Research Article

Phytochemical Profiling, Antioxidant, and Antityrosinase Activities of Bamboo Leaves in Thailand

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Abstract
Bamboo, an economic plant, has high genetic diversity and is distributed widely in many countries. The beneficial phytochemicals in bamboo leaves may vary according to genetic characteristics and harvesting season. Thus, in this study, the leaf phytochemical profiling of 27 bamboo genotypes popularly grown in Thailand was investigated during the summer and winter. The phytochemical contents in 60% ethanolic leaf extracts were analyzed using a spectrophotometer, thin layer chromatography (TLC), and reversed-phase high-performance liquid chromatography (RP-HPLC). Among the 27 genotypes, 11 had a high total phenolic and flavonoid content, as well as, high antioxidant and antityrosinase activity. Bamboo leaves were rich in natural antioxidants and antimelanogenic compounds, including chlorogenic acid, caffeic acid, isoorientin, orientin, *p*-coumaric acid, vitexin, isovitexin, and apigenin, and the major effective compound was different in each bamboo genotype. Moreover, harvesting bamboo leaves in the winter provided the highest quality yield. Therefore, both genotypes and harvesting seasons affect the leaf phytochemical profiling, and bamboo leaves appeared to be a valuable natural resource for the development of multiple pharmacological, cosmetic, and food supplement applications.

Keywords: Antioxidants, Antimelanogenic compounds, Bamboo Leaf Extract (BLE), Flavone C-glycoside (FCGs), Harvesting season

1 Introduction

Bamboo leaves are a rich source of antioxidative phytochemicals and have long been used as a traditional medicine in Japan and China [1]–[3]. BLE contains many effective compounds with nutraceutical potential and pharmacological functions, such as antimicrobial and antiviral, anti-inflammatory, antiatherosclerosis, and antityrosinase and anticancer effects, as well as improvement of obesity-related metabolic disorders; prevention of dementia, anxiety, and neuropsychiatric diseases; and improvement of radiation-induced genotoxicity [2]–[6]. Additionally, oral administration of BLE appeared to increase the levels of probiotics, modulate the cecal microbiome, alter the serum metabolome, and indirectly improve the antioxidant capacity and health status of Gallus gallus domesticus [3], [7]. Furthermore, the flavonoids present in BLE can be used as a potential therapeutic agent to alleviate skin aging in vitro and in vivo [8]. The major active ingredients of BLE are phenolic compounds that include several effective phytochemicals, such as flavonoids, lactones, and phenolic acids [1]. Examples of bamboo leaf flavonoids include FCGs (orientin, isoorientin, vitexin, and isovitexin), tricin, rutin, naringin, luteolin, apigenin, kaempferol, quercetin, rhamnetin, tamarixetin, anthocyanins, and other flavonoid derivatives, whereas the phenolic acids that are enriched in BLE are chlorogenic acid, caffeic acid, *p*-coumaric acid, and fumaric acid [2], [5], [9]. Thus, BLE could act as a natural antioxidant in light of its potent antioxidant activities, as well as a candidate for alleviating age-related diseases.

However, the phytochemical constituents,

antioxidant activity, and antityrosinase activity of BLE may vary depending on several factors, including plant genotypes and environmental factors [10]–[13]. The study revealed that the total flavonoid content (TFC), total phenolic acid content (TPA), and antioxidant activity of BLE (AOB) of 21 species of *Phyllostachys* varied among different genotypes and that AOB was positively correlated with TFC and TPA [10]. Additionally, the AOB and active phytochemical content of the leaves of two bamboo species were affected by the harvesting season, with orientin exhibiting the strongest correlation with the antioxidant capacity of bamboo leaves [12]. Various bamboo species (more than 1400 species in the subfamily Bambusoideae, family Poaceae) are distributed throughout the world [14]. In Thailand, approximately 80–100 bamboo species from 15–20 genera are distributed throughout the country [2]. However, there is a lack of information about their phytochemical constituents and their biological activities; therefore, this research aimed to study the effects of genotype and season variation on the phytochemical profiling, antioxidant activity, and antityrosinase activity of BLE from 27 bamboo genotypes that are popularly grown in Thailand using spectrophotometry, TLC, and RP-HPLC.

2 Materials and Methods

2.1 *Plant materials and extraction method*

The leaves of 27 different bamboo genotypes were collected from the Plant Genetic Conservation Project under the Royal Initiative of Her Royal Highness Princess Maha Chakri Sirindhorn, at Sirikit Dam, Tha Pla District, Uttaradit province, Thailand. All plant materials were confirmed and identified using the reference book, namely "Bamboo of Thailand" (Table 1) [15].

