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Contributed Paper

Antioxidant Activity and Starch-digesting Enzyme Inhibition of Selected Thai Herb Extracts

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ABSTRACT

The objective of this study was to screen the selected Thai herb extracts for antioxidant activity and potential inhibition of starch-digestive enzymes. Ethanolic extracts of eight Thai herbs, leaves of *Coccinia grandis,* leaves and fruits of *Aegle marmelos* Corr., fruits of *Momordica charantia*, leaves and sheaths of *Moringa oleifera* Lam., leaves of *Lagerstroemia speciosa* L., and bark of *Cinnamomum verum* J.Presl, were selected for evaluation in terms of their polyphenol compounds, antioxidant activity, and *in vitro* potential inhibition against α-amylase and α-glucosidase. The contents of the total phenolic compounds, flavonols, and flavonoids ranged from 20.31 mg gallic acid (GA)/g extract to 391.80 mg GA/g extract, from 5.22 mg quercetin (QE)/g extract to 52.39 mg QE/g extract, and from 5.15 mg QE/g extract to 108.74 mg QE/g extract, respectively. A high correlation between the total phenolic content and the total flavonoid content was observed. The antioxidant activities were expressed as the concentration of extract required to exhibit 50% inhibition of DPPH and ABTS radical scavenging activities, ranging from 0.04 mg/mL to 3.48 mg/mL and from 0.03 mg/mL to 1.74 mg/mL, respectively. The leaves of *L.speciosa* with cold extraction had the highest total phenolic content, total flavonoid content, and antioxidant activity (both DPPH and ABTS methods). The antioxidant activity correlated well with the flavonoid and the phenolic contents. The potential inhibition (IC_{50}) against human saliva α-amylase varied from 0.57 mg/mL to >5 mg/mL, porcine pancreas α-amylase varied from 0.19 mg/mL to >5 mg/mL, and yeast α-glucosidase varied from 0.04 mg/mL to >5 mg/mL. The leaves of *L.speciosa* with cold extraction had the highest potential of yeast α-glucosidase inhibition. As for α-amylase inhibition, *C.verum* bark extracted using hot method showed the best activity. The inhibitory activity of these herb extracts against the starch-digesting enzymes displayed significant correlation with phenolic content, flavonoid content, and antioxidant activity. In conclusion, the results indicated that *L.speciosa* leaves and *C.verum* bark extracts could be important Thai herbs with high potential antioxidant and starch-digestive inhibition activities.

Keywords: Thai herb extract, antioxidant activity, starch-digesting enzyme inhibitory activity

1. INTRODUCTION

Thailand is a tropical country with a wide diversity of herbs. Herbs are plants that are used in fresh or dry form, to add aroma and flavor in food or drink products. Thai herbs have been found to be good sources of nature phenolic phytochemicals, including flavonoids [1]. Many literature studies have reported that Thai herbs have potent antioxidant properties [1-2] and activity related to anti-diabetes [3-5]. Currently, one therapeutic approach to control or decrease postprandial hyperglycemia is the inhibition of α-amylase and α-glucosidase, resulting in aggressive delay of carbohydrate digestion and glucose absorption [6].

Moreover, diabetes is frequently associated with development of micro and macro vascular diseases, including neuropathy, nephropathy, retinopathy, and cardiovascular diseases, which are linked to oxidative stress, and administration of antioxidants has been considered as part of treatments [7]. Phenolic compounds, with their antioxidant properties, present in no fewer than 3,000 plant species including some Thai herbs have been studied [1, 8]. Many researches found that the phenolic contents of plants and their antioxidant capacity showed a good correlation [1, 8-9]. Recently, studies have shown that Thai plants exhibit enzyme inhibitory activity *in vitro*. However, there is very little data to elucidate the relationship between the chemical compositions with the antioxidant activity and their activity toward starch-digestive enzyme inhibition [5, 10].

The following Thai herbs were selected for this investigation: *Coccinia grandis* (Ivy gourd), *Aegle marmelos* Corr. (Bael), *Momordica charantia* (Bitter gourd), *Moringa oleifera* Lam. (Drumstick), *Lagerstroemia speciosa* L. (Banaba), and *Cinnamomum verum* J.Presl (Cinnamon). They are worldwide used not only as a food, but also in traditional treatment with multiple biological activities against diabetes. Cinnamon and Bael are widely popular and the old ingredients used for foods. In Asian traditional medicines, Cinnamon, *Lspeciosa*, *M.oleifera* Lam., and *M.charantia,* are presented as healing herb to treat diabetes with a long history of use in India, China, including Northern Thailand [4, 7]. *C.grandis* and *M.charantia* had a potential effect on antioxidant activity and *in vitro* potential inhibition against α-amylase and α-glucosidase and have been used in Thailand [5, 8]. Cinnamon and *L.speciosa* have been reported that there were the natural α-amylase and α-glucosidase inhibitors [6]. *M.oleifera* leaf extract had the α-glucosidase inhibitory activity and lipid-lowering property [3]. In addition, the antioxidant activity of Cinnamon parts was reported [6, 20]. To our knowledge there are few data on antioxidant properties and starch-digesting enzyme inhibition of these herbs especially their relations.

