International Journal of Agricultural Technology 2023 Vol. 19(2):639-654
Available online http://www.ijat-aatsea.com
ISSN 2630-0192 (Online)

Antioxidant activities and α-glucosidase inhibitory potential of crude extracts from five cultivars of *Antidesma thwaitesianum* Müll. Arg.

Sangsila, A.[1], Promden, W.[1], Chumroenphat, T.[2], Butekup, L.[3], Panchan, R.[4], Plaetita, W.[5], Pranakhon, R.[5], Sributta, I.[5], Pinta, W.[5] and Jorjong, S.[5*]

[1]Division of General Science, Faculty of Education, Buriram Rajabhat University, Buriram 31000, Thailand; [2]Aesthetic Sciences and Health Program, Faculty of Thai Traditional and Alternative Medicine, Ubon Ratchathani Rajabhat University, Ubonratchathani 34000, Thailand; [3]Department of Biotechnology, Faculty of Technology Mahasarakham University, Maha Sarakham 44150, Thailand; [4]Department of Agricultural Technology, Faculty of Technology, Mahasarakham University, Maha Sarakham 44150, Thailand; [5]Faculty of Natural Resources, Rajamangala University of Technology Isan, Sakon Nakhon Campus, Sakon Nakhon 47160, Thailand.

Sangsila, A., Promden, W., Chumroenphat, T., Butekup, L., Panchan, R., Plaetita, W., Pranakhon, R. Sributta, I., Pinta, W. and Jorjong, S. (2023). Antioxidant activities and α-glucosidase inhibitory potential of crude extracts from five cultivars of *Antidesma thwaitesianum* Müll. Arg. International Journal of Agricultural Technology 19(2):639-654.

Abstract The α-glucosidase inhibitory potential, total phenolic and flavonoid contents for antioxidant activities of 30 crude extracts of various parts of *Antidesma thwaitesianum* including leaves, flowers, immature green fruits, mature red fruits, ripe black fruits, and seeds were investigated. The results showed that the α-glucosidase inhibitory activity was not observed in the ripe black fruits and seeds of all cultivars. The high α-glucosidase inhibitory potential was detected in the leaves and flowers of all the tested cultivars, with the IC_{50} ranged of 0.22 – 2.63 μg/mL. Notably, the highest α-glucosidase inhibitory activity was found in the Sang Koh 2 flowers (IC_{50} = 0.22 μg/mL), followed by the flowers of Kam Lai (IC_{50} = 0.35 μg/mL) and Phu Phan Thong (IC_{50} = 0.35 μg/mL). While the total phenolics were predominant in the immature green and ripe red fruits (515.10 – 1230 mg GAE/100 g crude extract) the total flavonoids were found in all parts of cultivars (123.53 – 490.83 mg CE/100 g crude extract). It is indicated by the DPPH, $ABTS^{+\bullet}$ and FRAP values, all cultivars showed a good source of antioxidant agents. Hence, this plant species is an ideal antihyperglycemic agent for treating diabetes.

Keywords: Mao Luang (*Antidesma thwaitesianum* Müll.), Phytochemicals, Alpha-glucosidase

Introduction

Diabetes mellitus is a metabolic disorder of multiple etiology reckoned as one of the crucial causes of death in the globe owing to acute side effects (Sohrabi *et al*., 2022). It is characterized by chronic

[*] **Corresponding Author**: Jorjong, S.**; Email**: sujitar_9@hotmail.com

hyperglycemia with disturbance of carbohydrate, fat, and protein metabolism (Dilworth *et al.,* 2021) caused by inherited or acquired deficiency in insulin secretion, resulting in an elevated blood glucose level (Dirir *et al*., 2022; Sanni *et al*., 2020). The long-term high levels of blood glucose in patients with diabetes lead to diverse disorders like microvascular complications or destruction of very small blood vessels in the body causing serious kidney, eye, nerve and heart diseases (Sohrabi *et al*., 2022; Thungtak *et al*., 2021). This disease is a major public health problem globally and the proportion of people with type 2 diabetes mellitus is increasing in most countries, with approximately 90% of people with diabetes having type 2 diabetes mellitus. It is expected that the number will rise to 700 million by 2045 (Pitchalard *et al*., 2022). Like many other countries in Southeast Asia, Thailand has experienced an increased prevalence of diabetes with an increase from 7.7% in 2004 to 8.3% in 2020 (Zaman *et al*., 2021). This epidemic of diabetes can be a result from population aging, dietary preferences, lifestyle alterations, and even genetic/epigenetic predisposition (Chao *et al*., 2018).

Food carbohydrate like starch and dextrin is the major source of human blood sugar and the α-glucosidase in the gastrointestinal tract is responsible for catalyzing the cleavage of α-1,4 glycosidic bonds of carbohydrate to produce glucose, which is absorbed by small intestine and then moves into blood circulation (Chen *et al*., 2022; Khadayat *et al*., 2020). For this reason, inhibition of the α-glucosidase activity is reckoned one therapeutic approach for treating diabetes (Sohrabi *et al*., 2022). In modern medicine, many α-glucosidase inhibitors are therefore globally utilized to inhibit α-glucosidase in the brush border of the small intestine for slowing down the breakdown of carbohydrates and lowering postprandial hyperglycemia (Kao *et al*., 2016). However, they are costly and have adverse side effects, including kidney injury, gastrointestinal disturbances (flatulence, abdominal pain, and diarrhea), metabolic effect (such as weight gain), and drug resistance (Horii *et al*., 2022; Mwakalukwa *et al*., 2020). Thus, there is an urgent need to search for more effective and safe antidiabetics from natural sources (Chen *et al*., 2022; Wang *et al*., 2019).

Antidesma thwaitesianum Müll. Arg. is a tropical plant belonging to the family Euphorbiaceae (Natewong *et al*., 2022), which is most diverse in Southeast Asia (Tinchan *et al*., 2022). It grows well in various soil types, particularly in dipterocarp forests, and is naturalized in Australia, Africa, islands in the Pacific Oceans, and tropical Asia including in Thailand, where it typically thrives in the northeastern region of the country (Butkhup and Samappito, 2008; Jorjong *et al*., 2018). This plant is rich in bioactive compounds like polyphenols and flavonoids (Suravanichnirachorn *et al*., 2018) which provide several physiological benefits, including antioxidant, anti-apoptotic, antidiabetic, anti-inflammatory, anti-carcinogenic, and anti-hypertensive activities (Natewong *et al*., 2022; Puangpronpitag *et al*., 2011;

Tinchan *et al*., 2022). Therefore, it is of great interest to look for the α-glucosidase inhibitory potential of this plant.

