

Antioxidant and Antidiabetic Effects of *Garcinia schomburgkiana* Extracts and Fermented Juices

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ABSTRACT

This study investigated total phenolic contents, antioxidant activities, and antidiabetic activities in flesh, seed and leaf extracts, and the fermented juices of *Garcinia schomburgkiana* Pierre (Madan). Seed extract showed the highest total phenolic content, radical scavenging activity and amylase inhibitory activity, whereas the leaf extract had the strongest ferrous ion chelating activity and inhibitory activities on glucosidase and lipase. The very strong positive correlation between total phenolic content and radical scavenging activity, and between ferrous ion chelating and glucosidase inhibitory activities were significantly found ($r = 1.000$ and 0.998 at $P < 0.05$, respectively). Moreover, the Madan juice revealed the highest total phenolic content at 72 hour fermentation. However, production of radical scavenging and ferrous ion chelating activities of fermented juices increased at the start of fermentation. Additionally, amylase inhibitory activity increased during 24 hour fermentation, whereas glucosidase and lipase inhibitory activity reached maximal levels during 17 day and 48 hour fermentation, respectively. The total phenolic contents of the fermented juices were very strongly negatively correlated with radical scavenging activity, and ferrous ion chelating activity, significantly ($r = -0.869$ and -0.937 at $P < 0.05$, respectively). In addition, the very strong positive correlation between radical scavenging activity and ferrous ion chelating activity was significantly found ($r = 0.804$, $P < 0.05$). Our

results indicated that *G. schomburgkiana* Pierre is a potential nutraceutical source, and its fermented juice can be further improved as a healthy fruit drink.

Keywords: Antidiabetic activity, antioxidant activity, *Garcinia schomburgkiana* Pierre

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INTRODUCTION

Obesity is a major non-communicable disease (NCD) which is becoming a worldwide concern. It is also a cause of other NCDs (i.e. cardiovascular risk factors, hypertension, and hyperinsulinemia including type II diabetes mellitus) and death. In Thailand, the prevalence of obesity shows an increasing trend, with approximately 33% and 43% of males and females affected, respectively (Teerawattananon & Luz, 2017). Moreover, the age-adjusted prevalence of people with diabetes in Thailand increased from 7.7% in 2004 to 9.9% in 2014 (Aekplakorn et al., 2018). Nowadays, one of the most common NCDs associated with obesity is type II diabetes mellitus, which is a metabolic dysfunction characterized by insulin resistance, insufficient insulin, inappropriate glucagon secretion, hyperglycemia, and glucotoxicity. Type II diabetes mellitus is a critical problem of global public health (Janghorbani et al., 2013), especially in developing countries. In a recent report, it has been predicted that there will be a further increasing trend of 382 million people with diabetes in 2013 rising to 592 million in 2035 (Guariguata et al., 2014). Meanwhile, several therapeutic methods have been developed to decrease hyperglycemia in Thai patients with type II diabetes mellitus. Many reports have focused on key enzymes in type II diabetes mellitus such as lipase, α -glucosidase, and α -amylase. The α -glucosidase and α -amylase enzymes showed functions involving polysaccharide catabolism and starch hydrolysis which lead to a lower increased blood glucose level after a mixed

carbohydrate diet (Kwon et al., 2006). For the lipase enzyme, it shows the effect of lowering the increased plasma triglyceride levels after high fat diets (Zhang et al., 2008). Moreover, the presence of bioactive compounds found in plants (i.e. phenolic agents) may be of medicinal significance (i.e. antioxidant activities). Antioxidants are a major inhibitor in glycoside hydrolase activities, glycation reaction, and production of advanced glycation end-products (AGEs) (Adefegha et al., 2015). Previous study has been revealed that correlation between total phenolic content, antioxidant activity, and amylase inhibitory activity of bioprocessed local fruit extracts (pineapple and guava) are investigated to improve the value of fruit wastes (Sousa & Correia, 2012).

Finding novel antidiabetic drugs from plant extracts with fewer side effects, therefore, may be useful for human health worldwide. *Garcinia schomburgkiana* Pierre is a local fruit tree known as Madan that is commonly found in the central and southern regions of Thailand, especially in Nakhon Nayok province. Its leaves and fruit can be consumed either raw or cooked, but this is of limited popularity due to its sourness (Subhadrabandhu et al., 2001), and its seeds are usually discarded as waste during fruit processing. However, *G. schomburgkiana* Pierre has been reported to have high phytochemical content, high total phenolic content and high antioxidant property in root and branch acetone extract (Meechai et al., 2016a, 2016b, 2016c). Promoting consumption of the processed fruit could be an excellent way to improve the economic viability of Madan, with products such as healthy fruit drink and fruit compote.

