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Research Article

Evaluation of Aqueous Extract from *Cosmos caudatus* Leaves in Alloxan-Induced Diabetic Rats

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Abstract

Kenikir (*Cosmos caudatus*), often consumed as a vegetable in Southeast Asia, possesses therapeutic benefits for several diseases, including diabetes mellitus. However, there has been limited investigation of the aqueous extract of *C. caudatus* for this disease model. This study aimed to examine the glucose-lowering effect of *C. caudatus* aqueous extract in an alloxan-induced diabetes model in rats. Ultrasound-assisted extraction was performed to obtain an aqueous extract of *C. caudatus* leaves. Qualitative phytochemical screening was carried out to identify the presence of bioactive compounds. Male Wistar rats were allocated to the following experimental groups: non-diabetic, diabetic without treatment, and diabetic treated with glibenclamide or aqueous *C. caudatus* extract at concentrations of 25%, 50%, or 100%. Diabetes was induced by intraperitoneal injection of 150 mg/kg alloxan. Random blood glucose and body weight were monitored before (Day 0) and after treatment (Days 3 and 7). There was a trend of weight loss in diabetic rats compared to non-diabetic rats, though the difference was not statistically significant. After 7 days of treatment, there was a comparable decrease in the blood glucose of diabetic rats treated with 50% or 100% of aqueous *C. caudatus* extract and those treated with glibenclamide. Qualitative phytochemical screening indicated the presence of steroid, saponin, phenol, and flavonoid compounds. The total phenolic content was 38.48 mg GAE/g and IC_{50} DPPH antioxidant activity was 375.64 ppm. This study demonstrated that an aqueous extract of *C. caudatus* exhibits a blood glucose-lowering effect in an alloxan-induced diabetic rat model.

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INTRODUCTION

Diabetes mellitus, a chronic metabolic disorder characterized by impaired insulin action resulting from either ineffective insulin utilization or insufficient insulin production, leads to persistent hyperglycemia[1]. The escalating global prevalence of diabetes poses a significant public health concern. In Indonesia, the estimated number of individuals aged 20-79 years living with diabetes reached 19.5 million in 2021 and is alarmingly projected to increase by 46.67% by 2045[2]. Poorly managed diabetes can lead to severe micro- and macrovascular complications, affecting the nervous system, kidneys, and retina[1].
Current pharmacological interventions for diabetes include insulin therapy and various classes of oral antidiabetic drugs, such as α-glucosidase inhibitors (e.g., acarbose), GLP-1 receptor agonists, SGLT2 inhibitors (e.g., dapagliflozin), DPP-4 inhibitors (e.g., sitagliptin), insulin sensitizers (e.g., metformin and pioglitazone), and insulin secretagogues (e.g., glibenclamide). However, these synthetic agents are often associated with undesirable side effects, including weight gain, hypoglycemia, dizziness, weakness, tremors, and gastrointestinal disturbances (nausea, diarrhea, vomiting, and

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flatulence)[3,4]. This limitation has fueled a growing interest in exploring complementary and alternative therapies, particularly those derived from medicinal plants, which are perceived by many as having potentially fewer side effects[4].

The use of herbal medicine is a widespread practice in many developing nations, with approximately 80% of the population relying on traditional remedies, including plant-based treatments, as their primary healthcare approach in some communities[5,6]. One such plant of interest is *Cosmos caudatus*, commonly known as '*kenikir*' in Indonesia and '*ulam raja*' (king's salad) in other parts of Southeast Asia[7]. Belonging to the Asteraceae family, this plant is recognizable by its distinctive pink or purple flowers[8] and is found in regions spanning Southern Mexico, South America, and Southeast Asia, where it is frequently consumed in salads. Traditionally, *C. caudatus* has been employed to manage various health conditions, including hypertension, diabetes, and inflammatory disorders[7-9].

Previous studies have demonstrated that ethanol and methanol extracts of *C. caudatus* possess antioxidant properties and can inhibit α-glucosidase and α-amylase enzymes, both of which are drug targets for diabetes mellitus[10-12]. Phytochemical analyses of *C. caudatus* have revealed the presence of a diverse array of bioactive compounds, including phenolic acids, phenylpropanoids, and flavonoids[13]. Notably, quercetin and its derivatives are abundant flavonoids identified in the ethanol extract of *C. caudatus*[11], and prior research has demonstrated the blood glucose-lowering effects of quercetin administration in animal models of diabetes mellitus[14]. Despite the promising evidence supporting the therapeutic potential of *C. caudatus*, there remains a significant gap in the scientific literature regarding the investigation of its aqueous extract specifically for the management of diabetes mellitus. Therefore, this study aimed to evaluate the anti-diabetic effect of an aqueous extract prepared from *C. caudatus* leaves in an experimental animal model.

