetogenic actions to promote glucose homeostasis [52]. Rosmarinic acid has been documented to possess an antidiabetic effect through regeneration of pancreatic β -cells, increased glycogen content in skeletal muscle, enhanced activity of glycogen synthase, and suppression of glycogen phosphorylase [40, 41]. Syringic acid exhibits its antidiabetic effect by lowering the activities of glucose-6 phosphatase and fructose-1,6-bisphosphatase, as well as enhancing the activities of glycolytic enzymes to modulate carbohydrate metabolism [55].

Fatty acids that were identified using LC-MS analysis included palmitoleic acid, linoleic acid, and oleic acid. Palmitoleic acid (omega-7 monounsaturated fatty acid) exerts its antidiabetic effect by enhancing insulin sensitivity [56]. Linoleic acid (omega-6 fatty acid) exerts its antidiabetic effect through inhibition of the protein tyrosine phosphatase linked to insulin resistance [57]. Oleic acid (omega-9 fatty acid) protects against insulin resistance through the regulation of genes associated with the phosphatidylinositol 3-kinases signaling pathway [58].

The ethanol extract had a better antidiabetic effect than the aqueous extract, although it had fewer phytochemicals. This could be due to the ability of ethanol to extract both polar and non-polar compounds. The non-polar compounds could also have increased the antidiabetic effect.

CONCLUSIONS

This investigation concluded that ethanol and aqueous seed extracts of *C. maxima* at dosages of 200 and 400 mg/kg bw have potent antidiabetic effects following alloxan-induced diabetes in rats. The antidiabetic effect of the two extracts was associated with the phytochemicals that were detected using LC-MS analysis. The findings of this study can be utilized as a lead in developing novel antidiabetic drugs.

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AUTHOR CONTRIBUTIONS

PN, RW, and HO conceived and designed the study. SM and PN conducted experiments and analyzed data. RW, HO, SM, AM, and LA interpreted the data and supervised the project. All authors participated in drafting the manuscript.

CONFLICTS OF INTEREST

There is no conflict of interest among the authors.

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