Therefore, this study aimed to screen the selected Thai herb extracts from two extraction methods (hot and cold extractions). The comparison of the results was done in terms of total phenolic content, total flavonoid content, antioxidant activity, and starch-digesting enzyme (α-amylase and α-glucosidase) inhibitory activity. The results will be provided with theoretical database and technical support for further functional product applications.

2. MATERIALS AND METHODS

2.1 Chemicals

Three enzymes, α-amylase from human saliva (Type XIII-A), α-amylase from porcine

pancreas (Type VI-B), and α-glucosidase from *Saccharomyces cerevisiae* (Type I) were bought from Sigma-Aldrich Chemical Co., USA, and used for the inhibition of the starch-digestive enzyme. Acarbose drug, 2-chloro-4-nitrophenol-α-D-maltotrioside (CNPG3), *p*-nitrophenyl-α-D-glucopyranoside (*p*NPG), phosphate buffer, gallic acid (GA), quercetin (QE), 2,2-diphenyl-1-picrylhydrazyl (DPPH), and 2,2-azino-bis(3-ethylbenzothia zoline-6-sulfonic acid) diammonium salt (ABTS) were also purchased from Sigma-Aldrich Chemical Co., USA. Folin-Ciocalteu's phenol reagent, potassium thiocyanate (KSCN), potassium persulfate ($K_2S_2O_8$), and sodium hydroxide (NaOH) were purchased from Loba Chemie Pvt. Ltd., India. Sodium carbohydrate anhydrous (Na_2CO_3) was obtained from Ajax Finechem, Australia. Sodium acetate (CH_3COONa), aluminum chloride ($AlCl_3$), and sodium nitrite ($NaNO_2$) were bought from OReC Quality Reagent Chemical, New Zealand. Other chemicals such as ethanol, dimethylsulfoxide (DMSO), and sodium azide (NaN_3) were ordered from RCI Labscan Ltd., Thailand. All the reagents and chemicals used were of analytical grade.

2.2 Herbal Preparation

10 kg of each selected Thai herbs were bought from a local market in Chiang Mai and Lampang provinces, Thailand during August and November, 2013. They included the following: leaves of *Coccinia grandis* (Tamleung), leaves and fruits of *Aegle marmelos* Corr. (Matoom), fruits of *Momordica charantia* (Mara-khee-nok), leaves and sheaths of *Moringa oleifera* Lam. (Marum), leaves of *Lagerstroemia speciosa* L. (Inthanin-nam), and bark of *Cinnamomum verum* J.Presl (Obchuey-tes). The fresh samples were cut into small pieces and dried using a hot air oven (Memmert 600, Memmert GmbH +Co.KG, Germany) at 50±2 °C for 15-20 hours. The dried sample (5-10% moisture content, [11]) was ground into powder (particle size of 100 μm) with an Ultra Centrifugal Mill ZM200 (Retsch, Germany). The ground herb sample was subsequently packed in aluminum foil bags under reduced pressure and stored at -20 °C until extraction.

2.3 Extraction Processes

The extractions were conducted according to the adapted methods [5, 12] as follows:

Cold extraction: Thai herb powder was soaked in 80% ethanol/distilled water (a ratio of sample to solvent, 1:30 w/v) at room temperature (30±2 °C) for 24 hours (shaking speed 100-150 rpm).

Hot extraction: Thai herb powder was refluxed in 80% ethanol/distilled water (a ratio of sample to solvent, 1:10 w/v) at 95±2 °C for 30 minutes. This boiling process was repeated three times. The extracts from three times were combined together.

The extracts were filtered through Whatman no.1 filter paper. The clear solution was then concentrated to dryness under reduced pressure at 40±2 °C using a rotary evaporator (Buchi R210, Switzerland). The concentrated extract was lyophilized in a freeze-dryer (Benchtop, Labconco, UK), ground using a mortar (Princess Royal Grinder, Netherlands), and passed through a 100-mesh sieve to achieve uniform particle size (100 μm). All of them were packed in an aluminum foil bag and stored at -20 °C before analysis.

Extraction was done in triplicate. Yield of dried extracts was calculated by the following equation, Equation (1).

$$\%Yield = (WE/WS) \times 100, \quad (1)$$

where WE = weight of crude extract and WS = weight of dried herb sample.

The moisture content of the dried sample was determined gravimetrically according to the AOAC method 960.18 [11].

The extracts were prepared according to their percent composition (w/v) provided by the SI (International system of units) base unit. The samples were immediately dissolved in 10% DMSO before analysis.

2.4 Total Phenolic Content (TPC) Assay

The total phenolic content was measured using the Folin-Ciocalteu method [13], where gallic acid aqueous solution (with the concentration in the range of 0-100 mg/mL) was used as the standard. A volume of 200 µL of the extracted sample solution (1 mg/L) or the gallic acid standard (0-100 mg/L) was added into a test tube with 1.0 mL of diluted 10-fold Folin-Ciocalteu's reagent in water, and mixed thoroughly. The mixture was allowed to stand at room temperature for 5 minutes. Then, 800 µL of 7.5% (w/v) Na_2CO_3 in water was added to the mixture and mixed gently. After the sample was left at room temperature in darkness for 30 minutes for stabilization, and a blue color was formed, the absorbance was measured by Thermo Spectronic Biomate 5 UV/Vis spectrophotometer (Scinteck Instruments, USA) at 765 nm.

