

ALPHA-GLUCOSIDASE INHIBITORY AND ANTIOXIDANT ACTIVITIES FROM ETHANOL EXTRACTS OF SIX THAI VEGETABLES

Prakit Chaithada^{1,*}, Patchareeya Rattanapan¹, Amita Supawatin¹
Saowanee Maungchanburee² and Juthamas Supapan¹

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Abstract

The objective of this research was to determine the alpha-glucosidase inhibition and antioxidant activities by DPPH and ABTS assay of Thai vegetables. Six kinds of local vegetables including *Coccinia grandis* L., *Centella asiatica* L., *Diplazium esculentum* (Retz.) Sw., *Tiliacora triandra* (Colebr.) Diels, *Emilia sonchifolia* (L.) DC and *Carissa carandas* L. were extracted by ethanol using soxhlet extraction. Analysis of alpha-glucosidase inhibitory and the antioxidant activity by DPPH and ABTS assay, the results showed that the ethanolic extracts from *Tiliacora triandra* (Colebr.) Diels (TT) and *Carissa carandas* L. (CC) showed the strongest antioxidant activity and alpha-glucosidase inhibitory. *Tiliacora triandra* (Colebr.) Diels and *Carissa carandas* L. extracts exhibited alpha-glucosidase inhibitory with IC₅₀ of 115.24±5.91 and 212.65±4.09 ppm, respectively. *Tiliacora triandra* (Colebr.) Diels inhibited DPPH and ABTS radicals with IC₅₀ of 37.35±1.70 and 62.42±2.80 ppm, respectively. *Carissa carandas* L. extracts showed the inhibition of DPPH and ABTS radicals with IC₅₀ of 76.92±1.55 and 78.54±3.53 ppm, respectively. *Emilia sonchifolia* (L.) DC and *Centella asiatica* L. extracts showed moderate antioxidant activity and alpha-glucosidase inhibitory, while *Coccinia grandis* L. could not show both activities.

Keywords: Alpha-glucosidase, antioxidant activity, Thai vegetables

Introduction

Currently, investigation about “free radicals” get a lot of attention. Free radicals are species that consist of one single electron, created from the cell metabolism process and they affect the deterioration of cells. If free radicals are not inhibited by antioxidant substances, they will react with the biomolecules in the body, which is a major cause of many diseases. Free radical reactions in the human

body are harmful to the cardiovascular system, immune system and brain, induce diseases such as heart disease, high blood pressure, cancer, diabetes and degeneration of the nervous system (Fan *et al.*, 2017).

Free radicals can be controlled with antioxidants, which acts to prevent the oxidation process that causes free radicals. The most antioxidants

¹ General science, Faculty of Education, Nakhon Si Thammarat Rajabhat University, 80280, Thailand. Tel. 0-7539-2040; Fax. 0-7537-7440; E-mail: prakitch0106@gmail.com

² Biomedical sciences, Faculty of Medicine, Prince of Songkla University, Thailand.

* Corresponding author

can be found in natural vegetables and fruits that can be easily eaten locally. The previous research reported that eating fruits and vegetables that contain phenolic compounds may help the body to use it to neutralize free radicals and help reduce the risk of various diseases (Friedman *et al*, 2017). Total flavonoids content, total phenolic content and the antioxidant activity of various plant extracts depends on the extraction method, extraction solvent, botanical origin and harvesting season (Rebaya *et al.*, 2015; Chaithada *et al.*, 2018; Nascimento *et al.*, 2018).

Alpha-glucosidase is an enzyme that is important for the digestion of carbohydrates into monosaccharides. For diabetic patients, inhibiting the activity of alpha-glucosidase is one way to control blood sugar levels. Acarbose, a drug used in the treatment of diabetic patients Type 2 with mechanisms to inhibit the enzyme alpha-glucosidase in the small intestine. To reduce the rate of breakdown of carbohydrates to glucose, resulting in lower levels of sugar in the bloodstream (Ellappan *et al.*, 2013).

Nowadays, growing vegetables in modern industrial agriculture, especially with non-native vegetables, often have diseases and pests. Farmers can solve the problem by using insecticides, which can cause harm to the health of consumers. The researcher is interested in study local vegetables which are non-toxic because they are resistant to diseases and insects, therefore suitable for the climate grown. Farmers do not need chemicals. Folk vegetables are therefore non-toxic vegetables.