A myriad of studies have focused on exploring phytochemical contents in the edible parts of *A. thwaitesianum*, especially mature and ripe fruits (Chinprahast *et al*., 2020; Natewong *et al*., 2022; Tinchan *et al*., 2022), with few studies carried out in other plant parts (Dechayont *et al*., 2017; Jorjong *et al*., 2018). Most importantly, the α-glucosidase inhibitory potential of various parts of this plant has not yet been reported so far. Therefore, this study was carried out for the first time to quantify the total phenolic and flavonoid contents, antioxidant activities and α-glucosidase inhibitory activities of various parts (leaves, flowers, immature green fruits, mature red fruits, ripe black fruits, and seeds) of five cultivars of *A. thwaitesianum*. The correlation among the α-glucosidase inhibitory activities, total phenolic and flavonoid contents as well as the antioxidant activities were also determined using Pearson's correlations. The information obtained in this study should provide a foundation for future studies on development of natural and safe diet-derived antidiabetic agents.

Materials and methods

Plant materials and extract preparation

Five cultivars of *A. thwaitesianum*, namely Kam Lai, Sang Koh 2, Fah Prathan, Phu Song, and Phu Phan Thong, were collected from Mao Luang farms in Sakon Nakhon province, Northeast Thailand. Different parts of *A. thwaitesianum*, including leaves, flowers, immature green fruits, mature red fruits, ripe black fruits, and seeds, were freshly collected, rinsed with distilled water, oven-dried at 65 ℃ for 72 hr to dryness and milled to powder using an electrical grinder.

Powder samples of *A. thwaitesianum* were extracted in dark flasks using the maceration method (Jorjong *et al*., 2015) at a sample to solvent ratio of 1:3 (w/v) with ethanol (Fischer; 95%). The mixtures were extracted at ambient temperature for 3 days. The extraction was repeated twice for each sample. The extracts were combined, filtered through Whatman No.1 filter paper, and ethanol solvents were removed at 45 ℃ using a rotary vacuum evaporator. All crude extracts were stored in dark bottles at –20 ℃ until analysis.

α-Glucosidase inhibition assay

The α-glucosidase inhibitory activity of the extracts was determined based on the method of (Promyos *et al*., 2020) with some modifications in a 96-well microplate containing 10 μL of each of the extracts prepared in DMSO (10 mg/mL), 10 μL of *S. cerevisiae* α-glucosidase (1.0 U/mL, CAS

No.9001-42-7, Sigma-Aldrich) and 50 µL of 2.5 mM *p*-nitrophenyl-α-D-glucopyranoside (PNP-G) in 130 µL of 100 mM phosphate buffer (pH 6.8). For the control, A 10-µL volume of DMSO was used in place of the extracts. Acarbose (20 mg/mL) was used as the standard drug in this procedure. The reaction was monitored at a wavelength of 405 nm at 37 °C for 10 min using a UV/Vis absorbance spectrophotometer microplate reader (FLUOstar® Omega, BMG LABTECH). The percentage of inhibition was calculated using the following equation:

$$\text{Inhibition (\%)} = [(V_{0C} - V_{0T})/V_{0C}] \times 100$$

where V_{0C} denotes the initial velocity of the control reaction with enzyme while V_{0T} represents the initial velocity of the extracts with enzyme.

Half-maximal inhibition concentration (IC_{50})

As the crude extracts of *A. thwaitesianum* leaves, flowers, green fruits, and red fruits exhibited high inhibitory activities against α-glucosidase enzyme, these extracts were chosen for estimating the half-maximal inhibition concentration or IC_{50}. The crude extracts of leaves and flowers were prepared at concentrations of 0.195 – 12.5 µg/mL. Meanwhile, the crude extracts of immature green and mature red fruits were prepared at concentrations of 50 - 400 µg/mL. To estimate the IC_{50} of the extracts, percentage inhibition of each assay was plotted on Y-axis against the corresponding concentrations (X-axis) to find the slope of the plotted curve and to generate the linear formula to quantify the IC_{50}. Then the IC_{50} values were estimated using Microsoft Office Excel (Probit analysis) by applying the formula obtained.

Antioxidant activity test

The antioxidant capacity of the extracts was determined with the 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging assay, ferric reducing antioxidant power (FRAP) assay, and 2,2-azino-bis-3-ethylbenzothiazoline-6-sulfonic acid ($ABTS^{+\bullet}$) assay as reported by (Jorjong *et al*., 2015). The total phenolic content was determined by the Folin Ciocalteu method described by (Singleton and Rossi, 1965) and (Kähkönen *et al*., 1999). The total flavonoid content was assessed which based on the method of (Dewanto *et al*., 2002).

Statistical analysis

All tests were carried out in triplicate. The averages and standard errors of the data were calculated using Microsoft Excel. The differences were analyzed by SPSS statistical package version 17.0 using Duncan's multiple range test. Pearson's correlation analysis was also performed to understand the interrelation among α-glucosidase inhibitory activities, antioxidant activities, and total phenolic and flavonoid contents. The values with $p < 0.05$ were considered statistically significant.

Results

α-Glucosidase inhibitory activity

Five cultivars of *A. thwaitesianum* were investigated and 30 extracts were assessed for the α-glucosidase inhibitory potential and the results are shown in Table 1. It was observed that all the screened cultivars and the majority of the extracts demonstrated a significant capability to inhibit α-glucosidase. Acarbose employed as a standard α-glucosidase inhibitor could inhibit 50.60% of the enzyme activity. The crude extracts of various parts of *A. thwaitesianum* were screened for their potential to inhibit the activity of α-glucosidase enzyme, each at a concentration of 100 µg/mL, in comparison with acarbose (1000 µg/mL) and the results are given in Table 1. It was evident that all the studied cultivars had high degrees of α-glucosidase inhibitory activities. The leaf and flower crude extracts in particular exhibited high α-glucosidase inhibitory activities, ranging from 95.55% in the Phu Song flowers to 101.82% in the Sang Koh 2 leaves. The high α-glucosidase inhibitory potential was also observed in the immature green fruit crude extracts of some cultivars, with the maximum value in Kam Lai (99.19%) and the lowest in Fah Prathan (10.70%). It is interesting to note that none of the crude extracts prepared from the ripe black fruits and seeds possessed the α-glucosidase inhibitory potential.

