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Inhibitory Effects on α-Amylase and α-Glucosidase and Phytochemical Profiling of the Aerial Part of *Canna x generalis* L.H Bailey & E.Z Bailey

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ABSTRACT

Canna x generalis L.H Bailey & E.Z Bailey, Cannaceae, has been traditionally used to treat different diseases. However, the scientific evidence about its chemical composition and biological activities is minimal. This study aimed to evaluate for the first time the α-amylase and α-glucosidase inhibitory effects and phytoconstituents of the aerial part of *C. generalis*. Aerial *C. generalis* was extracted with ethanol, and then successively fractionated with *n*-hexane, ethyl acetate and water. The total extract and fractions were evaluated for their α-amylase and α-glucosidase inhibitory effects. The qualitative phytochemicals, the total phenolic content (TPC) and total flavonoid content (TFC) were assessed. The results showed that all extracts showed inhibitory effects against both α-amylase and α-glucosidase in a dose dependent manner. The ethyl acetate fraction exhibited the strongest α-amylase and α-glucosidase inhibitory effects (IC_{50}, 48.0 ± 4.48 and 78.0 ± 4.61 µg/mL for the α-amylase and α-glucosidase inhibition assay, respectively, $p < 0.05$). The ethyl acetate fraction also had the highest TPC and TFC compared to the others ($p < 0.05$). Moreover, the aerial part of *C. generalis* contained various phytoconstituents. Therefore, *C. generalis*, particularly, its ethyl acetate fraction could serve as a promising natural source for searching bioactive compounds that inhibit the two key enzymes, α-amylase and α-glucosidase. In addition, this interesting plant could be a potential candidate for the development of supplementary products for controlling hyperglycemia.

Keywords: α-amylase, α-glucosidase, *Canna x generalis*, flavonoid, phenolic, diabetes

Introduction

Diabetes is characterized by hyperglycemia resulting from deficiency in and insensitivity to insulin. This chronic disease usually leads to various complications.[1] Diabetes has become a major cause of death globally.[2] Moreover, type 2 diabetes accounts for nearly 90% of all diabetes patients. It is caused by the failure of pancreatic beta cells to produce insulin, thus glucose cannot be taken up from the blood into the cells, causing high blood glucose level. The two key enzymes linked to type 2 diabetes are α-amylase and α-glucosidase. Inhibition of these enzymes will reduce postprandial blood glucose levels. Acarbose and miglitol are the two synthetic inhibitors of α-amylase and α-glucosidase commonly used for managing diabetes. However, gastrointestinal side effects such as abdominal distension, bloating and diarrhea occur frequently. Therefore, exploring effective therapeutic methods with less side effects for diabetes is urgent. Screening inhibitors of α-amylase and α-glucosidase from medicinal plants has received much attention.[3-7]

Canna x generalis L.H Bailey & E.Z Bailey (CG) is a medicinal plant belonging to the *Canna* genus. In traditional medicine, *Canna* plants have been commonly used to treat various conditions such as diarrhea, hepatitis, dysentery, gonorrhea, bruises, pain, and heart diseases.[8,9] However, the scientists have not paid much attention to this genus.

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Several studies have reported pharmacological effects of *C. warszewiczii* and *C. edulis* such as antimalarial, antibacterial, antithrombotic, antioxidant and antiinflammatory activity. Bioactive flavonoids and phenolic compounds were isolated from *C. edulis*.[8,10-13] In addition, Xie *et al*. documented the potential inhibition of α-glucosidase of lignan from *C. edulis* ker residue.[14] However, scientific data on phytochemicals and biological effects of CG are minimal. The study by Mahmoud *et al*. reported the therapeutic potential against ulcerative colitis of CG rhizomes and identified its major phytoconstituents.[15] Recently, we documented the antithrombotic and antioxidant activity, isolated several bioactive compounds from aerial CG.[16] Nevertheless, the therapeutic potential of CG in managing diabetes has not been studied yet. This study aimed to evaluate the inhibitory effects on α-amylase and α-glucosidase, phytochemicals, total flavonoid content (TFC) and total phenolic content (TPC) of extracts from the aerial part of CG.