MATERIALS AND METHODS

Materials

Fresh *C. caudatus* plants were procured from a local traditional market in Palembang, Indonesia. Upon arrival at the laboratory, the plants were carefully sorted to select healthy leaves, which were then weighed to determine their initial wet weight. Taxonomic identification of the plant species was formally confirmed by the Department of Biology, Faculty of Mathematics and Natural Sciences, Universitas Sriwijaya, and a voucher specimen was assigned Certificate No. 396/UN9.1.7/4/EP/2024 for future reference.

Methods

Plant extraction

Fresh *C. caudatus* leaves were harvested and air-dried under controlled ambient conditions for a period of 7 to 9 days until a constant weight was achieved, allowing for the determination of dry biomass. The dried leaves were then pulverized into a fine powder using an electric blender to increase the surface area for efficient extraction. For the aqueous extraction, 10 grams of the powdered *C. caudatus* leaves were accurately weighed and transferred into a beaker containing 100 mL of distilled water, resulting in a plant material to solvent ratio of 1 : 10 (w/v). This mixture was then subjected to ultrasound-assisted extraction using a sonicator operating at a frequency of 40 kHz for a duration of 30 minutes, while maintaining a controlled temperature of 30°C[15]. Following sonication, the resulting suspension was filtered through a sterile filter cloth to separate the solid residue from the liquid extract. The obtained filtrate, representing the 100% *C. caudatus* aqueous extract, was then directly utilized or further diluted with distilled water to prepare 50% and 25% extract concentrations through serial dilution, as required for subsequent experimental procedures.

Animal experiments

This study was conducted following approval from the Medical and Health Research Ethics Committee, Faculty of Medicine, Universitas Sriwijaya (Ethical approval No. 104-2024). Healthy male Wistar rats, aged 3-4 months, were procured from a reputable animal farm in Palembang, Indonesia, and their health status was verified by a certified veterinarian from the local Department of Food Security and Livestock. Upon arrival, the rats were housed in the Animal House facility of the Faculty of Medicine, Universitas Sriwijaya, under controlled conditions (temperature: 25 ± 2°C; 12-hour light/dark cycle) with free access to standard rodent chow and water, provided twice daily. A seven-day acclimatization period was implemented to minimize stress. Diabetes mellitus was experimentally induced in the rats via a single intraperitoneal

injection of alloxan monohydrate at a dosage of 150 mg/kg BW. Random blood glucose levels were subsequently measured using a portable glucometer, requiring a maximum of 10 µL of blood obtained from the tail vein[16]. Rats exhibiting sustained blood glucose levels exceeding 200 mg/dL were classified as diabetic and selected for the subsequent treatment phase[17]. Utilizing a randomized block design, the diabetic rats were stratified into the following treatment groups: a non-diabetic control group, a diabetic control group (untreated), a positive control group receiving 5 mg/kg BW of glibenclamide via oral gavage daily, and three treatment groups receiving aqueous *C. caudatus* leaf extract at concentrations of 25%, 50%, and 100% (w/v), respectively, also administered daily via oral gavage[18,19]. Blood glucose levels were meticulously monitored and recorded at baseline (day 0), and subsequently on days 3 and 7 post-treatment initiation[20-22].

Phytochemical screening

Qualitative phytochemical analyses were conducted to ascertain the presence of key bioactive constituents within the test sample. Specifically, these assays aimed to identify the presence of alkaloids, steroids, flavonoids, saponins, and phenol hydroquinone, utilizing established colorimetric and precipitation reactions. The resulting data provided a preliminary profile of the chemical constituents potentially contributing to the observed biological activities.