Table 1. α-Glucosidase inhibitory activity of acarbose and crude extracts of different parts of *A. thwaitesianum*

Cultivars	**α-Glucosidase inhibitory activity (%) at 100 µg/mL**					
	Leaf	**Flower**	**Green fruit**	**Red fruit**	**Black fruit**	**Seed**
Kam Lai	99.87^{a}	99.62^{a}	99.19^{a}	89.28^{a}	n.d.	n.d.
Sang Koh 2	101.82^{a}	99.42^{a}	97.79^{a}	13.79^{b}	n.d.	n.d.
Fah Prathan	101.47^{a}	99.83^{a}	10.70^{c}	n.d.	n.d.	n.d.
Phu Song	98.13^{a}	95.55^{a}	79.09^{b}	16.64^{b}	n.d.	n.d.
Phu Phan Thong	100.21^{a}	97.23^{a}	91.57^{a}	n.d.	n.d.	n.d.
Acarbose(1000 µg/mL)	50.60					

The difference superscript letters in each column indicate the statistical significance between cultivars ($p<0.05$) and n.d.: not detected.

Table 2. α-Glucosidase inhibitory activity of the leaf and flower crude extracts of *A. thwaitesianum* at different concentrations

Cultivars	α-Glucosidase inhibitory activity (%)						
	0.195 µg/mL	0.39 µg/mL	0.78 µg/mL	1.56 µg/mL	3.13 µg/mL	6.25 µg/mL	12.5 µg/mL
Leaf extract							
Kam Lai	n.d.	1.73 ± 0.96	20.65 ± 10.87	58.82 ± 24.31	82.14 ± 21.30	92.58 ± 10.12	97.75 ± 3.2
Sang Koh 2	n.d.	12.81 ± 5.47	37.54 ± 12.90	72.68 ± 20.49	90.38 ± 13.08	96.70 ± 5.15	98.74 ± 1.5
Fah Prathan	n.d.	8.52 ± 3.05	0.48 ± 11.36	70.43 ± 21.12	89.12 ± 13.56	96.56 ± 5.67	98.71 ± 1.0
Phu Song	n.d.	7.64 ± 3.24	14.56 ± 3.97	43.30 ± 17.67	74.89 ± 23.02	90.35 ± 15.81	95.89 ± 6.5
Phu Phan Thong	n.d.	13.73 ± 6.26	38.47 ± 16.00	73.25 ± 21.19	90.06 ± 13.31	96.89 ± 5.63	97.86 ± 1.9
Flower extract							
Kam Lai	18.82 ± 4.10	55.91 ± 3.06	89.41 ± 7.37	98.90 ± 0.21	99.74 ± 0.39	99.39 ± 0.77	-
Sang Koh 2	39.67 ± 5.60	78.00 ± 6.88	95.21 ± 5.86	100.49 ± 2.35	99.70 ± 0.59	99.62 ± 0.27	-
Fah Prathan	10.02 ± 2.76	37.18 ± 5.45	74.53 ± 12.10	95.27 ± 2.79	99.49 ± 0.57	99.12 ± 0.94	-
Phu Song	15.51 ± 6.04	48.57 ± 9.80	84.49 ± 8.59	97.04 ± 2.55	100.12 ± 0.48	99.90 ± 0.18	-
Phu Phan Thong	17.24 ± 2.66	57.07 ± 7.07	88.13 ± 10.36	98.24 ± 1.89	100.60 ± 2.03	99.33 ± 0.34	-

n.d.: not detected; - : exceeding 100%.

Half-maximal inhibition concentration (IC_{50})

After the screening tests for the α-glucosidase inhibitory potential, the ripe black fruits and seeds were excluded from the study because they possessed no α-glucosidase inhibitory activities. The leaves, flowers, immature green fruits, and mature red fruits were further tested for their α-glucosidase inhibitory potential at various concentrations. The leaf and flower crude extracts were evaluated at a concentration range of 0.195 – 12.50 µg/mL. Meanwhile, the concentrations used for the immature green fruit and mature red fruits ranged from 50 µg/mL to 400 µg/mL. As presented in Table 2, the leaf crude extracts of the cultivars Sang Koh 2 and Phu Phan Thong appeared to possess stronger inhibitory activities than the other three cultivars at a concentration range of 0.39 – 12.50 µg/mL. Notably, inhibitory activity was observed at a concentration of 1.95 µg/mL for all the studied cultivars. Again, at a concentration range of 0.19 – 6.25 µg/mL the highest inhibitory activity was noted for the flower crude extracts of the cultivar Sang Koh 2, followed by Kam Lai and Phu Phan Thong. Interestingly, applied at 12.50 µg/mL the flower crude extracts of all the studied cultivars showed the α-glucosidase inhibitory activities exceeding 100%, suggesting that the flower crude extracts were far more effective than the leaf crude extracts. As shown in Table 3, the α-glucosidase inhibitory activities were found in the immature green fruit crude extracts of most of the studied cultivars, except for the cultivars Fah Prathan. Notably, the mature red crude extracts of the cultivars Kam Lai and Sang Koh 2 demonstrated the inhibitory potential. At a concentration of 50 µg/mL, only the mature red fruit crude extracts of the cultivar Kam Lai possessed the inhibitory activity.

The mean half-maximal inhibition concentration (IC_{50}) against α-glucosidase of the crude extracts of various parts of *A. thwaitesianum* and acarbose is presented in Table 4. In this study, the IC_{50} value of acarbose included as a standard α-glucosidase inhibitor was 2126.92 µg/mL. According to the IC_{50} vales, the crude extracts of all the studied cultivars were more effective in inhibiting the activity of α-glucosidase enzyme than acarbose, with the Sang Koh 2 flowers being most effective, followed by the Kam Lai and Phu Phan Thong flowers. The leaf crude extracts of all the studied cultivars were also considered as a promising inhibitor against α-glucosidase. Meanwhile, the immature green fruit and mature red fruit crude extracts of some cultivars were effective against α-glucosidase.