The absorbance of each reference standard was plotted as a linear graph and calculated to obtain a linear equation. The contents of the phenolic acid, evaluated using the obtained linear equation, were expressed as mg GA equivalent/g of dried sample.

2.5 Total Flavonoid Content (TFC) Assays

The total content of flavonoids was determined by using the spectrophotometric method [14] based on the comparison between the two adapted procedures in terms of the formation of aluminum-flavonoid complexes. Quercetin aqueous solution in the concentration range of 50-500 µM was chosen as the standard compound.

Procedure 1: An aliquant of 10% (w/v) $AlCl_3$ in water (0.1 mL) was added to 1 mL of the sample solution or standard, and, subsequently, 0.1 mL of 1.0 M CH_3COONa in water was added. The mixture was vigorously shaken, and then, after incubation at room temperature for 10 minutes, subjected to spectral analysis at 425 nm using the Thermo Spectronic Biomate 5 UV/Vis spectrophotometer.

Procedure 2: A volume of 1 mL of the standard or sample was mixed with 0.1 mL of 5% (w/v) $NaNO_2$ in water, and after 5 minutes, 0.1 mL of $AlCl_3$ aqueous solution (10%, w/v) was added. The mixture was mixed, and 6 minutes later, it was neutralized with 0.5 mL of 1 M NaOH aqueous solution. The mixture was left for 10 minutes at room temperature and then subjected to spectral analysis at 425 nm.

The contents of the flavonoid were evaluated using the above method as TPC, and the result was expressed as mg QE equivalent/g of dried sample.

2.6 Antioxidant Activity Assays

2.6.1 DPPH free radical scavenging activity

This activity was measured using a spectrophotometer according to the DPPH assay [15-16]. A volume of 60 µM of the DPPH solution in methanol was prepared fresh daily. The DPPH radical stock solution (2000 µL) was added to 200 µL of different concentrations of the sample solution. The mixture was placed in a dark room (30±2 °C) for 30 minutes and monitored at 517 nm using Thermo Spectronic Biomate 5 UV/Vis spectrophotometer.

2.6.2 ABTS free radical scavenging activity

This activity was also assessed using the ABTS assay [16-17], with slight modifications. ABTS radical cation ($ABTS^+$) was generated by reacting 1 portion of ABTS stock aqueous solution (7 mM) with 1 portion of $K_2S_2O_8$ (2.45 mM) after incubation in the dark at room temperature for 12-16 hours. The freshly prepared $ABTS^+$ solution was diluted with distilled water to obtain absorbance at 734 nm of 0.70±0.05 verified by a spectrophotometer before using. The $ABTS^+$ solution (2000 μL) was added into the sample solution (200 μL) with different concentrations. The absorbance reading at 734 nm was recorded exactly 6 minutes after the initial mixing and standing in the dark at room temperature using Thermo Spectronic Biomate 5 UV/Vis spectrophotometer.

L-ascorbic acid (1.0 mg/mL) was used as the positive control. A reagent blank with 10% DMSO (no sample) reading, AB, was taken. The decrease in the absorbance of the sample solution after incubation, AS, was also measured. The %inhibition values of the DPPH and the ABTS radical scavenging activities were then calculated in comparison with the blank as Equation (2) and expressed as IC_{50}, the concentration of the sample required to produce 50% inhibition of free radical scavenging activity.

$$\%Inhibition = [(AB-AS)/AB]\times 100, \quad (2)$$

2.7 Starch-digesting Enzyme Inhibition Assays

2.7.1 α-Amylase inhibitory activity

The CNPG3 was used as the substrate. A modified assay of α-amylase activity [18] was used for the sample solution (50 μL) at different concentrations; 50 μL of 0.02 M phosphate buffer (pH 6.9) containing α-saliva amylase solution (10.0 U/mL) or α-pancreas amylase solution (2.0 U/mL) was used, and incubated at 37 °C for 10 minutes. After pre-incubation, 450 μL of the reagent consisting of 0.15 mM CNPG3, 0.5 M KSCN, and 15 mM NaN_3 in 0.02 M phosphate buffer was added to each tube. The reaction mixturewas incubated at 37 °C for 20 minutes, and then the absorbance was measured with Thermo Spectronic Biomate 5 UV/Vis spectrophotometer.

2.7.2 α-Glucosidase inhibitory activity

The α-glucosidase activity was determined by the spectrophotometric method [19]. For this assay, the sample solution (50 μL) was taken at different concentrations along with 100 μL of 0.02 M phosphate buffer (pH 6.9) containing α-glucosidase solution (1.0 U/L), and incubated at 37 °C for 10 minutes. After pre-incubation, 50 μL of 3 mM *p*NPG solution in 0.02 M phosphate buffer was added to each tube. The reaction mixtures were incubated at 37 °C for 10 minutes. The reaction was then stopped by adding 2 mL of 0.1 M Na_2CO_3 before measurement with Thermo Spectronic Biomate 5 UV/Vis spectrophotometer.

Acarbose was also used as a positive control. The blank reaction was prepared using the same procedure without the extract or acarbose. The absorbance of the yellow-colored *p*-nitrophenol (*p*NP) released from CNPG3 or *p*NPG was also measured at 405 nm by using Thermo Spectronic Biomate 5 UV/Vis spectrophotometer. The results were calculated as %inhibition of enzyme activity, as given in Equation (3), and expressed as the concentration of each herb extract that brought about 50% inhibition of enzyme activity (IC_{50}).

$$\%Inhibition = [(AB-AS)/AB]\times 100, \quad (3)$$

where AB = absorbance reading of the blank and AS = absorbance reading of the sample or the control.