Coccinia grandis L. (CG) has a common name that Ivy gourd. The hydromethanolic extract of the leaves of CG showed effective reducing power, free radical scavenging activity and metal chelating ability (Umamaheswari *et al.*, 2008). The aqueous extract of *Centella asiatica* L. (CA) has a local name that gotu kola, showed increase of DPPH scavenging activity and against mouse melanoma (B16F1), human breast cancer (MDA MB-231) and rat glioma (C6) cell lines (Pittella *et al.*, 2009). Paco fern is a common name of *Diplazium esculentum* (Retz.) Sw. (DE). Chloroform and methanol extracts from its leaves exhibit antioxidant activity in cupric ion reducing capacity assay. Moreover, it exhibits antibacterial activity with MIC values in the range of 1.6-12.5 mg/ml (Akter *et al.*, 2014). *Tiliacora triandra* (Colebr.) Diels (TT) has a common name that bamboo grass. The methanolic extract of TT showed good antioxidant activity and affects the stability of lymphocyte and HeLa DNA without significant cytotoxicity at the cellular level (Chaveerach *et al.*, 2016). Red tassel flower is a common name of *Emilia sonchifolia* (L.) DC (ES). The n-hexane extract of ES showed the presence of terpenoids, which can

be used as a bioactive source of natural antioxidants (Sophia *et al.*, 2011). In which 5 plants including Ivy gourd, gotu kola, paco fern, bamboo grass and red tassel flower are popularly used by villagers for cooking or as vegetables. *Carissa carandas* L. (CC) had a common name that karanda. The leaves of CC are commonly boiled to relieve diarrhea, sore ears, and sore throat, while the Karanda's fruits are popularly eaten. From the study, it was found that native vegetables are vegetables that have high nutritional value. There are many medicinal properties. If consumed regularly, it may be able to prevent, treat and cure illness. The objective of this research was to determine the alpha-glucosidase inhibition and antioxidant activities of the ethanolic extracts of six local vegetables in Nakhon Si Thammarat province.

Materials and Methods

Chemicals and Reagents

Purified water from a distilling apparatus (SI Analytics D82000, Germany) was used for all solution preparations. All reagents were of analytical grades. The standard of total phenolic content (gallic acid), total flavonoid content (quercetin), alpha-glucosidase inhibitory (acarbose) and antioxidant activities (Trolox) were purchased from Merck Ltd. The solvent and chemical reagents including ethanol, alpha-glucosidase, 4-nitrophenyl- α -D-glucopyranoside (pNPG), phosphate buffer, 2,2-diphenyl-1-picrylhydrazyl (DPPH), 2,2'-azinobis(3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt (ABTS), potassium peroxodisulfate ($K_2S_2O_8$), Folin-Ciocalteu's reagent and sodium carbonate (Na_2CO_3) were obtained from Merck Ltd.

Sample Preparation

The leaves of CG, CA, DE, TT and ES and the fruits of CC were collected in December 2018 from Nakhon Si Thammarat Province, Southern Thailand. The samples were dried at room temperature for 5-7 days and grinding to increase surface area. The 10 g of samples were extracted in triplicate with 50 ml of ethanol using soxhlet extractor. The extracts were evaporated under reduced pressure to obtain 6 crude extracts. All crude extracts were calculated as the weight percentage.

Determination of Total Phenolic Content (TPC)

The total phenolic content of the extract was determined by the Folin-Ciocalteu method with some modifications (Shoib and Shahid, 2015). An amount of 0.5 ml of crude extract (1 mg/ml) was mixed with 2.5 ml of 10% Folin-Ciocalteu reagent. After 5 min, 2 ml of 7.5% Na_2CO_3 was added. After

incubation for 1 h in the dark, the absorbance was measured at 765 nm. All of the tests were performed in a triplet. The total phenolic content was calculated from the calibration curves of gallic acid, and the results were expressed as mg of gallic acid equivalent (GAE) per g of crude extract using the following formula:

$$\text{Total phenolic content (TPC)} = \frac{\text{GAE} \times \text{V} \times \text{D}}{\text{W}}$$

whereas: V is the volume of the extract solution in ml, W is the weight of the extract in g and D is dilution factor.