Table 3. α-Glucosidase inhibitory activity of the green and red fruit crude extracts of *A. thwaitesianum* at different concentrations

Cultivars	**α-Glucosidase inhibitory activity (%)**			
	50 µg/mL	**100 µg/mL**	**200 µg/mL**	**400 µg/mL**
Green fruit extract				
Kam Lai	n.d.	32.25±8.88	76.07±14.10	76.07±16.10
Sang Koh 2	n.d.	31.16±6.48	66.19±12.63	66.19±11.63
Fah Prathan	n.d.	n.d.	n.d.	n.d.
Phu Song	n.d.	31.16±7.50	37.39±5.06	36.36±6.19
Phu Phan Thong	n.d.	28.03±0.05	n.d.	46.08±1.35
Red fruit extract				
Kam Lai	12.92±1.58	27.66±1.76	60.42±3.15	91.29±2.22
Sang Koh 2	n.d.	27.16±0.29	36.73±1.53	63.37±6.95
Fah Prathan	n.d.	n.d.	n.d.	n.d.
Phu Song	n.d.	n.d.	n.d.	n.d.
Phu Phan Thong	n.d.	n.d.	n.d.	n.d.

n.d.: not detected.

Table 4. Mean half-maximal inhibition concentration (IC50) against α-glucosidase of the crude extracts of different parts of *A. thwaitesianum*

Cultivars	**IC_{50} (µg/mL)**			
	Leaf	**Flower**	**Green fruit**	**Red fruit**
Kam Lai	1.32 ± 0.31^{a}	0.35 ± 0.16^{b}	115.04±23.06	148.84±4.47
Sang Koh 2	0.94 ± 0.15^{a}	0.22 ± 0.02^{a}	147.11±24.80	276.69±44.60
Fah Prathan	1.05 ± 0.18^{a}	0.48 ± 0.03^{c}	n.d.	n.d.
Phu Song	2.63 ± 1.29^{b}	0.40 ± 0.04^{b}	146.11	n.d.
Phu Phan Thong	0.93 ± 0.19^{a}	0.35 ± 0.02^{b}	439.03	n.d.
Acarbose	2,126.92 ± 287.90			

The difference superscript letters in each column indicate the statistical significance between cultivars ($p<0.05$) and n.d.: not detected.

Total phenolic and flavonoid contents

Total phenolic and flavonoid contents in different parts of *A. thwaitesianum* are given in Tables 5 and 6. As expected, the contents of total phenolic compounds and flavonoids varied considerably across cultivars and plant parts. For most of the studied cultivars, the immature green and mature red fruits were more abundant in total phenolics than leaves and flowers, with the highest contents found in the Sang Koh 2 immature green fruits (1230 mg GAE/ 100 g crude extract), followed by the Phu Phan Thong immature green fruits (1055.90 mg GAE/ 100 g crude extract) and the Kam Lai mature red fruits (982 mg GAE/ 100 g crude extract) as shown in Table 5. The total phenolic contents in the leaves and flowers were in a range of 314.44 – 662.75 mg GAE/ 100 g crude extract. For the total flavonoid contents, most of the studied cultivars had high levels of total flavonoids in the leaves, with the values ranging from 319.15 mg CE/ 100 g crude extract to 490.83 mg CE/ 100 g crude extract. However, the lowest amount of total flavonoids was observed in the Phu Phan Thong leaves (123.53 mg CE/ 100 g crude extract). The Sang Koh 2 leaves contained the highest level of total flavonoids (490.83 mg CE/ 100 g crude extract), followed by the Phu Phan Thong immature green fruits (465.64 mg CE/ 100 g crude extract) and the Kam Lai mature red fruits (457.88 mg CE/ 100 g crude extract). Notably, the flowers of all the studied cultivars harbored total flavonoids less than 280 mg CE/ 100 g crude extract (Table 6).

Table 5. Total phenolic content of the crude extracts of different parts of *A. thwaitesianum*

Cultivars	Total phenolic content (mg GAE/ 100 g crude extract)			
	Leaf	Flower	Green fruit	Red fruit
Kam Lai	351.23^{b}	330.15^{bc}	920.0^{b}	982.00
Sang Koh 2	477.83^{a}	662.75^{a}	1230.0^{a}	801.22
Fah Prathan	332.46^{b}	315.68^{c}	948.7^{b}	841.81
Phu Song	314.44^{b}	373.90^{b}	515.1^{c}	772.74
Phu Phan Thong	342.29^{b}	376.65^{b}	1055.9^{b}	825.26

The difference superscript letters in each column indicate the statistical significance between cultivars ($p<0.05$) and n.d.: not detected.

Table 6. Total flavonoid content of the crude extracts of different parts of *A. thwaitesianum*

Cultivars	Total flavonoid content (mg CE/ 100 g crude extract)			
	Leaf	Flower	Green fruit	Red fruit
Kam Lai	399.54^{b}	168.73^{b}	303.89^{b}	457.88^{a}
Sang Koh 2	490.83^{a}	277.23^{a}	273.20^{b}	155.30^{c}
Fah Prathan	362.75^{c}	267.73^{a}	304.74^{b}	280.92^{b}
Phu Song	319.15^{d}	221.80^{ab}	285.70^{b}	289.09^{b}
Phu Phan Thong	123.53^{e}	142.64^{b}	465.64^{a}	241.39^{bc}

The difference superscript letters in each column indicate the statistical significance between cultivars ($p<0.05$).

Antioxidant activities

The crude extracts of various parts of *A. thwaitesianum* were assessed for their antioxidant activities as DPPH radical scavenging activity, $ABTS^{+\bullet}$ scavenging effect, and FRAP as shown in Table 7. The results unraveled that as suggested by the DPPH values, the leaves and flowers of all the studied cultivars had high levels of antioxidant activities, with the highest activities found in the leaves. The maximum DPPH value was noted in the Phu Phan Thong leaves (86.91%), followed by the Sang Koh 2 leaves (86.89%) and the Fah Prathan leaves (86.63%). By contrast, the immature green and mature red fruits of all the studied cultivars possessed low levels of antioxidant activities, with the DPPH values ranging from 14.61% in the Sang Koh 2 mature red fruits to 32.01% in the Kam Lai immature green fruits. Based on the $ABTS^{+\bullet}$ levels, high levels of antioxidant activities were observed in the flowers and immature green fruits of most of the studied cultivars, with the highest value of 75.80% in the Phu Song flowers, followed by the Sang Koh 2 flowers (67.16%) and the Phu Phan Thong flowers (66.26%). The mature red fruits of all the studied cultivars had relatively low levels of antioxidant activities, with the $ABTS^{+\bullet}$ values not exceeding 8.17%. The leaves of the studied cultivars possessed moderate levels of antioxidant activities, showing the $ABTS^{+\bullet}$ values in a range of 21.91 – 46.90%. Again, as indicated by the FRAP values, high levels of antioxidant activities were found in the flowers and immature green fruits, with the highest value of 145.28 mg Fe(II)/ g extract in the Phu Song immature green fruits, followed by the Phu Phan Thong immature green fruits (108.23 mg Fe(II)/ g extract) and the Fah Prathan flowers (81.04 mg Fe(II)/g extract). Moderate and low levels of antioxidant activities were observed in the leaves and mature red fruits, respectively.