2.8 Statistical Analysis

The results are presented as the mean ± standard deviation (at least three replicate experiments). The analysis of variance (ANOVA) and significant differences between the means using Duncan's multiple range test (DMRT) and T-test were performed using SPSS (Version 16, SPSS Inc., Chicago, USA) at a significant level of $p<0.05$. The correlation coefficients (r^2) between two variables were also determined at significant levels of $p<0.01$ and $p<0.05$.

3. Results and Discussion

3.1 Herbal Extraction Yield and Total Phenolic Content (TPC)

Polyphenol compounds such as phenolic acid, flavonoids, and tannins, which possess diverse biological activities, widely occur in the plant kingdom [16]. These compounds can be related to their antioxidant activity [16, 20].

The total phenolic contents in the various herb extracts were determined using the spectrometric method, according to the Folin-Ciocalteu phenol reagent analysis, and calculated as gallic acid (GA) equivalent. The total phenolic contents of samples extracted under ethanolic conditions (both room temperature and boiling temperature) are shown in Table 1; they widely ranged from 20.31 mg GA/g extract to 391.80 mg GA/g extract, also showing significant variation ($p<0.05$). Our comparative results of eight samples indicate that their TPC decreased in the following order: leaves of *Lagerstroemia speciosa* L. > bark of *Cinnamomum verum* J.Presl. > fruits of *Aegle marmelos* Corr. > leaves of *Aegle marmelos* Corr. > leaves of *Moringa oleifera* Lam. > leaves of *Coccinia grandis* > fruits of *Momordica charantia* > sheaths of *Moringa oleifera* Lam.

For determining the total flavonoid content, the formation of aluminum-flavonoid complexes was measured by two mostly applied spectrophotometric methods. The amount of total flavonoid content was calculated as quercetin standard as it is widely found in plants [14]. The extracts contained TFC in the range of 5.22-52.39 mg QE/g extract for procedure 1 and in the range of 5.15-108.74 mg QE/g extract for procedure 2 (Table 1). These also showed significant variations ($p<0.05$). The highest flavonoid content was in the *Lagerstroemia speciosa* L. leaf extract, whereas the lowest flavonoid content was observed in the *Momordica charantia* fruit extract. In this result, *Coccinia grandis* leaf extract showed higher total phenolic content than *Momordica charantia* fruit extract according to Chanwitheesuk and coworkers [8].

The differences in the amounts of bioactive compounds may be affected by the origins of the plants [5]. The amount of polyphenols was depend on the extraction method [16]. Cold extraction was more effective in extracting phenols and flavonoids, whereas hot extraction was more effective in extracting alkaloids and phytosterols [20].

In all the eight samples, the yield of extract was found to vary from 17.69% (leaves of *Coccinia grandis,* with cold extraction) to 36.08% (leaves of *Moringa oleifera* Lam., with hot extraction), as shown in Table 1. provide a higher yield compared to the herbs extracted using the cold extraction method (17.69-32.29%). Similar results have been found by Lin and coworkers [21], who reported that temperature was an important factor when extracting the tea herb. The results achieved by them indicate that significantly higher yields were obtained in the extraction of green tea with hot water than with cold water.

Table 1. Yield and phenolic and flavonoid contents of selected Thai herbs with the different conditions of extraction.

Thai herb extract/part	Extraction method	Yield (%)	TPC (mg GA/g sample)	TFC(mg QE/g sample)	
				Procedure 1	Procedure 2
Coccinia grandis, leaves	cold	17.69±1.51^{H,b}	53.96±1.75^{J,ns}	18.87±0.53^{E,ns}	24.61±1.44^{G,ns}
	hot	22.44±0.18^{H,a}	52.74±1.45^{H,ns}	16.65±1.44^{F,ns}	19.18±3.20^{H,ns}
Aegle marmelos Corr., leaves	cold	19.05±1.17^{E,a}	95.63±2.88^{I,a}	36.83±0.82^{D,ns}	47.50±2.11^{D,a}
	hot	24.36±1.31^{FG,b}	83.79±3.41^{FG,b}	36.04±1.14^{D,ns}	41.00±0.55^{E,b}
Aegle marmelos Corr., fruits	cold	28.15±1.20^{DE,ns}	133.15±3.96^{D,ns}	11.44±0.53^{G,ns}	46.39±1.83^{D,a}
	hot	31.24±1.48^{BC,ns}	137.03±2.28^{D,ns}	9.69±1.30^{H,ns}	40.86±0.54^{E,b}
Momordica charantia, fruits	cold	27.92±1.24^{DE,b}	25.85±1.19^{I,ns}	5.69±0.55^{IJ,ns}	9.69±0.72^{IJ,a}
	hot	35.85±0.25^{A,a}	27.42±1.34^{I,ns}	5.63±0.19^{J,ns}	5.15±0.10^{K,b}
Moringa oleifera Lam., leaves	cold	32.29±0.26^{B,b}	74.96±5.68^{G,ns}	52.39±1.03^{A,a}	41.53±0.69^{E,a}
	hot	36.08±1.01^{A,a}	77.76±2.04^{FG,ns}	45.02±1.18^{C,b}	32.04±0.74^{F,b}
Moringa oleifera Lam., sheaths	cold	27.75±0.64^{E,b}	25.57±1.06^{I,a}	9.18±0.45^{H,a}	11.71±0.38^{I,a}
	hot	29.98±0.79^{CD,a}	20.31±0.52^{I,b}	7.25±0.58^{I,b}	6.39±0.57^{JK,b}
Lagerstroemia speciosa L., leaves	cold	21.34±1.16^{H,b}	391.80±21.31^{A,a}	50.14±1.16^{B,ns}	108.74±3.58^{A,a}
	hot	25.41±0.60^{F,a}	340.65±14.80^{B,b}	49.91±1.17^{B,ns}	92.23±2.44^{B,b}
Cinnamomum verum J.Presl, bark	cold	23.53±0.67^{FG,ns}	174.71±30.27^{C,ns}	5.22±0.27^{J,ns}	66.71±6.39^{C,ns}
	hot	25.64±0.69^{F,ns}	161.65±34.70^{C,ns}	5.79±0.57^{IJ,ns}	66.14±1.94^{C,ns}