Alkaloid[23]: The sample was initially dissolved in a 2 N sulfuric acid solution. Subsequently, this acidic solution was subjected to qualitative analysis using three distinct alkaloid-precipitating reagents: Dragendorff's reagent, Meyer's reagent, and Wagner's reagent. A positive result for alkaloids was indicated by the formation of a characteristic precipitate with each reagent: a yellowish-white precipitate with Meyer's reagent, a brown precipitate with Wagner's reagent, and a red-to-orange precipitate with Dragendorff's reagent.

Steroid[24]: The sample was initially dissolved in 2 mL of chloroform within a clean test tube. Subsequently, 10 drops of acetic anhydride and 3 drops of concentrated sulfuric acid were carefully added to this solution. A positive reaction for the presence of the target analyte was visually determined by a sequential color change, specifically the formation of a red solution that transitioned through blue and ultimately to green upon reagent addition.

Flavonoid[25]: A defined aliquot of the sample was initially introduced to 1 mL of a 1% (w/v) NaOH solution. A positive reaction was visually determined by the development of a yellow coloration within the mixture. Subsequently, 1 mL of a 1% (v/v) HCl solution was added to the same mixture, and the disappearance of the yellow color, resulting in a colorless solution, further confirmed the presence of the targeted analyte based on this specific chemical transformation.

Saponin[26]: Briefly, 3 mL of hot distilled water was added to the sample, allowed to cool to room temperature, and then vigorously shaken for 10 seconds. A positive reaction for saponins was indicated by the formation of a persistent foam layer ranging from 1 to 10 cm in height that remained stable following the addition of one drop of 2N HCl.

Phenol hydroquinone[27]: Briefly, 1 g of the finely ground sample was extracted with 20 mL of 70% ethanol under controlled conditions to ensure optimal compound solubility. Subsequently, one milliliter of the resulting ethanolic extract was treated with two drops of a 5% $FeCl_3$ solution; the development of a green or blue-green coloration visually indicated the presence of phenolic constituents within the analyzed material.

Total phenolic content

The total phenolic content of the *C. caudatus* sample was quantitatively determined using the Folin–Ciocalteu assay, following a modified protocol. Briefly, the sample was mixed with 2.5 mL of 50% Folin-Ciocalteu reagent and incubated for 5 minutes, after which 2.5 mL of 5% Na_2CO_3 solution was added to adjust the pH. The resulting mixture was then incubated in the dark at room temperature for 60 minutes, and the absorbance was measured at 765 nm using a spectrophotometer. The total phenolic content was subsequently calculated against a gallic acid standard curve and expressed as mg of gallic acid equivalents per g of dry weight (mgGAE/g).

Antioxidant activity

The antioxidant capacity of C. *caudatus* sample was quantitatively determined by assessing its free radical scavenging activity using the 1,1-diphenyl-2-picrylhydrazyl (DPPH) assay, following a previously established protocol[28]. Briefly, a stock solution of *C. caudatus* leaf powder was prepared by dissolving 0.025 g in 25% methanol, which was subsequently diluted to create a series of test solutions ranging from 100 to 500 ppm in a final volume of 5 mL. The assay involved reacting 2 mL

of a freshly prepared DPPH methanolic solution (0.008 g in 50 mL methanol) with each *C. caudatus* solution, and the absorbance was measured at 517 nm using a spectrophotometer to quantify the scavenging activity, calculated using the **Equation 1**.

$$\%\text{antioxidant} = \frac{\text{absorbance of sample} - \text{absorbance of blank}}{\text{absorbance of blank}} \times 100 \qquad [1]$$

Total protein content

The total protein content of the *C. caudatus* sample was quantified by determining the total nitrogen content using the Kjeldahl method, following established protocols[29]. Briefly, 0.015 g of finely powdered *C. caudatus* sample was accurately weighed into a Kjeldahl flask. To this, a digestion mixture consisting of 1 g selenium in 2 mL of concentrated H_2SO_4 was carefully added. The flask was then heated under controlled conditions for 1 hour, or until the digest became a clear, pale green solution, indicating complete digestion. Following digestion, the flask was allowed to cool to room temperature. The digested sample was then transferred to a distillation apparatus, and the flask was rinsed thoroughly with 5-6 aliquots of 1-2 mL of distilled water, with the washings collected in the distillation flask. A receiving Erlenmeyer flask containing a known volume of H_3BO_3 solution and 2-4 drops of methyl red indicator was placed under the condenser of the distillation apparatus. Subsequently, 8-10 mL of a NaOH-$Na_2S_2O_3$ solution was added to the distillation flask to liberate ammonia. Approximately 40 mL of the distillate, containing the liberated ammonia, was collected in the receiving Erlenmeyer flask. The volume of the solution in the Erlenmeyer flask was then adjusted to 50 mL with distilled water. Finally, the ammonia trapped in the H_3BO_3 solution was quantified by titration with a standardized 0.01 N HCl solution until a persistent grey endpoint was reached. The nitrogen content was calculated based on the volume of HCl used in the titration, and the total protein content was subsequently determined by multiplying the nitrogen content by a conversion factor, as described in **Equations 2** and **3**.

