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Research Article

Inhibitory Activities of Thai Culinary Vegetables against Key Enzymes Relevant to Diabetes Mellitus and the Kinetics of Enzyme Inhibitions

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Diabetes mellitus (DM) is one of the most challenging noncommunicable diseases, as it causes significant costs for medical treatment as well as high morbidity and mortality rates. Dietary plants with antidiabetic properties have been explored as an alternative to synthetic medicines to treat DM because of their safety and nutrition. Hence, the objective of the present study was to determine the inhibitory activities of twenty commonly consumed Thai culinary vegetables against α-glucosidase and α-amylase. All vegetables were extracted using deionized water, ethanol, and hexane at 150 rpm and 30°C for 24 hours. The enzyme inhibitory activities were performed using a colorimetric assay. Diverse results for α-glucosidase and α-amylase inhibitory activities were found for all vegetable extracts. The most potent anti-α-glucosidase activity was obtained from the ethanolic extract of *Leucaena leucocephala* (Lamk.) de Wit with the half maximal inhibitory concentration (IC_{50}) of 13.39 ± 0.14 μg/mL, followed by the aqueous and ethanolic extracts of *Polygonum odoratum* Lour with IC_{50} of 25.60 ± 0.42 and 49.03 ± 0.72 μg/mL, respectively. All the samples exhibited mixed, noncompetitive, and uncompetitive inhibition. It can be concluded that the α-glucosidase and α-amylose inhibitory effects of the investigated extracts may be an indicator of antidiabetic potency, and these extracts might potentially be beneficial as functional components for postprandial hyperglycemia treatment.

1. Introduction

Diabetes mellitus (DM), a modern lifestyle-related disease, has been classified as one of the most challenging global public health problems. According to the World Health Organization (WHO), an estimated 463 million adults were living with diabetes as of 2021, and the number is projected to rise to 578 million by 2030 [1]. In addition, diabetes is a leading cause of disability and mortality, accounting for an estimated 4.2 million deaths annually [2]. According to the International Diabetes Federation (IDF), an estimated 6.0 million adults in Thailand were living with diabetes as of 2021, representing a prevalence of 10.6% [2]. Furthermore, an estimated 2.4 million adults in Thailand were estimated to have undiagnosed diabetes. The prevalence of diabetes in Thailand is projected to increase in the coming years, with an estimated 6.7 million adults living with diabetes by 2030. Diabetes is one of the leading causes of death in Thailand, and it is responsible for an estimated 62,000 deaths annually [2]. Undoubtably, the rising number of DM patients every year generates tremendous expenses in medical care as well as high morbidity and death rates.

DM is a chronic health condition that occurs when the body does not produce enough insulin or does not use the insulin effectively. Insulin is a hormone produced by the pancreas that plays a key role in the regulation of glucose

metabolism. It helps the body use glucose for energy and store excess glucose in the liver and muscles for future use. Insulin helps cells take up glucose from the blood, preventing it from reaching excessively high levels. It also stimulates the liver to take up and store glucose, helping to maintain stable blood glucose levels [3]. Without insulin, too much glucose can build up in the bloodstream, resulting in several health complications. To control blood glucose levels, most DM patients take synthetic medicines, which have an inhibitory effect against the activity of the enzymes, especially α-amylase and α-glucosidase which break down carbohydrates in the gut, leading to a slowdown of glucose absorption into the bloodstream. The digestion process is initiated by α-amylase by breaking down starches into smaller oligosaccharides and disaccharides, which are then further broken down by α-glucosidase to release glucose and other simple sugars. These monosaccharides are then absorbed into the bloodstream. By acting on the final step of carbohydrate digestion, α-glucosidase directly influences the rate at which glucose enters the bloodstream [4]. As a result, glucose levels can be maintained at a more consistent level, preventing spikes or drops in blood sugar. Nevertheless, these drugs often have unpleasant side effects such as flatulence, inflation, diarrhea, nausea, and loss of appetite. Therefore, a long-term use of these medications would constitute a burden to DM patients and reduce their quality of life [5]. To reduce the side effects caused by the synthetic antidiabetic medications and to lower the cost of medical treatments, many bioactive compounds from natural sources, especially plants, have been investigated.