$^{A-J}$ Different capital letters indicate significant difference between means in the same column (DMRT, $p<0.05$).

$^{a-b}$ Different lowercase letters indicate significant difference between means in the same extract with different processes (T-test, $p<0.05$).

^{ns} No significant effect in the same extract with different processes.

3.2 Antioxidant Activity

The antioxidant activity based on the concentration of herb extract required to bring about 50% inhibition of DPPH and ABTS radical scavenging activity (IC_{50}) also showed significant variation, ranging from 0.04 mg/mL to 3.48 mg/mL and from 0.03 mg/mL to 1.74 mg/mL, respectively (Table 2). Similar results showed that the leaves of *Lagerstroemia speciosa* L. exhibited the highest antioxidant activity, while the sheaths of *Moringa oleifera* Lam. showed the lowest by both the DPPH and the ABTS methods.

Typical chemical compounds that possess antioxidant activity have been characterized as vitamin C or vitamin E in many previous researches. They were found to act as a chain-breaking scavenger for peroxy radicals. It was reasonable to use vitamin C as a tested control. Phenolic and flavonoid compounds as the widespread group of natural compounds, possess the antioxidant activities including radical scavenging property. Antioxidant activity of herbs is mainly contributed by the phytochemicals present in them. The position of hydroxyl groups and other features in the phytochemical structure

are important for their antioxidant and free radical scavenging activities [21]. In general, the presence of 3-hydroxy group of flavonoid giving the structure in ring C, is responsible for enhancement of antioxidant activity. In addition, the combined presence of a 3-hydroxy group with a 2, 3-double bond in flavonoid structure increases the resonance stabilization for radical electron delocalization, improves the radical scavenging activity [16].

The ethanolic extracts of *Lagerstroemia speciosa* L. and *Cinnamomum verum* J.Presl. exhibited the high levels of phenolic compounds providing the strong antioxidant activity. No significant difference was found between *Lagerstroemia speciosa* L., *Cinnamomum verum* J.Presl., and vitamin C. However, their total phenolic contents presented a significant difference: *Lagerstroemia speciosa* L. contained the highest total phenolic content, over 2-fold greater than cinnamon bark. Their antioxidant capacity levels exhibited no significant difference. This may due to the high concentration of total flavonoids (65.52 mg QE/g extract) observed in Thai cinnamon [4]. This result concluded that the extract from *Lagerstroemia speciosa* L. leaf, *Cinnamomum verum* J.Presl. bark, *Aegle marmelos* Corr. fruit and leaf, *Moringa oleifera* Lam. leaf and *Coccinia grandis* leaf expressed the low scavenging activities as DPPH (IC_{50}=0.04-0.66 mg/mL) and ABTS (IC_{50}=0.03-0.65 mg/mL) resulting the good radical scavenger. In contrast, the extract from *Momordica charantia* fruit and *Moringa oleifera* Lam. sheath inhibited the DPPH and ABTS free radicals at high concentrations (IC_{50}=2.66-3.48 mg/mL and IC_{50}=0.04-0.66 mg/mL, respectively), could serve as the weak free radical inhibitor.

Table 2. IC_{50} (mg/mL) of antioxidant activity in selected Thai herbs with the different conditions of extraction.

