

## Sap Phytochemical Compositions of Some Bananas in Thailand

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Banana sap has some special properties relating to various phenomena such as browning of fruits after harvesting, permanent staining of cloth and fibers, and antioxidant and antibleeding properties. Analysis of banana sap using high-performance liquid chromatography–electrospray ionization–mass spectrometry (HPLC-ESI-MS) indicated the presence of phenolic and aromatic amino compounds of interest due to their special properties. With the online positive electrospray ionization mode (ESI), the possible structures of specific compounds were determined from the fragmentation patterns of each particular ion appearing in the mass spectra. The major compounds revealed from the sap of banana accessions, namely, *Musa balbisiana*, *Musa laterita*, *Musa ornata*, and *Musa acuminata*, and some cultivars were apigenin glycosides, myricetin glycoside, myricetin-3-*O*-rutinoside, naringenin glycosides, kaempferol-3-*O*-rutinoside, quercetin-3-*O*-rutinoside, dopamine, and *N*-acetylserotonin. The results indicated that there was a variety of phenolic and aromatic amino contents in many banana species. These compounds were reported to relate with biological activities. Moreover, the identities of these phytochemical compositions may be used as markers for banana diet, the assessment of physiochemical status, or the classification of banana clones.

**KEYWORDS:** Banana sap; polyphenols; flavonoids; *Musa*; LC-ESI-MS

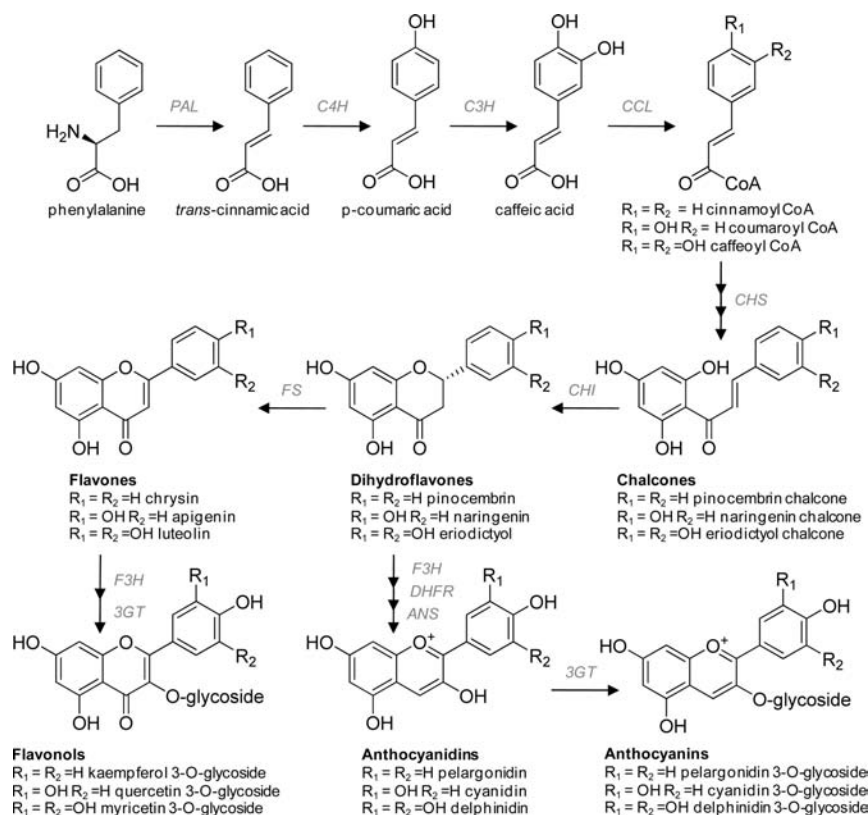
### INTRODUCTION

Banana (*Musa* spp.), a group of monocot plants in the family Musaceae, originated in South and Southeast Asia. Thailand is among the centers of their origins (1). Banana from these regions has been introduced to secondary loci such as Africa, Latin America, and the Pacific. Subsequently, it has been developing until modern times. Banana not only is a nutritious fruit for carbohydrates, vitamins, and minerals but also contains many secondary metabolites (2). Phenolic components, a group of secondary metabolites, play various roles in plants ranging from responses to physical stresses and protection against herbivores and diseases to bioactive compounds (3). In terms of human and livestock health, polyphenols were previously regarded as anti-nutritive because they can form complexes with proteins, leading to the reduction of digestibility (4). However, in recent years, these compounds have been highlighted for human health and widely used due to their possible health benefits. For example, banana extract has been used to treat diarrhea (5), to stop bleeding and promote wound healing (6), and as an antimicrobial or anti-inflammatory agent (7). In traditional uses, banana extract can be used as a mordant or brown color dye for clothes and fibers (8) and as an agent for converting animal hides to leather (9).