Plants are rich in many secondary metabolites possessing potent biological properties to maintain blood glucose levels [6]. Thus, many plants have recently been investigated for their inhibitory effects against α-amylase and α-glucosidase, including bitter gourd (*Momordica charantia*) [7], Java plum (*Syzygium cumini*) [8], turmeric (*Curcuma longa*) [9], and king of bitters (*Andrographis paniculata*) [10]. Some have been studied and provided valid scientific evidence, while others have not been scientifically demonstrated. To pursue the finding, in this study, twenty commonly consumed Thai culinary vegetables in the northeastern region of Thailand with herbal remedy backgrounds in diabetes management were screened and examined for their effect on α-amylase and α-glucosidase inhibition *in vitro*. The knowledge gained from this study would be useful to identify potential culinary vegetables with α-amylase and α-glucosidase inhibitory activity as functional foods for postprandial hyperglycemia management as well as help establish the scientific validity of folk medicine.

2. Materials and Methods

2.1. Chemicals and Reagents. The 3, 5-dinitrosalicylic acid, α-amylase from *Aspergillus oryzae*, α-glucosidase from *Saccharomyces cerevisiae*, *p*-nitrophenyl-α-D-glucopyranoside, and starch were purchased from Sigma-Aldrich (USA). Other chemicals were purchased from Fisher Scientific (USA).

2.2. Sample Preparation. Twenty Thai culinary vegetables were studied, namely ma klam (*Adenanthera pavonina* L), king daeng (*Alpinia purpurata* (Vielle.) Schum), phak khom (*Amaranthus lividus* L), sadao (*Azadirachta indica* A. Juss. var. siamensis Valeton), phak pang (*Basella alba* L), phak kard hin (*Brassica juncea* (L.) Czern), tam-leung (*Coccinia grandis* (L.) Voigt), fucktong (*Cucurbita moschata* Decne), phak naam (*Lasia spinosa* (L.) Thwaites), kra thin (*Leucaena leucocephala* (Lamk.) de Wit), ka-yang (*Limnophila aromatica*), kan jong (*Limnocharis flava* (L.) Buchenau), buap (*Luffa acutangula* (Linn.) Roxb), phak tob Thai (*Monochoria hastata* (L.) Solms), ma rum (*Moringa oleifera* Lam), cha phlu (*Piper sarmentosum* Roxb), phak peaw (*Polygonum odoratum* Lour), khae (*Sesbania grandiflora* (L.) Desv), mek (*Syzygium gratum* (Wight) S.N. Mitra var. gratum), and buap ngo (*Trichosanthes anguina* Linn). Fresh vegetables were obtained from three representative markets in Kalasin Province from January to May 2022. At each market, 1-2 kg of samples were collected from three representative outlets. A single composite sample for each representative market was prepared by combining about 500 g of a homogenized single sample of the same vegetable variety from three representative outlets and then homogenizing again to obtain a uniform single composite sample. The characteristics of vegetables are presented in Table 1.

2.3. Plant Extraction. Ten grams of freeze-dried vegetables were macerated using extraction solvents with different polarity including deionized water, ethanol, and hexane, in a ratio of 1 : 10 in a 30°C water bath shaker for 24 hours at 150 rpm. Vegetable debris was removed by centrifugation, and the vegetable extract was obtained after the removal of the extraction solvent by rotary evaporation. The vegetable extract was kept in darkness at 4°C for further analysis.

2.4. α-Glucosidase Inhibitory Assay. The α-glucosidase inhibitory properties were analyzed using the method, explained by Kim et al. with minor modifications [11]. Briefly, 50 μL of 10 mg/mL vegetable extract was preincubated with 0.1 M phosphate buffer, pH 6.8 containing α-glucosidase for 10 min. After preincubation, 1 mM *p*-nitrophenyl-α-D-glucopyranoside solution in 0.1 M phosphate buffer, pH 6.8 was added and further incubated at 37°C for 10 min. The reaction was stopped by adding 1 mL of 0.1 M sodium carbonate. The α-glucosidase inhibitory activity was followed by the measurement of absorbance at 405 nm. The α-glucosidase inhibitory property was expressed as the percentage of α-glucosidase inhibition and calculated according to the following equation:

$$\text{Percentage of inhibition } (\%) = \frac{A - B}{A} \text{ x } 100, \quad (1)$$

where A and B were the absorbance values for the control and sample, respectively. A control was prepared using the same procedure to replace the vegetable extract with distilled water. The experiment was conducted in five replicates.

