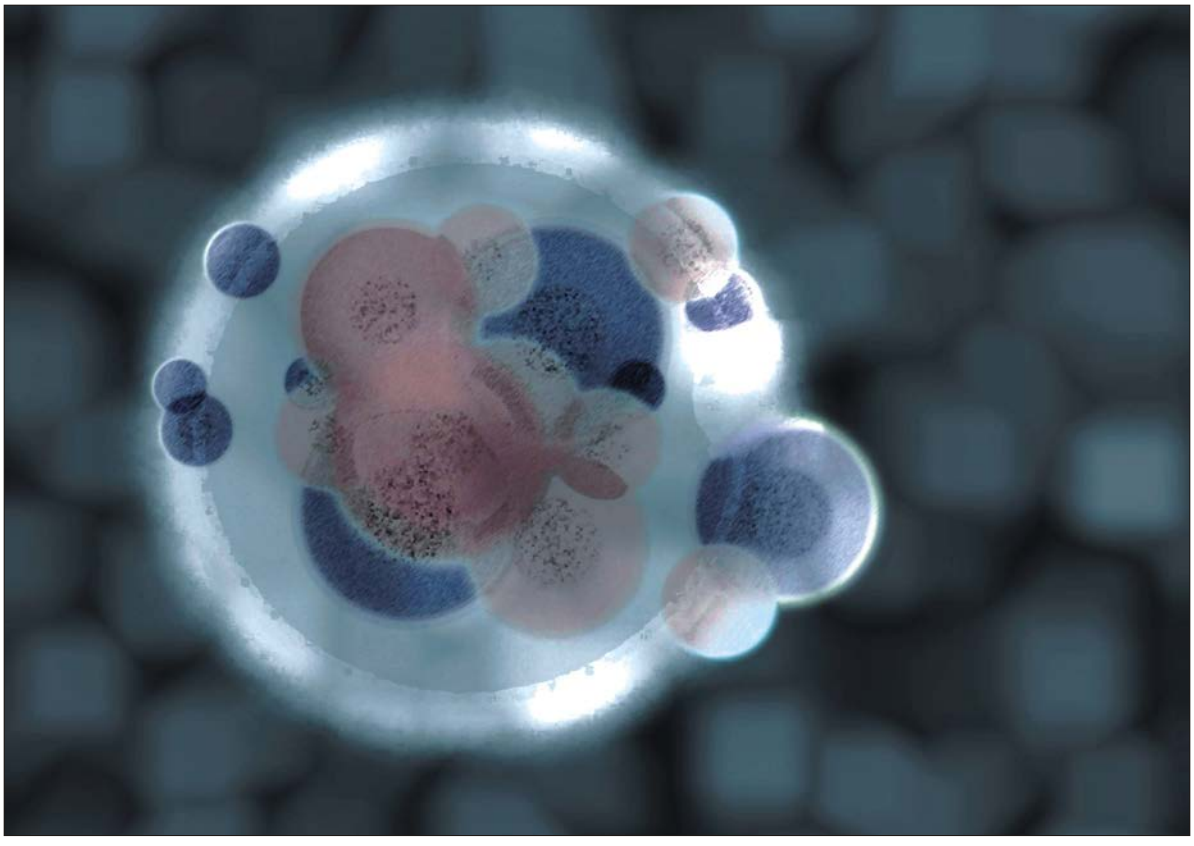

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Research Article
In vitro α-amylase and α-glucosidase Inhibitory Activities of *Coccinia grandis* Aqueous Leaf and Stem Extracts

