Songklanakarin J. Sci. Technol.
43 (5), 1499-1506, Sep. - Oct. 2021

http://www.sjst.psu.ac.th

Original Article

Phytochemical profile, antioxidant activity, and inhibition of α-amylase and α-glucosidase for banana central pseudo-stem juice

Intira Koodkaew*, Khwanruedee Chaiyawan, and Pimchanok Satapoomin

Department of Botany, Faculty of Liberal Arts and Science,
Kasetsart University, Kamphaeng Saen Campus, Kamphaeng Saen, Nakhon Pathom, 73140 Thailand

Received: 6 April 2020; Revised: 22 October 2020; Accepted: 16 December 2020

Abstract

The use of natural products to inhibit α-amylase and α-glucosidase is a strategy in the treatment of diabetes. This study investigated the phytochemical profile and antioxidant activity of, and α-amylase and α-glucosidase inhibition by banana central pseudo-stem juice (BPJ) from four cultivars [Kluai Hom Thong (KHT), Kluai Hak Muk (KHM), Kluai Namwa (KNW), and Kluai Namwa Dam (KND)], and two parts (basal and distal), at two ages (3 and 5 months). The phytochemical content and antioxidant activity in each part of BPJ were age-dependent. KND had the most polyphenol, while KHT had the most flavonoid, ascorbic acid, and highest antioxidant activity. The basal part of the five month-old KHM tends to have the most potential for treating diabetes because it has a high level of α-amylase (52.89±2.65%) inhibition and the strongest α-glucosidase inhibition (66.91±2.26%) among the investigated samples. This information may be used as the starting point for the development of BPJ-derived products.

Keywords: α-amylase inhibitor, cultivars, α-glucosidase inhibitor, *Musa*

1. Introduction

Diabetes mellitus (DM) is a highly prevalent disease with significant morbidity and mortality worldwide, due to a modern lifestyle with high levels of carbohydrate consumption. The International Diabetes Federation estimated that the number of people aged 20-79 years with diabetes will increase from 415 million in 2015 to 642 million in 2040 (Ogurtsova *et al.*, 2017). Type 2 DM is a metabolic syndrome associated with hyperglycemia, insulin resistance and relative insulin deficiency (Olokoba, Obateru, & Olokoba, 2012). A therapeutic approach to manage type 2 DM is to decrease postprandial hyperglycemia. The enzymes α-amylase and α-glucosidase are important in the digestion of carbohydrates and help intestinal absorption (Yang *et al.*, 2019). Therefore, the inhibition of these two carbohydrate-hydrolyzing enzymes can effectively control the postprandial increase of blood glucose level in diabetic patients. At present, synthetic hypoglycemic agents such as acarbose, miglitol, and voglibose are being used for therapy, but they have limitations and produce some serious side effects (Cheng & Fantus, 2005). A possible strategy is to use natural products from plants as they can have less adverse effects than many synthetic agents.

In the past decade, phytochemicals such as α-amylase and α-glucosidase inhibitors have been widely researched. Plant phenolic compounds, including flavonoids and tannin, have high antioxidant activities and could be considered for the control of the early stages of hyperglycemia via inhibiting α-amylase and α-glucosidase activities (Asgar, 2013). Phenolic compounds from *Musa* spp. flowers show strong α-glucosidase inhibition (Sheng *et al.*, 2014). Moreover, phytosterols isolated from *Musa* spp. flowers have been shown to inhibit α-amylase and α-glucosidase activities as well as glycation of protein and sugar (Sheng *et al.*, 2017).

Bananas are herbaceous monocotyledonous plants in the genus *Musa* belonging to the family Musaceae, which has a wide distribution and whose species provide an abundant natural resource globally. Most cultivated bananas are triploid ($2n = 3x = 33$), with genome constitutions of AAA (mainly the sweet dessert bananas), AAB or ABB (mainly, but not exclusively, starchy plantains eaten after cooking)

*Corresponding author
Email address: faasirk@ku.ac.th

(Heslop-Harrison & Schwarzacher, 2007). All parts of the banana have been reported to have pharmacological activities (Mathew & Negi, 2017), including the anti-diabetic property of the pseudo-stem (Bhaskar, Shobha, Sambaiah, & Salimath, 2011; Nguyen *et al.*, 2017).