Table 1: The characteristics of the selected vegetables.

Scientific name	Common name	Thai name	Part of use
Adenanthera pavonina L	Red sandalwood tree	Ma klam	Leaf
Alpinia purpurata (Vielle.) schum	Red ginger	King daeng	Rhizome
Amaranthus lividus L	Purple amaranth	Phak khom	Leaf
Azadirachta indica A. Juss. var. siamensis Valeton	Siamese neem	Sadao	Leaf
Basella alba L	Malabar spinach	Phak pang	Leaf
Brassica juncea (L.) Czern	Chinese mustard	Phak kard hin	Leaf
Coccinia grandis (L.) Voigt	Ivy gourd	Tam-leung	Leaf
Cucurbita moschata Decne	Pumpkin	Fucktong	Flower
Lasia spinosa (L.) thwaites	—	Phak naam	Leaf
Leucaena leucocephala (Lamk.) de Wit	Pearl wattle	Kra thin	Young shoot
Limnophila aromatica	Rice Paddy herb	Ka-yang	Leaf
Limnocharis flava (L.) Buchenau	Sawah lettuce	Kan jong	Leaf
Luffa acutangula (Linn.) Roxb	Angled loofah	Buap	Fruit
Monochoria hastata (L.) solms	Monochria	Phak tob Thai	Flower
Moringa oleifera Lam	Drumstick tree	Ma rum	Leaf
Piper sarmentosum Roxb	—	Cha phlu	Leaf
Polygonum odoratum Lour	Vietnamese coriander	Phak peaw	Leaf
Sesbania grandiflora L Desv	Vegetable hummingbird	Khae	Flower
Syzygium gratum (Wight) S.N. Mitra var. gratum	—	Mek	Leaf
Trichosanthes anguina Linn	Snake gourd	Buap ngo	Fruit

2.5. α-Amylase Inhibitory Assay. The α-amylase inhibitory property was analyzed using the method, explained by Kidane et al. with minor modifications [12]. Briefly, 50 μL of 10 mg/mL vegetable extract was preincubated with 0.02 M phosphate buffer, pH 6.9, containing α-amylase for 10 min. After preincubation, 1% starch in phosphate buffer, pH 6.9 was added and further incubated for 10 min. The reaction was stopped by adding 1 mL of the 3, 5-dinitrosalicylic acid reagent, then incubated in boiling water for 5 min and cooled to room temperature. The α-amylase inhibitory activity was followed by the measurement of absorbance at 540 nm. The α-amylase inhibitory property was expressed as the percentage of α-amylase inhibition and calculated according to the equation:

$$\text{Percentage of inhibition } (\%) = \frac{A - B}{A} \text{ x } 100, \qquad (2)$$

where A and B were the absorbance values for the control and sample, respectively. A control was prepared using the same procedure, replacing the vegetable extract with distilled water. The experiment was conducted in five replicates.

2.6. Determination of the IC_{50}. The vegetable extracts with more than 50% of α-glucosidase inhibitory or 50% of α-amylase inhibitory were selected for the evaluation of IC_{50} value. The IC_{50} is defined as the concentration of vegetable extract that could reduce the α-glucosidase activity by 50% which was only determined for the vegetable extract with inhibition ≥50%. The IC_{50} was obtained graphically for the plot of percentage of inhibition versus concentration [11]. The experiment was conducted in triplicate.

2.7. Kinetics of Enzyme Inhibition. In the enzyme-kinetic measurement, an inhibition assay was performed according to the protocol described by Kim et al. Inhibition modes of selected vegetable extracts against α-glucosidase were determined by increasing concentration of *p*-nitrophenyl-α-D-glucopyranoside solution in the absence or presence of selected vegetable extracts. The experiment was conducted in triplicate. The type of inhibition of the vegetable extracts was determined by a Lineweaver-Burk plot [11].