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Abstract

Background: *Coccinia grandis* (ivy gourd) an edible plant widely consumed in many Southeast Asian countries has been used traditionally for the treatment of diabetes mellitus. However, its role in the regulation of carbohydrate-digesting enzymes, especially α-amylase and α-glucosidase has not been clearly established. **Materials and Methods:** The aqueous leaf and stem extracts of *Coccinia grandis* (the CGL and the CGS extracts, respectively) were prepared and their actions toward *in vitro* activities of α-amylase and α-glucosidase were examined. The activities of α-amylase and α-glucosidase were indicated by the amounts of maltose and p-nitrophenol generated in the reactions, respectively. **Results:** The CGL and the CGS extracts possessed an α-amylase inhibitory action with the IC_{50s} of 8.09±0.72 and 8.06±1.27 mg mL^{-1}, respectively. The CGL extract produced a mixed inhibition against α-amylase, whilst the CGS extract inhibited the enzyme in an uncompetitive manner. The CGL and the CGS extracts exhibited an inhibitory action against α-glucosidase enzyme with the IC_{50s} of 77.66±9.16 μg mL^{-1} and 0.75±0.11 mg mL^{-1}, respectively. Both extracts acted as mixed enzyme inhibitors against α-glucosidase. **Conclusion:** These results support the traditional use of *Coccinia grandis* in diabetic patients, especially for the control of postprandial plasma glucose level. However, further studies on identification of the active phytochemicals acting as enzyme inhibitors and *in vivo* carbohydrate-digesting enzyme inhibition are required to verify its potential clinical use in diabetes mellitus.

Key words: Diabetes mellitus, postprandial plasma glucose, *Coccinia grandis*, α-amylase, α-glucosidase

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Diabetes Mellitus (DM) is a major chronic metabolic disorder affecting at least 400 million of people worldwide[1]. Dysfunctions in insulin secretion and/or insulin actions contribute to the pathogenesis of DM. An uncontrolled elevation of plasma glucose level culminates in both acute and chronic diabetic complications, which can ultimately lead to either disability or premature death. The management of DM thus mainly focuses on the control of plasma glucose level. In addition to a control of Fasting Plasma Glucose (FPG) level, a regulation of Postprandial Plasma Glucose (PPG) (after-meal) level is also crucial for the preventions of morbidity and mortality in diabetic patients[2]. Postprandial hyperglycemia is associated with a destruction of pancreatic beta-cell and the developments of both micro and macro-vascular complications[3,4]. The PPG level depends on digestion and absorption of dietary carbohydrates from the gastrointestinal tract. The functions of carbohydrate-digesting enzymes, especially α-amylase and α-glucosidase, thus play a major role in the regulation of PPG level[5]. The breakdown of α-glycosidic bond in starch requires the function of pancreatic α-amylase, whilst intestinal α-glucosidase is essential for the digestions of oligosaccharides and disaccharides. The inhibitions of these two enzyme activities are thus proposed as effective approaches for controlling the PPG level. Although, an inhibitor of both α-amylase and α-glucosidase, i.e., acarbose is currently available for the treatment of DM, its clinical use is still limited in some developing countries due to its relatively high cost when compare to other commonly used anti-diabetic agents such as glibenclamide and metformin. Medicinal plants with inhibitory activities against carbohydrate-digesting enzymes thus provide an interesting alternative for controlling the PPG level.

Coccinia grandis (ivy gourd) an edible plant in Curcubitaceae family has been used in Ayuravedic medicine for the treatment of various disorders such as microbial infections, gastrointestinal disorders, inflammation and also DM[6]. *Coccinia grandis* juice obtained from leaves and stems is used as a folk medicine for the treatment of DM. In Thailand, both fresh and cooked *Coccinia grandis* leaves and stems are widely consumed as a vegetable dish, especially in a form of clear soup. It is reported that the methanolic and ethanolic leaf extracts of the plant significantly decreased the FPG levels in streptozotocin-induced diabetic rats and alloxan-induced diabetic rats, respectively[7,8]. The ethanolic leaf extract of the plant produced significant reductions of both FPG and PPG levels in the type 2 diabetic patients[9]. From the phase 1 clinical trial, the PPG level in the volunteers, given a meal containing 20 g of *Coccinia grandis* leaves was lower than that of the control group[10]. The methanolic leaf extract of the plant showed the inhibitory actions against α-amylase and α-glucosidase enzymes *in vitro*[11]. However, it is still not clear whether the aqueous leaf and stem extracts of the plant, prepared in the similar manner to its cooking method for a Thai dish, exert their inhibitory actions against the carbohydrate-digesting enzymes. This study thus aimed to investigate the effects of *Coccinia grandis* aqueous leaf and stem extracts on *in vitro* α-amylase and α-glucosidase enzyme activities.