Determination of Total Flavonoids Content

The total flavonoids content of the extract was determined by the aluminium chloride colorimetry method with some modifications (Bag *et al.*, 2015). Briefly, 0.5 ml of crude extract (5 mg/ml) was mixed with 2 ml in an appropriate solvent, 0.1 ml of 10% aluminum chloride, 0.1 ml of 1 M potassium acetate and diluted with distilled water in a 5 ml volumetric flask. The absorbance was determined at 415 nm. The data of total flavonoids content were expressed as mg of quercetin equivalents (QE) per g of crude extract using the following formula:

$$\text{Total flavonoids content (TFC)} = \frac{\text{QE} \times \text{V} \times \text{D}}{\text{W}}$$

whereas: V is the volume of the extract solution in ml, W is the weight of the extract in grams and D is dilution factor.

Alpha-glucosidase Inhibitory Activity

All six extracts at different concentrations (50-1,000 ppm) 20 µl mixed with 0.1 U/ml solution of alpha-glucosidase, 20 µl and 50 mM phosphate buffer solution (pH = 6.8), volume 100 µl into the 96-well plate hole, then warm the mixed solution at 37°C for 10 min, then add 20 µl of 2 mM 4-nitrophenyl-α-D-glucopyranoside (pNPG) solution, warm the solution at 37°C for 10 min then stop reaction with 40 µl of 1 mM Na₂CO₃ and then measuring the absorbance of the solution at a wavelength of 405 nm with a microplate reader and calculate the percent inhibition of the enzyme alpha-glucosidase by comparing with standard acarbose.

DPPH Radical Scavenging Activity

The radical scavenging activity of the extracts was determined according to the literature method (Aksoy *et al.*, 2013) with slightly modifications. Take six plant extracts at various concentrations (50-1,000 ppm) in the volume of 2 ml in a test tube. Then add 0.4 ml of 0.3 mM DPPH solution and 1.6 ml of methanol to the test tube. Then shake the

substance together with the vortex mixture. Keep in the dark at room temperature for 30 min. The absorbance is measured at the wavelength of 517 nm by a spectrophotometer. By using the methanol solution as blank and calculate the percentage of free radical DPPH in comparison with the standard Trolox substance. All tests were carried out in triplicate. The antioxidant activity of the samples was defined as:

$$\text{DPPH scavenging activity(\%)} = \left[\frac{\text{Abs}_{\text{control}} - \text{Abs}_{\text{sample}}}{\text{Abs}_{\text{control}}} \right] \times 100$$

whereas: Abs_{control} is the absorbance of the control (DPPH solution without the test sample), and Abs_{sample} is the absorbance of the test sample. The mean of sample concentration needed for inhibition of the initial radical-scavenging activity by 50% or IC₅₀ was calculated based on the dose-response plot of DPPH absorbance versus concentration of extract samples. Trolox served as the standard substance.

ABTS Radical Scavenging Assay

Free radical scavenging activity of the samples were analyzed by investigating their ability to scavenge the ABTS⁺ using the method as described previously in the literature (Zhen *et al.*, 2016; Yuan *et al.*, 2018). The 7 mM ABTS⁺ was mixed with 2.45 mM potassium persulfate, and this mixture was stored in the dark at room temperature for 12-16 h to create stable, darkblue-green radical solution. The solution was then diluted with 20 mM sodium acetate buffer (pH 4.5) to an absorbance of 0.70±0.02 at 734 nm to form the test reagent. After the addition of 0.5 ml of plant extract at various concentrations (50-1,000 ppm) to 1.5 ml of diluted ABTS⁺ solution, the absorbance was measured at 30 min after the initial mixing with UV-Vis spectrophotometer. An appropriate solvent blank was run in each assay. All the measurements were carried out at least three times. The scavenging activity of ABTS radicals was calculated using the following equation:

$$\text{ABTS scavenging activity(\%)} = \left[\frac{\text{Abs}_{\text{control}} - \text{Abs}_{\text{sample}}}{\text{Abs}_{\text{control}}} \right] \times 100$$

whereas: Abs_{control} is absorbance of the ABTS solution without sample and Abs_{sample} represents absorbance of the ABTS solution with tested samples. Trolox was used as a standard compound.