3. Results

3.1. Screening of In Vitro α-Glucosidase and α-Amylase Inhibitory Activity. The α-glucosidase inhibitory activities of the vegetable extracts, intentionally chosen for their Thai remedial background in diabetes management are presented in Table 2. Using different extraction solvents to extract anti-α-glucosidase agents from each vegetable resulted in different anti-α-glucosidase activity levels. Nine out of twenty vegetables showed an inhibitory effect against α-glucosidase, whereas eleven showed no inhibition. Hexane did not appear to be a suitable solvent to extract anti-α-glucosidase substances. Most of the α-glucosidase inhibitory activities were discovered from the extracts using water and ethanol as extraction solvents. *L. leucocephala* (Lamk.) de Wit and *P. odoratum* Lour generated the most promising vegetable extracts with very high anti-α-glucosidase activity. The α-glucosidase inhibitory activity of the ethanolic extract from *L. leucocephala* (Lamk.) de Wit was 96.10 ± 1.64% and the aqueous and the ethanolic extract of *P. odoratum* Lour produced the anti-α-glucosidase activity with values of 93.47 ± 1.66% and 96.19 ± 1.15%, respectively. Moderate α-glucosidase inhibitory effects were also obtained from *S. gratum* (Wight) S.N. Mitra var. gratum, which gave lower anti-α-glucosidase activity when compared to the formers. Its inhibition rates against α-glucosidase were 55.12 ± 1.71% from the aqueous extract and 53.11 ± 1.44% from the ethanolic extract, respectively. The ethanolic extract from *C. grandis* (L.) Voigt showed

Table 2: The α-glucosidase and α-amylase inhibitory activities of vegetable extracts.

Scientific name	Percentage of inhibition against α-glucosidase (%)			Percentage of inhibition against α-amylase (%)		
	Water	Ethanol	Hexane	Water	Ethanol	Hexane
Adenanthera pavonina L	nd	nd	nd	nd	nd	nd
Alpinia purpurata (*Vielle.*) schum	nd	nd	nd	nd	nd	nd
Amaranthus lividus L	nd	nd	nd	nd	nd	nd
Azadirachta indica A. Juss. var. siamensis Valeton	nd	nd	nd	nd	nd	nd
Basella alba L	nd	nd	nd	nd	nd	nd
Brassica juncea (L.) Czern	nd	nd	nd	nd	nd	nd
Coccinia grandis (L.) Voigt	nd	67.30 ± 1.39	nd	nd	35.12 ± 3.06	nd
Cucurbita moschata Decne	nd	nd	nd	nd	nd	nd
Lasia spinosa (L.) thwaites	nd	nd	nd	nd	nd	nd
Leucaena leucocephala (Lamk.) de Wit	22.40 ± 1.54	96.10 ± 1.64	nd	nd	31.81 ± 3.19	nd
Limnophila aromatica	nd	nd	3.69 ± 1.62	nd	nd	nd
Limnocharis flava (L.) Buchenau	nd	nd	nd	nd	nd	nd
Luffa acutangula (Linn.) Roxb	nd	15.56 ± 0.79	nd	nd	nd	nd
Monochoria hastata (L.) solms	nd	24.69 ± 2.76	nd	nd	nd	nd
Moringa oleifera Lam	nd	nd	nd	nd	nd	nd
Piper sarmentosum Roxb	12.69 ± 1.40	6.66 ± 1.91	nd	nd	nd	nd
Polygonum odoratum Lour	93.47 ± 1.66	96.19 ± 1.15	nd	nd	7.67 ± 1.05	nd
Sesbania grandiflora (*L.*) Desv	nd	nd	nd	nd	nd	nd
Syzygium gratum (Wight) S.N. Mitra var. gratum	55.12 ± 1.71	53.11 ± 1.44	6.17 ± 2.92	nd	22.42 ± 0.16	nd
Trichosanthes anguina Linn	22.84 ± 2.40	17.40 ± 1.03	nd	nd	nd	nd

Remarks: All data was expressed as mean ± standard deviation (S.D.). Nd indicates "not detected."

67.30 ± 1.39% of inhibition, but no inhibition was found from the aqueous or hexane extract. Besides, other vegetable extracts produced either no or insufficient anti-α-glucosidase activity; their inhibitory activities were less than 25% of inhibition against α-glucosidase activity.