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Nowadays, the very large number of compounds from various plant extracts could be analyzed by reverse-phase high-performance liquid chromatography (HPLC). More than 8,000 phenolic compounds have been identified in plants (10). Naturally, they are flavonoids, which include flavanones, flavones, flavanols, flavonols, isoflavones, and anthocyanins. The flavonoids are the largest group of phenolics. Over 4,000 chemical structures have been elucidated (4). The other groups of phenolics are phenolic acids, lignans, and stilbenes (11). The majority of pigments in most flowers and fruits are anthocyanins in the flavonoid group (12). Flavonoids are synthesized via a specific flavonoid pathway using phenylalanine as the precursor (Figure 1). Flavonoid biosynthesis has been extensively studied in several plant species. In this study, preliminary analysis of the chemical compositions in sap, as the representative fluid of the whole plant, revealed high levels of phenolic and aromatic amino compounds together with significant amounts of lipids, sugars, and proteins (data not shown). To unveil the phytochemical contents, sample preparation was needed.

A major problem to retain sap properties was an enzymatic browning and thickening of banana sap due to an oxidation reaction catalyzed by polyphenol oxidase (PPO) enzyme (13). To stabilize the sap, a preservative solution containing NaCl, ascorbic acid, citric acid, and Na<sub>2</sub>S<sub>2</sub>O<sub>5</sub> was required (14, 15). Characterization of phenolic and amino compounds was determined in *Musa balbisiana* Colla, *Musa laterita* Cheesman, *Musa ornata*



**Figure 1.** Schematic presentation of the flavonoid biosynthesis pathway, modified from Fowler et al. (37), with emphasis on the chalcones, dihydroflavonols, flavones, flavonols, anthocyanidins, and anthocyanins. Enzyme abbreviations: PAL, phenylalanine ammonium-lyase; C4H, cinnamate 4-hydroxylase; C3H, cinnamate 3-hydroxylase; CCL, coumaroylCoA ligase; CHS, chalcone synthase; CHI, chalcone isomerase; F3H, flavonols 3-hydroxylase; DHFR, dihydroflavonols reductase; ANS, anthocyanin synthase; 3GT, 3-glycosyl transferase; FS, flavone synthase.

Roxb., *Musa acuminata* Colla, and some cultivars by reverse-phase HPLC and MS, respectively, to clarify special properties of banana sap, specific physicochemical roles, and phytochemical classification to each group of bananas and, also, to apply as food markers in human and animal consumption.

## MATERIALS AND METHODS

**Plant Materials.** For each accession, banana sap samples were collected from cut rachis, after the male bud appeared from the different plants in the same clump, two or three separated times in about one or two years after sucker planting. All bananas were gathered from a private ex situ germplasm collection in Nakorn Pathom, Thailand. Voucher specimens were identified on the basis of Simmonds (1) and Cheesman (16) and deposited at the Suan Luang Rama IX Herbarium, Bangkok, Thailand. Details of the 22 accessions used, shown in Table 1, were based on Atawongsa (17), Wongniam (18), and Kitdamrongsont (19).