Thai herb extract/part	Extraction method	DPPH assay	ABTS assay
Coccinia grandis,	cold	$0.66\pm0.03^{C,ns}$	$0.52\pm0.03^{F,a}$
leaves	hot	$0.66\pm0.03^{C,ns}$	$0.65\pm0.03^{G,b}$
Aegle marmelos Corr.,	cold	$0.37\pm0.01^{B,ns}$	$0.17\pm0.01^{C,ns}$
leaves	hot	$0.35\pm0.01^{B,ns}$	$0.19\pm0.01^{CDE,ns}$
Aegle marmelos Corr.,	cold	$0.68\pm0.03^{C,ns}$	$0.14\pm0.01^{ABC,ns}$
fruits	hot	$0.65\pm0.02^{C,ns}$	$0.14\pm0.01^{ABC,ns}$
Momordica charantia,	cold	$3.48\pm0.14^{F,b}$	$1.74\pm0.05^{J,ns}$
fruits	hot	$3.00\pm0.11^{E,a}$	$1.64\pm0.15^{J,ns}$
Moringa oleifera Lam.,	cold	$0.43\pm0.03^{B,ns}$	$0.28\pm0.01^{DE,ns}$
leaves	hot	$0.43\pm0.02^{B,ns}$	$0.29\pm0.02^{E,ns}$
Moringa oleifera Lam.,	cold	$2.66\pm0.22^{D,ns}$	$1.38\pm0.03^{I,b}$
sheaths	hot	$2.76\pm0.04^{D,ns}$	$1.01\pm0.20^{H,a}$
Lagerstroemia speciosa L.,	cold	$0.04\pm0.01^{A,a}$	$0.03\pm0.01^{A,a}$
leaves	hot	$0.05\pm0.01^{A,b}$	$0.04\pm0.01^{AB,b}$
Cinnamomum verum J.Presl,	cold	$0.12\pm0.01^{A,a}$	$0.15\pm0.02^{ABC,ns}$
bark	hot	$0.14\pm0.01^{A,b}$	$0.16\pm0.03^{BC,ns}$
Positive control: ascorbic acid		0.04 ± 0.01^{A}	0.03 ± 0.01^{A}

[A-G] Different capital letters indicate significant difference between means in the same column (DMRT, $p<0.05$).
[a-b] Different lowercase letters indicate significant difference between means in the same extract with different processes (T-test, $p<0.05$).
[ns] No significant effect in the same extract with different processes.

3.3 Starch-digesting Enzyme Inhibition

Comparison between the inhibition effects of %starch-digesting enzyme activities in selected Thai herb extracts with regard to hot extraction and cold extraction is shown in Table 3. When comparison is made between the IC_{50} values of Thai herb extracts, it can be observed that the highest yeast α-glucosidase inhibitory activity was in the leaf extract from *Lagerstroemia speciosa* L. with IC_{50} of 0.0381-0.0517 mg/mL extract solution. The finding showed that the bark extract from *Cinnamomum verum* J.Presl. had the most effective human saliva α-amylase and porcine pancreas α-amylase inhibitor (IC_{50}=0.5709-0.6607 mg/mL extract solution and IC_{50}=0.1901-0.3225 mg/mL extract solution, respectively). While the other extracts showed the high concentrations for starch-digestive enzymes inhibition activities, with IC_{50} values >5 mg/mL. However, the inhibitory activities of all the Thai herb extracts were less potent than that of the acarbose drug against saliva α-amylase, pancreatic α-amylase, and intestinal α-glucosidase (IC_{50}=0.0070-0.0339 mg/mL extract solution). According to the results, both *Lagerstroemia speciosa* L. leaf extract and *Cinnamomum verum* J.Presl. bark extract expressed more specific for intestinal α-glucosidase inhibition than for saliva and pancreatic α-amylase inhibitions with non-significant difference from acarbose. In contrast, acarbose was found to be a more specific inhibitor of pancreatic α-amylase and intestinal glucosidase than of saliva α-amylase. The finding in Cinnamon bark agreed with the report of Adisakwattana et al. [4]. In addition, acarbose was a competitive inhibitor of α-glucosidase as well as a mixed-noncompetitive inhibitor of α-amylase [25].

When comparison was carried out between the different extraction methods, the present study revealed that cold extraction was more effective for starch-digesting enzyme inhibition of *Lagerstroemia speciosa* L. leaf extract, while hot extraction was more effective for the α-amylase inhibitory activity of *Cinnamomum verum* J.Presl. bark extract. Therefore, it could conclude that *Lagerstroemia speciosa* L. leaf and *Cinnamomum verum* J.Presl. bark extracts contained the good starch-digestive enzymes inhibitors.

Table 3. IC_{50} (mg/mL) of starch-digesting enzyme inhibition in selected Thai herbs with the different conditions of extraction.

Thai herb extrac/part	Extraction method	Human saliva α-amylase	Porcine pancreas α-amylase	Yeast α-glucosidase
Coccinia grandis, leaves	cold	> 5	> 5	> 5
	hot	> 5	> 5	> 5
Aegle marmelos Corr., leaves	cold	> 5	> 5	> 5
	hot	> 5	$4.94{\pm}0.40^{E}$	> 5
Aegle marmelos Corr., fruits	cold	> 5	> 5	$3.53{\pm}0.53^{C,b}$
	hot	> 5	> 5	$2.61{\pm}0.45^{B,a}$
Momordica charantia, fruits	cold	> 5	> 5	> 5
	hot	> 5	> 5	> 5
Moringa oleifera Lam., leaves	cold	> 5	$4.74{\pm}0.33^{E}$	> 5
	hot	> 5	> 5	> 5
Moringa oleifera Lam., sheaths	cold	> 5	> 5	> 5
	hot	> 5	> 5	> 5
Lagerstroemia speciosa L., leaves	cold	$1.05{\pm}0.20^{C,a}$	$0.63{\pm}0.07^{C,a}$	$0.04{\pm}0.01^{A,a}$
	hot	$3.02{\pm}0.31^{D,b}$	$1.57{\pm}0.09^{D,b}$	$0.05{\pm}0.01^{A,b}$
Cinnamomum verum J.Presl, bark	cold	$0.66{\pm}0.01^{B,ns}$	$0.32{\pm}0.04^{B,b}$	$0.12{\pm}0.01^{A,a}$
	hot	$0.57{\pm}0.10^{B,ns}$	$0.19{\pm}0.03^{B,a}$	$0.14{\pm}0.01^{A,b}$
Positive control: acarbose		$0.03{\pm}0.01^{A}$	$0.01{\pm}0.01^{A}$	$0.01{\pm}0.01^{A}$

$^{A-E}$ Different capital letters indicate significant difference between means in the same column (DMRT, $p<0.05$).