The pseudo-stem is a part of the banana that looks like a trunk, with the central pseudo-stem being consumed as a vegetable in many countries, including Thailand. The pseudo-stem of bananas is known for its many ethnomedicinal uses (Camacho-Corona *et al.*, 2008). Phytochemicals, namely phenolic compounds, sugar, and phytosterol, have been reported as found in banana pseudo-stem (Bhaskar *et al.*, 2012; Nguyen *et al.*, 2017; Sheng *et al.*, 2017). In addition, antioxidant properties of the pseudo-stem of *Musa* sp. var. *elakki bale* have been reported by Bhaskar, Mahadevamma, Chilkunda, and Salimath (2012). Recently, Nguyen *et al.* (2017) reported inhibitory activity against α-amylase and α-glucosidase of freeze-dried stem juices from *Musa* × *paradisiaca* L. Although there have been some reports on the antioxidant and anti-diabetic abilities of the banana pseudo-stem, there has is no prior detailed study of different banana cultivars, and plant parts at various ages. Moreover, Thailand is believed to have a great diversity of banana species and cultivars. Therefore, the objective of this study was to compare the phytochemical profiles, antioxidant properties, and α-amylase and α-glucosidase inhibitory activities of various parts of four banana cultivars grown in Thailand at different ages.

2. Materials and methods

2.1 Preparation of plant materials

Pseudo-stems from four cultivars of banana—namely, (1) *Musa* AAA group cv 'Kluai Hom Thong' (KHM, *M. acuminata*), (2) *Musa* ABB group cv 'Kluai Hak Muk' (KHM, *M.* × *paradisiana*), (3) *Musa* ABB group cv 'Kluai Namwa' (KNW), and (4) *Musa* ABB group cv 'Kluai Namwa Dam' (KND)—were kindly provided by the Central Laboratory and Greenhouse complex, Kasetsart University Kamphaeng Saen Campus, Nakhon Pathom, Thailand. Banana pseudo-stems aged three (3) and five (5) months were collected and divided into basal (up to 50 cm from the bottom) and distal (50 cm and above from the basal end) parts. The layers of a freshly cut pseudo-stem were unwrapped to access the central white core of the stem. Consequently, plant materials were cut into small pieces (about 100 g) and crushed using a pestle and a mortar. The slurry was filtered through a cheesecloth and then the juice was centrifuged at 12,000 rpm for 10 min to remove fine particles. The supernatant was collected as banana central pseudo-stem juice (BPJ) and used to assess the parameters discussed below.

2.2 Determination of total phenolic compounds content

The total phenolic compounds content was determined using the Folin-Ciocalteu method (Singleton & Rossi, 1965) with analytical grade gallic acid as the standard. The results are expressed as milligrams of gallic acid equivalent (GAE) per gram fresh weight.

2.3 Determination of total flavonoid content

The total flavonoid content was investigated following the method of aluminium chloride colorimetric assay described by Zhishen, Mengcheng, and Jianming (1999) using analytical grade quercetin as the standard. The results are expressed as milligrams of quercetin equivalent (QE) per gram fresh weight.

2.4 Determination of total tannin content

The total tannin content was determined using polyvinyl-polypyrrolidone (PVPP) to precipitate tannins (Makkar, Bluemmel, Becker, & Becker, 1993). After precipitation, the non-tannin phenolic compounds fraction was determined using the Folin-Ciocalteu method as described above. The total tannin content was calculated as the difference between the total phenolic compounds content and the non-tannin phenolic compounds content in the BPJ.

2.5 Determination of ascorbic acid content

Ascorbic acid was determined using colorimetric assay according to the method of Li, Tang, Xu, Lin, and Han (2012). Ascorbic acid was used as the standard.

2.6 Determination of total soluble sugar content

The total soluble sugar content was examined using phenol-sulfuric acid assay (Dubios, Gilles, Hamilton, Rebers, & Smith, 1956). Glucose was used as the standard.