However, the health effect of processing on the activities of lipase, amylase, and glycosidase, including antioxidant capacities of this fruit, are little known and poorly understood. Therefore, our major goals focused on the effect of fermented juice and extracts from *G. schomburgkiana* Pierre as an antioxidant and antidiabetic drug. This work was carried out in Ongkarak district, Nakorn Nayok province.

MATERIALS AND METHODS

Chemicals

Orlistat (Sigma), triolein (Sigma-Aldrich), sodium dihydrogen phosphate dehydrate (Sigma-Aldrich), ethanol absolute (Merck), Di-potassium hydrogen phosphate anhydrous (Merck), 4-nitrophenyl-alpha-D-glucopyranoside (PNPG) (Sigma), 3,5-dinitrosalicylic acid (Sigma), DTNB (5,5'-dithiobis, 2-nitrobenzoic acid) (Sigma), ethanol solution (Sigma), Tween 40 (Merck), α -amylase from porcine pancreas (Sigma-Aldrich), α -glucosidase from *Saccharomyces cerevisiae* (Sigma-Aldrich), lipase from *Candida Antarctica* (Sigma-Aldrich), reduced glutathione solution (GSH) (Sigma), acarbose (Sigma), sodium potassium tartrate (Merck), phenolphthalein indicator (Merck).

Sample Collection

Leaves and fruits of *G. schomburgkiana* Pierre were collected from Ongkarak district, Nakorn Nayok province, then washed with distilled water and kept in a plastic bag at 4°C until used.

Aqueous Fermented Extracts

Fresh fruit samples of *G. schomburgkiana* Pierre without seeds were cut into small pieces. After that, they were mixed with distilled water at ratios of 1:1, 1:2, 1:3, and 1:4. The mixtures were combined with brown sugar 2% (w/v), and then left at room temperature to ferment. Each fermented juice was collected in 50 ml polyethylene tubes at 0 hours, 24 hours, 48 hours, 72 hours, 10 days, 17 days, and 24 days, and kept at -20°C until used.

Plant Extracts

Seed, flesh and leaf samples of *G. schomburgkiana* Pierre were cut into small pieces, incubated at 50°C for about 24 hours and then milled using a homogenizer. After that, 50 g of the powder was added by 95% ethanol and mixed, then incubated at 37°C for 14 hours with shaking. The extracts were filtered through cheesecloth, and all filtrates were evaporated at 45°C for 2 hours by vacuum evaporator (IKA^a RV10), weighed and adjusted the volume with 95% ethanol solvent to the final concentration of 1 g/ml. This was then diluted to 1:1000, 1:100, 1:50, and 1:10 with 95% ethanol solvent, and kept at -20°C until used. Each sample was extracted in duplicate (Thummajitasakul et al. 2014; Thummajitsakul & Silprasit, 2017).

Total Phenolic Contents

Total phenolic contents were determined with the Folin–Ciocalteu colorimetric technique. Each extract (300 μ l) was

combined with 1.5 ml of Folin-Ciocalteu reagent and left for 5 min. After that, 1.2 ml of sodium carbonate (7.5% w/v) was added and incubated for 30 min at room temperature. The absorbance of each reaction was then measured at wavelength 765 nm by a spectrophotometer (Model T60UV). Each assay was carried out in duplicate. Gallic acid was used to generate a calibration curve ($y=5.32x-0.02$; $R^2=0.96$) (Deetae et al., 2012). Total phenolic contents were then shown in unit of mg gallic acid equivalent per gram extract.

ABTS Method

ABTS [2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) diammonium] radical cation was prepared from 7 mM ABTS solution (10 ml) and 140 mM potassium persulfate (179 μ l), then incubated for 12-16 hours in the dark at room temperature. After that, the ABTS radical cation was diluted with distilled water until its absorbance reached 0.700 ± 0.050 at 734 nm. Then 20 μ l of each extract was reacted with 3.9 ml of the diluted ABTS radical solution for 6 min in the dark at room temperature, and the absorbance was quickly measured at 734 nm. Each assay was performed in duplicate. The antioxidant capacity was calculated using the below formula (Deetae et al., 2012).

$$\% \text{ Antioxidant capacity} = \frac{(A_{\text{ABTS}} - A_{\text{sample}})}{A_{\text{ABTS}}} \times 100 \quad (1)$$

where A_{ABTS} was the absorbance of the ABTS^{•+} solution without a sample, and A_{sample} was the absorbance of the ABTS^{•+} solution with a sample.