**Reagents and Standards.** All reagents including HPLC and MS grade solvents were purchased from Fisher Scientific (Waltham, MA), whereas standards catechin and dopamine hydrochloride were from Sigma-Aldrich (St. Louis, MO).

**Sample Collection.** Sap from freshly cut rachis was collected in 80% ethanol containing 100 mM NaCl, 0.2 mM ascorbic acid, 40 mM citric acid, 0.1 mM Na<sub>2</sub>S<sub>2</sub>O<sub>5</sub>, 0.25% Triton X-100, and 0.2 mM EDTA as antioxidants and inhibitors of PPO. The sap sample was heated at 80 °C for 30 min and then centrifuged at 11250g for 15 min at 25 °C. The supernatant was collected and concentrated by an evaporation process (rotary evaporator, Buchi Rotavapor R-205 (Flawil, Switzerland)). The remainder was resolubilized with 80% ethanol to 2 mL, flushed with N<sub>2</sub> gas, and stored at 4 °C under airtight and dark conditions for further analysis.

The stored samples were filtered through a 0.45 μm nylon membrane (Millex, Millipore (Billerica, MA)). Total phenolic and aromatic amine amounts of all samples were equalized to 0.01 mmol equivalent to catechin standard by Prussian Blue Assay (4) prior to injection into HPLC.

**HPLC Analysis.** The analysis was carried out for each accession three times from different batches of sample preparations with HPLC (Waters 717+ HPLC system equipped with pump model 600 and photodiode array detector (PDA) model 2996 (Milford, MA)). An aliquot of 50 μL of sample was injected into a reverse phase column (3.0 × 250 mm, 5 μm, Waters Spherisorb ODS2 Cartridge) using the solvents 0.1% TFA in acetonitrile (A) and 0.1% TFA in water (B). A flow rate of 1.0 mL/min was used, and UV wavelengths of 280, 320, and 370 nm (1.2 nm bandwidth) were used to detect the compounds. The analysis was performed at 25 °C in the linear gradient elution mode, using 8% A to 60% A in 50 min and then up to 95% within 5 min. For the next 10 min, the eluant was returned to 8% A to equilibrate the column at the initial condition for the next injection.

**Mass Spectrometry.** Mass spectral data were obtained by the MS system (Bruker Daltonics 3300+ (Bremen, Germany)) with electrospray ionization in positive mode (ESI+) with a quadrupole ion trap mass analyzer (resolution  $m/z$  1.0). The ion parameters were 3 kV for capillary and 10 psi for nebulizer. The desolvation temperature was 200 °C, and the electrospray probe flow was adjusted to 1.5 L/min. The voltage settings were as follows: skimmer, 40 V; cap exit, 121.0 V; Oct1 DC, 9.0 V; Oct2 DC, 1.10 V; trap drive, 40; Oct RF, 100 Vpp; lens 1, -10 V; and lens 2, 35 V. Continuous mass spectra were recorded over the range  $m/z$  100–1000, and time accuracy was 200 ms.

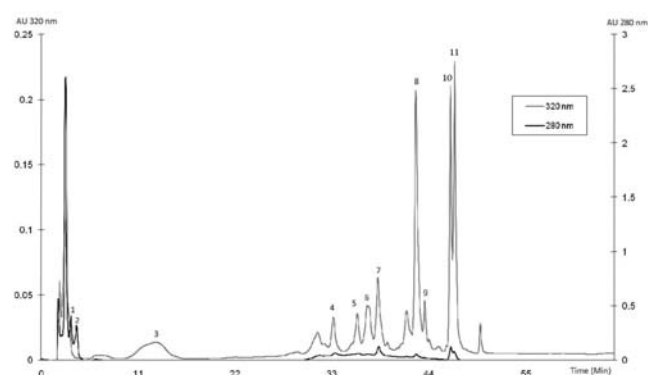
## RESULTS AND DISCUSSION

**HPLC-UV Photodiode Array Detection (PDA).** Eleven peaks were characterized from all samples (Figure 2). The sap samples from each accession were collected two to three times. Each accession from different collecting times had the same peak pattern on HPLC chromatogram, but absolute phytochemical concentrations and amounts of collected saps varied due to uncertainty of absolute sap volume in each collection and uncertainty of the remaining amount after sample preparation. However, the steady proportion level of these compounds may reflect needs to maintain

