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α-AMYLASE AND α-GLUCOSIDASE INHIBITING EFFECTS OF *CANNA EDULIS* KER GAWL RHIZOME

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SUMMARY

Screening inhibitors of α-amylase and α-glucosidase from medicinal plants is a topic of interest in the world. *Canna edulis* Ker Gawl is an important rhizome plant belonging to the genus *Canna* of the family Cannaceae. It is traditionally used to treat many conditions such as gonorrhea, pain, bruises, diarrhea, hepatitis, chest pain and heart diseases. The potential of this plant in the treatment and prevention of diabetes has rarely been documented. This study reports, for the first time, α-amylase and α-glucosidase inhibiting effects and the phytochemical profile of the rhizome part of *C. edulis*. *C. edulis* rhizome was extracted with 96% ethanol, and then successively fractionated with *n*-hexane, ethyl acetate and water. α-amylase and α-glucosidase inhibiting assays were performed to investigate the enzyme inhibitory activities of the total extract and fractions. Qualitative phytoconstituents were analyzed using chemical reactions. The results showed that all extract and fractions had α-amylase and α-glucosidase inhibiting effects in a dose dependent manner. The ethyl acetate fraction exhibited the strongest inhibiting activities against both enzymes (IC_{50}, 45.24 ± 2.90 and 90.09 ± 6.70 μg/mL for α-amylase and α-glucosidase inhibiting assays, respectively) and its effects were significantly higher than the positive control ($p < 0.05$). The water fraction showed the lowest effects. *C. edulis* rhizome contained abundant secondary metabolites including cardiac glycosides, cholesterols, glycosides, flavonoids, sterol and triterpenes, tannins, steroids, saponins, coumarins and proteins. All these secondary metabolites, except for saponin, were found in the ethyl acetate fraction. *C. edulis* rhizome could serve as a potential candidate to find novel anti-α-amylase and anti-α-glucosidase agents and to develop supplementary products for controlling hyperglycemia.

Keywords: α-amylase, α-glucosidase, *Canna edulis,* diabetes, phytochemicals, rhizome

INTRODUCTION

Type 2 diabetes mellitus (T2DM) is a chronic metabolic disorder that occurs when the blood glucose level is too high as a result of insufficient secretion of insulin from the β-cells of the pancreatic or cell resistance to the action of insulin. The global prevalence of T2DM is increasing because of unhealthy diet, aging, smoking and hyperlipidemia

(Diabetes American Association, 2009). Major used therapies for the treatment of T2DM are to enhance endogenous insulin production, improve the insulin action at target tissues, or inhibit carbohydrate hydrolyzing enzymes. However, there is significant concern about side effects associated with current drug therapies such as abdominal pain, diarhea, and flatulence. Therefore, investigation of alternative antidiabetic therapies has achieved much attention among scientists in the world. Medicinal plants are promising natural sources for discovering safe, inexpensive and effective alternative remedies for treatment and prevention of diabetes (Ghauri *et al*., 2021; Gong *et al*., 2020; Nguyen *et al*., 2020).

Canna edulis Ker Gawl is an important rhizome plant belonging to the genus *Canna* of the family Cannaceae. It is traditionally used to treat many conditions such as gonorrhea, pain, bruises, diarrhea, hepatitis, chest pain and heart diseases (Nguyen *et al*., 2020; Vu *et al.* 2019). Several studies reported antioxidant, antiplatelet and anticoagulant activities, as well as the inhibitory effects on pepsin, lipase, lactoglobulin digestibility and tributyrin hydrolysis of *C. edulis* and its phytoconstituents (Nguyen *et al*., 2020; Zhang *et al*., 2011). However, the potential of this plant in the treatment and prevention of diabetes has rarely been documented. Recently, Xie *et al*. reported the anti-α-D-glucosidase effect of lignin isoated from *C. edulis* rhizome, indicating that this compound could be a promissing candidate for the treatment of T2DM (Xie *et al*., 2017). This study reported, for the first time, the inhibitory effects on α-amylase and α-glucosidase enzymes of *C. edulis* rhizome.