FIC Method

The ferrous ion chelating activity was measured by mixing each sample extract (1 ml) with 0.1 mM FeSO₄ (1 ml) and 0.25 mM ferrozine (1 ml). This was left at room temperature for 10 min in the dark, following which the absorbance of each reaction was measured at 562 nm. EDTA was used as a standard solution. Each reaction was performed in duplicate. The percentage of metal chelating ability was calculated according to the below formula (Deetae et al., 2012).

$$\% \text{ Metal chelating capacity} = 1 - \frac{(A_{\text{sample}} - A_{\text{sample blank}})}{(A_{\text{water}} - A_{\text{water blank}})} \times 100 \quad (2)$$

where A_{sample} and $A_{\text{sample blank}}$ were the absorbance of a sample with ferrozine and without ferrozine, respectively.

A_{water} and $A_{\text{water blank}}$ were the absorbance of distilled water with ferrozine, and without ferrozine, respectively.

Lipase Inhibitory Activity

Lipase inhibitory activity was determined by mixing each extract (900 μ l), 1% triolein in Tween 40 (1 ml), and 50 mM sodium phosphate buffer pH 8.0 (400 μ l). This was then incubated at 37°C for 30 min. After that, 0.15 unit/ml lipase solution (400 μ l) was gently mixed, before incubation at

37°C for 1 hour. For the blank solution, sodium phosphate buffer was used in place of enzyme solution, then each reaction was mixed with 95% ethanol (1 ml), and titrated with 0.025 N NaOH until the phenolphthalein indicator changed to pink (Jagdish et al., 2013; Wrolstad et al., 2005). Orlistat was used as a positive control. Each extract was performed in duplicate. The percentage of lipase inhibition was calculated using the below formula.

$$\% \text{ Lipase inhibition} = \frac{[(A-a)-(B-b)]}{(A-a)} \times 100 \quad (3)$$

where A and B were the volume of NaOH used until the end-point was reached, for titration of distilled water and extract with lipase, respectively.

a and b were the volume of NaOH used until the end-point was reached, for titration of distilled water and extract without lipase, respectively.

Amylase Inhibitory Activity

Amylase inhibitory activity was measured by mixing between 12 units/ml amylase solution (100 µl) with each extract (100 µl) and incubating at 25°C for 30 min. Then, 100 µl of 1% starch solution was added, and left at 25°C for 3 min. After that, 100 µl of DNS reagents (96 mM 3, 5-dinitrosalicylic acid and 5.3 M sodium potassium tartrate in 2 M sodium hydroxide) were added and incubated at 85°C for 15 min, then immediately cooled at 4°C. Additionally,

distilled water (900 µl) was added and mixed, and the absorbance was detected at 540 nm (Wang et al., 2018). Acarbose was used as a positive control. Each extract was carried out in duplicate. The percentage of amylase inhibition was calculated using the following formula.

$$\% \text{ Amylase inhibition} = 1 - \frac{(A_{\text{sample}} - A_{\text{sample blank}})}{(A_{\text{water}} - A_{\text{water blank}})} \times 100 \quad (4)$$

where A_{water} and A_{sample} were the absorbance of distilled water and sample extract with amylase, respectively.

$A_{\text{water blank}}$ and $A_{\text{sample blank}}$ were the absorbance of distilled water and sample extract without amylase, respectively.

Glucosidase Inhibitory Activity

Glucosidase inhibitory activity was determined in a reaction of 3 mM glutathione (25 µl), 67 mM potassium phosphate buffer pH 6.8 (250 µl), 0.3 unit/ml glucosidase (25 µl), and each extract (100 µl) at 37°C for 10 min. After that, 10 mM PNPG (25 µl) was mixed and incubated at 37°C for 10 min, then 0.1 M sodium carbonate (400 µl) was added with gently shaking. The absorbance was measured at 400 nm (Elya et al., 2012). Acarbose was used as positive control. The percentage of glucosidase inhibition was calculated following formula.

$$\% \text{ Glucosidase inhibition} = 1 - \frac{(A_{\text{sample}} - A_{\text{sample blank}})}{(A_{\text{water}} - A_{\text{water blank}})} \times 100 \quad (5)$$

where A_{water} and A_{sample} were the absorbance of distilled water and sample extract with glucosidase, respectively.