Statistical Analysis

Experimental data was subjected to one-way analysis of variance (ANOVA) and Tukey's multiple-comparison tests using SPSS software at 95% significance level (P≤0.05).

Table 1. Weight percentage of extraction, total flavonoids content, total phenolic content, alpha-glucosidase inhibitory activity and antioxidant activities of six crude extracts

Plant sample	%Yield	Total flavonoids content (mgQE/g DW)	Total phenolic content (mgGAE/g DW)	Alpha-glucosidase Inhibitory IC ₅₀ (ppm)	DPPH Scavenging IC ₅₀ (ppm)	ABTS scavenging IC ₅₀ (ppm)
CG	19.16±1.77	20.35±0.83 ^d	27.43±1.77 ^d	>1,000 ^e	>1,000 ^f	>1,000 ^e
CA	19.45±3.70	74.60±0.25 ^a	133.89±6.15 ^b	645.28±10.88 ^d	172.14±4.35 ^c	315.24±8.05 ^c
DE	16.63±3.52	27.52±0.90 ^c	29.75±10.74 ^d	>1,000 ^e	898.16±4.35 ^e	452.25±7.58 ^d
TT	17.10±2.81	29.44±3.92 ^{bc}	64.98±2.53 ^c	115.24±5.91 ^a	37.35±1.70 ^a	62.42±2.80 ^a
ES	15.24±4.35	33.89±0.49 ^b	60.34±13.08 ^c	412.56±7.81 ^c	212.22±4.18 ^d	321.45±8.67 ^c
CC	24.05±3.32	72.36±2.09 ^a	252.17±2.98 ^a	212.65±4.09 ^b	76.92±1.55 ^b	78.54±3.53 ^{ab}
Acarbose				125.42±2.57 ^a	-	-
Trolox				-	75.68±0.73 ^b	82.65±5.96 ^b

^{a-f} significant ($P \leq 0.05$) differences within a column.

Results and Discussion

Yields of Extraction

All six specimens of plants were cut into small pieces and dried, then finely ground. Every 10 g of six local vegetable powder was extracted with a soxhlet extractor by using ethanol as a solvent for 4 h. When the extracts were evaporated by rotary evaporator, the yields of extraction are shown in Table 1. The ethanolic extract of CC had the highest percentage of extraction at 24.05±3.32%, indicating that CC was likely to contain highly polar compounds, followed by CA and CG with the percentage of extraction. 19.45±3.70% and 19.16±1.77%, respectively.

Total Flavonoids Content and Total Phenolic Content

The flavonoids compounds from plants always show a wide range of biological activities. The analysis of total flavonoids content of all six plant extracts by the aluminium chloride colorimetry method, it was found that CA had the highest total flavonoids content of 74.60±0.25 mg QE/g DW, followed by CC, ES, TT, DE, and CG with total flavonoids content of 72.36±2.09, 33.89±0.49, 29.44±3.92, 27.52±0.90 and 20.35±0.83 mg QE/g DW respectively (Table 1). No significant ($p > 0.05$) difference of CA and CS extracts was found in the total flavonoids content. Flavonoid compounds, including flavones, flavanols and condensed tannins, are plant secondary metabolites are a lot of bioactive substances. Flavonoids inhibit chelating oxygen production involved in free radical production, scavenge reactive species, and regulate and prevent antioxidant defenses (Agati *et al.*, 2012; Baba and Malik, 2015). The phenolic compounds of some plants are an important ingredient in human food because of the antioxidant activity and the ability to relieve tissue damage caused by stress caused by chronic reactions (Rio *et al.*, 2013). The analysis of total phenolic content by Folin-Ciocalteu reagent method, was found that CC showed the highest of

total phenolic content with 252.17±2.98 mg GAE/g DW, followed by CA, TT, ES, DE, and CG had total phenolic content of 133.89±6.15, 64.98±2.53, 60.34±13.08, 29.75±10.74 and 27.43±1.77 mg GAE/g DW, respectively (Table 1). The CC extracts exhibited a high total phenolic content consistent with previous research of Sarma *et al.* (2015) using 70% methanol for extraction (188.75±1.42 mg GAE/g). The study results showed that it can be used as a dietary supplement and medicine.