Table 2 also shows the α-amylase inhibitory activities of the vegetable extracts. It was interesting that most of the vegetable extracts were unable to inhibit α-amylase. Few of them, including the ethanolic extracts from *C. grandis* (L.) Voigt, *L. leucocephala* (Lamk.) de Wit, *P. odoratum* Lour, and *S. gratum* (Wight) S.N. Mitra var. gratum occupied inadequate anti-α-amylase activity. Their α-amylase inhibitory activities were measured at 35.12 ± 3.06%, 31.81 ± 3.19%, 7.67 ± 1.05%, and 22.42 ± 0.16%, respectively.

These screening results indicated that *L. leucocephala* (Lamk.) de Wit, *P. odoratum* Lour, *S. gratum* (Wight) S.N. Mitra var. gratum, and *C. grandis* (L.) Voigt appeared to be good potential sources of anti-α-glucosidase agents. Therefore, these vegetable extracts were selected for further analysis on IC_{50} determination and study for the kinetics of enzyme inhibition.

3.2. The IC_{50} and Kinetics of Enzyme Inhibitions. In the α-glucosidase inhibition assay, the ethanolic extract of *L. leucocephala* (Lamk.) de Wit was the most influential on α-glucosidase inhibitory activity with an IC_{50} value of 13.39 ± 0.14 μg/mL, while the extracts from *P. odoratum* Lour and *C. grandis* (L.) Voigt could inhibit α-glucosidase with IC_{50} values ranging from 25.60 ± 0.42 to 82.74 ± 1.39 μg/mL, respectively. The IC_{50} values of the aqueous and ethanolic extracts of *S. gratum* (Wight) S.N. Mitra var. gratum were 516.92 ± 5.08 and 542.50 ± 0.90 μg/mL, respectively (Table 3).

To determine the inhibition mechanism of selected vegetable extracts with high inhibitory activity against α-glucosidase, the inhibitory kinetics of the vegetable extracts were measured at various concentrations of substrate, and the data were exported using the method of Lineweaver-Burk plot. Table 4 shows the K_m and V_{max} values of the vegetable extracts towards α-glucosidase. Compared to the uninhibited reaction (reaction containing α-glucosidase without inhibitor), a decrease in V_{max} was found for all vegetable extracts, but the effects of the vegetable extracts on K_m values were different. The K_m values were reduced in the presence of an aqueous extract of *S. gratum* (Wight) S.N. Mitra var. gratum and the ethanolic extract of *C. grandis* (L.) Voigt. The K_m values were increased in the presence of the ethanolic extract of *S. gratum* (Wight) S.N. Mitra var. gratum and *P. odoratum* Lour. However, the aqueous extract of *P. odoratum* Lour and the ethanolic extract of *L. leucocephala* (Lamk.) de Wit did not change the K_m values of the reactions. These results demonstrated the mode of inhibition for the vegetable extracts on α-glucosidase. The aqueous extract of *S. gratum* (Wight) S.N. Mitra var. gratum and the ethanolic extract of *C. grandis* (L.) Voigt exhibited an uncompetitive inhibition mode. The ethanolic extract of *S. gratum* (Wight) S.N. Mitra var. gratum and *P. odoratum* Lour were in a mixed inhibition mode, while the aqueous extract of *P. odoratum* Lour and the ethanolic extract of *L. leucocephala* (Lamk.) de Wit demonstrated a noncompetitive inhibitor mode.

4. Discussion

There is growing interest in developing novel and potential antidiabetic properties with minimal adverse effects that can be derived from plants that have known, scientifically

Table 3: The IC_{50} and kinetics for enzyme inhibition of the vegetable extracts.

Scientific name	Extraction solvent	IC_{50} (μg/mL)
Syzygium gratum (Wight) S.N. Mitra var. gratum	Ethanol	542.50 ± 0.90
	Water	516.92 ± 5.08
Coccinia grandis (L.) Voigt	Ethanol	82.74 ± 1.39
Polygonum odoratum Lour	Ethanol	49.03 ± 0.72
	Water	25.60 ± 0.42
Leucaena leucocephala (Lamk.) de Wit	Ethanol	13.39 ± 0.14

Remarks: All data was expressed as mean ± standard deviation (S.D.).

Table 4: Kinetic parameters for α-glucosidase inhibition of the vegetable extracts.