2.7 Determination of antioxidant activity

The antioxidant activity was investigated using the 2,2-diphenyl-1-picrylhydrazyl (DPPH) method (Brand-Williams, Cuvelier, & Berset, 1995). The DPPH-scavenging activity was calculated using the equation: DPPH-scavenging activity (%) = $[(A_0–A_1)/A_0] \times 100$, where A_0 is the absorbance of the control and A_1 is the absorbance of the test sample.

2.8 Inhibition of α-amylase enzyme

The inhibition effect of BPJ on α-amylase enzyme was investigated according to Wickramaratne, Punchihewa, and Wickramaratne (2016) with some modifications. Briefly, 250 µL of the BPJ was mixed with 250 µL of α-amylase (1 U/mL in 40 mM phosphate buffer, pH 6.9) and then the mixture was incubated at 30°C for 10 min. After incubation, 250 µL of 1% starch solution was added to the mixture and incubated at 30°C for 5 min. The reaction was terminated by adding 250 µL of 3,5-dinitrosalicylic acid agent solution and incubating in a water bath at 90°C for 5 min. After the solution had cooled, 5 mL of distilled water was added and the absorbance was measured at 540 nm using a UV-Vis spectrophotometer. The percentage inhibition of α-amylase activity was calculated using the equation: α-amylase inhibition (%) = $[(A_0-A_1)/A_0] \times 100$, where A_0 is the absorbance of the control (phosphate buffer instead of the plant sample) and A_1 is the absorbance of the sample solution.

2.9 Inhibition of α-glucosidase enzyme

The inhibition effect of BPJ on α- glucosidase enzyme was determined according to Kazeem, Adamson, and Ogunwande (2013). The BPJ (50 µL) was pre-incubated with 100 µL of the α-glucosidase solution (1 U/mL in 20 mM phosphate buffer, pH 6.9) at 37°C for 10 min in a water bath. Then, 50 µL of 3 mM *p*-nitrophenyl glucopyranoside in the same buffer was added to start the reaction and the absorbance at 405 nm was immediately monitored using the UV-Vis spectrophotometer at 0 and 7 min. The percentage inhibition of α-glucosidase activity was calculated using the equation: α-glucosidase inhibition (%) = $[(\Delta A_0-\Delta A_1)/\Delta A_0] \times 100$, where ΔA_0 is the change of absorbance at 405 nm of the control (phosphate buffer instead of the plant sample) and ΔA_1 is the change of absorbance at 405 nm with the sample.

2.10 Statistical analysis

All assays were performed in triplicate. All data are expressed as mean ± standard error (SE). Data were subjected to statistical analysis using the R program package (R Core Team, 2015). Two-way analysis of variance (ANOVA) was carried out using cultivars and parts as main factors. When the interaction between cultivars and parts was significant, data were subjected to one-way ANOVA. Multiple comparison tests were performed using Duncan's multiple range test, with $p<0.05$ considered as a statistically significant difference. Finally, in comparisons between ages t-tests were performed.

3. Results and Discussion

3.1 Extraction yields

The BPJ yield after extraction ranged from 41.00±0.58% to 52.00±3.51% (at 3 months) and from 37.33±2.90% to 61.00±0.58% (at 5 months) as shown in Table 1.

Table 1. Extraction yields of banana central pseudo-stem juice

Cultivar	Part	Extraction yield (%)	
		3 month	5 month
Kluai Hom Thong (KHT)	Basal	47.00±7.73^{ns}	57.67±1.33ab
	Distal	41.00±0.58	37.33±2.90d
Kluai Hak Muk (KHM)	Basal	45.33±2.60	61.00±0.58a
	Distal	50.00±7.02	56.00±3.46ab
Kluai Namwa (KNW)	Basal	52.00±3.51	42.33±1.20cd
	Distal	42.67±3.53	49.67±1.76a-d
Kluai Namwa Dam (KND)	Basal	47.00±3.60	45.33±7.12b-d
	Distal	49.00±5.86	52.67±2.33a-c

Data are expressed as mean ± SE (n = 3). Means with different letters in the same column are significantly different ($p < 0.05$), ns means non-significant.