**Table 1.** Banana Accessions in Groups Based on Similar Chromatograms in both Peak Numbers and Retention Times

chromatogram profile	accession no. <sup>a</sup>	source province
<i>M. acuminata</i> subsp. <i>siamea</i> 1 <sup>b</sup>	96	Loei
	247	Kanchanaburi
<i>M. acuminata</i> subsp. <i>siamea</i> 2 <sup>b</sup>	7	Nakhon Ratchasima
	132	Nakhon Ratchasima
	147	Prachuap Khiri Khan
<i>M. acuminata</i> subsp. <i>siamea</i> with yellow bracts <sup>b</sup>	27	Kamphaeng Phet
	173	Nakhon Ratchasima
	177	Chanthaburi
<i>M. acuminata</i> subsp. <i>malaccensis</i> 1 <sup>c</sup>	113	Nakhon Sithammarat
	141	Surat Thani
<i>M. acuminata</i> subsp. <i>malaccensis</i> 2 (Kra Isthmus) <sup>c</sup>	107	Chumphon
	111	Krabi
	139	Phangnga
<i>M. balbisiana</i>	117	Surat Thani
	135	Phetchaburi
	178	Chanthaburi
<i>M. laterita</i>	57	Kamphaeng Phet
	240	Kanchanaburi
<i>M. ornata</i>	45	Kamphaeng Phet
<i>Musa</i> (AA) 'Kluai Nuea Thong' <sup>d</sup>	68	Kamphaeng Phet
<i>Musa</i> (ABB) 'Kluai Namwa Suan' <sup>d</sup>	166	Nakhon Pathom
<i>Musa</i> (AAA) 'Kluai Nak Yak' <sup>d</sup>	189	Kanchanaburi

<sup>a</sup> Accession numbers are assigned by S. Swangpol and J. Somana as described in Atawongsa (17), Wongniam (18), and Kitdamrongson (35). <sup>b</sup> *M. acuminata* subsp. *siamea* can be divided into three groups: types 1 and 2 and that with yellow bracts (34). <sup>c</sup> *M. acuminata* subsp. *malaccensis* can be divided into two forms: typical and Kra Isthmus (17). <sup>d</sup> *Musa* cultivars (18).



**Figure 2.** Chromatograms at 280 and 320 nm of phenolic compositions in mixed samples: 1, dopamine; 2, *N*-acetylserotonin; 3, caffeoylquinic acid; 4, myricetin-3-*O*-rutinoside; 5, myricetin glycoside; 6, kaempferol-3-*O*-rutinoside; 7, quercetin-3-*O*-rutinoside; 8, naringenin glycoside I; 9, apigenin glycoside I; 10, naringenin glycoside II; 11, apigenin glycoside II. (Unlabeled peaks are unknown artifacts.)

some specific physiological functions in each banana accession. Analyses from different accessions in the same subspecies also showed similar chromatograms peak numbers, amount proportions between each peak, and retention times with special remarks for *M. acuminata* subsp. *siamea* and *M. acuminata* subsp. *malaccensis* (Table 1). Because of the differences in the chromatograms of sap and anthocyanin profiles in male bracts of

*M. acuminata* subsp. *siamea*, this subspecies can be divided into three groups, type I, type II, and yellow bract, and *M. acuminata* subsp. *malaccensis* can be divided into a typical form and Kra Isthmus form (19). The same compounds were identified from different samples by comparing their retention times and UV spectra. For structure identification, a particular peak compound was interpreted on the basis of its UV spectrum (not shown) and its MS<sup>n</sup> fragmentation pattern (Table 2).