Materials and Methods

Plant collection and identification

CG was collected in Thai Nguyen province, Vietnam, and identified by Dr. Do Van Hai, Institute of Ecology and Biological Resources, Vietnam Academy of Science and Technology in November, 2021. The plant was deposited at the Department of Life Sciences, University of Science and Technology of Hanoi, Vietnam Academy of Science and Technology with a voucher number of CG.A.TN02.

Chemical materials

Chemicals used in this study were dimethyl sulfoxide (DMSO), α-amylase, α-glucosidase, acarbose, p-nitrophenyl-α-glucopyranoside (pNPG), $NaNO_2$, $AlCl_3$ from Sigma-Aldrich. In addition, acetic acid, sodium carbonate, ethanol (EtOH), Na_2CO_3, acetic acid, *n*-hexane and ethyl acetate (EtOAc) were obtained from China.

Plant extraction
The aerial part of CG was cleaned, dried at room temperature and powdered. Then, the powder (1.0 kg) was extracted with 96% EtOH four times at room temperature, and filtered. The solvent was evaporated to obtain the total ethanol extract (CG.Et, 350 g). Then the total extract (100g) was then successively fractionated with *n*-hexane, ethyl acetate, and water. After being filtered and evaporated under reduced pressure to completely remove the solvent, three fractions of *n*-hexane (CG.H, 7.4 g), ethyl acetate (CG.EA, 3.78g), and water (CG.W 45g) were obtained. The extracts were stored at 4 - 6 °C for further use. The percentage yield for each extract was calculated using the formula: Yield (%) = $m_f/m \times 100$, where m_f is the mass of dried fractions and m is the mass of the dried material before extraction.

Determination of α -amylase inhibitory activity
α-Amylase inhibition activities of the total extract and fractions of the aerial part of CG were determined following the previous method described by Wu *et al*. with some modifications.[17] The extracts were dissolved in DMSO and diluted to obtain final concentrations of 5, 25, 50, 100, 200 µg/mL. *α*-Amylase (0.5 U/mL) was prepared in phosphate buffer (pH = 6.9). Then, 50 µL *α*-amylase (0.5 U/mL) was mixed with samples at different concentrations and 250 µL phosphate buffer (pH = 6.9). After incubation of the mixture for 20 min at 37 °C, 250 µL starch solution (1 mg/mL) was added, and the mixture was re-incubated for 20 min, followed by the addition of 250 µL acetic acid 50% to stop the reaction. The new mixture was then centrifuged at 3000 rpm at 4 °C. The absorbance of the supernatant was measured at 595 nm using a UV – visible spectrophotometer. The positive control was acarbose. Percentage inhibition of enzyme activity was calculated as below:

Inhibitory effect (%) = $(OD_{control} - OD_{sample})/OD_{control}$
$OD_{control}$ and OD_{sample} are defined as the absorbance of control and tested sample, respectively. IC_{50}, which is the concentration of samples required to inhibit 50% of enzyme activity, was determined.

Determination of α -glucosidase inhibitory activity
The inhibitory effect against *α*-glucosidase of extracts was assayed as described previously by Zhang *et al*. with some modifications.[18] The extracts were dissolved in DMSO and diluted with phosphate buffer 0.1M (pH = 6.8). The enzyme *α*-glucosidase was prepared at a concentration of 0.5 U/mL in phosphate buffer 0.1 M (pH = 6.8). Then, 50 µL of extracts at different concentrations and 130 µL of phosphate buffer 0.1 M were mixed with 20 µL of enzyme *α*-glucosidase. The mixtures were incubated at 37 °C for 10 min. The reaction was initiated with the addition of pNPG. The mixtures were further incubated for 60 min at 37 °C. The reaction was stopped with the addition of 80 µL Na_2CO_3 0.2 N. The absorbance was measured at 405 nm using an ELISA plate reader (Bio-rad). Acarbose was used as a positive control. Percentage inhibition of enzyme activity of tested samples was calculated as below:

Inhibitory effect (%) = $(A_{control} - A_{sample})/A_{control}$
$A_{control}$ and A_{sample} are defined as the absorbance of control and tested sample, respectively. IC_{50}, which is the concentration of samples required to inhibit 50% of enzyme activity, was determined.

Phytochemical screening
The qualitative phytochemical assessment of aerial CG fractions was performed by chemical reactions using the methods previously described by Jagessar.[19] The presence of secondary metabolites in the samples, including cholesterols, cardiac glycosides, glycosides, tannins, flavonoids, sterol and triterpenes, steroids, proteins, saponins and coumarins were identified.