$$\%\text{nitrogen} = \frac{\text{ml HCl} \times \text{N HCl} \times 14.00 \times 100}{\text{mg sample}} \qquad [2]$$

$$\%\text{protein} = \%\text{nitrogen} \times 6.25 \qquad [3]$$

Data analysis

Statistical analyses were conducted using GraphPad Prism version 9. Prior to inferential testing, the normality of the data distribution for each variable was assessed using the Shapiro-Wilk test. A p-value greater than 0.05 was considered indicative of a normally distributed dataset. For normally distributed data, parametric tests were employed: paired t-tests were used for comparisons within the same group across different time points or conditions, while one-way Analysis of Variance (ANOVA) was utilized for comparisons between multiple independent groups. Where ANOVA yielded statistically significant differences, a post-hoc Bonferroni was applied to account for multiple comparisons and identify specific pairwise differences. In all statistical tests, a p-value of less than 0.05 was considered to indicate statistical significance.

RESULTS AND DISCUSSION

Weight loss

Analysis of body weight changes revealed no statistically significant difference between the diabetic and non-diabetic rat groups at baseline and following the seven-day treatment period ($p > 0.05$). However, a discernible trend of moderate weight loss was observed in the diabetic rats over the course of the experiment, while the non-diabetic control group exhibited a modest increase in body weight (**Figure 1**). Furthermore, no significant difference in weight loss was detected among the untreated diabetic rats and those treated with either glibenclamide or the *C. caudatus* extract ($p > 0.05$). The observed trend in the diabetic control group warrants consideration in future, longer-term investigations.

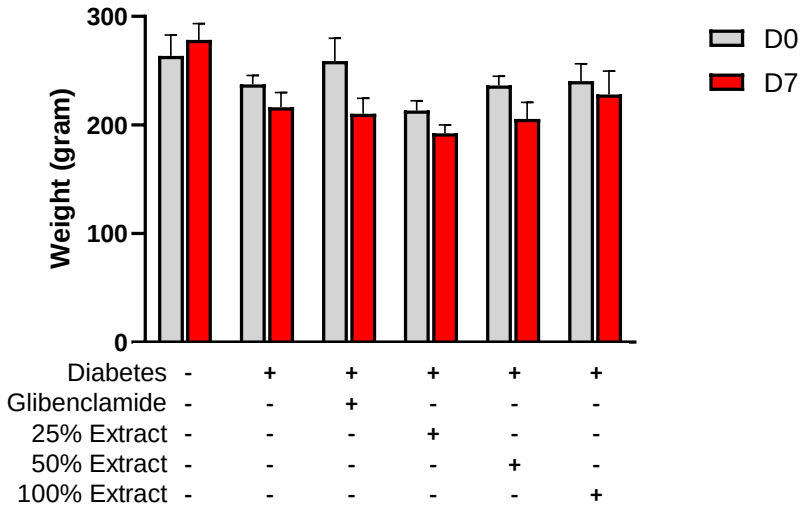

Figure 1. Rat body weight before (D0) and after seven days (D7) of treatment.