Table 1: Investigated leaves of 27 different bamboo genotypes

No.	Genera	Plant Scientific Name	Local Name	Abbreviation
1	*Bambusa*	*B. beecheyana Munro*	Pai Kim Sung	PKS
2		*B. lako* Widjaja	Pai Dum Timor	PDT
3		*B. longispiculata* Gamble ex Brandis	Pai Lummalok	PLM
4		*B. oliveriana* Gamble	Pai Hang Chang	PHC
5		*B. vulgaris* Schrader ex Wendland cv. Vittata McClure	Pai Lueang	PLU
6		*B. vulgaris* Schrader ex Wendland cv. Wamin McClure	Pai Namtao	PNT
7		*Bambusa* sp.	Pai Donwan	PDW
8		*Bambusa* sp.	Pai Kiewkhao Sa-ming	PKKS
9		*Bambusa* sp.	Pai Pong	PP
10	*Dendrocalamus*	*D. asper* (Schult.) Backer	Pai Tong Luemlang	PTLL
11		*D. copelandii*	Pai Matarwor	PMTW
12		*D. copelandii* (Gamble ex Brandis) N.H.Xia & Stapleton	Pai Mun Moo	PMM
13		*D. giganteus Munro*	Pai Giant	PG
14		*D. latiflorus Munro*	Pai Sang Kum	PSK
15		*D. membranaceus Munro*	Pai Sang Nuan	PSN
16		*D. sericeus*	Pai Sang Mon	PSM
17		*Dendrocalamus* sp.	Pai Dam Kwan	PDK
18		*Dendrocalamus* sp.	Pai Hea	PH
19		*Dendrocalamus* sp.	Pai Pakking	PPK
20		*Dendrocalamus* sp.	Pai Phamon	PPM
21		*Dendrocalamus* sp.	Pai Pok Pahma	PPP
22		*Dendrocalamus* sp.	Pai Yak Muang Nan	PYMN
23	*Phyllostachys*	*P. nigra* (Lodd. Ex Lindl.) Munro	Pai Dum	PD
24		*Phyllostachys* sp.	Pai Laii	PL
25	*Schizostachyum*	*S. pergracile* (Munro) R.B.Majumdar	Pai Khaolam	PKL
26		*Schizostachyum* sp.	Pai Khaolam Kab Deang	PKLD
27	*Thyrsocalamus*	*T. liang* Sungkaew & W.L. Goh	Pai Liang	PLI

Fresh leaves were randomly harvested in the hot season (March), with four biological replicates. Whole leaves from the primary and secondary branches at more than 2 m above the ground were harvested. After phytochemical analysis, the 11 bamboo genotypes that contained the highest content of phytochemicals and the strongest antioxidant activity were harvested again in the winter (December) from the same bamboo clump. All samples were dried in an oven at 60 °C for 3 days and subsequently extracted with 60% ethanol [13]. Briefly, dried leaf samples were ground with liquid nitrogen, 3 mL of 60% ethanol was added to 1 g of leaf powder, and the mixture was vortexed for 30 s before incubating in a water bath at 60 °C for 20 min. The supernatant was collected after a 10 min centrifugation step and re-extracted with 3 mL of solvent four times. The supernatants were pooled into one tube, and the volume was adjusted to 10 mL with 60% ethanol, followed by storage at 4 °C before further analysis.

2.2 *Determination of the total phytochemical content*

The total phenolic content (TPC), TFC, and total monomeric anthocyanin content (MAC) of BLE were estimated using UV–VIS spectrophotometry (Optizen 3220UV, Korea) [13]. Briefly, the Folin–Ciocalteu colorimetric method was used for the determination of the TPC of BLE. The absorbance was measured at 765 nm, and gallic acid was used as a standard. TPC was expressed as milligram of gallic acid equivalent per gram of sample dry weight (mg GAE gDW^{-1}). The TFC was analyzed using the aluminum chloride colorimetric method. The absorbance was measured at 415 nm, and the results were expressed as milligram of quercetin equivalent per gram of sample dry weight (mg QE gDW^{-1}). Additionally, the MAC was determined using the pH differential method. The absorbance of each reaction from pH 1.0 and pH 4.5 was measured at 510 and 700 nm using cyanidin-3-O-glucoside as a standard. All analyzed samples were from four biological replicates, and all analyses were performed using two technical replicates.