$A_{\text{water blank}}$ and $A_{\text{sample blank}}$ were the absorbance of distilled water and sample extract without glucosidase, respectively.

Data Analysis

All tests were analyzed in duplicate, at minimum. Data were expressed as mean \pm SD, and significant differentiation between mean values was evaluated by one-way variance analysis (ANOVA). The principal component analysis (PCA) with varimax rotation was used to determine correlations among variables. Linear regression analysis ($R^2 = 0.74-1.00$) was applied to estimate EC50 values (effective concentration of each extract needed to scavenge ABTS radicals, chelate ferrous ions, or inhibit enzymes by 50%), which were consequently adjusted to $1/EC50$ (Thummajitsakul et al., 2014). All statistics were calculated via Paleontological statistic program version 3.16 (Hammer et al., 2001) and PSPP program version 0.10.5 (Pfaff et al., 2013).

RESULTS AND DISCUSSION

Nowadays it is well known that amylase and glucosidase are enzymes for carbohydrate digestion in the digestive system. Amylase is responsible for breaking down 1, 4-glycosidic bonds of polysaccharides to disaccharides, whereas glucosidase digests the disaccharides to monosaccharides, which leads to an increase of the postprandial plasma glucose. Therefore, inhibition of these enzyme activities can help to delay

carbohydrate digestion or extend time in digestion, which causes a reduction in glucose absorption and postprandial hyperglycemia (Yilmazer-Musa et al., 2012). In addition, lipase is an enzyme responsible for fat digestion and absorption, which consequently leads to hyperlipidemia (Kershaw et al., 2006). Therefore, lipase is also a key enzyme in preventing obesity and obesity-related diseases, inhibition of which can help to delay lipid absorption (Padwal & Majumdar, 2007).

In our study, the results demonstrated that total phenolic content, radical scavenging activity, ferrous ion chelating activity, and antidiabetic potential were found in 95% ethanol extracts of seed, flesh, leaf, and fermented juices from *G. schomburgkiana* Pierre. Total phenolic contents of seed, leaf, and flesh extracts were 110.03 ± 21.60 , 60.89 ± 8.55 , 6.21 ± 2.85 mg gallic acid/g extract, respectively. The EC50 values of each extract and fermented juice for radical scavenging, ferrous ion chelating, and enzyme inhibitory activities were adjusted to $1/EC50$. The higher $1/EC50$ values indicated higher biological activities. The corresponding order of the $1/EC50$ of radical scavenging activities was 0.1335, 0.0724, and 0.0011 for seed, leaf, and flesh extracts, respectively. However, ferrous ion chelating activity showed the highest $1/EC50$ value in leaf extract (0.5128), followed by seed extract (0.0525), and flesh extract (0.0287). Additionally, amylase inhibitory activities were observed in seed, flesh and leaf extracts that showed $1/EC50$ values in order 0.0277, 0.0192 and

0.0186, whereas the order of glucosidase and lipase inhibitory activity was leaf extract (0.0801 and 0.0450), seed extract (0.0675 and 0.0309), and flesh extract (0.0658 and 0.0208) (Table 1).

Table 1

Total phenolic contents, radical scavenging activities, ferrous ion chelating activities, inhibitory activities on amylase, glucosidase and lipase of 95% ethanol extracts of seed, flesh and leaf from *G. schomburgkiana* Pierre

Biological activities	95% ethanol extracts		
	Seed	Flesh	Leaf
Total phenolic contents (mg gallic acid/ g extract)	110.03±21.60	6.21±2.85	60.89±8.55
Radical scavenging activities			
EC50 (1/EC50)	7.49±0.92 (0.1335)	859.86±45.72 (0.0011)	13.81±1.91 (0.0724)
ferrous ion chelating activities			
EC50 (1/EC50)	19.03±11.62 (0.0525)	34.85±24.70 (0.0287)	1.95±0.85 (0.5128)
Amylase inhibition			
EC50 (1/EC50)	36.15±1.72 (0.0277)	52.06±0.01 (0.0192)	53.60±2.03 (0.0186)
Glucosidase inhibition			
EC50 (1/EC50)	14.81±6.19(0.0675)	15.19±4.85 (0.0658)	12.48±0.21 (0.0801)
Lipase inhibition			
EC50 (1/EC50)	32.33±7.95 (0.0309)	48.09±1.67 (0.0208)	22.21±8.36 (0.0450)