Correlation coefficients between α-glucosidase inhibitory activities and antioxidant activities as well as total phenolic and flavonoid contents

Pearson's correlation analysis was performed to comprehend the interrelation among α-glucosidase inhibitory activities, antioxidant activities, total phenolic contents, and total flavonoid contents (Table 8). In this study, α-glucosidase inhibitory activities (GIA) were highly ($p \leq 0.01$) positively correlated with DPPH values. In contrast, FRAB values displayed a strong negative correlation with $ABTS^{+\bullet}$ values.

Table 7. Antioxidant activity of the crude extracts of different parts of *A. thwaitesianum*

Cultivars	Antioxidant activity			
	Leaf	**Flower**	**Green fruit**	**Red fruit**
DPPH radical scavenging activity (%)				
Kam Lai	85.91^{ab}	84.00^{a}	32.01	15.19
Sang Koh 2	86.89^{a}	84.57^{a}	29.63	14.61
Fah Prathan	86.63^{a}	75.54^{b}	28.43	27.54
Phu Song	85.47^{b}	60.40^{d}	27.38	15.70
Phu Phan Thong	86.91^{a}	69.95^{c}	25.97	18.73
$ABTS^{+\bullet}$ scavenging effect (%)				
Kam Lai	46.90^{a}	45.51^{c}	55.49^{a}	4.78^{bc}
Sang Koh 2	22.39^{c}	67.16^{b}	54.81^{ab}	2.81^{c}
Fah Prathan	32.60^{b}	34.39^{d}	53.70^{bc}	2.87^{c}
Phu Song	21.91^{c}	75.80^{a}	52.29^{c}	8.17^{a}
Phu Phan Thong	39.03^{ab}	66.26^{b}	52.26^{c}	6.27^{ab}
FRAP (mg Fe(II)/ g extract)				
Kam Lai	56.46^{ab}	61.79^{b}	36.04^{c}	21.94^{c}
Sang Koh 2	37.62^{c}	42.08^{c}	36.72^{c}	32.64^{a}
Fah Prathan	52.91^{b}	81.04^{a}	30.49^{c}	29.99^{ab}
Phu Song	40.65^{c}	35.87^{c}	145.28^{a}	28.39^{b}
Phu Phan Thong	60.44^{a}	63.55^{b}	108.23^{b}	28.19^{b}

The difference superscript letters in each column indicate the statistical significance between cultivars ($p<0.05$) and n.d.: not detected.

Table 8. Pearson's correlations among α-glucosidase inhibitory activity, total phenolic content, total flavonoid content, and antioxidant activity of the ethanolic extracts of *A. thwaitesianum*

	GIA	DPPH	$ABTS^{+\bullet}$	FRAP	TPC	TFC
GIA	1					
DPPH	**0.90***	1				
$ABTS^{+\bullet}$	-0.75	-0.49	1			
FRAP	0.47	0.26	**-0.87***	1		
TPC	0.19	0.41	0.48	-0.57	1	
TFC	0.43	0.20	-0.14	-0.12	0.49	1

GIA: α-Glucosidase inhibitory activity; DPPH: 2,2-diphenyl-1-picrylhydrazyl radical scavenging assay, FRAP: ferric reducing antioxidant power assay; $ABTS^{+\bullet}$: 2,2-azino-bis-3-ethylbenzothiazoline-6-sulfonic acid assay; TPC: total phenolic content; TFC: total flavonoid content.
* Correlation is significant at the $p \leq 0.05$ level.
** Correlation is significant at the $p \leq 0.01$ level.

Discussion

The substantial increasing prevalence of diabetes is reckoned one of the most challenging public health problems. In spite of several novel antidiabetic drugs being available to treat hyperglycemia, treatment is far from ideal since people with diabetes remain unable to obtain optimal control as a result of side effects of medications or unacceptable rates of hypoglycemia, or perhaps they experience the complications of diabetes irrespective of the availability of these therapies (Khoo, 2017). Accordingly, a plethora of studies have focused on seeking more effective and safe antidiabetic agents from natural sources targeting to the inhibition of α-glucosidase, a key enzyme involved in post prandial hyper glycemia (Krishnan *et al*., 2022; Xie *et al*., 2021; Ye *et al*., 2022). A number of plant species from all over the globe have been explored for their α-glucosidase activities as well as antioxidant capacities, including some species in the genus *Antidesma* (Elya *et al*., 2012; Mauldina *et al*., 2017; Nguyen *et al*., 2012; Quiming *et al*., 2016). However, the α-glucosidase inhibitory potential and antioxidant activities of the species *A. thwaitesianum*, which is native and widely cultivated in many areas of Northeast Thailand has not yet been reported so far.