2.3 *Antioxidant activities*

First, the 2,2-diphenyl-1-picrylhydrazyl (DPPH) free radical scavenging assay was performed [13]. The results were expressed as the DPPH inhibition rate (%), 50% inhibitory concentration (IC_{50}), and Trolox equivalent antioxidant capacity (TEAC; mM Trolox gDW^{-1}). Second, a ferric reducing antioxidant power (FRAP) assay was performed to confirm the reliability of the results [16]. The FRAP solution was prepared by mixing 300 mM acetate buffer (pH 3.6), 10 mM 2,4,6-tris(2-pyridyl)-(S)-triazine, and 20 mM $FeCl_3 \cdot 6H_2O$ (10: 1: 1 v/v/v). Then, 0.15 mL of BLE was added to 2.85 mL of FRAP solution, followed by vortexing and incubation in a water bath at 37 °C for 30 min. The absorbance was measured at 593 nm, and the FRAP value was calculated using $FeSO_4 \cdot 7H_2O$ as a standard.

2.4 *Antityrosinase activities*

The tyrosinase inhibitory activity of BLE was measured [5]. Briefly, the mixture of 2.5 mM L-3,4-dihydroxyphenylalanine (L-DOPA) (0.5 mL) and 0.1 M sodium phosphate buffer pH 7.0 (0.9 mL) was boiled for 10 min before adding 100 µL of 5.0 mg mL^{-1} BLE. Then, 50 µL of 0.5 mg/mL tyrosinase (134 enzyme units) was added and mixed, and the absorbance at 475 nm was measured after 10 min of incubation. Standard compounds including kojic acid, butylated hydroxytoluene (BHT), and ascorbic acid at a concentration of 0.5 mg mL^{-1} were used to compare the antityrosinase activity of the mixture.

2.5 *Determination of FCGs using TLC*

The TLC technique was used for the rapid screening of FCG in BLE from 27 bamboo genotypes. Four FCGs were used as standards, and their stock solution was prepared at a concentration of 1 mg mL^{-1} as follows: isoorientin and isovitexin were dissolved in methanol, whereas orientin and vitexin were dissolved in methanol/water (90 : 10 v/v). A plate precoated with silica gel 60 F254 (Merck, Germany) was used to perform TLC according to the previous protocol [13].

2.6 *Phytochemical analysis using RP-HPLC*

BLE from the five bamboo genotypes that exhibited the highest phytochemical content and antioxidant activity and are popularly grown in Thailand were selected for RP-HPLC analysis [17]. The BLE was

evaporated and redissolved in methanol to obtain a final concentration of 10 mg mL^{-1}. The extract was then filtered using a 0.22 µm syringe filter, and 10 µL of the filtrate was injected into the RP-HPLC system. An Agilent HPLC 1260 Infinity II Bio-Inert system (Agilent Technologies, Germany) coupled with a binary solvent manager, an autosampler, a Waters 2996 Photodiode Array Detector, and an Agilent 5 TC-C18 (2) (250 × 4.6 mm) column were used in this study. The mobile phase was acetonitrile (A) and acetic acid/water (0.8: 100, v/v) (B). The gradient elution program was as follows: 0–2 min, A 10%, B 90%; 2–6 min, A 10%–14%, B 90%–86%; 6–16 min, A 14%–17%, B 86%–83%; 16–23 min, A 17%–19%, B 83%–81%; and 23–28 min, A 19%–40%, B 81%–60%. The flow rate was 1.0 mL min^{-1} at 25 °C, and detection was performed at 335 nm. Chlorogenic acid, caffeic acid, isoorientin, orientin, *p*-coumaric acid, vitexin, isovitexin, and apigenin were dissolved in methanol and were used as standards for this analysis. All samples were analyzed in triplicate.

2.7 *Statistical analysis*

The significance of the differences in the mean values and the standard deviation (or standard error in Table 2) were analyzed using Duncan's multiple range test of the SPSS software at $p < 0.05$.

3 Results and Discussion

3.1 *Bamboo leaves are rich in natural antioxidants and antimelanogenic compounds*

Oxidative stress and free radicals are generally known to be detrimental to human health and contribute to a broad spectrum of chronic diseases, such as cardiovascular disease, neurological disease, rheumatoid arthritis, diabetes, cancers, and other aging-related diseases [1], [18]. Antioxidants modulate oxidative stress by suppressing the formation of free radicals and reducing the initiation of free radicals through several mechanisms. Numerous compounds in BLE have antioxidant activity and consequently exert their potential of alleviating chronic diseases and age-related diseases [2], [3], [5], [16]. Moreover, it has been reviewed that the ethanolic extract from the leaves of bamboo is not toxic [19]. In this study, we found that BLE from different genotypes exhibited varying antioxidant ability and antityrosinase activity (Figure 1).