A recent study of ethanolic extract of *Garcinia mangostana* Linn., which revealed hypoglycaemic activity by the increase of the number of insulin-producing β -cells in rat (Taher et al., 2016), support our result. Generally, appropriate reactive oxygen species (ROS) are useful for many biological processes in the human body such as immune function, gene expression and cellular responses. However, an excess ROS can attack insulin-producing pancreatic β -cells, which is one of the major causes of type II diabetes mellitus (Tangvarasittichai, 2015). Moreover, obesity can trigger an increase in ROS, which is linked to the development of obesity-related disease (Marseglia et al., 2015).

In addition, our result indicated the moderate level of ABTS radical scavenging activities and total phenolic contents in ethanolic extract from *G. schomburgkiana* leaf. Similarly, previous study shows low total phenolic contents (58.84, 37.68, and 97.97 mg of gallic acid/g extract), and moderate ABTS radical scavenging activities (151.51, 31.21, and 93.19 mg of g extract) have been found in dichloromethane, acetone, and methanol extracts of *G. schomburgkiana* leaf, respectively (Meechai et al., 2016a). However, acetone extracts of roots and branches of this plant show high phenolic contents, antioxidant capacity, and radical scavenging activity (Meechai et al., 2016a, 2016b).

Moreover, parts of *G. schomburgkiana*, namely bark, branch, root, wood, and fruit have been reported as natural sources of phenolics such as xanones, flavonoid, biphenyls, depsidones, biflavonoids, and benzophenones (Ito et al., 2013; Le et al., 2016; Meechai et al., 2016c; Mungmee et al., 2013; Sukandar et al., 2016; Vo et al., 2012). These phenolics reveal many biological capacities such as antidiabetic and antiobesity activities (Liu et al., 2015; Trinh et al., 2017; Vinayagam & Xu, 2015). Therefore, several extracts from plant sources are used as a phenolic reservoir in effective interaction or inhibition on the key enzymes which are linked to type II diabetes and obesity. Interestingly, seed extract showed lower ferrous ion chelating activities, glucosidase inhibitory activity, and lipase inhibitory activity in comparison to leaf extracts, whereas total phenolic content, radical scavenging activity, and amylase inhibitory activity were higher. Based on previous studies, seeds of several fruits consist of abundant phenolic contents and antioxidant activities (Babbar et al., 2011).

The PCA results of the ethanolic extracts revealed that total phenolic content, radical scavenging activity, ferrous ion chelating activity, and inhibitory activities on amylase, glucosidase, and lipase were grouped into 2 components with eigenvalues above 1. PC1 and PC2 accounted for 53.13% and 46.87% of the total variance, respectively. PC1 contained total phenolic content, radical scavenging activity, and amylase inhibitory activity, whereas PC2 contained

ferrous ion chelating activity, glucosidase inhibitory activity, and lipase inhibitory activity. It confirmed that the highest value of total phenolic content, radical scavenging activity, and amylase inhibitory activity were found in the seed extract, whereas the highest value of ferrous ion chelating activity, glucosidase inhibitory activity, and lipase inhibitory activity were found in the leaf extract (Figure 1A). Furthermore, the results showed that total phenolic content was very strongly correlated with radical scavenging activity ($r = 1.000$, $P < 0.05$), and ferrous ion chelating activity was very strongly correlated with glucosidase inhibitory activity, significantly ($r = 0.998$, $P < 0.05$).

It is possible that the antioxidant activities found in all extracts are contributed by the phenolic agents found in this plant, and that the glucosidase inhibition activities are provided by phytochemical constituents which exhibit ferrous ion chelating activities. It has been reported that dichloromethane, acetone, and methanol extracts of *G. schomburgkiana* contain many biological agents, namely benzoic acid, vanillin, citric acid, linoleic acid, oleic acid, protocatechuic acid, catechol, and phloroglucinol (Meechai et al., 2016c). Moreover, GC-MS analysis of phytochemical compounds in acetone extracts of *G. schomburgkiana* branch shows that antioxidant activity is contributed by phenolic compounds (isovanillic acid and 2,6-dihydroxy-4-methoxybenzophenone) and fatty acids in the extract (Meechai et al., 2016a).