3.2 Phytochemical contents

The results showed that the BPJ contained several classes of phytochemicals, namely phenolic compounds, flavonoids, tannins, ascorbic acid, and sugar, and the contents were influenced mainly by cultivar and its part, as well as their interaction (Tables 2 and 3). Regarding the combinations of cultivar and part, phenolic compounds were found in the BPJ, with concentrations of up to 10.01±0.05 mg GAE/g in the distal part of KND and the flavonoid content in BPJ ranged from 0.21±0.00 to 0.59±0.01 mg QE/g (Table 4). These findings agree with Bhaskar *et al.* (2012) who reported that pseudo-stem from *Musa* sp. var. *elakki bale* possessed polyphenols, and with Nguyen *et al.* (2017) who found that the stem juice from *Musa* × *paradisiaca* contained phenolic acids such as *p*-hydroxybenzoic acid, gallic acid, and ferulic acid. Phenolic compounds, including flavonoids, have been receiving much attention for controlling the digestibility of starch and for having high antioxidant activities (Asgar, 2013). The BPJ had a large amount of tannin compared to other phytochemicals, especially in BPJ from KND (Table 4).

Table 2. Analysis of variance and means comparison for phytochemicals content, antioxidant activity and enzyme inhibition in banana central pseudo-stem juice from different parts and cultivars at 3 months age

	Phenolic (mg GAE/g)	Flavonoid (mg QE/g)	Tannin (mg/g)	Ascorbic acid (mg/g)	Sugar (mg/g)	Radical scavenging (%)	α-Amylase inhibition (%)	α-Glucosidase inhibition (%)
Cultivar (C)								
KHT	3.52±0.28c	0.51±0.04a	1.45±0.25c	0.27±0.03a	0.43±0.05c	81.05±0.66a	25.60±1.17a	55.19±3.37b
KHM	2.73±0.04d	0.41±0.02c	0.89±0.02d	0.12±0.01d	0.45±0.03c	75.87±0.13c	20.56±1.24b	60.70±0.40a
KNW	3.63±0.18b	0.40±0.01c	1.92±0.14b	0.14±0.00c	1.13±0.08b	77.36±0.32b	15.86±1.49c	39.87±1.92d
KND	4.07±0.10a	0.45±0.03b	2.36±0.15a	0.16±0.00b	1.66±0.18a	73.45±0.32d	24.76±0.74a	45.63±3.99c
Part (P)								
Basal	3.58±0.20a	0.46±0.03a	1.79±0.20a	0.19±0.03a	1.09±0.19a	77.59±0.96a	19.88±1.57b	54.55±2.63a
Distal	3.40±0.17b	0.42±0.01b	1.52±0.19b	0.16±0.01b	0.74±0.12b	76.28±0.73b	23.52±0.97a	46.15±2.98b
Significance								
C	***	***	***	***	***	***	***	***
P	***	***	***	***	***	***	***	***
C×P	***	***	***	***	***	**	*	*
CV. (%)	0.86	4.10	1.99	1.76	8.88	0.62	9.74	8.65

Data are expressed as mean ± SE (n = 12 for parts; n = 6 for cultivars). Means with different letters in the same column are significantly different ($p < 0.05$). *; **, ***, significant at P<0.05, 0.01 and 0.001, respectively. Sample abbreviations are provided in Table 1.

Table 3. Analysis of variance and means comparison for phytochemicals content, antioxidant activity and enzyme inhibition in banana central pseudo-stem juice from different cultivars and parts at 5 months age

	Phenolic (mg GAE/g)	Flavonoid (mg QE/g)	Tannin (mg/g)	Ascorbic acid (mg/g)	Sugar (mg/g)	Radical scavenging (%)	α-Amylase inhibition (%)	α-Glucosidase inhibition (%)
Cultivar (C)								
KHT	4.71±0.50c	0.29±0.03bc	2.45±0.47c	0.16±0.00a	1.82±0.11c	74.00±1.70a	56.63±3.00a	49.34±1.29b
KHM	4.82±0.27c	0.27±0.03b	2.49±0.35c	0.10±0.01c	4.15±0.50b	69.03±0.22b	53.12±1.69b	62.17±2.35a
KNW	6.36±0.12b	0.32±0.03a	3.93±0.09b	0.16±0.04a	5.36±0.25a	61.17±1.25d	46.51±1.14c	59.13±2.92a
KND	8.23±0.82a	0.25±0.02b	6.38±0.70a	0.12±0.00b	5.52±0.43a	64.73±1.19c	51.62±0.81b	60.04±2.33a
Part (P)								
Basal	5.39±0.33b	0.23±0.01b	3.39±0.38b	0.11±0.01b	4.15±0.47b	65.04±1.46b	50.34±1.24b	61.66±2.10a
Distal	6.67±0.64a	0.33±0.01a	4.23±0.69a	0.16±0.01a	4.27±0.54a	69.42±1.59a	53.60±1.87a	53.67±1.39b
Significance								
C	***	***	***	***	***	***	***	***
P	***	***	***	***	*	***	*	***
C×P	***	*	***	***	**	***	**	*
CV. (%)	2.28	6.27	3.71	5.84	14.25	1.37	5.50	6.28