Among 27 bamboo extracts, BLE from 11 bamboo genotypes, i.e., PPP, PG, PLI, PKLD, PSN, PDK, PHC, PMM, PSM, PMTW, and PDW, had an IC_{50} of <0.56 mg mL^{-1}. The BLE from these 11 genotypes also showed high TEAC (0.80–1.34 mM Trolox gDW^{-1}), FRAP (82.85–95.97 mM Fe(II) gDW^{-1}), and antityrosinase inhibition (81.11%–97.33%) values and corresponded to the TPC (43.13–46.48 mg GAE gDW^{-1}) and TFC (4.13–4.32 mg QE gDW^{-1}) (Figures 1 and 2). However, the antioxidant activity was not correlated with the MAC found in small amounts in BLE (Figure 2).

Previously, based on the DPPH scavenging method, the IC_{50} of the BLE of nine bamboo genotypes harvested in Thailand ranged approximately from 0.7 to 4.5 mg mL^{-1} [13], whereas that of a 70% ethanolic extract from the leaves of *Gigantochloa atter*, *Dendrocalamus asper*, and *Gigantochloa verticillata* collected in Indonesia was 1.82, 1.02, and 0.57 mg mL^{-1}, respectively [20]. There has been reported that the ethanolic BLE of 20 species of *Phyllostachys* comprised abundant flavonoids (8.96–28.06 mg rutin equivalent gDW^{-1}) and phenolic acids (12.38–31.89

Table 2: The concentrations of eight effective phytochemicals in bamboo leaf extracts

BLE	Phytochemicals Content (mg/g)*							
	Chlorogenic Acid	Caffeic Acid	Isoorientin	Orientin	*p*-coumaric acid	Vitexin	Isovitexin	Apigenin
PLI	0.20 ± 0.01^{b}	0.22 ± 0.02^{c}	0.17 ± 0.01^{b}	2.82 ± 0.16^{b}	4.12 ± 0.32^{b}	2.42 ± 0.26^{c}	0.14 ± 0.00^{c}	0.30 ± 0.01^{a}
PKLD	0.35 ± 0.02^{b}	0.86 ± 0.18^{a}	0.93 ± 0.05^{a}	13.82 ± 1.59^{a}	0.62 ± 0.03^{cd}	11.65 ± 0.06^{b}	0.29 ± 0.01^{a}	ND
PSN	1.01 ± 0.06^{a}	0.77 ± 0.03^{a}	0.13 ± 0.01^{b}	1.57 ± 0.03^{b}	0.14 ± 0.01^{d}	4.01 ± 0.40^{c}	ND	0.27 ± 0.02^{ab}
PHC	0.31 ± 0.02^{b}	0.50 ± 0.04^{b}	ND	2.43 ± 0.2^{b}	5.27 ± 0.27^{a}	17.67 ± 1.82^{a}	0.11 ± 0.01^{c}	0.25 ± 0.02^{b}
PSM	0.96 ± 0.10^{a}	0.16 ± 0.01^{c}	0.13 ± 0.01^{b}	0.76 ± 0.08^{b}	0.97 ± 0.03^{c}	2.33 ± 0.32^{c}	0.18 ± 0.02^{b}	0.29 ± 0.01^{ab}

*Mean ± standard error (n = 3); ND = not detected; Different letters indicate significant difference of each phytochemical in 5 BLE ($p < 0.05$).

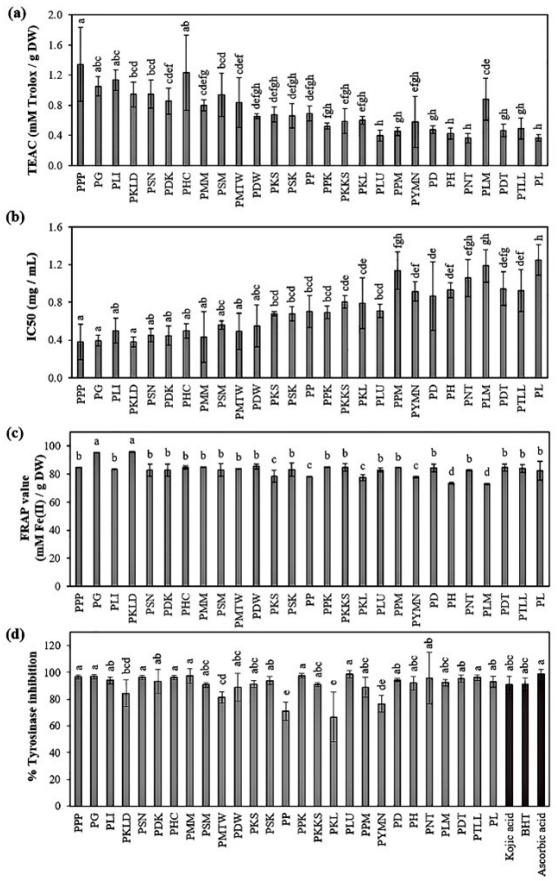

Figure 1: (a) Determination of TEAC, (b) IC_{50}, (c) FRAP value, and (d) %Tyrosinase inhibition in BLE of 27 bamboo genotypes. Data represent mean ± SD. (n = 4); Different letters indicate significant differences.