Blood glucose level

Initial random blood glucose (RBG) measurements in the alloxan-induced diabetic rat model reached a hyperglycemic state of approximately 500 mg/dL (**Figure 2A**). Following three days of treatment, all diabetic groups exhibited significantly elevated RBG levels (505.1 ± 35.55 mg/dL) compared to the normoglycemic control group (103 ± 2.97 mg/dL; **Figure 2B**). Subsequently, a time-dependent reduction in RBG levels was observed in diabetic rats treated with glibenclamide (298 ± 63.88 mg/dL at day 3 and 211.30 ± 80.86 mg/dL at day 7) and the 50% *C. caudatus* extract (255.50 ± 71.68 mg/dL at day 3 and 221.70 ± 56.50 mg/dL at day 7). The 100% *C. caudatus* extract also demonstrated a reduction in RBG (301 ± 85.40 mg/dL at day 3 and 361.50 ± 61.49 mg/dL at day 7). Statistical analysis at day 7 revealed that both glibenclamide and the 50% *C. caudatus* extract significantly lowered RBG levels ($p < 0.05$) compared to the untreated diabetic group (467.50 ± 45.36 mg/dL). Notably, the reduction achieved by the 50% extract was comparable to that of glibenclamide. In contrast, the 25% *C. caudatus* extract did not elicit a significant change in RBG levels throughout the treatment period (513.80 ± 59.44 mg/dL at day 3 and 447.80 ± 28.16 mg/dL at day 7; $p > 0.05$; **Figure 2B** and **2C**), suggesting a dose-dependent effect of the extract. These findings indicate that the aqueous extract of *C. caudatus*, particularly at concentrations of 50% and 100%, possesses significant hypoglycemic activity in this experimental model, warranting further investigation into the underlying mechanisms and identification of the active compounds responsible for this effect.

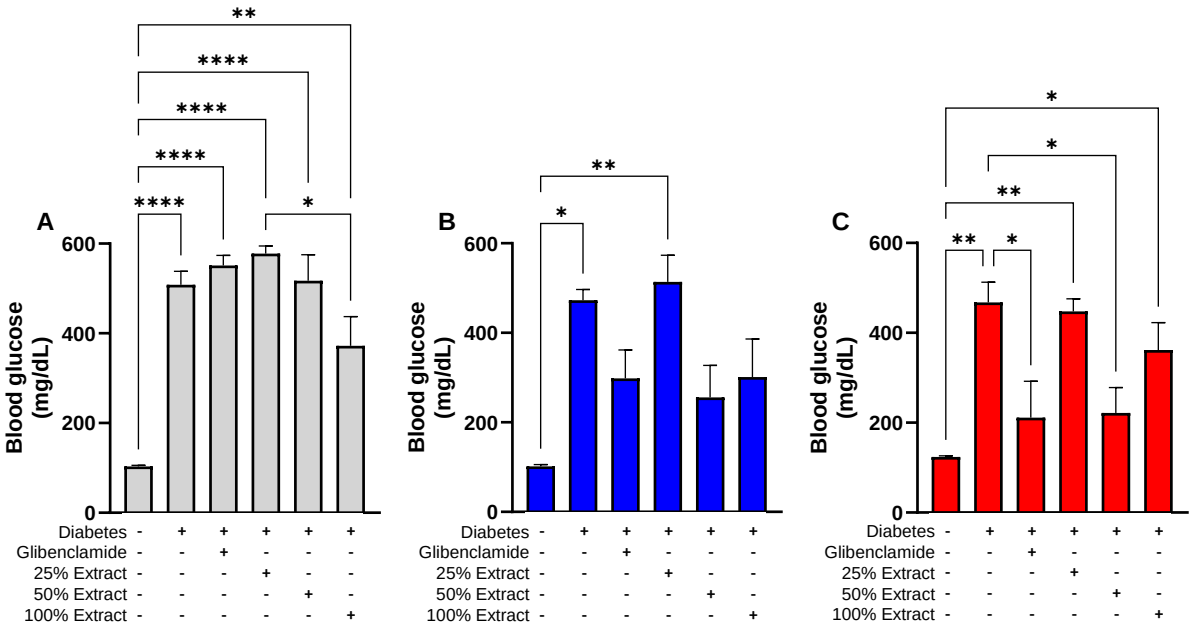

Figure 2. Blood glucose (**A**) before and after (**B**) 3 days or (**C**) 7 days of treatment period. *p <0.05; **p <0.01; ***p <0.001; ****p <0.0001.

Characterization of crude aqueous extract

Qualitative phytochemical screening of the aqueous extract of *C. caudatus* leaves revealed the presence of several bioactive secondary metabolites, including steroids, saponins, flavonoids, and phenol hydroquinones (**Table I**). Quantitative analysis of the dried leaves demonstrated a total phenolic content (TPC) of 38.48 mgGAE/g. Furthermore, the extract exhibited weak antioxidant activity, as determined by the DPPH assay, with an IC_{50} value of 375.64 ppm. This antioxidant capacity, visually represented in **Figure 3** alongside the potent standard ascorbic acid (IC_{50} 6 ppm), suggests the presence of compounds capable of scavenging free radicals. Additionally, the total protein content of the dried *C. caudatus* leaves, determined using the Kjeldahl method, was found to be 18.41%.