Data are expressed as mean ± SE (n = 12 for parts; n = 6 for cultivars). Means with different letters in the same column are significantly different ($p < 0.05$). *; **, ***, significant at P<0.05, 0.01 and 0.001, respectively. Sample abbreviations are provided in Table 1.

Table 4. Phytochemicals contents in banana central pseudo-stem juice by combination of cultivar and part.

Cultivar	Part	3 month					5 month				
		Phenolic (mg GAE/g)	Flavonoid (mg QE/g)	Tannin (mg/g)	Ascorbic acid (mg/g)	Sugar (mg/g)	Phenolic (mg GAE/g)	Flavonoid (mg QE/g)	Tannin (mg/g)	Ascorbic acid (mg/g)	Sugar (mg/g)
KHT	Basal	4.14±0.01b	0.59±0.01a	2.02±0.00c	0.35±0.00a	0.52±0.06d	3.61±0.01g	0.23±0.00de	1.40±0.01f	0.16±0.00b	1.59±0.04d
	Distal	2.91±0.02f	0.43±0.01cd	0.89±0.01ef	0.20±0.00b	0.33±0.03d	5.81±0.02d	0.35±0.02ab	3.49±0.03de	0.16±0.00b	2.05±0.07cd
KHM	Basal	2.65±0.01h	0.37±0.00e	0.85±0.00f	0.11±0.00h	0.50±0.01d	5.42±0.05e	0.22±0.01e	3.27±0.02e	0.07±0.00d	5.07±0.60ab
	Distal	2.81±0.01g	0.45±0.01c	0.93±0.02e	0.14±0.00f	0.40±0.03d	4.22±0.02f	0.33±0.02b	1.72±0.03f	0.12±0.00c	3.22±0.10c
KNW	Basal	3.22±0.02e	0.38±0.01e	1.60±0.00d	0.13±0.00g	1.29±0.01b	6.13±0.08c	0.26±0.01cd	4.07±0.00c	0.08±0.00d	4.70±0.26b
	Distal	4.04±0.02c	0.41±0.01cd	2.25±0.01b	0.14±0.00e	0.96±0.05c	6.58±0.13b	0.38±0.01a	3.79±0.00cd	0.23±0.01a	6.33±0.45a
KND	Basal	4.29±0.02a	0.50±0.02b	2.69±0.01a	0.17±0.00c	2.04±0.08a	6.40±0.14b	0.21±0.00e	4.81±0.01b	0.12±0.01c	5.23±0.27ab
	Distal	3.85±0.02d	0.39±0.01de	2.02±0.01c	0.16±0.00d	1.27±0.05b	10.01±0.05a	0.28±0.02c	7.94±0.01a	0.11±0.00c	5.49±0.49ab

Data are expressed as mean ± SE (n = 3). Means with different letters in the same column are significantly different ($p < 0.05$). Sample abbreviations are provided in Table 1.

The sugar content of BPJ was investigated because consumption of sugar-rich foods can increase postprandial blood glucose and insulin response. In this study, the amount of sugar in BPJ (less than 2.10 mg/g), as shown in Table 4, was remarkably lower than that in fruit juice as reported by Serpen (2012). The low sugar content of BPJ could be beneficial to diabetic patients.

Cultivar of BPJ affected its phytochemical profile. KND had the most phenolic compounds, tannins, and sugar at both of the two ages, whereas the most flavonoids and ascorbic acid were found in KHT at 3 months age, and in KNW at 5 months age (Tables 2 and 3). These results agree with Jorjong, Butkhup, and Samappito (2015) who reported that the variation in phytochemicals of *Antidesma bunius* L. was affected by cultivar.