MATERIALS AND METHODS

Materials

The plant was collected in Son La province, Vietnam and identified with the scientific name as *Canna edulis* Ker Gawl. A voucher specimen (No. CE.TN01) was kept at the Department of Life Sciences, University of Science and Technology of Hanoi, Hanoi, Vietnam.

Chemicals used were dimethyl sulfoxide (DMSO), α-amylase, α-glucosidase, acarbose, and p-nitrophenyl-α-glucopyranoside (pNPG) from Sigma-Aldrich, acetic acid, sodium carbonate, ethanol (EtOH), *n*-hexane and ethyl acetate (EtOAc) from China.

Preparation of *C. edulis* rhizome extracts

The raw rhizome part was cleaned, cut into pieces, dried at room temperature, and ground into fine powder. The powder (1.2 kg) was macerated in 96% EtOH three times at room temperature, and the solvent was evaporated. The total ethanol extract (CER.Et) was fractionated with *n*-hexane, EtOAc, and water. Each fraction was extracted three times. After evaporating the solvents, the fractions of *n*-hexane (CER.H, 2.5 g), ethyl acetate (CER.EA, 3.5 g), and water (CER.W, 55.5 g) were obtained and kept at 4 - 6°C for further use.

Determination of α-amylase inhibiting effect

The α-amylase inhibiting effects of the total extract and fractions of *C. edulis* rhizome were evaluated using the previously described method by Wu *et al*. (2022) with some modifications. The extracts were dissolved in DMSO and diluted to obtain

final concentrations of 5, 25, 50, 100, 200 µg/mL. The enzyme α-amylase (0.5 U/mL) was prepared in phosphate buffer (pH = 6.9). Then, mixutures of 50 µL α-amylase (0.5 U/mL), 250 µL phosphate buffer (pH = 6.9) and samples at different concentrations were prepared and incubated for 20 min at 37°C. Then, 250 µL starch solution (1 mg/mL) was added, and the mixtures were re-incubated for 20 min. Finally, 250 µL acetic acid 50% was added to stop the reaction. After being centrifuged at 3000 rpm at 4°C, the absorbance of the supernatant was measured at 540 nm using a UV – visible spectrophotometer. The positive control was acarbose. Percentage inhibition of enzyme activity was calculated as below:

$$\text{Inhibitory effect (\%)} = (A_{control} - A_{sample})/A_{control}$$

$A_{control}$ and A_{sample} are defined as the absorbance of the control and tested sample, respectively. IC_{50}, which was the concentration of samples required to inhibit 50% of enzyme activity, was determined.

Determination of α-glucosidase inhibiting effect

The inhibitory effect against α-glucosidase of extracts was assayed as described previously by Zhang *et al*. (2014) with some modifications. The extracts were dissolved in DMSO and diluted with phosphate buffer 0.1 M (pH = 6.8). The enzyme α-glucosidase 0.5 U/mL was prepared in phosphate buffer 0.1 M (pH = 6.8). Mixtures of 50 µL extracts at different concentrations, 130 µL phosphate buffer 0.1 M and 20 µL of enzyme α-glucosidase were prepared and incubated at 37°C for 10 min. Then, pNPG was added to initiate the reaction. The mixtures were further incubated for 60 min at 37°C. Finally, the addition of 80 µL Na_2CO_3 0.2 N was done to stop the reaction. Measurement of the absorbance at 405 nm was performed with an ELISA plate reader (Bio-rad). Acarbose was applied as the positive control. Percentage inhibition of enzyme activity of tested samples was calculated as below:

$$\text{Inhibitory effect (\%)} = (OD_{control} - OD_{sample})/OD_{control}$$

$OD_{control}$ and OD_{sample} are defined as the absorbance of control and tested sample, respectively. IC_{50}, which was the concentration of samples required to inhibit 50% of enzyme activity, was also determined.

Phytochemical screening for secondary metabolites

The qualitative phytochemical assessment for secondary metabolites of fractions of *C. edulis* rhizome was performed by chemical reactions following the methods previously described by Jagessar (Jagessar, 2017; Le *et al*., 2020). The presence of following secondary metabolites was identified: cardiac glycosides, cholesterols, glycosides, flavonoids, sterols and triterpenes, tannins, steroids, saponins, coumarins and proteins.