**Identification of Phytochemical Structure.** Characterization of phytochemical components from banana sap based on UV spectra from PDA of HPLC data and determined structures from MS<sup>n</sup> fragmentation pattern are compared to previous studies in Table 2. Among the phenolics, for instance, hydroxycinnamic acids, dopamine, and *N*-acetylserotonin were detected. With regard to flavanones, isoflavones, and flavonols, fragmentation of the *O*-glycosidic bonds provided information about the nature of their glycosides by subtraction of the fragment mass from the protonated molecular ion (M + H)<sup>+</sup> mass. ESI-MS spectra showed a high-intensity peak corresponding to the protonated aglycone moiety (A + H)<sup>+</sup>.

**Hydroxycinnamic Acids.** The hydroxycinnamic acid structure contains the cinnamoyl system, which is responsible for the band I absorption (310–325 nm) seen in the UV spectra. Caffeoylquinic acid showed its *m/z* 355 and fragment corresponding to cinnamoyl molecule (cinnamoyl ion)<sup>+</sup> due to the loss of esterified quinic acid at *m/z* 163 and 147, respectively (20, 21).

**Flavanones.** The protonated molecular ions of two apigenin glycosides were *m/z* 679 and 721. Apigenin was detected as aglycone with protonated molecular ion at *m/z* 271. The loss of *m/z* 162 of both glycosides at MS<sup>3</sup> fragmentation highly suggested the hexose residue, possibly glucose (22). Naringenin glycoside established the protonated molecular ion at *m/z* 674. MS<sup>2</sup> of *m/z* 674 showed the *m/z* 351 and 309 fragments. Naringenin exhibited the protonated molecular ion at *m/z* 273 at MS<sup>3</sup> fragmentation. The *m/z* 309 was suggested to be the rutinoside residue. This fragmentation pattern was the same as for *m/z* 716 (23).

**Flavonols.** Flavonols are distinguished from flavan-3-ol and dihydrochalcones by their UV spectra. Flavan-3-ol and dihydrochalcones typically exhibit an absorption band around 240–280 nm (band II) and do not present an absorption band at region 350–370 nm (band I). Band I is considered to be associated with the B-ring, cinnamoyl system, and band II with the A-ring, benzoyl system. In flavonols with a substituted 3-hydroxyl group, band I is in the region of 328–357 nm. Flavan-3-ols and dihydrochalcones are readily distinguished from flavonols by their UV spectra; the former typically exhibit an intense band II (275–285 nm) and do not present band I as a result of their lacking any conjugation between the A- and B-rings. Three naringenin derivatives, aglycone kaempferol, quercetin, and myricetin, were detected with the glycosides. Kaempferol-3-*O*-rutinoside, quercetin-3-*O*-rutinoside, and myricetin-3-*O*-rutinoside were identified. The protonated molecular ions at MS<sup>3</sup> fragmentation were *m/z* 287, 303, and 319, respectively (20, 24). The loss of *m/z* 308 of each flavonol was referred to the rutinosyl group, a disaccharide of glucose and rhamnose. The rutinosyl cation, which was revealed as the *m/z* 309 fragment, followed by cleavage of the glycosidic bond between glucose and rhamnose: first, loss of *m/z* 146 (rhamnosyl), followed by a loss of *m/z* 162 (glucosyl). Moreover, myricetin glycoside was found in the sap of *M. balbisiana*. The parent protonated molecular ion was *m/z* 633, the daughter molecular ion of the MS<sup>2</sup> was *m/z* 481 and that of the MS<sup>3</sup> was *m/z* 319. The loss of *m/z* 162 was again suggested to be a glucose residue.

**Dopamine and *N*-Acetylserotonin.** These are well-known animal neurotransmitters and hormones. These two compounds were eluted at an early part of the HPLC chromatogram. The protonated molecular ions were *m/z* 154 and 220, respectively (25).