Table I. Qualitative phytochemical screening.

Secondary metabolites	Result
Alkaloid	-
Steroid	+
Saponin	+
Flavonoid	+
Phenol hydroquinone	+

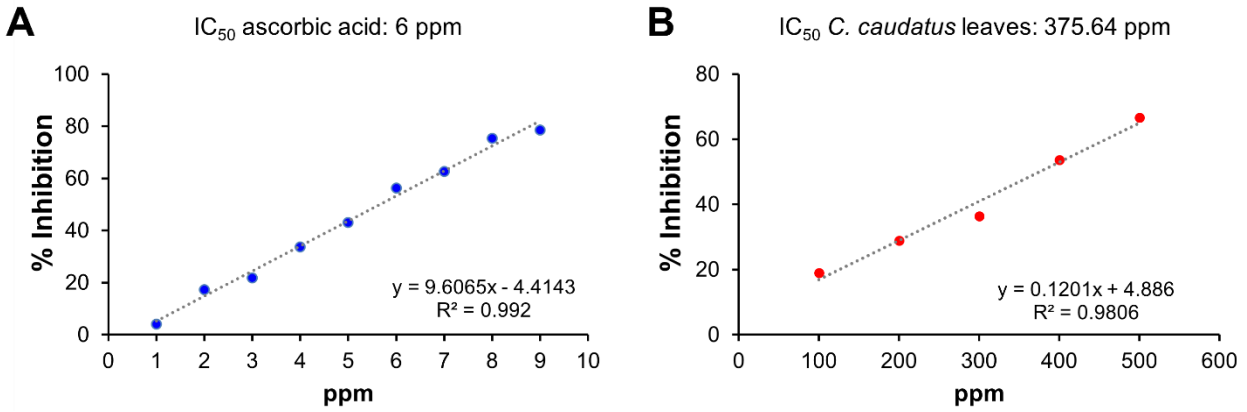

Figure 3. Comparison of antioxidant activity between (**A**) ascorbic acid and (**B**) *C. caudatus* leaves.

The present study demonstrated the hypoglycemic efficacy of a *C. caudatus* aqueous extract in an alloxan-induced diabetic rat model. Oral administration of both the 50% and 100% concentrations of an aqueous extract derived from 10 g of dried *C. caudatus* leaves resulted in a reduction of blood glucose levels, comparable to that observed in the glibenclamide-treated positive control group, following a once-daily dosing regimen. Notably, after seven days of treatment, blood glucose levels in the diabetic groups, including those receiving the plant extract and glibenclamide, remained elevated above the normoglycemic threshold (<200 mg/dL), suggesting that the observed effect was significant but not curative within this timeframe. The reduction in blood glucose levels following *C. caudatus* extract administration appeared to be gradual, as evidenced by the progressive decrease observed at days 3 and 7. This suggests that a more prolonged treatment period may be necessary to achieve a more pronounced and potentially normalizing effect on blood glucose. Intriguingly, our findings revealed a U-shaped dose-response relationship, with the 50% extract concentration exhibiting a greater hypoglycemic effect than both the 25% and 100% concentrations. This non-linear dose-response is a phenomenon reported in other studies investigating the biological activities of plant extracts and their constituent metabolites. This could be attributed to the complex interplay of multiple metabolites within the *C. caudatus* extract, where synergistic interactions at optimal concentrations (50%) contribute to the blood glucose-lowering effect. However, at higher concentrations (100%), these interactions may shift towards antagonism or exhibit inhibitory effects on the overall hypoglycemic activity[30-32]. Therefore, a comprehensive identification and quantification of the various metabolites and other bioactive components within the herbal extract are crucial for establishing the optimal therapeutic window and understanding the underlying mechanisms of action.