Regarding the parts, plant part choice also affected the phytochemical profile. The content of phytochemicals in each part of BPJ was dependent on age of the plant. The basal part of BPJ at 3 months age had higher contents of all phytochemicals than the distal part, whereas the distal part had higher phytochemicals contents than the basal part at 5 months age (Tables 2 and 3). This may have been due to alterations in the physiology of the central pseudo-stem as a consequence of maturation. The phytochemical composition in BPJ was influenced by cultivar, part, and age, which in turn influences the bioactivities.

3.3 Antioxidant activity

Antioxidant activity was measured using DPPH assay. The DPPH· method measures reduction of radicals in solution in the presence of a hydrogen-donating antioxidant, which is one of the most important antioxidant mechanisms. There were significant main effects of cultivar and part, and an interaction of cultivar × part on the antioxidant activity (Tables 2 and 3). BPJ from all samples had antioxidant activity ranging from 58.72±0.72% to 82.50±0.14%. KHT had the highest radical scavenging activity at both ages of 3 and 5 months (Table 5). This finding agrees with the report of Bhaskar *et al.* (2012) who claimed that the pseudo-stem fraction of *Musa* sp. var. *elakki bale* had a high amount of antioxidants. The comparison between parts for each age

Table 5. Antioxidant activity of banana central pseudo-stem juice by combination of cultivar and part

Cultivar	Part	Radical scavenging (%)	
		3 month	5 month
KHT	Basal	82.50±0.14a	70.21±0.21b
	Distal	79.61±0.22b	77.79±0.29a
KHM	Basal	76.02±0.23e	69.16±0.31b
	Distal	75.72±0.09e	68.91±0.35bc
KNW	Basal	77.80±0.51c	58.72±0.72e
	Distal	76.91±0.21d	63.62±1.16d
KND	Basal	74.03±0.37f	62.09±0.21d
	Distal	72.88±0.19g	67.37±0.12c

Data are expressed as mean ± SE (n = 3). Means with different letters in the same column are significantly different ($p < 0.05$). Sample abbreviations are provided in Table 1.

showed the same trend as the phytochemical profile (Tables 2 and 3). This shows a relationship between the phytochemicals and antioxidant activity. From the current results, the BPJ had stronger antioxidant properties and also high secondary metabolites contents. Among the investigated cultivars, KHT had the highest radical scavenging activity and also large amounts of phenolic compounds, flavonoids, and ascorbic acid. Phenolic compounds, flavonoids, tannins, and ascorbic acid are known to be effective hydrogen donors, which gives them excellent antioxidant ability (Rice-Evans, Miller, & Paganga, 1997). It can be claimed that the antioxidant activity of BPJ resulted from these compounds that were detected in the BPJ.

3.4 Inhibition of α-amylase and α-glucosidase enzymes

One promising method in diabetes treatment is to retard the elevation of postprandial blood sugar levels by inhibiting the carbohydrate-digesting enzymes. The BPJ was found to exert moderate inhibition of α-amylase and α-glucosidase activities, which varied with cultivar, plant part, and plant age. Regarding cultivars, the highest inhibitory activities of α-amylase and α-glucosidase were found for KHT and KHM, respectively, at both ages (Tables 2 and 3). KHT is in the AAA group (*M. acuminata*), which is different from the three other cultivars, and KHM is in the ABB group (*M.* × *paradisiana*) as are KNW and KND. This suggests that the inhibitory activity of the two starch digestive enzymes depends on the species and cultivar. Interestingly, this is the first known report about the anti-diabetic property of pseudo-stem juice of *M. acuminata*. As regards the parts, BPJ from ages 3 and 5 months showed the same trend, with the distal part having a higher α-amylase inhibitory activity than the basal part, but with the basal part having a higher α-glucosidase inhibitory activity than the distal part (Tables 2 and 3). Regarding the interaction between cultivar and part, the BPJ had much stronger inhibition activity against α-glucosidase than against α-amylase. The strongest inhibition of α-amylase and α-glucosidase was found for the distal part of KHT and the basal part of KHM, respectively (Table 6). However, the most effective BPJ to be used in treatment of diabetes should be the basal part of KHM at 5 months age, because it had the strongest α-glucosidase inhibition (66.91±2.26%) and also a high level of α-amylase inhibition (55.82±2.65%), followed by the basal part of KND at 5 months age (Table 6).