MATERIALS AND METHODS

Preparation of *Coccinia grandis* aqueous leaf and stem extracts: *Coccinia grandis* was collected from the plantation in Maha Sarakham province, Thailand. The authentication of the plant was performed by Assistant Professor Dr. Wanida Caichompoo, a botanist at the Faculty of Pharmacy, Mahasarakham University, Thailand. A voucher is collected at the Herbarium of Pharmaceutical Chemistry and Natural Product Research Unit, the Faculty of Pharmacy, Mahasarakham University (code: MSU.PH-CUR-CG-01). The leaves and stems of the plant were sorted out, washed thoroughly with tap water and cut into small pieces. The specimens were then dried at 60°C for 24 h in a hot-air oven and ground. The ground samples were boiled for 20 min, filtered twice with gauze and subsequently evaporated by using a spray dryer (Labplant, UK). The percentage yields of 3.75 and 4.58% w/w were achieved from the leaf and stem extracts of the plants, respectively. The extracts were kept freezing at -20°C until use.

Total phenolic content assay: The total phenolic content was determined by using the method of Attard[12]. An aliquot of the sample (1 mL) was mixed thoroughly with 5 mL of Folin-Ciocalteu (diluted with deionized water, 1:10) and 4 mL of 1 M Na_2CO_3. The mixture was incubated at room temperature for 20 min. The Optical Density (OD) of the mixture was subsequently measured at the wavelength of 630 nm. The standard solutions with varying concentrations of gallic acid were prepared in methanol. The total phenolic content was calculated from the gallic acid standard curve and expressed as mg of Gallic Acid Equivalent (GAE)/g of extract (dry weight).

Total flavonoid content assay: The total flavonoid content assay was performed according to the method of

Marinova *et al.*[13]. An aliquot of sample (1 mL) was added with 5 mL deionized water. The $NaNO_2$ (5%) at the volume of 0.3 mL was added and the mixture was then shaken vigorously and left at room temperature for 5 min. After that, 0.3 mL of 10% $AlCl_3$ was added into the mixture, mixed thoroughly and incubated at room temperature for further 5 min. Then, 2 mL of 1 M NaOH was added into the mixture and the total volume of the mixture was adjusted into 10 mL with deionized water. The mixture was mixed well and then the OD of the mixture was measured at the wavelength of 510 nm. The standard solutions with varying concentrations of (+)-catechin were prepared in methanol. The total flavonoid content was calculated from the (+)-catechin standard curve and expressed as mg of (+)-catechin equivalent/g of extract (dry weight).

Alpha-amylase enzyme activity assay: The α-amylase enzyme activity assay was performed according to the method of Ali *et al.*[14] with slight modifications. Briefly, 40 µL of sample at the designated concentration was incubated with 200 µL of freshly prepared porcine pancreatic α-amylase (4 unit mL^{-1}) at 25°C for 5 min. The mixture was subsequently added with potato starch solution (0.5% w/v, 400 µL) as well as deionized water (160 µL) and incubated at 25°C for further 3 min. A 200 µL volume of the mixture was then mixed with 100 µL of 3,5-dinitrosalicylic acid (DNS) color reagent solution (96 mM DNS and 5.31 M sodium potassium tartrate in 2 M NaOH) and then heated at 85°C for 15 min. The OD of the mixture was measured at the wavelength of 540 nm. The exact absorbance of the mixture was obtained by subtracting the absorbance of the blank from the absorbance of the colored sample. The concentration of maltose, generated from α-amylase catalyzing reaction was calculated from the maltose standard curve and used as an index of α-amylase enzyme activity. Reaction (%) was obtained from the following equation:

$$\text{Reaction (\%)} = \frac{\text{Mean maltose in sample}}{\text{Mean maltose in negative control}} \times 100$$

Percentage of inhibition was calculated as 100-% reaction. The concentration-inhibitory response curve was plotted by using GraphPad Prism software version 6.0.

Alpha-glucosidase enzyme activity assay: The α-glucosidase enzyme activity was studied following the protocol of Elya *et al.*[15] with minor modifications. Concisely, 40 µL of sample, 460 µL of phosphate buffer (100 mM, pH 6.8) and 125 µL of yeast α-glucosidase enzyme (0.15 unit mL^{-1}) was mixed and incubated at 37°C for 5 min. The mixture was then added with 250 µL of p-nitrophenyl-α-D-glucopyranoside (p-NPG; 5 mM) and 125 µL of phosphate buffer (100 mM, pH 6.8) and incubated for further 15 min at 37°C. Afterwards, 200 µL of the mixture was taken out and added with 400 mL of Na_2CO_3 (200 mM), the solution was then left at room temperature for further 5 min. The mixture was then diluted with 600 µL of deionized water and the OD of the final mixture was measured at the wavelength of 405 nm. The absorbance of the colored sample was subtracted by the absorbance of the blank in order to obtain the exact absorbance of the mixture. The amount of p-nitrophenol released from α-glucosidase-catalyzing reaction was used as an index of α-glucosidase enzyme activity. The percentage of inhibition was calculated by the following equation:

$$\text{Inhibition (\%)} = \frac{\text{OD of negative control} - \text{OD of sample}}{\text{OD of negative control}} \times 100$$

The concentration-inhibitory response curve was generated by using GraphPad Prism software version 6.0.

Statistical analysis: The percentage of enzyme inhibition was expressed as mean±standard error of mean (SEM), whilst the median inhibitory concentration (IC_{50}), the total phenolic content and the total flavonoid content were expressed as mean±standard deviation (SD). The statistical analysis was performed by using one-way analysis of variance (ANOVA) followed by the Bonferroni *post hoc* test. Statistically significant differences were indicated at $p<0.05$.

RESULTS

Total phenolic content and the total flavonoid content of *Coccinia grandis* aqueous leaf and stem extracts: Total phenolic content and total flavonoid content of *Coccinia grandis* aqueous leaf and stem extracts are shown in Table 1.

Table 1: Total phenolic content and the total flavonoid content of the *Coccinia gradis* aqueous leaf (CGL) extract and the *Coccinia grandis* aqueous stem (CGS) extract

Extracts	Total phenolic content (mg of GAE/g of extract)	Total flavonoid content mg of (+)-catechin equivalent/g of extract
CGL extract	87.26±0.05	4.22±0.01
CGS extract	203.38±0.13	4.48±0.01

Table 2: Percentage of inhibition of the CGL extract against α-amylase enzyme activity

Concentration (mg mL^{-1})	Inhibition (%)
30	111.74±5.05*
20	94.61±6.02*
10	61.80±6.61*
7.5	38.05±4.04*
5	29.00±6.41*
1	15.04±1.09
0.5	12.35±3.85
0	0.00±0.02

*p<0.05, when compared with negative control (deionized water), One-way ANOVA, Bonferroni *post hoc* test, n = 4, CGL: *Coccinia grandis* leaf extract

Table 3: Percentage of inhibition of the CGS extract against α-amylase enzyme activity

Concentration (mg mL^{-1})	Inhibition (%)
30	119.67±9.58*
20	86.48±3.24*
10	60.13±5.39*
5	26.17±3.93*
1	17.32±0.80
0.5	10.19±4.52
0.25	14.98±5.04
0	0.00±0.00

*p<0.05, when compared with negative control (deionized water), One-way ANOVA, Bonferroni *post hoc* test, n = 4, CGS: *Coccinia grandis* stem extract

Table 4: Changes of V_{max} and K_m of α-amylase enzyme in the presence of the test agents

Agents	ΔV_{max} (Maltose (%w/v) min^{-1})	ΔK_m (Starch (%w/v))
CGL extract (8 mg mL^{-1})	-0.0381	+0.2461
CGS extract (8 mg mL^{-1})	-0.0822	-0.1223
Acarbose (200 mg mL^{-1})	-0.0577	+0.0383

CGL: *Coccinia grandis* leaf, CGS: *Coccinia grandis* stem, ΔK_{max}: Changes of maximal rate reaction, ΔK_m: Changes of the substrate concentration causing half maximal rate of reaction

Effects of *Coccinia grandis* aqueous leaf and stem extracts on *in vitro* α-amylase activity: The aqueous extract of *Coccinia grandis* leaves (CGL extract) at the concentrations of 5, 7.5, 10, 20 and 30 mg mL^{-1} significantly inhibited α-amylase activity ($p<0.05$) with the median inhibitory concentration (IC_{50}) of 8.09±0.72 mg mL^{-1} (mean±SD, n = 4). The extract at the concentration of 30 mg mL^{-1} produced the maximal enzyme inhibition at 111.74±5.05% (Table 2). The aqueous stem extract of *Coccinia grandis* (CGS extract) at the concentrations of 5, 10, 20 and 30 mg mL^{-1} significantly produced an inhibitory action against α-amylase activity ($p<0.05$, n = 4), with the IC_{50} of 8.06±1.27 mg mL^{-1} (mean±SD, n = 4). The maximal inhibitory activity of 119.67±9.58% was found at the concentration of 30 mg mL^{-1} (Table 3). Acarbose (200 μg mL^{-1}), a positive control, exhibited a significant inhibition against α-amylase activity with the percentage inhibition of 56.53±2.62 (n = 4).