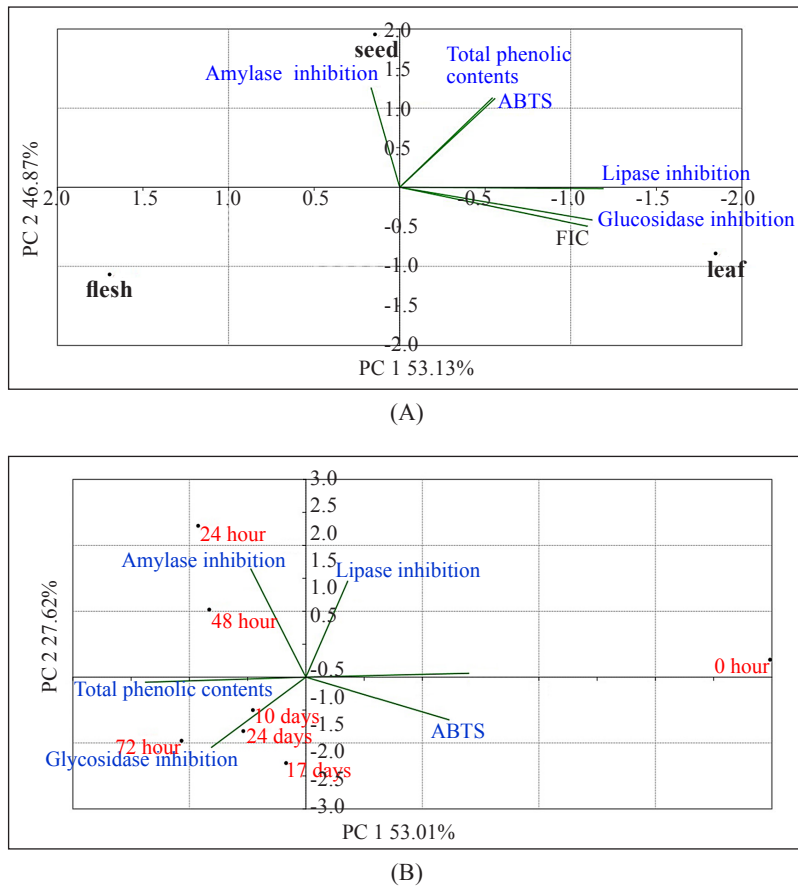


Figure 1. Principal component analysis (PCA). A is PCA of seed, flesh and leaf extracts of *G. schomburgkiana* Pierre. B is PCA of fermented juices of *G. schomburgkiana* Pierre. ABTS was expressed as radical scavenging activities, and FIC was expressed as ferrous ion chelating activities

In addition, it has been reported that several phenolic compounds (i.e. methyl 4-O-galloylchlorogenate, 4-O-galloylchlorogenic acid, methyl chlorogenate, dihydromyricetin, quercitrin, myricitrin, catechin, epicatechin, gallic acid) from plants have health-related benefits, and exhibit antioxidant properties (Parik & Patel, 2017) including inhibitory activities on amylase, glucosidase, and lipase (Kamiyama et al., 2010; Li et al., 2009; McDougall et al., 2005; Yang et al., 2014; Yilmazer-Musa

et al., 2012), which have key roles in the control of diabetic and obesity-related diseases in humans.

Moreover, our results also indicated that total phenolic content, antioxidant activity and antidiabetic activity were found in the fermented juice of *G. schomburgkiana* Pierre. Total phenolic contents of the fermented juices increased to a maximum at 1.06 ± 0.36 mg gallic/g fresh fruit after 72 hour fermentation, and then gradually decreased. However, the radical scavenging activity and ferrous ion chelating activity of the

fermented juice showed the highest 1/EC50 value at the start of fermentation (1/EC50 = 0.67×10^{-3} and 4.83×10^{-3} , respectively), and then they again increased to 0.47×10^{-3} and 0.80×10^{-3} on 17 and 24 days after the start of fermentation, respectively (Table 2). The results of amylase inhibitory activity showed the highest 1/EC50 level (11.38×10^{-3}) at 24 hour fermentation and then decreased. Glucosidase inhibitory activity in the fermented juice had maximal levels at 4.90×10^{-3} at 17 day fermentation and

then reduced, and lipase inhibitory activity had the highest value ($1/EC50 = 2.36 \times 10^{-3}$) at 48 hour fermentation and then declined (Table 3).