This study was implemented for the first time to assess the α-glucosidase inhibitory potential and quantify the total phenolic and flavonoid contents as well as antioxidant activities of 30 crude extracts of various parts (leaves, flowers, immature green fruits, mature red fruits, ripe black fruits, and seeds of five *A. thwaitesianum* cultivars. Most of the crude extracts of the studied cultivars had high potential to inhibit α-glucosidase at a concentration of 100 µg/mL, with the significantly high inhibitory capacities and observed in the leaf and flower crude extracts (95.55 – 101.82%). The immature green fruit crude extracts of some cultivars were also effective in suppressing α-glucosidase, with the highest value of 99.19%. The crude extracts of the ripe black fruits and seeds had no inhibitory activities. The crude extracts were further tested for the α-glucosidase inhibitory potential at varied concentrations, except those possessing no inhibitory activities. Only the leaf crude extracts of the cultivars Sang Koh 2 and Phu Phan Thong had a strong inhibitory activity at a concentration range of 0.39 – 12.50 µg/mL while the leaf crude extracts of all the studied cultivars displayed inhibitory activity at a concentration of 1.95 µg/mL. The flower crude extracts of the cultivar Sang Koh 2 again showed the greatest inhibitory activity at a concentration range of 0.19 – 6.25 µg/mL, followed by Kam Lai and Phu Phan Thong, with the inhibitory activity higher than 100% when tested at a concentration of 12.50 µg/mL as observed for the flower crude extracts of all the studied cultivars. These results suggested that the flower crude extracts of *A. thwaitesianum* were far more effective in retarding the function of α-glucosidase than the leaf crude extracts. The α-glucosidase inhibitory activities were also found in most of the immature green fruit crude extracts of the studied cultivars, except the cultivar Fah Prathan. Meanwhile, only the mature red fruit crude extracts of some cultivars had the inhibitory activities. It was evident that the leaf and flower crude extracts of *A. thwaitesianum* in the present studies had stronger α-glucosidase inhibitory activities than the root extracts of *A. ghaesembilla* in one previous study (Nguyen *et al.*, 2012), which showed the percentage inhibition in a range of 15.5 – 85.7% applied at concentrations of 25 – 250 μg/mL. Moreover, the inhibitory potential of the immature green fruit extracts of *A. thwaitesianum* was comparable to that of *A. ghaesembilla*. Additionally, the fruit crude extracts of some cultivars of *A. thwaitesianum* demonstrated a greater inhibitory potential than the fruit crude extracts of *A. bunius* reported in an earlier study (Quiming *et al.*, 2016), which showed the percentage inhibition of around 25%.

The crude extracts of all the studied cultivars were more effective in inhibiting the activity of α-glucosidase than acarbose, with the Sang Koh 2 flowers being most effective (0.22 μg/mL), followed by the Kam Lai and Phu Phan Thong flowers (0.35 μg/mL). The leaf crude extracts of all the studied cultivars were also considered a promising inhibitor against α-glucosidase, with an IC_{50} range of 0.93 – 2.63 μg/mL. The immature green fruit and mature red fruit crude extracts of some cultivars were merely effective against α-glucosidase (IC_{50}: 115.04 – 439.03 μg/mL). The findings obtained in this study showed that as suggested by the IC_{50} values the leaf and flower crude extracts of *A. thwaitesianum* were 417-fold to 663-fold more effective than the root extracts of *A. ghaesembilla* (IC_{50} = 145.8 μg/mL) reported in an earlier study (Nguyen *et al*., 2012). Again, the leaf and flower crude extracts of all the studied cultivars of *A. thwaitesianum* showed higher inhibitory activities than the stem bark extracts of *A. bunius* in one previous study which had the IC_{50} value of 19.33 μg/mL (Mauldina *et al*., 2017). Moreover, the flower crude extracts of *A. thwaitesianum* exhibited a greater capability to repress α-glucosidase than the stem bark and leaf crude extracts of some species in the genus *Antidesma*, such as *A. bunius* (IC_{50}: 3.90 – 7.94 μg/mL), *A. celebicum* (IC_{50}: 2.34 – 3.93 μg/mL), *A. montanum* (IC_{50}: 2.83 μg/mL) and *A. neurocarpum* (IC_{50}: 4.22 μg/mL) (Elya *et al*., 2012).

Phenolics and flavonoids act as reducing agents, hydrogen donors, and are responsible for scavenging free radicals. These molecules present in plant extracts at high levels may contribute substantially to the antioxidant capacities (Cosmulescu *et al.*, 2017; Phuyal *et al*., 2020). The total phenolic and flavonoid contents of *A. thwaitesianum* differed greatly across cultivars and plant parts. For the majority of the studied cultivars, the total phenolics were predominant in the immature green and mature red fruits, with the maximum content of 1230 mg GAE/ 100 g crude extract in the Sang Koh 2 immature green fruits, followed by the Phu Phan Thong immature green fruits (1055.90 mg GAE/ 100 g crude extract) and the Kam Lai mature red fruits (982 mg GAE/ 100 g crude extract). The total phenolic contents in the leaves and flowers ranged between 314.44 mg GAE/ 100 g crude extract and 662.75 mg GAE/ 100 g crude extract. On the other hand, most of the studied cultivars showed high total flavonoid contents in the leaves.

However, the lowest total flavonoid content was detected in the Phu Phan Thong leaves (123.53 mg CE/ 100 g crude extract). The Sang Koh 2 leaves contained the highest total flavonoid content of 490.83 mg CE/ 100 g crude extract, followed by the Phu Phan Thong immature green fruits (465.64 mg CE/ 100 g crude extract) and the Kam Lai mature red fruits (457.88 mg CE/ 100 g crude extract). It is interesting to note that the flowers of all the studied cultivars possessed the total flavonoids less than 280 mg CE/ 100 g crude extract. The crude extracts of all parts of the studied cultivars possessed strikingly high contents of total phenolic and flavonoid contents when compared to those in the fresh and dried leaf extracts of the same plant species using the same extraction procedure, with total phenolic and flavonoid contents of 200.18 – 274.42 mg GAE/ g and 5.54 – 22.17 mg CE/ g, respectively (Dechayont *et al*., 2017). The total phenolics in this study were again higher than those in the leaf extracts of 15 *A. thwaitesianum cultivars* in our previous study (Jorjong *et al*., 2018), but the total flavonoids were comparable.