**Table 2.** Phytochemical HPLC-ESI-MS Data (*m/z*) Obtained from Banana Sap<sup>a</sup>

polyphenol	<i>t<sub>R</sub></i> (min)	(M + H) <sup>+</sup>	(M + H - 146) <sup>+</sup>	(A + H) <sup>+</sup>	(C + H) <sup>+</sup>	ref
<b>hydroxycinnamic acids</b>						
1. caffeoylquinic acid	13.0	355			163	20, 21
<b>flavones</b>						
2. apigenin glycoside I <sup>b</sup> ( <i>unknown</i> )	42.6	679		271		22
3. apigenin glycoside II <sup>c</sup> ( <i>unknown</i> )	46.9	721		271		22
4. naringenin glycoside I <sup>d</sup> ( <i>unknown</i> )	42.5	674		273		23
5. naringenin glycoside II <sup>e</sup> ( <i>unknown</i> )	46.5	716		273		23
<b>flavonols</b>						
6. myricetin-3- <i>O</i> -rutinoside	33.2	627	481	319		20, 24
7. myricetin glycoside <sup>f</sup> ( <i>unknown</i> )	35.4	663		319		20, 24
8. kaempferol-3- <i>O</i> -rutinoside	35.6	595	449	287		20, 24
9. quercetin-3- <i>O</i> -rutinoside	35.8	611	465	303		20, 24
<b>others</b>						
10. dopamine	3.5	154				standard
11. <i>N</i> -acetylserotonin	4.1	220				25

<sup>a</sup> (M + H)<sup>+</sup> = molecular ion, (A + H)<sup>+</sup> = aglycone ion, (C + H)<sup>+</sup> = cinnamoyl ion. <sup>b</sup>MS<sup>2</sup> (679), 433 (A + H + 162)<sup>+</sup>; MS<sup>3</sup> (433), 271. <sup>c</sup>MS<sup>2</sup> (721), 433 (A + H + 162)<sup>+</sup>; MS<sup>3</sup> (433), 271. <sup>d</sup>MS<sup>2</sup> (674), 351, 309; MS<sup>3</sup> (351), 273. <sup>e</sup>MS<sup>2</sup> (716), 351, 309; MS<sup>3</sup> (351), 273. <sup>f</sup>MS<sup>2</sup> (663), 481 (A + H + 162)<sup>+</sup>; MS<sup>3</sup> (481), 319.