The anti-diabetic properties pseudo-stem from some other varieties or cultivars of *Musa* have been reported, namely for *Musa* × *paradisiaca* (Nguyen *et al.*, 2017) and *Musa* sp. var. *elakki bale* (Bhaskar *et al.*, 2011). Furthermore, *M. paradisiaca* stem juice could reduce the blood glucose level of diabetic rats (Singh, Kesari, Rai, & Watal, 2007). A possible inhibition mechanism is that BPJ could bind with α-amylase and α-glucosidase to form a complex, which induces unfolding of the enzymatic structures of the two carbohydrate digestive enzymes (Wu *et al.*, 2018).

BPJ inhibited α-amylase and α-glucosidase enzymes, suggesting the presence of potential enzyme-inhibiting compounds in the extract. Previous studies have reported that polyphenols have potential to inhibit the α-amylase and α-glucosidase activities *in vitro* (Piparo *et al.*, 2008; Sheng *et al.*, 2014). In the structure of polyphenol, hydroxyl groups can form hydrogen bonds with the polar group of an enzyme and galloyl groups can bind the enzyme through hydrophobic association, which inactivates the enzyme (Asgar, 2013). The enzyme-inhibitory activities of BPJ might have resulted from the presence of phenolic compounds, flavonoids, and tannins. This is supported by Nguyen *et al.* (2017) who reported that *Musa* × *paradisiaca* stem juice contained several phenolic compounds (namely

Table 6. α-Amylase and α-glucosidase inhibitory activities of banana central pseudo-stem juice by combination of cultivar and part

Cultivar	Part	3 month		5 month	
		α-Amylase inhibition (%)	α-Glucosidase inhibition (%)	α-Amylase inhibition (%)	α-Glucosidase inhibition (%)
KHT	Basal	24.43±2.29ab	59.96±5.67a	50.09±1.24bc	51.34±1.00bc
	Distal	26.78±0.50a	50.43±1.37b	63.16±0.93a	47.34±1.81c
KHM	Basal	18.61±1.79d	61.42±0.44a	52.89±2.65b	66.91±2.26a
	Distal	22.51±0.82bc	59.99±0.27a	53.35±2.68b	57.42±0.28b
KNW	Basal	12.64±0.20e	42.56±2.79c	45.13±1.96c	64.63±2.51a
	Distal	19.09±0.78cd	37.18±1.87c	47.89±0.85bc	53.64±2.46bc
KND	Basal	23.82±0.80ab	54.27±1.20ab	53.26±0.73b	63.78±3.30a
	Distal	25.69±1.11ab	36.99±1.88c	49.98±0.30bc	56.29±1.48b

Data are expressed as mean ± SE (n = 3). Means with different letters in the same column are significantly different ($p < 0.05$). Sample abbreviations are provided in Table 1.

lupeol, ferulic acid, vanillic acid, *p*-hydroxybenzoic acid, *p*-coumaric acid, rutin, chlorogenic acid, gallic acid, caffeic acid, and nicotiflorin) and that these compounds possess anti-α-amylase and anti-α-glucosidase activities. In the current study, there was relatively high antioxidant activity in KHT through its high α-amylase and α-glucosidase inhibitory activities. This suggests that the ability of BPJ to inhibit α-amylase and α-glucosidase activities may have been related to antioxidant properties. This is supported by Arun, Thomas, Reshmitha, Akhil, and Nisha (2017) who claimed that dietary antioxidants can play a major role in the prevention and management of diabetes. Consequently, the present study revealed that the α-amylase and α-glucosidase inhibitory activities of BPJ were attributable to their phytochemical composition which is related to their antioxidant properties. This result agrees with Arun *et al.* (2017) who reported that the methanol extract of *M. paradisiaca* inflorescence was a good source of many important phenolic antioxidants, and that this extract exhibited significant antioxidant activity and inhibited both α-amylase and α-glucosidase.