$^{a-b}$ Different lowercase letters indicate significant difference between means in the same extract with different processes (T-test, $p<0.05$).

^{ns} No significant effect in the same extract with different processes.

3.4 Relation Between Chemical Compound Contents and Antioxidant Activities

Total phenolic contents and total flavonoid contents have been reported to be responsible for the antioxidant activities (DPPH and ABTS assays) of herb extracts, as shown in Table 4. The results revealed that the DPPH and the ABTS radical scavenging activities correlated with the results of total phenolic content and total flavonoid content (in both procedure 1 and procedure 2) at significant levels, $p<0.01$. A recent report demonstrated that some bioactive compounds present in herbs possess high total antioxidant activity, which is due to the presence of phenolic compounds.

As for the results, moderate and negative correlation coefficients were found between total phenolic content and DPPH radical scavenging activity (r^2=-0.600, $p<0.01$) and ABTS radical scavenging activity (r^2=-0.621, $p<0.01$). But the total phenolic content was found to correlate highly and positively of the total flavonoid content when procedure 2 was used (r^2=0.951, $p<0.01$). Moreover, the correlations between the total flavonoid content (procedure 2) and the antioxidant activities (in the DPPH and the ABTS assays) were observed to be slightly high and negative (r^2=-0.746 and r^2=-0.758, respectively, $p<0.01$). The DPPH radical scavenging activity exhibited high and positive correlation with the ABTS radical scavenging activity (r^2=0.958, $p<0.01$). Therefore, it can be concluded that an increase in the phenolic or the flavonoid compound in the herb extract reduces the dose of herb extract for the DPPH and the ABTS radical scavenging activities. It can be demonstrated that Thai herb extract which contained a high amount of polyphenol or flavonoids, also possessed a good antioxidant capacity.

Table 4. Correlations (r^2) between different antioxidant capacity parameters and chemical compound contents of various ethanolic herb extracts.

	TPC[a]	TFC[b](P1*)	TFC[b](P2*)	DPPH[c]	ABTS[d]
TPC	1.000	0.480**	0.951**	-0.600**	-0.621**
TFC (M1)	0.480**	1.000	0.532**	-0.553**	-0.525**
TFC (M2)	0.951**	0.532**	1.000	-0.746**	-0.758**
DPPH	-0.600**	-0.553**	-0.746**	1.000	0.958**
ABTS	-0.621**	-0.525**	-0.758**	0.958**	1.000

Note: r^2, correlation coefficient; [a]TPC, total phenolic content; [b]TFC, total flavonoid content; [c]DPPH radical scavenging activity; and [d]ABTS radical scavenging activity.

*P1, procedure 1; and P2, procedure 2. **Significance level at $p<0.01$.

3.5 Relation Between Chemical Compound Contents and Starch-digesting Enzyme Inhibitory Activities

The correlations between the chemical compound contents (TPC and TFC) and the starch-digesting enzyme inhibitory activities are shown in Table 5. This result shows that total phenolic content exhibits high correlation with total flavonoid content with procedure 2 (r^2=0.957, $p<0.01$). High and negative correlation was found between total phenolic content and starch-digesting enzyme inhibition (human saliva α-amylase r^2=-0.792, porcine pancreas α-amylase r^2=-0.780, and yeast α-glucosidase r^2=-0.855, $p<0.01$). The

correlation coefficient between total flavonoid content (procedure 2) and starch-digesting enzyme inhibitory activities was found to be slightly high and negative (human saliva α-amylase r^2=-0.811, porcine pancreas α-amylase r^2=-0.830, and yeast α-glucosidase r^2=-0.839, p<0.01). These results conclude that when there is an increase in the phenolic or the flavonoid compounds in herb extracts, the concentration of the herb extract for starch-digesting enzyme inhibitory activities decreases. Moreover, the inhibition of human saliva α-amylase activity presented highly positive correlation with porcine pancreas α-amylase inhibitory activity (r^2=0.942, p<0.01) and yeast α-glucosidase inhibitory activity (r^2=0.848, p<0.01). The inhibition of porcine pancreas α-amylase inhibitory activity presented highly positive correlation with yeast α-glucosidase inhibitory activity (r^2=0.781, p<0.01). In addition, insignificant correlations were observed between the total flavonoid content with procedure 1 and the inhibition of human saliva α-amylase and yeast α-glucosidase inhibitory activities.

Table 5. Correlations (r^2) between different starch-digested enzyme inhibition parameters and chemical compound contents of various ethanolic herb extracts.