Effects of *Coccinia grandis* aqueous leaf and stem extracts on α-amylase enzyme kinetics: Lineweaver-Burk plot was drawn from the α-amylase enzyme kinetics study in the absence and the presence of the extracts at the concentrations of their approximate IC_{50s}. At the concentration of 8 mg mL^{-1} (IC_{50}), the aqueous leaf and stem extracts of *Coccinia grandis* caused changes of both the maximal rate of reaction (V_{max}) and the concentration of substrate producing half-maximal rate of reaction (K_m) (n = 3). The CGL extract produced a decrease of V_{max} and an increase of K_m (Fig 1a, Table 4), whilst the CGS extract reduced both V_{max} and K_m (Fig. 1b, Table 4). Meanwhile, acarbose (200 μg mL^{-1}) decreased V_{max} but slightly increased K_m (Fig. 1c, Table 4).

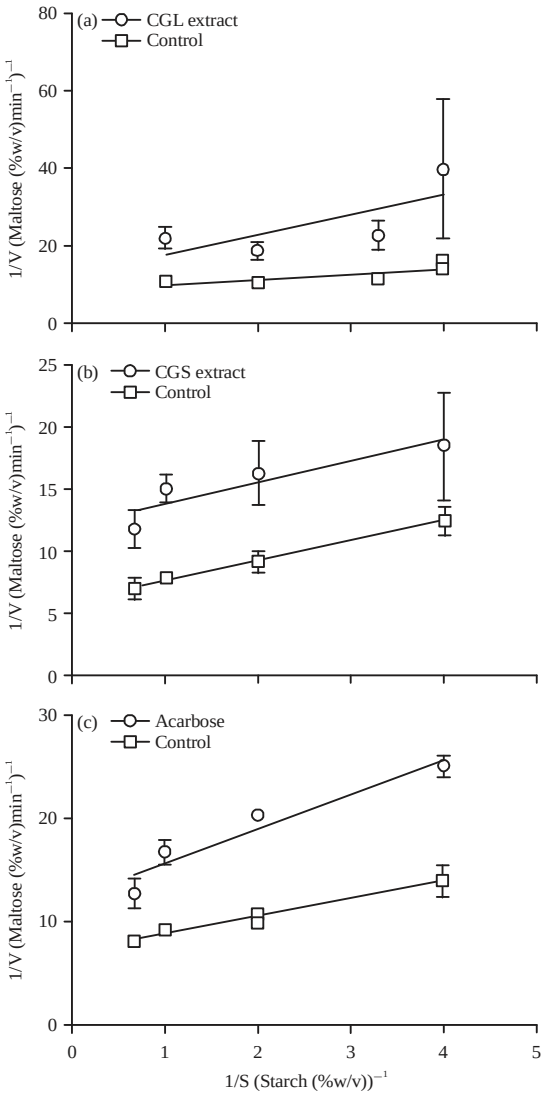

Fig. 1(a-c): Kinetics analysis of α-amylase inhibition by (a) CGL extract (8 mg mL^{-1}), (b) CGS extract (8 mg mL^{-1}) and (c) Acarbose (200 μg mL^{-1}). Lineweaver-Burk plot of the reaction against α-amylase in the presence or absence of the test agents (Mean±SD, n = 3)

Table 5: Percentage of inhibition of the CGL extract against α-glucosidase enzyme activity

Concentration (mg mL^{-1})	Inhibition (%)
5	118.84±4.42*
1	85.79±1.23*
0.5	75.10±2.03*
0.1	46.24±1.68*
0.05	39.10±0.93*
0.01	32.52±1.67*
0	0.00±0.00

*p<0.05, when compared with negative control (deionized water), One-way ANOVA, Bonferroni *post hoc* test, n = 4, CGL: *Coccinia grandis* leaf extract

Table 6: Percentage of inhibition of the CGS extract against α-glucosidase enzyme activity