Determination of total phenolic content (TPC)
The TPC of the total extract and fractions was measured using the Folin-Ciocalteu method as previously described by Singleton *et al*.[20] Extracts were dissolved and diluted in methanol. Then, 480 µL of the diluted Folin – Ciocalteu reagent and 480 µL Na_2CO_3 6% were added into 40 µl samples. The mixture was incubated at 40 °C in 15 min. The absorbance was measured at 765 nm by a microplate spectrophotometer. The experiments were done in triplicate. A standard curve of gallic acid at 200, 100, 50 and 25 µg/ml was used to estimate the TPC in the extracts. The TPC was expressed as mg of gallic acid equivalents (GAE/g).

Determination of total flavonoid content (TFC)
The plant extracts were dissolved and diluted in methanol. First, 240 µL of extracts was added to 40 µL of 5% $NaNO_2$ solution and the mixture was incubated for 6 min at 25 °C. Then, 40 µL of 10% $AlCl_3$ was added, followed by the addition of 400 µL of 1M NaOH and 280 µL of 30% ethanol. After incubation at room temperature for 15 min, the absorbance was measured at 510 nm. A standard curve of quercetin (0 - 200 µg/mL) was used to estimate the TFC in the samples. The TFC was expressed as mg of quercetin equivalents (QE/g).

Data analysis
All data were expressed as mean ± standard deviation (SD). Statistical analysis was performed using Statistical Package for Social Sciences (SPSS) 23.0 software. Independent sample t-test and ANOVA test were performed to assess the differences between samples. $P < 0.05$ was regarded as statistically significant.

Results and Discussion

Diabetes is increasing at an alarming rate in developing countries. This chronic disease is contributed by various factors including stress, lifestyle (dietary habits and lack of exercise) and family history. Controlling strategies of diabetes commonly target either the enzymes or hormone receptors to achieve optimal level of blood glucose as soon as possible after meal.[2,21] While the enzyme *α*-amylase breaks down long-chain carbohydrates, the enzyme *α*-glucosidase is crucial to catalyze the final stage of carbohydrate digestion. Therefore, inhibitors of both enzymes are effective in reducing the absorption of glucose and managing diabetes. Acarbose is an anti-diabetic drug commonly used to treat type 2 diabetes. It inhibits reversibly intestinal *α*-glucosidase, thus delays the glucose absorption from the intestines and improves postprandial hyperglycemia. However, its disadvantages are common gastrointestinal side effects.[3] In this study, inhibitory effects against the two key enzymes *α*-amylase and *α*-glucosidase of the total extract and fractions of aerial CG were thus determined, taking acarbose as a positive control.
The extraction of the aerial part of CG yielded the total ethanol extract (CG.Et, 35.0%) and 3 fractions: CG.H, 0.3%; CG.EA, 0.13%; CG.W, 1.5%.

α-Amylase inhibitory activity
As shown in the Figure 1, all extracts of aerial CG possessed the inhibitory effects against *α*-amylase. Their effects were dose-dependent in the tested range. Among extracts, the ethyl acetate fraction showed the highest *α*-amylase inhibitory activity ($p < 0.05$). Moreover, the inhibitory effect of the ethyl acetate fraction was significantly stronger than the positive control (IC_{50}, 48.0 ± 4.48 vs. 58.27 ± 10.78 µg/mL, $p < 0.05$). The *n*-hexane fraction exhibited the lowest inhibitory effect with an IC_{50} of 181.44 ± 31.15 µg/mL ($p < 0.05$). The water fraction also had a good *α*-amylase inhibitory activity, which is similar to the positive control ($p > 0.05$) (Table 1).

α-Glucosidase inhibitory activity
Figure 2 shows that the total extract and three fractions of the aerial part of CG had potential inhibitory effects against *α*-glucosidase, and their effects were all dose dependent in the tested range. Among the extracts, the ethyl acetate fraction had the most potent *α*-glucosidase inhibitory effect ($p < 0.05$), which was significantly stronger than the positive control acarbose (IC_{50}, 78.0 ± 4.61 vs. 122.57 ± 12.78 µg/mL, $p < 0.05$). Similar to the results obtained in the *α*-amylase assay, the *n*-hexane fraction exhibited the smallest activity with an IC_{50} of 322.21 ± 9.94 µg/mL compared to the others ($p < 0.05$). The water fraction and acarbose had similar *α*-glucosidase inhibitory activities (IC_{50}, 126.22 ±

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8.45 and 122.57 ± 12.78 µg/mL for CG.W and acarbose, respectively, $p > 0.05$).