The results from the fermentation process may involve in several mechanisms. Fermentation is a method of food preservation by microorganisms or enzymes based on converting carbohydrates to organic acids or alcohol, producing health-promoting bioactive agents, improving stability and functions of the bioactive

Table 2

Total phenolic contents, radical scavenging activities, and ferrous ion chelating activities of fermented juice from flesh of *G. schomburgkiana* Pierre

Fermentation time	Total phenolic contents (mg gallic/ g fresh fruits)	Radical scavenging activities		Ferrous ion chelating activities	
		EC50(mg fresh weight/ml)	1/EC50	EC50 (mg fresh weight/ml)	1/EC50
0 hour	0.33±0.07	1,501.36± 735.37	0.67×10^{-3}	207.13± 6.51	4.83×10^{-3}
24 hour	0.91±0.38	4,183.49± 636.99	0.24×10^{-3}	5,259.15± 530.19	0.19×10^{-3}
48 hour	1.02±0.34	5,595.61± 840.83	0.18×10^{-3}	1,628.77± 185.62	0.61×10^{-3}
72 hour	1.06±0.36	2,991.89± 364.10	0.33×10^{-3}	2,648.73± 830.94	0.38×10^{-3}
10 days	0.96±0.44	2,581.04± 637.19	0.39×10^{-3}	2,002.68± 85.03	0.50×10^{-3}
17 days	0.80±0.29	2,140.76± 532.54	0.47×10^{-3}	1,877.75± 330.11	0.53×10^{-3}
24 days	0.92±0.31	3,788.18± 488.48	0.26×10^{-3}	1,245.41± 240.40	0.80×10^{-3}

Table 3

Inhibitory activities on amylase, glucosidase and lipase by fermented juices from flesh of *G. schomburgkiana* Pierre

Fermentation time	Amylase inhibition		Glucosidase inhibition		Lipase inhibition	
	EC50 (mg fresh weight/ml)	1/EC50	EC50 (mg fresh weight/ml)	1/EC50	EC50 (mg fresh weight/ml)	1/EC50
0 hour	648.77± 87.46	1.54×10^{-3}	121,077	8.26×10^{-6}	479.79±11.90	2.08×10^{-3}
24 hour	87.89± 32.35	11.38×10^{-3}	587.47±17.74	1.70×10^{-3}	436.73±18.06	2.29×10^{-3}
48 hour	256.84± 123.69	3.89×10^{-3}	535.61±24.74	1.87×10^{-3}	424.17±27.56	2.36×10^{-3}
72 hour	559.02± 118.82	1.79×10^{-3}	249.92±144.91	4.00×10^{-3}	807.76±12.62	1.24×10^{-3}
10 days	219.52± 151.16	4.55×10^{-3}	603.18±9.64	1.66×10^{-3}	2142.12±449.22	4.67×10^{-4}
17 days	642.34± 48.97	1.56×10^{-3}	204.24±52.97	4.90×10^{-3}	711.90±76.97	1.40×10^{-3}
24 days	583.20± 23.21	1.71×10^{-3}	513.20±13.58	1.95×10^{-3}	1803.33±440.23	5.55×10^{-4}

agents (Tamang et al., 2009, 2016; Thapa & Tamang, 2015). Further to this, it has been reported that several isolates of natural lactic acid bacteria (i.e. *Lactobacillus brevis*, *Lactobacillus pentosus* and *Lactococcus lactis*) were found in pickled *G. schomburgkiana* Pierre (Madan) from local markets at Nakhon Nayok province and Nakhon Ratchasima province in Thailand (Chanprasert & Gasaluck, 2011). However, phenolic levels can be reduced in fermented juices by oxidation or polymerization with biomolecules (i.e. phytochemicals carbohydrates, lipids, and proteins) that induce precipitation of phenolic and biomolecules during fermentation (Chen et al., 2018; Manach et al., 2004; Parada & Aguilera, 2007; Zou et al., 2017). In contrast, radical scavenging activity, ferrous ion chelating activity, inhibitory activities on amylase, glucosidase and lipase of the fermented juices were higher during fermentation. These results can be explained by the reaction of enzymes and organic acid from bacteria or fruits which may help to release phenolics or antioxidants from complicated forms in fruit fiber into free forms. Similar evidence supports our results that total flavonoid content in papaya juice increases during fermentation, but phenolic compounds have the highest level at 30 hours and then decrease (Chen et al., 2018).