Concentration (mg mL^{-1})	Inhibition (%)
15	113.13±7.83*
10	103.89±2.97*
5	81.17±2.82*
1	49.18±3.34*
0.5	37.53±0.16*
0.25	33.87±2.96*
0	0.00±0.00

*p<0.05, when compared with negative control (deionized water), One-way ANOVA, Bonferroni *post hoc* test, n = 4**,** CGS: *Coccinia grandis* stem extract

Table 7: Changes of V_{max} and K_m of α-glucosidase enzyme in the presence of the test agents

Agents	ΔV_{max} (unit min^{-1})	ΔK_m (mM)
CGL extract (80 μg mL^{-1})	-0.0703	+0.5251
CGS extract (0.75 mg mL^{-1})	-0.0820	+0.4870
Acarbose (500 mg mL^{-1})	-0.0665	+2.1089

CGL: *Coccinia grandis* leaf, CGS: *Coccinia grandis* stem, ΔK_{max}: Changes of maximal rate reaction, ΔK_m: Changes of the substrate concentration causing half maximal rate of reaction

Effects of *Coccinia grandis* aqueous leaf and stem extracts on *in vitro* α-glucosidase activity: The CGL and the CGS extracts at every concentration tested significantly inhibited α-glucosidase activity with the IC_{50s} of 77.66±9.16 μg mL^{-1} and 0.75±0.11 mg mL^{-1}, respectively (mean±SD, n = 4). The maximal inhibitions against α-glucosidase activity were achieved at the concentrations of 5 and 15 mg mL^{-1} for the CGL and the CGS extracts, respectively (Table 5, 6). Acarbose (500 μg mL^{-1}) caused a significant inhibition against the enzyme with the percentage of inhibition of 40.87±2.25 (n = 4).

Effects of *Coccinia grandis* aqueous leaf and stem extracts on α-glucosidase enzyme kinetics: From the Lineweaver-Burk plot, both extracts at their approximate IC_{50s} (80 μg mL^{-1} and 0.75 mg mL^{-1} for the CGL and the CGS extracts, respectively) modified the α-glucosidase enzyme kinetics. The V_{max} was lowered whilst K_m was raised in the presence of either the CGL extract or the CGS extract (Fig. 2a, b, Table 7). Concurrently, acarbose (500 μg mL^{-1}) also caused changes of both V_{max} and K_m in a similar manner as those of the extracts (Fig. 2c, Table 7).