The crude extracts of *A. thwaitesianum* were assessed for their antioxidant activities, which were expressed as DPPH radical scavenging, $ABTS^{+\bullet}$ scavenging, and FRAP values. As indicated by the DPPH values, the leaf and flower crude extracts showed high antioxidant activities, with the highest activities in the leaves. The highest DPPH value of 86.91% was found in the Phu Phan Thong leaves, followed by the leaves of Sang Koh 2 (86.89%) and Fah Prathan (86.63%). As with $ABTS^{+\bullet}$ values, most of the studied cultivars exhibited high antioxidant activities in the flowers and immature green fruits, with the highest value of 75.80% in the Phu Song flowers, followed by the Sang Koh 2 flowers (67.16%) and the Phu Phan Thong flowers (66.26%). The leaves of the studied cultivars possessed moderate levels of antioxidant activities, showing the $ABTS^{+\bullet}$ values in a range of 21.91 – 46.90%. Moreover, according to the FRAP values, high antioxidant activities were observed in the flowers and immature green fruits, with the highest value of 145.28 mg Fe(II)/ g extract in the Phu Song immature green fruits, followed by the Phu Phan Thong immature green fruits (108.23 mg Fe(II)/g extract) and the Fah Prathan flowers (81.04 mg Fe(II)/g extract). These results showed that all parts of the studied cultivars displayed the antioxidant potential which was comparable to those reported in previous studies (Jorjong *et al*., 2018). However, the antioxidant activities of the crude extracts in the current study were much lower than those reported in an earlier study (Dechayont *et al*., 2017) measured in terms of FRAP (451.23 – 941.26 mg Fe(II)/g).

The interrelation among the α-glucosidase inhibitory activities, total phenolic and flavonoid contents, and antioxidant activities were also assessed using Pearson's correlations. In this study, α-glucosidase inhibitory activities (GIA) were highly ($p \leq 0.01$) positively correlated with DPPH values. In contrast, FRAB values displayed a strong negative correlation with ABTS$^{+\bullet}$ values.

In conclusion, the crude extracts of various parts of the five *A. thwaitesianum* cultivars possess high levels of the α-glucosidase inhibitory potential and provide a good source of total phenolics and flavonoids acting as antioxidant agents. Therefore, this plant species is an ideal antihyperglycemic agent for treating diabetes.

Acknowledgments

This research was funded by the Thailand Science Research and Innovation (TSRI) (grant No. FRB630010/0174-P3-03 or 63A173000024). The authors are very grateful to the Natural Products Laboratory, Division of General Science, Faculty of Education, Buriram Rajabhat University and the Faculty of Natural Resources, Rajamangala University of Technology Isan, Sakon Nakhon Campus for laboratory facilities.

References

Butkhup, L. and Samappito, S. (2008). An analysis on flavonoids contents in Mao Luang fruits of fifteen cultivars (*Antidesma bunius*), grown in Northeast Thailand. Pakistan Journal of Biological Sciences, 11:996-1002.

Chao, C.-T., Wang, J., Huang, J.-W. and Chien, K.-L. (2018). Acarbose use and liver injury in diabetic patients with severe renal insufficiency and hepatic diseases: a propensity score-matched cohort study. Frontiers in Pharmacology, 9:860.

Chen, S., Lin, B., Gu, J., Yong, T., Gao, X., Xie, Y., Xiao, C., Zhan, J. Y. and Wu, Q. (2022). Binding interaction of betulinic acid to α-glucosidase and its alleviation on postprandial hyperglycemia, In: Molecules.

Chinprahast, N., Boonying, J. and Popuang, N. (2020). Antioxidant activities of mamao luang (*Antidesma thwaitesianum* Müll. Arg.) fruit: extraction and application in raw chicken patties. Journal of Food Science, 85:647-656.

Cosmulescu, S., Trandafir, I. and Nour, V. (2017). Phenolic acids and flavonoids profiles of extracts from edible wild fruits and their antioxidant properties. International Journal of Food Properties, 20:3124-3134.

Dechayont, B., Itharat, A., Phuaklee, P., Chunthorng-Orn, J., Juckmeta, T., Prommee, N., Nuengchamnong, N. and Hansakul, P. (2017). Antioxidant activities and phytochemical constituents of *Antidesma thwaitesianum* Müll. Arg. leaf extracts. Journal of integrative medicine, 15:310-319.

Dewanto, V., Wu, X., Adom, K. K. and Liu, R. H. (2002). Thermal processing enhances the nutritional value of tomatoes by increasing total antioxidant activity. Journal of Agricultural and Food Chemistry, 50:3010-3014.

Dilworth, L., Facey, A. and Omoruyi, F. (2021). Diabetes mellitus and its metabolic complications: the role of adipose tissues. International journal of molecular sciences, 22:7644.

Dirir, A. M., Daou, M., Yousef, A. F. and Yousef, L. F. (2022). A review of alpha-glucosidase inhibitors from plants as potential candidates for the treatment of type-2 diabetes. Phytochemistry Reviews, 21:1049-1079.

Elya, B., Basah, K., Mun'im, A., Yuliastuti, W., Bangun, A. and Septiana, E. K. (20120. Screening of α-glucosidase inhibitory activity from some plants of Apocynaceae, Clusiaceae, Euphorbiaceae, and Rubiaceae. Journal of Biomedicine and Biotechnology, 2012:281078.

Horii, T., Oikawa, Y., Kunisada, N., Shimada, A. and Atsuda, K. (2022). Acute kidney injury in Japanese type 2 diabetes patients receiving sodium–glucose cotransporter 2 inhibitors: a nationwide cohort study. Journal of Diabetes Investigation, 13:42-46.

Jorjong, S., Butkhup, L. and Samappito, S. (2015). Phytochemicals and antioxidant capacities of Mao-Luang (*Antidesma bunius* L.) cultivars from Northeastern Thailand. Food Chemistry, 181:248-255.

Jorjong, S., Sakhunkhu, S. and Plaetita, W. (2018). Phenolic compounds and antioxidant capacities in leaves of fifteen Mao-Luang (*Antidesma thwaitesianum* Müll. Arg.) cultivars. International Journal of Agricultural Technology, 14:1293-1306.

Kähkönen, M. P., Hopia, A. I., Vuorela, H. J., Rauha, J. P., Pihlaja, K., Kujala, T. S., Heinonen, M. (1999). Antioxidant activity of plant extracts containing phenolic compounds. Journal of Agricultural and Food Chemistry, 47:3954-3962.

Kao, C., Wu, P., Wu, C., Chen, L., Chen, H., Wu, M. and Wu, V. (2016). Risk of liver injury after α-glucosidase inhibitor therapy in advanced chronic kidney disease patients. Scientific reports, 6:18996.

Khadayat, K., Marasini, B.P., Gautam, H., Ghaju, S. and Parajuli, N. (2020). Evaluation of the alpha-amylase inhibitory activity of Nepalese medicinal plants used in the treatment of diabetes mellitus. Clinical Phytoscience, 6:34.