The PCA result of the fermented juice showed that total phenolic content, radical scavenging activity, ferrous ion chelating activity, and inhibitory activities on amylase, glucosidase and lipase were also divided into 2 components. PC1 and PC2 accounted

for 53.01% and 27.62% of the total variance, respectively. PC1 contained total phenolic content, radical scavenging activity, and ferrous ion chelating activity, whereas PC2 contained amylase inhibitory activity, glucosidase inhibitory activity, and lipase inhibitory activity. The result confirmed that total phenolic content and glucosidase inhibitory activity were at the highest levels in the fermented juices after fermentation for 72 hours and 17 days respectively, whereas radical scavenging activity and ferrous ion chelating activity showed the highest level at 0 hour, including amylase and lipase inhibitory activities which had the highest value at 24 and 48 hour fermentation, respectively (Figure 1B). Furthermore, total phenolic content was, significantly, very strongly negatively correlated with radical scavenging activity ($r = -0.869$, $P < 0.05$) and ferrous ion chelating activity ($r = -0.937$, $P < 0.05$). In addition, radical scavenging activity was, significantly, very strongly positively correlated with ferrous ion chelating activity ($r = 0.804$, $P < 0.05$). It is possible that the presence of nonphenolic compounds in bioprocessed fruit may contribute to antioxidant activity, and hydrolysis of fruit by microorganism enzyme can produce complex mixture and bioactive agents that can disturb in antioxidant activity (Sousa & Correia, 2012). Moreover, the nonphenolic compounds (such as beneficial fatty acids) in acetone extracts of *G. schomburgkiana* branch and root may act as antioxidants (Meechai et al., 2016a).

Fermented fruits are functional foods with health-promoting properties for humans. Previously, several studies have reported that the presence of functional microorganisms in fermented food can promote human health by preventing non-communicable diseases such as cardiovascular disease, cancer, and diabetes (Swain et al., 2014). Overall, our study indicated that all ethanol extracts and fermented juices of *G. schomburgkiana* Pierre have interesting biological activities. The local fruit waste has also benefits as a natural source of bioactive agents such as phenolics, antioxidants, and antidiabetic agents. It also implies, however, that other phytochemical groups (i.e. alkaloids, terpenoids and hydroxycitric acid) in leaf extracts may help ferrous ion chelating activity and inhibitory activities of glucosidase and lipase. Earlier studies demonstrated that *Garcinia* sp. (i.e. *G. cambogia*, *G. atroviridis*, and *G. indica*) contains organic acid such as hydroxycitric acid, which displays anti-obesity activities (Chuah et al., 2013). Our studies, therefore, demonstrated for the first time that seeds generally discarded as waste had rich total phenolic content, radical scavenging activity, ferrous ion chelating activity, and antidiabetic potential, and that a distribution of the biological activities in different parts of *G. schomburgkiana* Pierre fruit was found. In addition, the nutritional values of this fruit can be improved by fermentation, allowing for further development as a healthy fruit drink.

CONCLUSION

We conclude that the total phenolic content, ferrous ion chelating activity, and radical scavenging activity including antidiabetic properties were found in seed, flesh, and leaf extracts, as well as the fermented juices of *G. schomburgkiana* Pierre. Seed extract showed the highest value of total phenolic content, radical scavenging activity, and amylase inhibitory activity, whereas the leaf extracts revealed the strongest ferrous ion chelating activity, and inhibitory activities on glucosidase and lipase. Furthermore, fermentation of *G. schomburgkiana* Pierre can provide nutraceutical capacities, namely radical scavenging activity, ferrous ion chelating activity, and inhibitory activities on amylase, glucosidase and lipase, which varied according fermentation periods. The extracts and fermented juices from *G. schomburgkiana* Pierre could, therefore, be developed as a tool to manage and prevent diabetic and obesity diseases (along with an appropriate healthy diet and physical activity), and the phytochemicals with antioxidant activities and antidiabetic activities in seeds of this plant should be studied further, including the efficacy of fermentation.

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