Phytochemical screening

The first-hand knowledge of the phytochemical constituents of the CG aerial part was provided by phytochemical analysis using standard established tests.[19] The results showed that the CG aerial part contained abundant secondary metabolites (Table 3). In the *n*-hexane extract, the presence of cholesterol, flavonoids, sterol and triterpenes, steroids and protein was observed, whereas the presence of cardiac glycosides, glycosides, tannins, saponins and coumarins was not observed. However, there were cardiac glycosides, glycosides, tannins, flavonoids, sterol and triterpenes, steroids, proteins and coumarins in the ethyl acetate fraction. In the water fraction, cardiac glycosides, tannins, flavonoids, sterol and triterpenes and proteins were observed. It could be seen that there were more tested secondary metabolites in the ethyl acetate fraction compared to the others. In the study by Mahmoud *et al*. on the CG rhizome ethanol extract, flavonoids, phenolic acids and phytosterols were found to be the major phytoconstituents of CG rhizomes.[15] Phytoconstituents play the key role in phytomedicine. The presence of various phytoconstituents in the plants is directly associated with the therapeutic effect of phytomedicine. Glycosides, flavonoids, steroids, coumarins, and triterpenes were reported to have various pharmacological activities. Moreover, these secondary metabolites also possess inhibitory effects against α-amylase and α-glucosidase.[22-25]

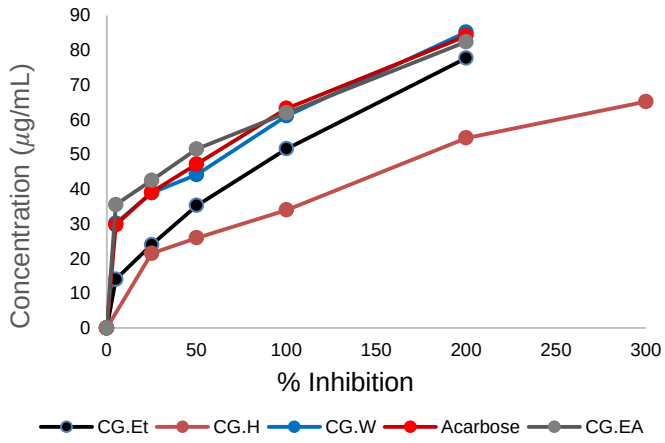

Figure 1: Inhibitory effect against α-amylase of acarbose, the total ethanol extract (CG.Et), *n*-hexane (CG.H), ethyl acetate (CG.EA) and water fraction (CG.W) of the aerial part of *C. generalis*

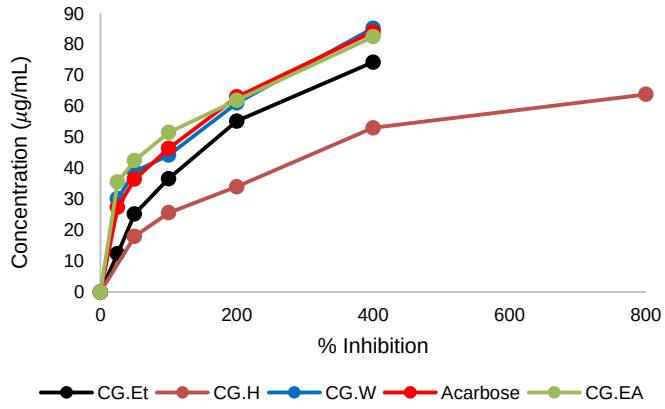

Figure 2: Inhibitory effect against α-glucosidase of acarbose, the total ethanol extract (CG.Et), *n*-hexane (CG.H), ethyl acetate (CG.EA) and water fraction (CG.W) of the aerial part of *C. generalis*