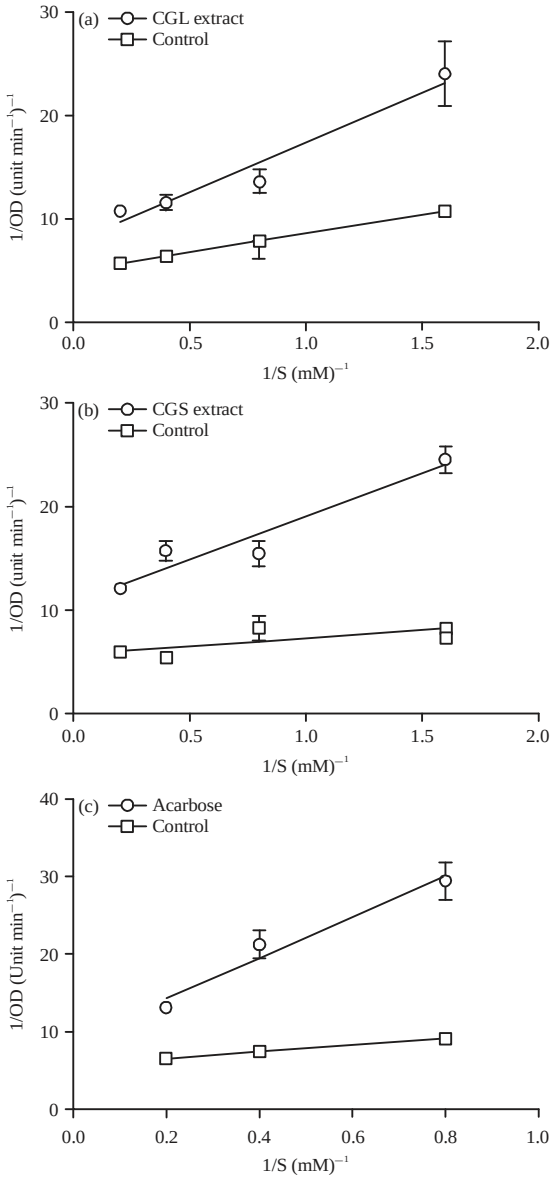

Fig. 2(a-c): Kinetics analysis of α-glucosidase inhibition by (a) CGL extract (80 μg mL^{-1}), (b) CGS extract (0.75 mg mL^{-1}) and (c) Acarbose (500 μg mL^{-1}). Lineweaver-Burk plot of the reaction against α-glucosidase in the presence or absence of the test agents (Mean±SD, n = 3)

DISCUSSION

From this study, the aqueous extracts of leaves and stems of *Coccinia grandis* (the CGL and the CGS extracts, respectively) possessed an α-amylase inhibitory action with the IC_{50s} of 8.09±0.72 and 8.06±1.27 mg mL^{-1}, respectively. Girish *et al.*[11] reported a more potent inhibitory action of the *Coccinia grandis* methanolic leaf extract with the IC_{50} of

8.17 μg mL^{-1}. It is possible that the active phytochemicals with α-amylase inhibitory action were extracted more with a less polar solvent. Several phytochemicals were reported to be present in the whole plant of *Coccinia grandis* such as docos-1-ol, docos-8-one, betulin and β-sitosterol[16]. Tamilselvan *et al.*[17] reported the presence of flavonoids, amino acids, glycosides, phenols, carbohydrates and alkaloids in the aqueous extract of the plant. Additionally, phenolic compounds, specifically caffeic acid and p-coumaric acid were detected in the aqueous leaf extract of the plant[18]. Phenolics and flavonoids were also present in both CGL and CGS extracts in this study (Table 1). The phytochemicals found in *Coccinia grandis* with documented α-amylase inhibition include β-sitosterol, betulin, saponins, flavonoids and caffeic acid[19-24].

The similar levels of total flavonoid content in the CGL and the CGS extracts were identified in this study (4.22±0.01 and 4.48±0.01 mg of GAE g^{-1} of extract, respectively). Since both extracts produced a comparable inhibitory activity against α-amylase enzyme (IC_{50s} of 8.09±0.72 and 8.06±1.27 mg mL^{-1}, respectively). It is likely that the active phytochemicals acting as an α-amylase inhibitor in the extracts are flavonoids. However, from the current results of the enzyme kinetics study, the two extracts inhibited α-amylase with different modes of action. Both extracts caused a decrease in V_{max}. However, they changed K_m in the opposite direction. The K_m was raised in the presence of the CGL extract but the CGS extract caused a depletion in K_m. These indicate that the CGL extract exhibited a mixed inhibitory action, whilst the CGS extract inhibited the enzyme in an uncompetitive manner. In this study, acarbose, a positive control, significantly inhibited α-amylase in a mixed inhibition manner which is in agreement with the study of Al Kazaz *et al.*[25]. It is reported that the different kinds of flavonoids exhibited different modes of inhibition against α-amylase[26,27]. Modifications of the chemical structure of flavonoids are linked with their α-amylase inhibitory activity[20,28]. Thus, it is expected that different kinds of flavonoids acted as the inhibitor against α-amylase in these two extracts. Additionally, other groups of phytochemicals apart from flavonoids may also play a role in the α-amylase inhibitory action of the extracts. Further study is still required to identify the exact active phytochemicals in *Coccinia grandis* acting against α-amylase activity.