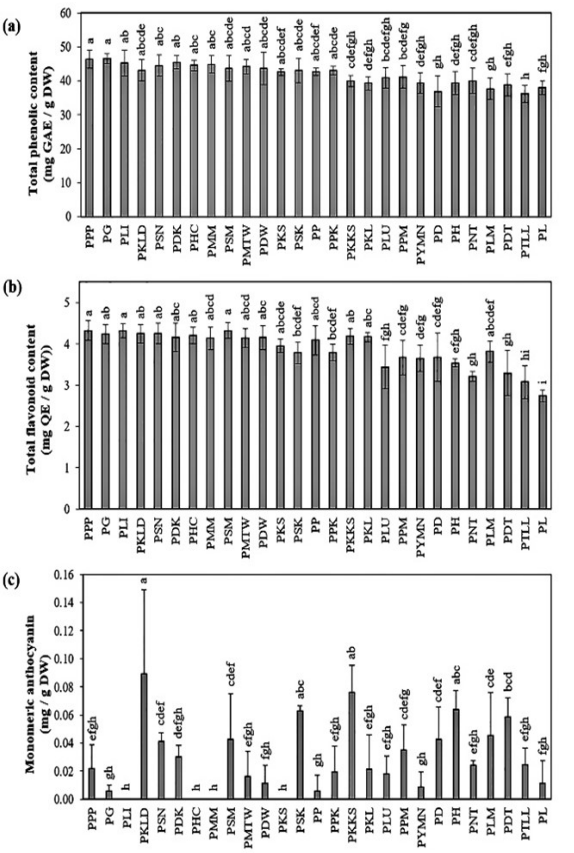

Figure 2: (a) Determination of TPC, (b) TFC, and MAC in BLE of 27 bamboo genotypes. Data represent mean ± SD. (n = 4); Different letters indicate significant differences.

mg GAE gDW^{-1}), whereas the IC_{50} of the BLE ranged from 0.52 to 2.83 mg mL^{-1} [10]. Additionally, the BLE of three medicinal bamboo genotypes harvested from different locations in China had an IC_{50} ranging from 1.25 to 10.17 mg mL^{-1} [21]. A lower IC_{50} indicates a higher antioxidant ability; thus, the BLE from the 11 genotypes examined in this study ($IC_{50} < 0.56$ mg mL^{-1}) exhibited high antioxidant activity compared with those in previous reports.

Moreover, the inhibitory effect of these samples (5 mg mL^{-1}) on tyrosinase activity, as assessed using L-DOPA as a substrate, was higher than 80% and was not significantly different from the standards, i.e., kojic acid, BHT, and ascorbic acid. Similarly, the BLE of *Phyllostachys bambusoides* at a concentration of 5 mg mL^{-1} inhibited tyrosinase activity and reduced melanin production [22]. Additionally, it was found that the BLE of *Phyllostachys nigra* var. henosis, which contains many types of flavonoids, such as catechin, chlorogenic acid, caffeic acid, and *p*-coumaric acid, exhibited antimelanogenetic activities by interfering with the phosphorylation of the intracellular protein kinase A/cAMP response element binding protein and subsequently suppressing the microphthalmia-associated transcription factor (MITF), thus resulting in the inhibition of melanogenic enzymes [23].

Our results suggest that the phytochemical content, antioxidant ability, and antityrosinase activity of the BLE were affected by genotypic variation and that the BLE may comprise diversified natural antioxidants

and skin-whitening compounds for the treatment of melanogenesis, such as isoorientin, ferulic acid, catechin, chlorogenic acid, caffeic acid, and *p*-coumaric acid [11], [23], [24]. Therefore, bamboo leaves are a rich source of natural antioxidants and antimelanogenic compounds that may potentially benefit food supplement, cosmetic, and pharmaceutical industries.

3.2 *The phytochemical profile of BLE is affected by genotype and harvesting season*

The phytochemical profile of BLE from 27 bamboo genotypes was rapidly investigated using a simple TLC technique [13]. Four active FCGs that are generally used as indicators for ensuring BLE quality, namely, orientin, isoorientin, vitexin, and isovitexin, were used as standards [25]. The FCG bands that appeared were assigned as having a very high content (++, yellow), high content (+, gray), and no detection (−, blue), and the data were clustered using MEV software, as shown in Figure 3. The results revealed that the genotype affected the profile of FCGs and that the major constituents in the leaves of most bamboo genotypes were orientin and isoorientin, similar to those reported by [11] and [25]. The total antioxidant capacity of orientin and isoorientin in a bamboo shavings extract was higher than that of vitexin and isovitexin [21], [26]. The BLE from the 11 bamboo genotypes that had the highest antioxidant capacity comprised at least two FCGs, i.e., orientin and isoorientin, with the exemption of PLI, which contained isoorientin and isovitexin, thus indicating that the leaves of these bamboos are a source of potent antioxidants (Figure 3).