Data analysis

Data were presented in the form of mean ± standard deviation (SD). Statistical analysis was performed using SPSS 23.0 software. The independent sample t-test and ANOVA test were used to compare the differences between samples. $P < 0.05$ was regarded as statistically significant.

RESULTS AND DISCUSSION

The rhizome part of *C. edulis* is shown in

Figure 1. The extraction of the rhizome part of *C. edulis* yielded the total ethanol extract (CER.Et, 20.0%) and 3 fractions: CER.H, 0.2%; CER.EA, 0.3%; CER.W, 4.6%.

Figure 1. *C. edulis* rhizome.

α-Amylase inhibiting activity

Figure 2A represents the percentage inhibition of α-amylase activity of the samples at different concentrations. It could be seen that all *C. edulis* rhizome extracts showed anti-α-amylase activity and their effects were dose-dependent in the tested range of concentrations. Among *C. edulis* rhizome extracts, the ethyl acetate fraction exhibited the strongest α-amylase inhibiting activity ($p < 0.05$). Moreover, the inhibitory effect against α-amylase of the ethyl acetate fraction was significantly higher than the one of acarbose - the positive control (IC_{50}, 45.24 ± 2.90 vs. 62.12 ± 6.60 µg/mL, $p < 0.05$). The lowest α-amylase inhibiting effect was observed for the water fraction with an IC_{50} of 179.88 ± 26.87 µg/mL ($p < 0.05$). The descending order of anti-α-amylase activity of *C. edulis* rhizome extracts was as follows: CER.EA > CER.Et > CER.H > CER.W (Table 1).

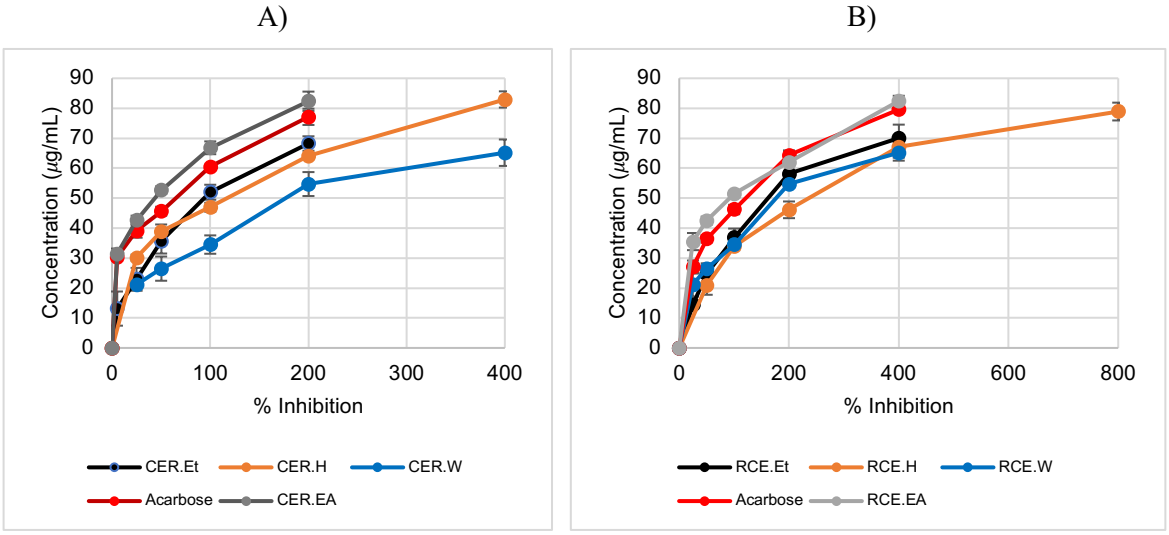

Figure 2. Inhibitory effect of *C. edulis* rhizome on A) α-amylase and B) α-glucosidase (CER.Et: total ethanol extract of *C. edulis* rhizome, CER.H: *n*-hexane fraction of *C. edulis* rhizome, CER.EA: ethyl acetate fraction of *C. edulis* rhizome, CER.W: water fraction of *C. edulis* rhizome).