	TPC[a]	TFC[b] (P1*)	TFC[b] (P2*)	Humansaliva α-amylase	Porcinepancreas α-amylase	Yeast α-glucosidase
TPC	1.000	0.530**	0.957**	-0.792**	-0.780**	-0.855**
TFC (M1)	0.530**	1.000	0.577**	-0.172	-0.333***	-0.102
TFC (M2)	0.957**	0.577**	1.000	-0.811**	-0.830**	-0.839**
Human saliva α-amylase	-0.792**	-0.172	-0.811**	1.000	0.942**	0.848**
Porcine pancreas α-amylase	-0.780**	-0.333***	-0.830**	0.942**	1.000	0.781**
Yeast α-glucosidase	-0.855**	-0.102	-0.839**	0.848**	0.781**	1.000

Note: r^2, correlation coefficient; [a]TPC, total phenolic content; and [b]TFC, total flavonoid content.
*P1, procedure 1; and P2, procedure 2. **Significance level at p<0.01. ***Significance level at p<0.05.

We know that flavonoids are a subset of phenolic compounds. Hence, it is expected that the total phenolic content relates to the total flavonoid content [1]. The reaction in procedure 1 exhibited the maximum absorbance at 415-425 nm, and flavonols form complexes with hydroxyl groups at similar wavelength [13]. Procedure 1 seems to be more specific for flavonol content [14]. In this study, the correlation between TPC and TFC in procedure 1 was a less significant correlation, but TFC in procedure 2 showed much higher correlation with TPC. When comparing TFC (procedure 1) with the potential inhibition against α-amylase and α-glucosidase, insignificant correlations were observed (r^2=0.102-0.333, p>0.05), while TFC (procedure 2) showed a highly significant correlation with the potential inhibition against α-amylase and α-glucosidase (r^2=0.811-0.839, p<0.05).

The correlation between DPPH and TPC, DPPH and TFC, ABTS and TPC, ABTS and TFC, and DPPH and ABTS showed good relationships, in agreement with the datas reported by Wongsa et al. [5] and Wojdylo et al. [16]. These results prove the importance of polyphenol compounds in the antioxidant behavior of herb extracts. However, a comparison of the potential inhibition against α-amylase with TFC (procedure 2) revealed more significant

correlations than TPC, but the potential inhibition against α-glucosidase with TPC demonstrated more significant correlations than TFC (procedure 2). All of these herb extracts are rich in polyphenols and flavonoids, many of which have shown efficacy in controlling the symptoms of diabetes. The polyphenol and flavonoid compounds may play a key role in the inhibition of starch digesting enzymes. Depending on the complex structure, these compounds react with proteins/enzymes and alter various properties of biopolymers such as the molecular weight, solubility and *in vitro* digestibility [22]. It had evidenced that the high concentration and the number as well as the position of hydroxyl groups decreased enzyme activities [22-23]. Normally, α-amylase binds longer polysaccharide substrates than α-glucosidase, while phytochemical compounds suppress α-amylase activity by competitive and reversible kinetic [24]. Polyphenols are the large group of structurally natural compound including gallic acid, which showed strong α-glucosidase inhibitory activity with the non-competitive mode [23]. In addition, flavonoids are abundant class of natural phenolic compounds; these include xanthones, flavanones, flavans, anthocyanins, and other structural motifs indicated their competitive inhibition. Paloma et al. [25] reported that the action mechanism for the α-amylase inhibitory capacity of flavonoids correlated the potency of these compounds with (1) the number of hydroxyl groups on the B ring of flavonoid skeleton and (2) the stabilized interaction between hydroxyl groups in position R6 and R7 of ring A and position of R4' and R5' of ring B of polyphenol ligands as well as the catalytic residues of the enzyme binding site. Also, the high inhibitory capacity is observed in flavonols and flavones groups. However, further work is needed to detail these fully potential interactions of bioactive compounds in herb/plants with α-amylase and α-glucosidase.

An investigation of the correlations between total phenolic content and antioxidant activity and starch-digesting enzyme inhibitory activity of herb extracts revealed significant correlations. A similar study by Manaharan and coworkers [26] reported that total phenolic content of 14 tropical plant extracts displayed a highly significant correlation with antioxidant ability (DPPH free radical scavenging activity). In addition, anti-hyperglycemic activity, in the cases of both α-glucosidase and α-amylase, also displayed a very strong correlation to antioxidant ability.

4. Conclusions

This study showed that there were differences in the phenolic contents, flavonoid contents, antioxidant activity, and inhibitory effects in starch-digesting enzyme activity between eight Thai herb extracts. Some herbs can be considered as good sources as natural antioxidants and hyperglycemia inhibitors since their extracts exhibit high contents of phenolic and flavonoid compounds and antioxidant activity, and their biological functions can prevent α-amylase and α-glucosidase activities. This study demonstrated that the cold extraction was more effective for extracting phenolics and flavonoids, resulting in antioxidant activity and starch-digesting enzyme inhibition, whereas the hot extraction provided a higher yield. Good significant correlations between the phenolic and the flavonoid contents of herb extracts and the antioxidant and starch-digesting enzyme inhibitory activities were found. The extracts from *Lagerstroemia speciosa* L. leaf and *Cinnamomum verum* J.Presl. bark displayed antioxidant and starch-digesting enzyme

inhibition activities comparatively higher than other herb extracts and close to the commercial anti-hyperglycemic drug acarbose.

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