Scientific name	Extraction solvent	K_m (mM)	V_{max} (μM/min)
α-glucosidase without inhibitor		0.39 ± 0.001	16.0 ± 0.002
Syzygium gratum (Wight) S.N. Mitra var. gratum	Ethanol	0.41 ± 0.001	4.99 ± 0.001
	Water	0.15 ± 0.001	5.98 ± 0.001
Coccinia grandis (L.) Voigt	Ethanol	0.06 ± 0.002	2.32 ± 0.001
Polygonum odoratum Lour	Ethanol	0.53 ± 0.001	6.41 ± 0.002
	Water	0.39 ± 0.001	5.71 ± 0.001
Leucaena leucocephala (Lamk.) de Wit	Ethanol	0.39 ± 0.001	7.13 ± 0.002

Remarks: All data was expressed as mean ± standard deviation (S.D.).

validated antidiabetic characteristics. Numerous phytochemicals, such as polyphenols, phenolic acids, stilbenes, lignin, glucosinolates, and carotenoids are abundant in plants. These phytoconstituents play a vital part in plant metabolism and offer considerable health advantages to slow the progression of diseases as well as prevent them from occurring. A decrease in developing metabolic syndromes, including DM, can eventually result from an increase in plant consumption, whether through direct ingestion or dietary supplements. Plants are thought to contain a variety of substances that have hypoglycemic effects driven by several different mechanisms, including insulin sensitization, insulin release, and carbohydrate-hydrolyzing enzyme inhibition [6, 13, 14].

In the present study, the inhibition of carbohydrate-hydrolyzing enzymes of regularly consumed vegetables in Thailand was the subject of investigation. The enzyme inhibitory property may be a practical method for regulating the control of blood glucose levels. The target enzymes in this investigation were the hydrolyzing enzymes: α-amylase and α-glucosidase. These enzymes are primarily responsible for the decomposition of starch into glucose. The inhibition of these enzymes results in a delay in glucose absorption into the blood vessels.

The inhibition of carbohydrate-hydrolyzing enzymes of sixty different vegetable extracts from different twenty vegetables was tested. It was discovered that anti-α-glucosidase and anti-α-amylase activities were affected by the type of vegetables and solvent utilized, which was in agreement with several previous studies [15–18]. Plants have a wide range of phytochemicals that are structurally different, resulting in distinct polarity [16, 17]. In this study, solvents with a wide range of polarity from nonpolar to polar were used to ensure that plant materials that differed in their polarity were sequentially extracted based on their polarity, and all components were presented in the screening study. It was found that only four out of twenty vegetables evaluated in this study had high anti-α-glucosidase activity (>50% of inhibition). This circumstance could be explained by the phytochemical components present in plants varying contingent on plant origin, plant genotype, geography, climate, soil fertility, and stress level. These elements affect how plants create bioactive compounds, which might vary in quantity and form [19–21]. In addition, the results of the current study suggested that the anti-α-glucosidase agents from these vegetable extracts were also likely composed of functional groups that appear to be hydrophilic with polarity indices between 5.2 (absolute ethanol) and 10.2 (water) because the anti-α-glucosidase activities were found in the ethanolic and aqueous extracts. Five out of the twenty vegetables evaluated, however, had minimal inhibitory effects on α-glucosidase. Additionally, a few of the vegetable extracts exhibited minor inhibitory effects on α-amylase. Therefore, it is probable that these vegetables may not contain effective or sufficient anti-α-glucosidase and anti-α-amylase agents. As mentioned previously, the type and concentration of bioactive chemicals found in various plants may vary.

Measuring enzyme inhibition at a fixed concentration provided rather limited information [22]. The IC_{50} value was therefore used to compare the efficiency of the vegetable extract to inhibit α-glucosidase in this study. The IC_{50} value means the concentration of vegetable extract that generates 50% inhibition under a particular assay condition, resulting in a difference in the IC_{50} value found among the conditions used [23].