α-Glucosidase inhibiting activity

Figure 2B represents the percentage inhibition of α-glucosidase activity of the samples at different concentrations. The results showed that the total extract and three fractions of *C. edulis* rhizome had potential α-glucosidase inhibiting effects, and their

effects were all dose-dependent in the tested range of concentrations. Moreover, the ethyl acetate fraction expressed the most potent inhibitory effect against α-glucosidase ($p < 0.05$). The anti-α-glucosidase activity of the ethyl acetate fraction was significantly stronger than the positive control acarbose (IC_{50}, 90.09 ± 6.70 vs. 115.52 ± 6.92 μg/mL, $p < 0.05$). Similar to the finding observed in the α-amylase test, the water fraction showed the lowest inhibitory effect against α-glucosidase with an IC_{50} of 314.47 ± 15.13 μg/mL compared to other extracts and the positivie control ($p < 0.05$). The *n*-hexane fraction had similar α-glucosidase inhibitory activities to acarbose (IC_{50}, 116.03 ± 13.35 and 115.52 ± 6.92 μg/mL for CER.H and acarbose, respectively, $p > 0.05$). The descending order of anti-α-glucosidase activity of *C. edulis* rhizome extracts was as follows: CER.EA > CER.H > CER.Et > CER.W (Table 1).

Table 1. IC50 values for α-amylase and α-glucosidase inhibitory effect.

	α-Amylase assay (μg/mL)	**α-Glucosidase assay (μg/mL)**
CER.Et	91.69 ± 3.86[b,c,d,e]	253.08 ± 13.29[b,c,d,e]
CER.EA	45.24 ± 2.90[a,c,d,e]	90.09 ± 6.70[a,c,d,e]
CER.H	112.39 ± 6.77[a,b,d,e]	116.03 ± 13.35[a,b,d,e]
CER.W	179.88 ± 26.87[a,b,c,e]	314.47 ± 15.13[a,b,c,e]
Acarbose	62.12 ± 6.60[a,b,c,d]	115.52 ± 6.92[a,b,c,d]

[a]: $p < 0.05$ compared to the total ethanol extract, [b]: $p < 0.05$ compared to the ethyl acetate extract, [c]: $p < 0.05$ compared to the *n*-hexane extract, [d]: $p < 0.05$ compared to the water extract, [e]: $p < 0.05$ compared to the positive control, CER.Et: total ethanol extract of *C. edulis* rhizome, CER.H: *n*-hexane fraction of *C. edulis* rhizome, CER.EA: ethyl acetate fraction of *C. edulis* rhizome, CER.W: water fraction of *C. edulis* rhizome.

Table 2. Phytochemical screening of the n-hexane (CER.H), ethyl acetate (CER.EA) and water fraction (CER.W) of *C. edulis* rhizome part.

Phytochemicals	**CER.H**	**CER.EA**	**CER.W**
Cholesterol	+	++	++
Cardiac glycosides	++	++	-
Glycosides	+	++	++
Tannins	-	++	++
Flavanoids	+	++	-
Sterols & Triterpenes	+	+	+
Steroids	++	++	-
Proteins	-	++	-
Saponins	-	-	+
Coumarins	-	+	+

Phytochemical screening of *C. edulis* rhizome fractions

The phytochemical screening using standard established tests (Jessgar, 2017) provided the first-hand knowledge of the phytoconstituents of *C. edulis* rhizome. The results showed that *C. edulis* rhizome contained all selected analyzed secondary metabolites (Table 2). In the *n*-hexane extract, the presence of cholesterols, cardiac glycosides, glycosides, flavonoids, sterols and triterpenes, and steroids was observed, whereas tannins, saponins, protein and coumarins were not present. However, all analyzed secondary metabolites, except for saponin, were found in the ethyl acetate fraction. In the water fraction, cholesterol, glycosides, tannins, sterols and triterpenes, saponin and coumarins were observed. It could be seen that there were more secondary metabolites in the ethyl acetate fraction compared to the others.

DISCUSSION

One important strategy in the treatment of diabetes is to reduce postprandial hyperglycemia (Ghauri *et al*., 2021). α-Glucosidase and α-amylase are two important enzymes to digest dietary starch, leading to increased glucose level in the blood. Therefore, inhibition of these enzymes will decrease postprandial blood glucose levels, and this is a very beneficial tool to manage T2DM. Acarbose is a common anti-diabetic drug used for the treatment of T2DM. This synthetic drug suppresses reversibly α-amylase and α-glucosidase in the intestines, thus reduces the glucose absorption and decreases postprandial hyperglycemia. However, gastrointestinal side effects commonly occur with the use of acarbose. Screening inhibitors of α-amylase and α-glucosidase from medicinal plants is a topic of interest in the world (Gong *et al.* 2020).