Table 1: IC_{50} values for α-amylase inhibitory effect

Sample	IC_{50} (µg/mL)
Total ethanol extract	93.32 ± 3.37
N-hexane fraction	181.44 ± 31.15
Ethyl acetate fraction	48.01 ± 4.88
Water fraction	64.18 ± 4.43
Acarbose	58.27 ± 10.78

Table 2: IC_{50} values for α-glucosidase inhibitory effect

Sample	IC_{50} (µg/mL)
Total ethanol extract	168.69 ± 28.12
N-hexane fraction	322.21 ± 9.94
Ethyl acetate fraction	88.93 ± 8.73
Water fraction	126.22 ± 8.45
Acarbose	122.57 ± 12.78

Table 3: Phytochemical screening of the *n*-hexane (CG.H), ethyl acetate (CG.EA) and water fraction (CG.W) of *C. generalis* aerial part

Phytochemicals	CG.H	CG.EA	CG.W
Cholesterol	+	-	-
Cardiac Glycosides	-	+	+
Glycosides	-	+	-
Tannins	-	+	+
Flavanoids	+	+	+
Sterol & Triterpenes	+	+	+
Steroids	+	+	-
Proteins	+	+	+
Saponins	-	-	-
Coumarins	-	+	-

Total phenolic content

As can be seen in the Figure 3, the TPC in the total extract and fractions of the CG aerial part ranged from 26.59 ± 0.24 to 86.04 ± 5.73 mg GAE/g, in which the highest TPC was found in the ethyl acetate fraction (86.04 ± 5.73 mg GAE/g, $p < 0.05$). However, the water extract had the lowest TPC compared to the others (26.59 ± 0.24 mg GAE/g, $p < 0.05$). Phenolic compounds are important secondary metabolites of plants. They play important roles in various physiological processes of plants such as flavor, coloring, plant quality, and stress resistance. They were also reported to possess a wide range of biological activities such as antibacterial, antioxidant, anticancer, antinflammatory, and prevention of diabetes, heart diseases and oxidative stress-related diseases.[26] The health benefit of phenolic constituents have gained great attention of researchers.[27] Moreover, phenolic compounds can be beneficial for health by lowering the risk of metabolic disorders, such as type 2 diabetes.[6] Many phenolic compounds in plants have been identified to possess inhibitory activities on α-amylase and α-glucosidase such as caffeic acid, chlorogenic acid, gallic, rutin, p-coumaric acid, genistein, tangeretin, pelargonidin, formononetin and delphinidin chloride.[28-30] The TPC in the ethanol extract of the CG aerial part was 35.38 ± 2.53 mg GAE/g, which is higher than the TPC in the ethanol extract of CG rhizomes (20.55 mg GAE/g) reported previously by Mahmoud *et al*.[15] Moreover, in this study, the highest TPC together with the strongest α-amylase and α-glucosidase inhibition effects observed in the ethyl acetate fraction suggest that the aerial CG might contain some natural

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phenolic inhibitors of *α*-amylase and *α*-glucosidase, which can help control postprandial hyperglycemia.

Total flavonoid content

Flavonoids are the most prevalent and well-studied polyphenolic compounds present in plants. The TFC in the total extract and fractions of the CG aerial part ranged from 6.33 ± 0.76 to 59.31 ± 0.75 mg GAE/g (Figure 3). Notably, the highest TFC was seen in the ethyl acetate fraction (59.31 ± 0.75 mg GAE/g, $p < 0.05$), which is similar to the TPC results. The water fraction contained the lowest TFC (6.33 ± 0.76 mg GAE/g, $p < 0.05$). The TFC in the total ethanol extract of the CG aerial part was 22.82 ± 1.80 mg GAE/g, which is higher than the TFC in the ethanol extract of CG rhizomes (6.74 mg QE/g) reported by Mahmoud *et al*.[15] Flavonoids have been demonstrated to possess broad bioactivities including antioxidant, antibacterial, antiinflammatory, and cardioprotective effects. They are also potent *α*-amylase and *α*-glucosidase inhibitors. These compounds have achieved a great interest. Different flavonoids including flavones, flavanones, flavonols, flavanols, isoflavones and anthocyanines were reported to be strong inhibitors of *α*-amylase and *α*-glucosidase.[31] The high TFC and potent inhibitory effects against *α*-amylase and *α*-glucosidase of the aerial CG, particularly, the ethyl acetate fraction, suggest that the aerial part of CG could be a good natural source for identification of bioactive flavonoids for the treatment and prevention of diabetes.