The IC_{50} results revealed that the aqueous and ethanolic extracts of *S. gratum* (*Wight*) *S.N. Mitra* var. *gratum* were approximately the same with values of 516.92 ± 5.08 and 542.50 ± 0.90 μg/mL, respectively. This result implied that

the active compounds found in these two extracts were possibly hydrophilic-like compounds, which corresponded to the study from Syabana et al. [24]. In their study, the leaf extract of *S. gratum* (*Wight*) *S.N. Mitra* var. *gratum* was found to be a potential source of α-glucosidase inhibitors, especially the fractions extracted by acetone-water 4 : 1 and 3 : 2. The IC_{50} of these two fractions were 24.8 and 31.8 μg/mL, respectively [24]. According to the study from Syabana et al. [24], the α-glucosidase inhibitory activity of *S. gratum* (*Wight*) *S.N. Mitra* var. *gratum* could be a result of myricetin-3-O-rhamnoside (myricitrin) and epigallocatechin-3-gallate (EGCG) [24]. Additionally, both aqueous extract and ethanolic extract from the leaves of *S. gratum* (*Wight*) *S.N. Mitra* var. *gratum* showed strong antioxidant and intercellular oxygen scavenging activity, according to the study from Senggunpri et al. [25]. The aqueous extract also showed a cytoprotective effect *in vivo*. The activity of heme oxygenase (HO-1), a potent cytoprotective enzyme in the antioxidant defense system, was significantly increased in the high-dose-treated C57BL/6J mice, and the expression of HO-1 gene had a tendency to increase when treated with the aqueous extract. The data extrapolated the benefit of *S. gratum* (*Wight*) *S.N. Mitra* var. *gratum* as a source of natural antidiabetic agents and antioxidants, and it could induce cytoprotective enzymes without toxicity being observed.

The IC_{50} values of the *P. odoratum* Lour extracts were 49.03 ± 0.72 μg/mL for the ethanolic extract and 25.60 ± 0.42 μg/mL for the aqueous extract, respectively. These results were supported by the study of Thongra-ar et al. [26], which specified that the ethanolic extract of *P. odoratum* Lour strongly inhibited α-glucosidase with an IC_{50} value of 9.82 ± 1.64 μg/mL [26]. Moreover, Dedvisitsakul and Watla-Iad [27] further reported the inhibitory effect of the ethanolic extract from *P. odoratum* Lour towards α-glucosidase with the IC_{50} of 0.66 ± 0.08 mg/mL. Their study also discovered that the ethanolic *P. odoratum* Lour extract demonstrated significant inhibitory activity towards the formation of advanced glycation end product (AGEs) which derived from glucose using a BSA-glucose system with the IC_{50} of 0.03 ± 0.01 mg/mL [27]. The phenolic compound (gallic acid and chlorogenic acid) and flavonoid (isorhamnetin) were believed to respond to the inhibitory effect of α-glucosidase in accordance with their phytochemical study [26]. The *in vivo* study from Deng et al. [28] also indicated saponins found in this vegetable presented antidiabetic activity, whereas flavonoids influenced antioxidant activity.

The ethanolic extract of *C. grandis* (L.) Voigt proved its IC_{50} against α-glucosidase at 82.74 ± 1.39 μg/mL. Likewise, the study by Pulbutr et al. indicated the IC_{50} value of the ethanolic extract against α-glucosidase at 77.66 ± 9.16 μg/mL [29]. The antidiabetic properties of the ethanolic extract were supported by the study from Astiti et al. [30], which identified the compounds responsible for the antidiabetic effect from the extract of *C. grandis* leaves, including rutin, kaempferol 3-*O*-rutinoside or nicotiflorin, kaempferol 3-*O*-robinobioside, quercetin 3-*O*-robinobioside, quercetin 3-*O*-β-D-apiofuranosyl-(1⟶2)-[α-L-rhamnopyranosyl-(1⟶6)]-β-D-glucopyranoside or CTN-986, kaempferol 3-*O*-β-D-apiofuranosyl-(1⟶2)-[α-L-rhamnopyranosyl-(1⟶6)]-β-D-glucopyranoside, and kaempferol 3-*O*-β-D-apiofuranosyl-(1⟶2)-[α-L-rhamnopyranosyl-(1⟶6)]-β-D-galactopyranoside.