3.5 Comparison between ages 3 and 5 months

In Thailand, the central pseudo-stem is commonly taken from a pre-flowering growth stage of banana for consumption. Therefore, bananas aged 3 and 5 months were selected and a t-test was performed to compare these two ages of BPJ. Older-aged BPJ had higher levels of phenolic compounds, tannins, and sugar compared to the age of 3 months, whereas the amount of flavonoids and ascorbic acid as well as the antioxidant activity were higher in the BPJ aged 3 months than at age 5 months (Table 7). These results indicate that the biosynthesis of polyphenols, especially of tannins, and the accumulation of sugar increased with maturation. Flavonoids and ascorbic acid presented at higher level at 3 months, maybe because these two compounds act as defensive compounds in young bananas and contribute to antioxidant activity. BPJ presented a higher α-amylase and α-glucosidase inhibition at age 5 months. This reveals that enzyme-inhibiting compounds were increased with maturation.

4. Conclusions

The profile of phytochemicals, antioxidant activity, and inhibition of α-amylase and α-glucosidase were influenced by cultivar, plant part and plant age. The contents of phytochemicals and antioxidant activity in each plant part was dependent on age. KND variety had the most phenolic compounds, tannins, and sugar, while KHT had the most flavonoids, ascorbic acid, and highest antioxidant activity. BPJ from older bananas inhibited α-amylase and α-glucosidase more than the younger one. The basal part of KHM at 5 months tended to be the potentially most effective one for diabetes treatment, because it had high levels of α-amylase and α-glucosidase inhibition. Moreover, this is the first report to show the anti-diabetic properties of pseudo-stem juice from *M. acuminata*. The obtained data could be utilized for pursuing beneficial health effects or for the development of BPJ-derived products with high levels of bioactive compounds.

Acknowledgements

This work was supported by the Research Promotion and Technology Transfer Center, Faculty of Liberal Arts and Science, Kasetsart University, Kamphaeng Saen Campus, Thailand.

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Table 7. Means comparison for phytochemicals contents, antioxidant activity and enzyme inhibition in banana central pseudo-stem juice by plant age

Parameter	Kluai Hom Thong			Kluai Hak Muk			Kluai Namwa			Kluai Namwa Dam		
	3 month	5 month		3 month	5 month		3 month	5 month		3 month	5 month	
Phenolic (mg GAE/g)	3.52±0.23	4.71±0.42	*	2.73±0.03	4.82±0.23	***	3.63±0.16	6.36±0.10	***	4.07±0.08	8.23±0.69	***
Flavonoid (mg QE/g)	0.51±0.03	0.29±0.02	***	0.41±0.01	0.27±0.02	**	0.40±0.01	0.32±0.02	*	0.45±0.02	0.25±0.01	***
Tannin (mg/g)	1.45±0.21	2.45±0.39	ns	0.89±0.02	2.50±0.30	***	1.92±0.12	3.93±0.08	***	2.36±0.13	6.38±0.59	***
Ascorbic acid (mg/g)	0.27±0.03	0.16±0.00	**	0.12±0.00	0.10±0.01	*	0.14±0.00	0.16±0.03	ns	0.16±0.00	0.12±0.00	***
Sugar (mg/g)	0.43±0.04	1.82±0.09	***	0.45±0.02	4.14±0.42	***	1.13±0.07	5.52±0.36	***	1.66±0.15	5.36±0.22	***
Radical scavenging (%)	81.05±0.56	74.00±1.44	**	75.87±0.11	69.03±0.18	***	77.36±0.27	61.17±1.06	***	73.45±0.27	64.73±1.00	***
α-Amylase inhibition (%)	25.60±0.99	56.63±2.54	***	20.56±1.05	53.12±1.43	***	15.86±1.26	46.51±0.96	***	24.76±0.63	51.62±0.69	***
α-Glucosidase inhibition (%)	47.59±2.85	49.34±1.09	ns	60.71±0.33	62.16±1.99	ns	39.87±1.63	59.13±2.47	***	45.63±3.37	60.04±1.96	*

Data are expressed as mean ± SE (n = 6). Ns, *; **, *** mean non-significant, significant at P<0.05, 0.01 and 0.001, respectively using t-test

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