This study documented novel findings on potent inhibitory effects on both ezymes *α*-amylase and *α*-glucosidase as well as phytoconstituents of aerial CG. Plants are a rich natural source of bioactive molecules.[32, 33,34] Many previous studies also reported the role of herbal extracts as *α*-amylase and *α*-glucosidase inhibitors. This clearly indicates that plant extracts have the potential to manage hyperglycemia.[6,21,30,33,34] Similar to the studies by Bhati *et al*. on *Cornus capitata*[5] and Nguyen *et al*. on *Codonopsis javanica*,[4] the ethyl acetate fraction was found to possess the strongest enzyme inhibitory activity among extracts. Moreover, Le *et al*. evaluated the antithrombotic activity of the aerial part of CG and reported that the ethyl acetate fraction had more substantial antiplatelet and anticoagulant activities than other fractions.[16] These activities of plant extracts could be explained by the phytoconstituents present in them.[5] In the study by Le *et al*. on CG rhizomes, further isolation of the ethyl acetate fraction led to the identification of bioactive compounds such as p-coumaric acid, protocatechuic aldehyde, astragalin, isoquercitrin, rutin, 4-ketopinoresinol, indole-3-carboxylic acid.[16] Currently, scientific evidence on phytochemicals and pharmacological effects of this interesting plant is very limited. This study indicates that CG, particularly, its ethyl acetate fraction could serve as a promising natural source for searching bioactive compounds that inhibit the two key enzymes, *α*-amylase and *α*-glucosidase. In addition, this interesting plant could be a potential candidate for the development of supplementary products for controlling hyperglycemia.

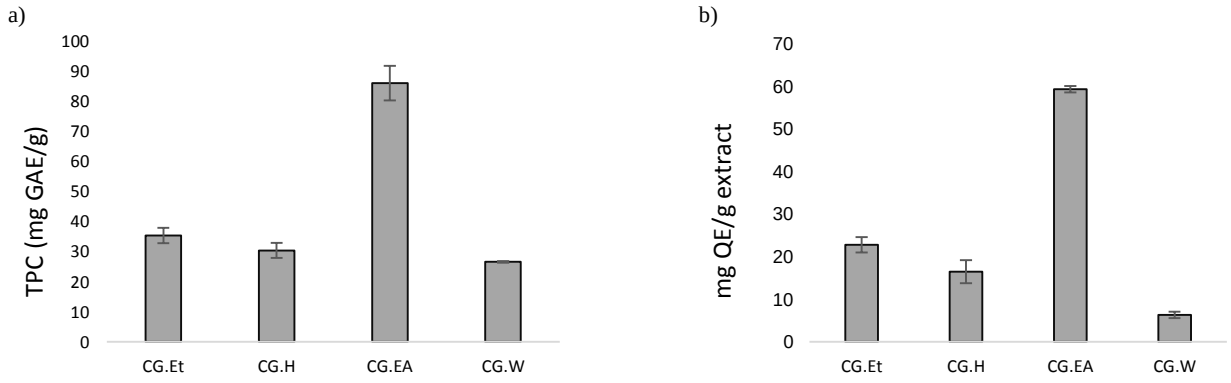

Figure 3: Total phenolic content (a) and total flavonoid content (b) of the total extract and fractions of aerial *C. generalis*
CG.Et: the total ethanol extract of aerial *C. generalis*, CG.H: the *n*-hexane fraction of aerial *C. generalis*, CG.EA: the ethyl acetate fraction of aerial *C. generalis*, CG.W: the water fraction of aerial *C. generalis*

Conclusion

This study demonstrated for the first time the inhibitory effects against the two key enzymes linked to type 2 diabetes, *α*-amylase and *α*-glucosidase, of the aerial part of CG. Moreover, the ethyl acetate fraction exhibited the strongest *α*-amylase and *α*-glucosidase inhibitory activity and highest TPC and TFC. The aerial part of CG contained various phytochemical constituents. This plant might be a promising candidate for drug discovery and development to manage diabetes. Further studies should be performed to find new bioactive molecules from this interesting plant.

Conflict of Interest

The authors declare no conflict 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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