The most efficient anti-α-glucosidase activity in this study was obtained from the ethanolic extract from *L. leucocephala* (Lamk.) de Wit with the lowest IC_{50} of 13.39 ± 0.14 μg/mL. In addition, the study by Renganathan et al. [31] revealed that *L. leucocephala* (Lam.) De Wit leaf extract inhibited enzyme activity in a dose-dependent manner. The study from Wan-Nadilah et al. [32] also found the *in vitro* α-glucosidase inhibitory activity from the seed of *L. leucocephala* (Lam.) De Wit with the IC_{50} of 30.80 ± 2.50 μg/mL. Parts of use in the study by Renganathan et al. [31] and the present study were not the same; their extracts were obtained from leaves, while the extracts in this study were from young shoots. This might have contributed to the difference in IC_{50} values. However, these data indicated that leaves, seeds, and young shoots of *L. leucocephala* (Lam.) De Wit were a good source of anti-α-glucosidase constituents. Moreover, *in silico* virtual screening was used to identify the phytochemicals involved in α-amylase enzyme inhibition, while hexadecenoic acid and oleic acid ((Z)-octadec-9-enoic acid) were identified as α-amylase inhibitors.

Line-weaver-Burk plots revealed that the inhibition modes of all samples might be mixed–uncompetitive and noncompetitive inhibition. A mixed inhibitor can either bind to the free enzyme or the enzyme-substrate complex which results in an alteration of K_m and V_{max} values (an increase in K_m and a decrease in V_{max}). For the uncompetitive inhibition and noncompetitive inhibition, the binding of these types of inhibitor can influence the binding of the substrate by changing the conformation of the enzyme. The uncompetitive inhibitor binds the enzyme-substrate complex, resulting in a decrease of K_m and V_{max}, while the noncompetitive inhibitor either binds to a free enzyme or the enzyme-substrate complex, which results in a decrease of V_{max} value and no change in K_m [11, 18, 23]. It is likely that the capacity of these extracts as mixed, uncompetitive, and noncompetitive inhibitors to bind to extensive areas of the enzyme other than the active site allows them to show a broader specificity of inhibition when compared to acarbose as a competitive inhibitor [11, 18, 23]. The explanation for various inhibition modes from the extracts could be as a result of the different bioactive constituents available in the extracts [18, 22, 23]. The various bioactive compounds presented in the extracts probably had different binding modes to α-glucosidase. Contrary to acarbose, these extracts may not be affected by increased quantities of the substrate, which is one advantage they have over acarbose. With increased carbohydrate meal consumption, higher dosages of acarbose as a competitive inhibitor would be necessary to have the same impact, but with the mixed, uncompetitive, and noncompetitive inhibition, the inhibitor would be effective at lower concentrations [18, 23, 33]. Moreover, the stronger inhibition activity of the α-glucosidase than the α-amylase activity of these extracts revealed their medicinal potential to prevent

some negative effects of utilizing synthetic α-glucosidase and α-amylase inhibitors. The side effects of using synthetic enzyme-inhibitor drugs can include abnormal bacteria fermentation of undigested carbohydrates in the colon because these drugs strongly inhibit α-amylase over α-glucosidase. Therefore, more potent inhibitors for enzymes should have a strong inhibitory effect on α-glucosidase and a moderate inhibitory effect on α-amylase, which can improve the management of postprandial hyperglycemia with the fewest side effects [34, 35].

The results of the current study showed the potential of vegetable extracts toward enzyme-hydrolyzing carbohydrates. As a result, individuals should be encouraged to consume more of these vegetables as an alternative course for diabetic prevention and treatment. However, more information on *in vivo* bioactivity and the absorption of these bioactive components needs to be established before deciding on their applications.

5. Conclusion

Vegetables that are frequently consumed in the northeastern region of Thailand showed a varying range of α-glucosidase and α-amylase inhibitory effects. Promising α-glucosidase inhibitory activities were reported from *L. leucocephala* (Lamk.) de Wit, *P. odoratum Lour*, *C. grandis* (L.) Voigt, and *S. gratum* (Wight) S.N. Mitra var. gratum. The results of this investigation suggest that these vegetables may be good dietary sources of extractable anti-α-glucosidase agents for preventing or managing postprandial hyperglycemia-induced complications. Nevertheless, this was an *in vitro* study with potential relevance concerning phytochemicals.

Data Availability

All the data presented are included in the article materials. Inquiries should be directed to the corresponding author.

Conflicts of Interest

The authors declare that there are no conflicts of interest.

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