Phytochemical analysis of the *C. caudatus* leaf extract in this study revealed the presence of key secondary metabolites, including steroids, saponins, flavonoids, and the specific phenol, hydroquinone. While the total phenolic content (38.48 mgGAE/g) was consistent with previously reported values (36.09–37.76 mgGAE/g)[33], the aqueous extract exhibited

notably weaker *in vitro* antioxidant activity (IC_{50} 375 ppm) compared to findings from other investigations[34,35]. This observation aligns with Cheng *et al.*'s study[11], which similarly reported the highest total phenolic content but the lowest DPPH radical scavenging activity in their aqueous *C. caudatus* extract compared to methanol or ethanol extracts. As highlighted by several studies, the efficiency of phenolic compound extraction and consequently the antioxidant activity of *C. caudatus* extracts are significantly influenced by the solvent and extraction method employed. The generally lower DPPH activity observed in crude aqueous extracts compared to ethanol-based extracts may be attributed to the differential solubility of phenolic compounds, with ethanol being a more effective solvent for a broader range of these bioactive molecules[36,37]. The potentially limited extraction of specific phenolic compounds by water, compared to ethanol, likely contributed to the weaker antioxidant capacity observed in our aqueous *C. caudatus* extract[33,37]. Prior characterization efforts have identified various phenolic compounds in *C. caudatus*, including quercitrin, rutin, kaempferol, luteolin, and apigenin, present in varying concentrations[15,37].

It is important to note that a direct correlation between total phenolic content and antioxidant activity is not always evident due to the diverse radical scavenging capacities of individual phenolic compounds. Certain polar phenolics, preferentially soluble in water, may exhibit a lower propensity to reduce DPPH radical cations, whereas flavonoids, often more soluble in organic solvents like methanol or ethanol, generally demonstrate higher DPPH scavenging efficacy[36]. This difference in individual compound activity could partially explain the lower antioxidant capacity of our aqueous extract. Notably, quercitrin, a quercetin glycoside reported as a major phenolic component in crude aqueous *C. caudatus* extracts, exhibits lower antioxidant and cytoprotective effects but higher water solubility compared to its aglycone, quercetin[38,39]. Furthermore, steric hindrance associated with the glycosidic moiety in quercitrin and similar compounds could potentially impede their ability to effectively scavenge DPPH radicals in the assay[33].

Considering the well-established role of oxidative stress in the pathogenesis of alloxan-induced diabetes, leading to the destruction of pancreatic β-cells[40-42], the observed blood glucose-lowering effect of our *C. caudatus* extract in this model warrants further consideration. While our extract exhibited relatively weak *in vitro* antioxidant activity, suggesting that direct radical scavenging might not be the sole mechanism of action, other pathways could be involved. For instance, non-phenolic compounds, such as specific amino acids, have been shown to stimulate insulin secretion from pancreatic β-cells, thereby contributing to reduced blood glucose levels[43,44]. Interestingly, *C. caudatus* leaves have been reported to possess a relatively high total protein content[45,46], suggesting a potential role for amino acids or even plant-derived peptides[47,48] in the observed hypoglycemic effect. Future research should therefore explore the contribution of non-phenolic constituents in *C. caudatus* to its anti-diabetic properties.

CONCLUSION

This study provides evidence that the aqueous extract of *C. caudatus* leaves possesses a significant hypoglycemic effect in an alloxan-induced diabetic rat model. Phytochemical analysis of the extract revealed the presence of several bioactive compound classes, including steroids, saponins, flavonoids, and phenol hydroquinones. The total phenolic content observed was consistent with values reported in previous investigations of this plant. However, the relatively weak antioxidant activity of the extract suggests that mechanisms beyond direct radical scavenging may contribute to the observed reduction in blood glucose levels in diabetic rats. Therefore, future research should focus on the isolation and identification of specific bioactive compounds within the *C. caudatus* extract and the elucidation of the precise molecular mechanisms underlying its anti-diabetic action. This will provide a more comprehensive understanding of its therapeutic potential for managing diabetes.

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Project administration: -
Resources: Ayeshah Augusta Rosdah, Subandrate
Software: -
Supervision: Ayeshah Augusta Rosdah, Nia Savitri Tamzil
Validation: Subandrate
Visualization: Subandrate
Writing - original draft: Ayeshah Augusta Rosdah, Nia Savitri Tamzil, Subandrate
Writing - review & editing: Ayeshah Augusta Rosdah, Nia Savitri Tamzil, Subandrate

DATA AVAILABILITY

None.

CONFLICT OF INTEREST

The authors declare no conflicts of interest related to this study.

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