Khoo, C. M. (2017). Diabetes Mellitus Treatment, In: International Encyclopedia of Public Health (Second Edition). Academic Press, Oxford, pp. 288-293.

Krishnan, V., Verma, P., Saha, S., Singh, B., Vinutha, T., Kumar, R. R., Kulshreshta, A., Singh, S.P., Sathyavathi, T., Sachdev, A. and Praveen, S. (2022). Polyphenol-enriched extract from pearl millet (*Pennisetum glaucum*) inhibits key enzymes involved in post prandial hyper glycemia (α-amylase, α-glucosidase) and regulates hepatic glucose uptake. Biocatalysis and Agricultural Biotechnology, 43:102411.

Mauldina, M. G., Sauriasari, R. and Elya, B. (2017). α-Glucosidase inhibitory activity from ethyl acetate extract of *Antidesma bunius* (L.) Spreng stem bark containing triterpenoids. Pharmacognosy magazine, 13:590-594.

Mwakalukwa, R., Amen, Y., Nagata, M. and Shimizu, K. (2020). Postprandial hyperglycemia lowering effect of the isolated compounds from olive mill wastes – an inhibitory activity and kinetics studies on α-glucosidase and α-amylase enzymes. ACS Omega, 5:20070-20079.

Natewong, S., Niwaspragrit, C., Ratanachamnong, P., Samatiwat, P., Namchaiw, P. and Jaisin, Y. (2022). Photo-protective and anti-inflammatory effects of *Antidesma thwaitesianum* Müll. Arg. fruit extract against UVB-induced keratinocyte cell damage, In: Molecules, p. 5034.

Nguyen, M. T. T., Nguyen, N. T., Nguyen, H. X., Huynh, T. N. N., Min, B. S. (20120. Screening of α-glucosidase inhibitory activity of vietnamese medicinal plants: isolation of active principles from *Oroxylum indicum*. Natural Product Sciences, 18:47-51.

Phuyal, N., Jha, P. K., Raturi, P. P., Rajbhandary, S. (2020). Total phenolic, flavonoid contents, and antioxidant activities of fruit, seed, and bark extracts of *Zanthoxylum armatum* DC. The Scientific World Journal, 2020:8780704.

Pitchalard, K., Wimolphan, P., Singkhon, O., Griffin Agazio, J. B. and Moonpanane, K. (2022). "Life is bitter and sweet": the lived experience of ethnic minority elders with type 2 diabetes mellitus in rural, Thailand. Asian Nursing Research, 16:155-161.

Promyos, N., Temviriyanukul, P. and Suttisansanee, U. (2020). Investigation of anthocyanidins and anthocyanins for targeting α-glucosidase in diabetes mellitus. Preventive nutrition and food science, 25:263-271.

Puangpronpitag, D., Yongvanit, P., Boonsiri, P., Suttajit, M., Areejitranusorn, P., Na, H.-K. and Surh, Y.-J. (2011). Molecular mechanism underlying anti-apoptotic and anti-inflammatory effects of Mamao (*Antidesma thwaitesianum* Müll. Arg.) polyphenolics in human breast epithelial cells. Food Chemistry, 127:1450-1458.

Quiming, N., Asis, J. L., Nicolas, M., Versoza, D., Alvarez, M. R. (20160. In vitro α-glucosidase inhibition and antioxidant activities of partially purified *Antidesma bunius* fruit and *Gynura nepalensis* leaf extracts. Journal of Applied Pharmaceutical Science, 6:97-101.

Sanni, O., Erukainure, O. L. and Islam, M. S. (2020). Chapter 20 - Dacryodes edulis: protective antioxidant effects on diabetes pathology, In: Pathology. Academic Press, pp. 205-212.

Singleton, V. L. and Rossi, J. A. (1965). Colorimetry of total phenolics with phosphomolybdic-phosphotungstic acid reagents. American Journal of Enology and Viticulture, 16:144.

Sohrabi, M., Binaeizadeh, M. R., Iraji, A., Larijani, B., Saeedi, M. and Mahdavi, M. (2022). A review on α-glucosidase inhibitory activity of first row transition metal complexes: a futuristic strategy for treatment of type 2 diabetes. RSC Advances, 12:12011-12052.

Suravanichnirachorn, W., Haruthaithanasan, V., Suwonsichon, S., Sukatta, U., Maneeboon, T. and Chantrapornchai, W. (2018). Effect of carrier type and concentration on the properties, anthocyanins and antioxidant activity of freeze-dried mao [*Antidesma bunius* (L.) Spreng] powders. Agriculture and Natural Resources, 52:354-360.

Thungtak, R., Wannapakhe, J. and Lapanantasin, S. (2021). Thai version of the questionnaire for diabetes-related foot disease (Thai Q-DFD): validity and reliability. Heliyon, 7:e07832.

Tinchan, P., Sirijariyawat, A., Prommakool, A., Phattayakorn, K., Pheungsomphane, S. and Tayuan, C. (2022). *Antidesma thwaitesianum* Müll. Arg. fruit juice, its phytochemical contents, antimicrobial activity, and application in chiffon cake. International Journal of Food Science, 2022:5183562.

Wang, R., Zhao, H., Pan, X., Orfila, C., Lu, W. and Ma, Y. (2019). Preparation of bioactive peptides with antidiabetic, antihypertensive, and antioxidant activities and identification of α-glucosidase inhibitory peptides from soy protein. Food Science & Nutrition, 7:1848-1856.

Xie, X., Chen, C. and Fu, X. (2021). Screening α-glucosidase inhibitors from four edible brown seaweed extracts by ultra-filtration and molecular docking. LWT, 138:110654.

Ye, C., Zhang, R., Dong, L., Chi, J., Huang, F., Dong, L., Zhang, M. and Jia, X. (2022). α-Glucosidase inhibitors from brown rice bound phenolics extracts (BRBPE): identification and mechanism. Food Chemistry, 372:131306.

Zaman, S. B., Gupta, R. D., Pramual, P., Khan, R. K., Sujimongkol, C., Hossain, N., Haider, M. R., Karim, M. N., Kibria, G. M. and Islam, S. M. S. (2021). The burden of chronic kidney disease among people with diabetes by insurance schemes: findings from a primary referral hospital in Thailand. Diabetes Epidemiology and Management, 4:100026.

(Received: 12 September 2022, accepted: 28 February 2023)