The present study demonstrated, for the first time, the inhibitory effects against both enzymes α-amylase and α-glucosidase of *C. edulis* rhizome, taking acarbose as the positive control. The total extract and three fractions all showed dose-dependent enzyme inhibiting effects. These findings clearly indicate that *C. edulis* extracts have the potential for management of hyperglycemia. The study by Singh *et al*. (2016) reported the anti-α-amylase and anti-α-glucosidase enzyme activities of *C. indica*, which is another species belonging to the same genus *Canna*. In this study, *C. indica* leaves powder was successively extracted with petroleum ether, chloroform, ethanol, and water. The ethanol extract of *C. indica* leaves was found to have maximum reduction in α-amylase and α-glucosidase enzyme activities, while the water extract showed minimum reduction in both ezyme activities (Singh *et al.*, 2016). In the present study on *C. edulis* rhizome, the solvents including ethanol, *n*-hexane, ethyl acetate and water were used for successive extraction process, and the water fraction also exhibited the lowest α-amylase and α-glucosidase inhibitory effects. However, the highest inhibition activities against α-amylase and α-glucosidase enzymes were found in the ethyl acetate fraction. In the studies by Bhati *et al*. (2019) on *Cornus capitate* and Nguyen *et al*. (2020) on *Codonopsisjavanica*, the ethyl acetate fraction was also found to possess the strongest α-amylase and α-glucosidase enzyme inhibitory activity among extracts. In addition, the ethyl acetate fractions of *C. edulis* rhizome and other *Canna* species including *C. indica* and *C. generalis* were reported to have strongest antiplatelet,

anticoagulant and antioxidant activity in our previous studies (Nguyen et al., 2020; Le *et al*., 2020; Le *et al*., 2022).

Moreover, the present study showed that *C. edulis* rhizome contained abundant secondary metabolites, especially the ethyl acetate fraction. *C. edulis* rhizome was also previously reported to contain high amount of polyphenolic and flavonoids (Mishra *et al*., 2012). It is noted that phytochemical constituents are the key of phytomedicine. The therapeutic effect of phytomedicine is directly associated with various phytoconstituents present in the plants. Secondary metabolites such as flavonoids, glycosides, coumarins, steroids, and triterpenes were reported to have various biological effects. Futhermore, these secondary metabolites also exhibit inhibitory effects against α-amylase and α-glucosidase (Khan *et al*., 2019; Patil *et al*., 2022; Takahama *et al*., 2018; Vento *et al*., 2009). This study indicated that the ethyl acetate fraction of *C. edulis* rhizome could serve as a potential source for searching new antidiabetic molecules. Moreover, this interesting rhizome plant might be useful for the development of supplementary products to control hyperglycemia. Further *in vitro* and *in vivo* studies should be done to confirm these important results and determine the phytochemicals responsible for α-amylase and α-glucosidase inhibitory activities.

CONCLUSION

This is the first experimental study to prove the inhibitory effects against α-amylase and α-glucosidase enzymes and investigate the phytochemical profile of the rhizome part of *C. edulis*. The total ethanol extract and fractions exhibited enzyme inhibition activities in a dose dependent manner. The ethyl acetate fraction possessed the strongest inhibitory effects against both α-amylase and α-glucosidase enzymes. Moreover, *C. edulis* rhizome contained abundant secondary metabolites including cardiac glycosides, cholesterols, glycosides, flavonoids, sterols and triterpenes, tannins, steroids, saponins, coumarins and proteins. All these secondary metabolites, except for saponin, were found in the ethyl acetate fraction. The present study indicated that *C. edulis* rhizome could be a promissory natural source for the discovery of novel antidiabetic molecules and the development of supplementary products for managing hyperglycemia. Further *in vitro* and *in vivo* studies should be performed to confirm the interesting results and investigate new bioactive molecules from this plant for treatment and prevention of diabetes.

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