Among these 11 genotypes, PLI, PKLD, PSN, PHC, and PSM, which are popularly grown in Thailand, were investigated for their effective phytochemical profiling using RP-HPLC (Table 2 and Figure 4). RP-HPLC is a precise and sensitive method that is commonly used for the simultaneous determination of the characteristic phytochemicals in bamboo leaves [13], [17], [21]. The results demonstrated that the major effective compound of each genotype was different (Table 2). Vitexin was the most abundant effective flavonoid in BLE of PSN, PHC, and PSM, which had vitexin content of 4.01, 17.67, and 2.33 mg g^{-1}, respectively, whereas orientin (13.82 mg g^{-1}) was enriched in PKLD and the major phenolic acid in BLE of PLI was *p*-coumaric acid (4.12 mg g^{-1}).

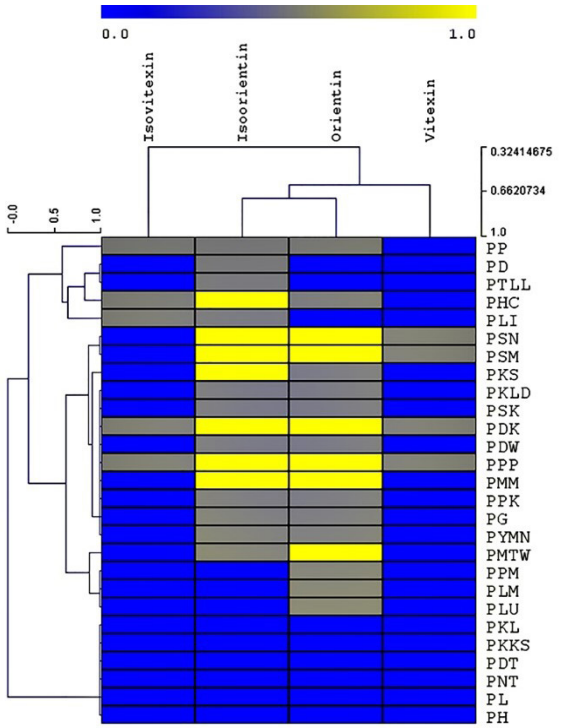

Figure 3: Comparative phytochemical profiling of FCGs appeared in BLE of 27 bamboo genotypes analyzed by TLC and the data was displayed as a heat map created by MEV software. Each row represents an individual bamboo genotype and each column represents an individual FCG. Yellow, gray, and blue indicate a very high content, high content, and no detection of FCGs, respectively.

In this study, eight effective phytochemicals, including chlorogenic acid, caffeic acid, isoorientin, orientin, *p*-coumaric acid, vitexin, isovitexin, and apigenin, were analyzed (Table 2).

Among these ingredients, caffeic acid exhibited stronger antioxidant ability than chlorogenic acid, ferulic acid, orientin, isoorientin, *p*-coumaric acid, vitexin, and isovitexin [26]. The chlorogenic acid content was highest in BLE of PSN (1.01 mg g^{-1}) and PSM (0.96 mg g^{-1}), whereas apigenin was richest in PLI, PSN, and PSM (approximately 0.3 mg g^{-1}). BLE of PHC had the highest content of *p*-coumaric acid (5.27 mg g^{-1}) and vitexin (17.67 mg g^{-1}). Notably, BLE of PKLD contained the highest content of caffeic acid (0.86 mg g^{-1}), isoorientin (0.93 mg g^{-1}), orientin (13.82 mg g^{-1}), and isovitexin (0.29 mg g^{-1}),