The CGL and the CGS extracts produced an inhibitory action against α-glucosidase enzyme with the IC_{50s} of 77.66±9.16 μg mL^{-1} and 0.75±0.11 mg mL^{-1}, respectively. The α-glucosidase inhibitory action of the CGL extract was thus approximately 10 times higher than that of the CGS extract. Wongsa *et al.*[18] also reported a high efficacy of the *Coccinia grandis* aqueous leaf extract with the maximal inhibition (approximately 100%) found at the concentration of 5 mg mL^{-1}. The methanolic leaf extract of *Coccinia grandis* were found to inhibit α-glucosidase with the lower potency (IC_{50} of 1.8 mg mL^{-1})[29]. The active phytochemicals acting against α-glucosidase enzyme are hence likely to be extracted more with water. The total phenolic content of the CGL extract found in this study (87.26±0.05 mg GAE g^{-1} extract) was slightly higher than that reported earlier by Wongsa *et al.*[18] (53.44±0.29 mg GAE g^{-1} extract). The difference in extraction methods may be responsible for this discrepancy. A moderate correlation between total phenolic content and potential inhibition against α-glucosidase was also described previously ($r = 0.49$)[18]. However, various culinary herbs from different plant families, such as Curcubitaceae, Alliaceae, Leguminosae and Zingiberaceae were investigated in their study. The total phenolic content of the CGS extract (203.38±0.13 mg GAE g^{-1} extract) was approximately two times higher than that of the CGL extract in this study. Accordingly, the total phenolic content of the extracts is unlikely to be related to their α-glucosidase inhibitory action. A comparable amount of total flavonoid content also disputes a role of flavonoids in α-glucosidase inhibition of the extracts. Thus, phytochemicals other than phenolic compounds and flavonoids might be accountable for the α-glucosidase inhibitory action of the extracts. Besides flavonoids, other compounds including β-sitosterol, betulin and triterpenoid saponins are phytochemicals identified in *Coccinia grandis* with the evidence of α-glucosidase inhibition[23,24,30,31].

In the current study, acarbose significantly inhibited α-glucosidase with an obvious increase in K_m but a trivial decrease in V_{max}. Acarbose was reported to inhibit α-glucosidase in a competitive inhibition manner[32]. Both CGL and CGS extracts produced their α-glucosidase inhibition in a mixed inhibition manner with a decrease of V_{max} and an increase of K_m (Table 7). The degrees of changes in V_{max} and K_m caused by both extracts were also comparable. The same but not yet identified, phytochemicals were probably responsible for the α-glucosidase inhibition of the two extracts. In the presence of a mixed enzyme inhibitor, the maximal rate of reaction is decreased even at higher concentrations of the substrate. Additionally, when there is the same amount of the substrate, the rate of reaction is also decreased. A mixed enzyme inhibitor thus offers an advantage for the clinical use, since the rate of carbohydrate digestion is reduced no matter what amount of carbohydrate is consumed. The aqueous extracts of other medicinal plants, such as *Morinda lucida* and *Vauquelinia corymbosa*, also exhibited a mixed inhibitory mode of action against α-glucosidase[33,34].

The CGL and the CGS extracts significantly inhibited both α-amylase and α-glucosidase activities. However, the α-glucosidase inhibitory action of the extracts was apparently higher than their α-amylase inhibitory action (approximately 100 and 10 times higher for the CGL and the CGS extracts, respectively). Since various types of carbohydrate, including polysaccharides, oligosaccharides and disaccharides are present generally in typical meals. Inhibitions of both enzymes would provide advantage over an inhibition of each enzyme alone. *Coccinia grandis* was reported to produce its anti-diabetic action via various mechanisms of action including inhibition of glycogenolysis, inhibition of gluconeogenesis, stimulation of cellular glucose uptake and also induction of insulin secretion[10,35,36]. The α-amylase and α-glucosidase inhibitions of the CGL and the CGS extracts shown in this study thus additionally support the traditional use of *Coccinia grandis* for the treatment of diabetes mellitus, especially for the control of PPG level. However, further *in vivo* study is necessary to confirm their inhibitory actions against the carbohydrate-digesting enzymes.

CONCLUSION

The aqueous leaf and stem extracts of *Coccinia grandis* produced the inhibitory actions against both α-amylase and α-glucosidase enzymes *in vitro*. These partly explain the anti-diabetic mechanisms of this medicinal plant and affirm its traditional use for the treatment of diabetes mellitus, especially for the management of postprandial hyperglycemia.

SIGNIFICANT STATEMENTS

- The aqueous leaf and stem extracts of *Coccinia grandis* (CGL and the CGS extracts, respectively) significantly inhibited α-amylase with the IC_{50s} of 8.09±0.72 μg mL^{-1} and 8.06±1.27 mg mL^{-1}, respectively
- The CGL and the CGS extracts also possessed an α-glucosidase inhibitory action with the IC_{50s} of 77.66±9.16 and 0.75±0.11 mg mL^{-1}, respectively
- These results support the traditional use of *Coccinia grandis* in diabetic patients, especially for the control of postprandial plasma glucose level

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