Alpha-glucosidase Inhibitory Activity

Activity of alpha-glucosidase inhibitory activity was performed in optimal conditions for the enzyme. The optimal conditions include pH 6.8, temperature 37°C, enzyme concentration 0.1 U/ml, and 4-nitrophenyl- α -D-glucopyranoside (pNPG) substrate concentration 2 mM. The absorbance was measured at a wavelength of 405 nm using a microplate reader. The result showed that a standard acarbose had IC₅₀ of 125.42±2.57 ppm. This test was performed on six plant extracts, which the TT showed the highest of alpha-glucosidase inhibitory activity with IC₅₀ of 115.24±5.91 ppm, followed by CC, ES, and CA with IC₅₀ of 212.65±4.09, 412.56±7.81, and 645.28±10.88 ppm, respectively. The CG and ED extracts did not inhibit alpha-glucosidase activity as shown in Table 1. The presence of alpha-glucosidase inhibitory activity may be influenced by the chemical constituents present in the TT leaves. Its extract contained oxoanobine as the main constituent and had anticancer activity. Methanolic extracts had the highest cytotoxic activity against lung cancer cells (NCI-H187), while water extracts had the highest activity against oral cancer (KB) (Rattana *et al.*, 2016). New fatty acids, 5,7-dihydroxy-6-oxoheptadecanoic acid, was isolated from 95% ethanolic extract from the aerial part of TT, which showed excellent of alpha-glucosidase inhibitory activity with IC₅₀ of 11.58±0.32 μ M (Makinde *et al.*, 2020).

Antioxidant Activities

All the crude extracts were analyzed for the antioxidant activity by the DPPH method, the samples must be diluted to obtain the concentration of 5, 10, 25, 50, 100, 200, 500, and 1,000 ppm and be measured. The absorbance using spectrophotometer using Trolox as standard was found that TT had the highest activity with IC_{50} of 37.35 ± 1.70 ppm, followed by CC, CA, ES, DA, and CG with IC_{50} of 76.92 ± 1.55 , 172.14 ± 4.35 , 212.22 ± 4.18 , 898.16 ± 4.35 and $>1,000$ ppm, respectively which were statistically significant differences ($P \leq 0.05$) as shown in Table 1. The investigation of antioxidant activity by the ABTS assay was consistent with the DPPH assay. The standard Trolox showed IC_{50} of 82.65 ± 5.96 ppm. The IC_{50} of TT and CC showed strong antioxidant activity, while CA, ES, and DE exhibited moderate antioxidant activity (Table 1). The chemical constituents of the extract of CC include carisol, α -amyryn epimer, linalool, β -caryophyllene, carissone, carissic acid, carindone, ursolic acid, carinol, ascorbic acid, lupeol and β -sitosterol, as well as volatile flavors: isoamyl alcohol, isobutanol, and β -caryophyllene (Singh and Uppal, 2015). These compounds may result in CC extracts showing very good antioxidant activities. These results showed that the ethanolic extract from CC with the highest total phenolic content (252.17 ± 2.98 mgGAE/g) was consistent with the antioxidant activity of DPPH and ABTS assay. The ethanolic extract of TT exhibited excellent antioxidant activity, but had moderate total phenolic and total flavonoids content, suggesting that not only functions in antioxidants but also found that other substances can affect the antioxidant as well. As previously reported, the methanolic extract of TT also contained other chemical constituents such as vitamin E, phytol 1-cyclohexenylacetic acid, oleamide, oleic acid, and others which can have antioxidant effects (Chaveerach *et al.*, 2016).

Conclusions

In the present study, it was found that the ethanol extract of TT and CC showed the strongest antioxidant activity and alpha-glucosidase inhibitory activity compare with standard substance. The results of antioxidant activities are consistent based on DPPH and ABST methods. Therefore, such powerful biological activity will be an area of interest for phytochemical investigation and the resources that have pharmacological activity.

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