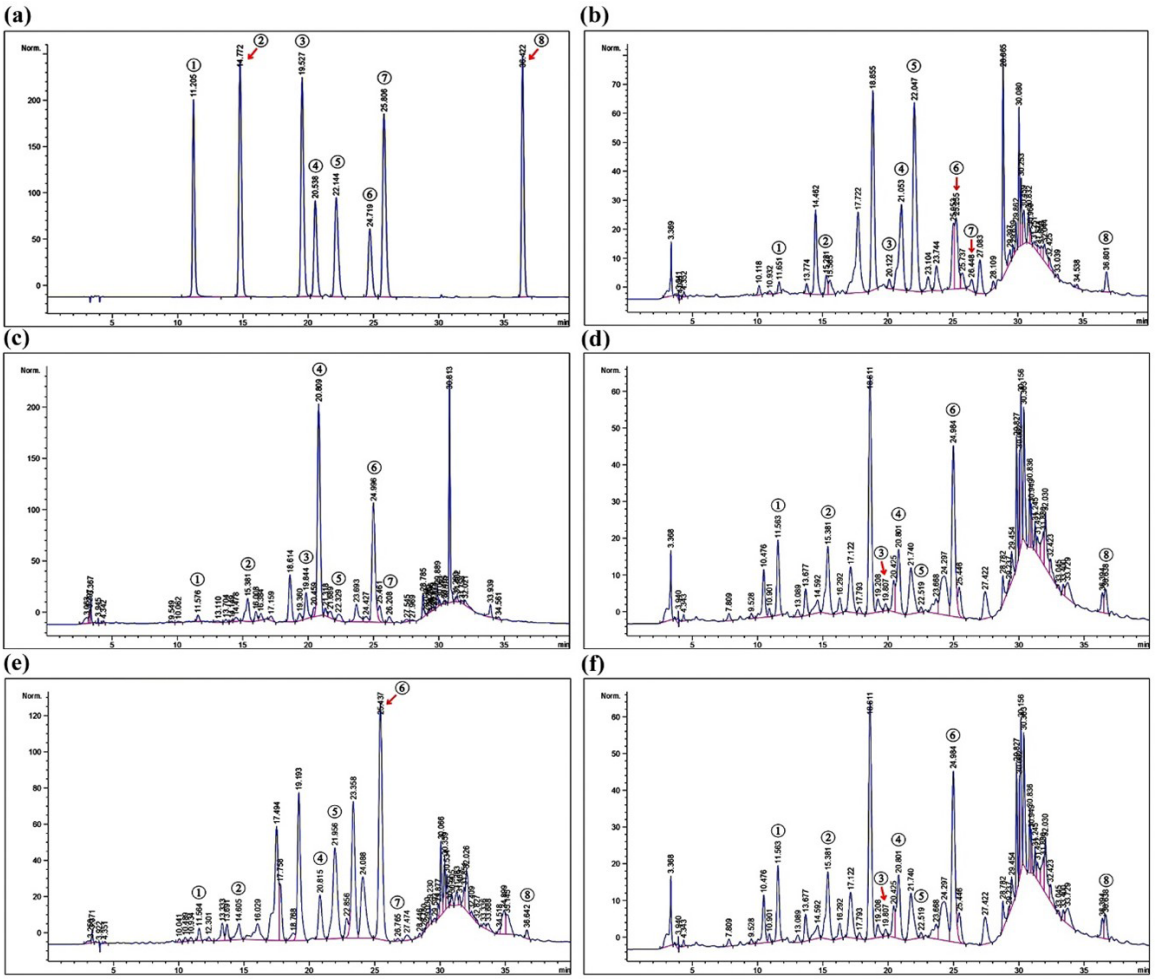

Figure 4: (a) Chromatogram of standards and (b) BLE from PLI, (c) PKLD, (d) PSN, (e) PHC, and (f) PSM. Peak numbers: (1) chlorogenic acid, (2) caffeic acid, (3) isoorientin, (4) orientin, (5) *p*-coumaric acid, (6) vitexin, (7) isovitexin, and (8) apigenin.

corresponding to the lowest IC_{50} (0.38 mg mL^{-1}).

However, the unidentified peak in Figure 4 suggested that BLE may comprise other active phytochemicals, as described in a previous study that showed that quercetin, rutin, luteolin, kaempferol, and tricin from BLE can be analyzed using liquid chromatography–tandem mass spectrometry [9]. Therefore, the BLE of the selected bamboo genotypes exhibited an abundance of strong antioxidants, among which different major active compounds contributed to its power for scavenging free radicals.

Not only genotype but also environmental factors and harvesting season affect plant phytochemical profiling [12], [27], [28]. Here, the leaves of the 11 bamboo genotypes that exhibited a high phytochemical content and high antioxidant capacity were again collected during the winter for the comparative study of their phytochemical content. The results demonstrated that the leaf samples collected in the winter had a higher amount of TPC, TFC, and FRAP values compared with those collected in the summer (Figure 5). The weather in Thailand consists of 6 months of rainfall during the rainy season (June to October), 3 months of dry and cooling breezes during the winter (November to February), and 3 months of heat during the summer (March to

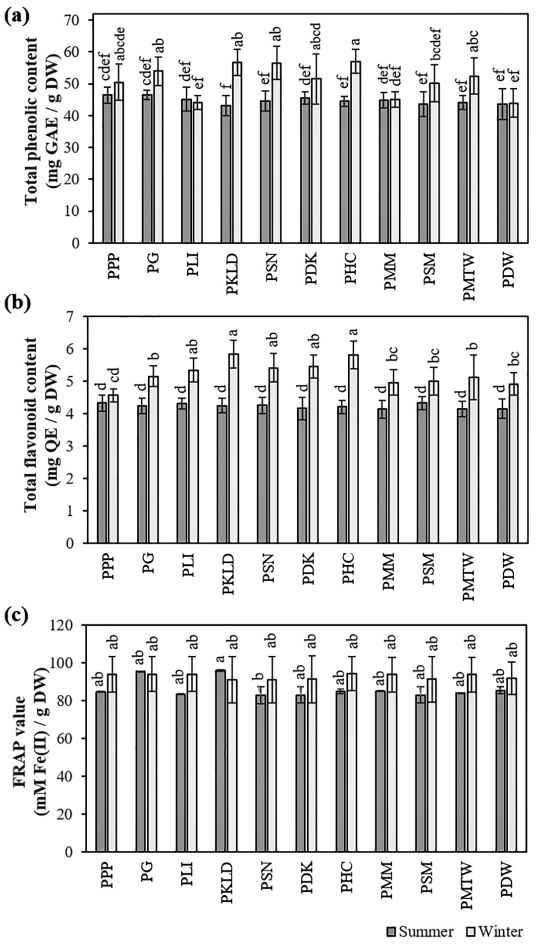

Figure 5: (a) Determination of total phenolic content, (b) Total flavonoid content, and (c) FRAP value of leaf extracts from 11 bamboo genotypes harvested during summer and winter. Data represent mean ± SD. (n = 4); Different letters indicate significant differences.

May). The average temperature in Thailand ranges from 18 °C to 38 °C. The samples from the summer were harvested in March, in which the average temperature was 30 °C–32 °C and the average precipitation was approximately 10–50 mm, whereas the samples from the winter were harvested in December, in which the average temperature was approximately 22 °C–24 °C and the average precipitation was approximately 1–10 mm (data not shown). These data suggest that the samples from the winter had a much stronger exposure to both drought and cold stress than the summer samples.

Previous studies reported that the TFC in seedlings of *Zea mays* L. was increased under water-deficit stress [9] and that cold stress induced the accumulation of both phenolics and flavonoids in Brassica rapa [28]. Moreover, under cold stress (4 °C), the seedlings of *Solanum lycopersicum* L. exhibited the highest quantities of TPC and TFC and strongest antioxidant capacity compared with those under heat stress treatments (45 °C and 50 °C) [27]. Taken together, these results indicate that the cold-stressed plants contain the highest amount of benzoic acid and quercetin [27]. Although some studies have shown that drought and cold stress affect the growth, flowering, photosynthesis, and protein expression of bamboo, evidence of changes in the effective antioxidant compounds in bamboo leaves was rarely found [29], [30].

In this study, the TPC and TFC levels in the winter samples, which experienced both drought and cold stress, were higher than those found in the leaves collected during the summer. Accordingly, the BLE from two bamboo species, namely, *Pleioblastus kongosanensis f. aureostriatus* and *Shibataea chinensis*, showed the highest level of total active components and antioxidant activity in winter, among which chlorogenic acid, caffeic acid, and isoorientin tended to increase in December to February of the next year [12]. Therefore, bamboo leaves harvested in the winter showed the highest quality yield.

4 Conclusions

Bamboo leaves are rich in natural antioxidants and anti-melanogenic compounds. TLC technique can be rapidly used for screening the quality of BLE while RP-HPLC is an accurate and sensitive method for determining the characteristic phytochemical compounds in BLE. BLE consists of more or less eight effective phytochemicals including chlorogenic acid, caffeic acid, isoorientin, orientin, *p*-coumaric acid, vitexin, isovitexin, and apigenin and the major effective compound of each bamboo genotype was different. Both genotypes and harvested seasons affect the phytochemical profiling in bamboo leaves and the phytochemical contents in samples harvested in the winter are higher than those found in the summer. Therefore, bamboo leaves are a rich source of natural antioxidants and could benefit the food supplement, pharmaceutical, and cosmetic

industries in the future.

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Author Contributions

S.K.: investigation, methodology, data curation; S.M.: conceptualization, investigation, data analysis, writing an original draft, reviewing and editing, funding acquisition, project administration. All authors have read and agreed to the published this version of the manuscript.

Conflicts of Interest

The authors declare no conflict of interest.

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