**Table 3.** Phytochemical Contents in Banana Sap

scientific name <sup>b</sup>	accession no. <sup>b</sup>	identifiable content <sup>a</sup> (%)										
		1	2	3	4	5	6	7	8	9	10	11
<i>Musa acuminata</i> subsp. siamea 1	96	18.55	31.45	0.00 <sup>c</sup>	28.50	0.00	0.00	21.50	0.00	0.00	0.00	0.00
	247	17.22	32.79	0.00	22.50	0.00	0.00	27.50	0.00	0.00	0.00	0.00
<i>Musa acuminata</i> subsp. siamea 2	7	15.97	33.93	0.00	2.69	0.00	2.89	1.15	2.94	0.00	21.96	18.46
	132	15.39	34.26	0.00	4.37	0.00	8.94	2.98	4.77	0.00	17.38	11.92
	147	18.00	32.00	0.00	24.00	0.00	23.50	1.20	1.00	0.00	0.10	0.20
<i>Musa acuminata</i> subsp. siamea (yellow bracts)	27	28.50	21.50	0.00	5.50	0.00	0.00	0.00	0.00	5.50	24.00	15.00
	173	37.67	19.09	0.00	17.54	0.00	0.00	0.00	0.00	18.06	2.84	4.80
	177	17.62	34.76	0.00	23.81	0.00	0.00	0.00	0.00	23.81	0.00	0.00
<i>Musa acuminata</i> subsp. malaccensis 1 (typical form)	113	24.50	25.50	0.00	8.50	0.00	17.50	15.50	0.00	0.00	0.00	8.50
	141	30.00	20.00	0.00	8.00	0.00	8.00	34.00	0.00	0.00	0.00	0.00
<i>Musa acuminata</i> subsp. malaccensis 2 (Kra Isthmus form)	107	25.50	24.50	0.00	13.50	0.00	0.00	5.50	31.00	0.00	0.00	0.00
	111	33.00	17.00	0.00	41.00	0.00	0.00	4.50	4.50	0.00	0.00	0.00
	139	21.50	28.50	0.00	45.00	0.00	0.00	5.00	0.00	0.00	0.00	0.00
<i>Musa balbisiana</i>	117	0.00	0.00	45.00	10.00	45.00	0.00	0.00	0.00	0.00	0.00	0.00
	135	0.00	0.00	40.00	20.00	40.00	0.00	0.00	0.00	0.00	0.00	0.00
	178	0.00	0.00	24.00	75.00	1.00	0.00	0.00	0.00	0.00	0.00	0.00
<i>Musa laterita</i>	57	25.00	25.00	0.00	39.50	0.00	0.00	0.00	0.00	0.00	10.50	0.00
	240	24.50	25.50	0.00	40.50	0.00	0.00	0.00	0.00	0.00	9.50	0.00
<i>Musa ornata</i>	45	25.50	24.50	0.00	21.00	0.00	0.00	29.00	0.00	0.00	0.00	0.00
<i>Musa</i> (AA)	68	53.73	0.00	0.00	15.05	0.00	0.00	3.82	0.00	19.88	0.00	7.52
<i>Musa</i> (ABB)	166	51.81	0.00	0.00	16.58	0.00	0.00	3.64	0.00	20.72	0.00	7.25
<i>Musa</i> (AAA)	189	50.20	0.00	0.00	16.06	0.00	0.00	3.87	0.00	22.59	0.00	7.28

<sup>a</sup> Calculated from absorbance units by combination of signals from both 280 and 320 nm. 1, dopamine; 2, *N*-acetylserotonin; 3, caffeoylquinic acid; 4, myricetin-3-*O*-rutinoside; 5, myricetin glycoside; 6, kaempferol-3-*O*-rutinoside; 7, quercetin-3-*O*-rutinoside; 8, naringenin glycoside I; 9, apigenin glycoside I; 10, naringenin glycoside II; 11, apigenin glycoside II.

<sup>b</sup> The same references as in Table 1. <sup>c</sup> Not detectable or <0.005%.

Dopamine was identified by comparison to the standard dopamine as well as by the spiking technique.

**Determination of Phytochemical Contents in Banana Sap.** The phytochemical contents in bananas was based on HPLC profiles monitored at 280, 320, and 370 nm (bands I and II). By inhibiting the functions of PPO, flavonoids should not undergo polymerization or become condensed tannins. Groups of accession numbers, which define the same species or subspecies, showed similar chromatograms in both HPLC and MS profiles. The identified compounds and proportions are shown in Table 3.

These results are consistent with phytochemical studies and can account for some special properties. Dopamine was previously reported in the banana latex vessels and parenchyma cells during the green tip stage to yellow stage of fruit ripening (26). Dopamine, mostly over 15% in the sap (Table 3), is a potent vasoconstrictor, which clarifies the stop-bleeding properties of banana sap in ethnobotanical uses (27, 28). *N*-Acetylserotonin was also reported in many foods including banana (26). *N*-Acetylserotonin and a further metabolite, serotonin, were suggested to be involved in melatonin production in the ripening period. However, actual

biological functions of dopamine and *N*-acetylerotonin in bananas are still unclear. Caffeoylquinic acid or chlorogenic acid is an important intermediate in lignin biosynthesis and mainly contributes antioxidant activity in many plants (29). Naringenin was believed to have a role as an anti-estrogen and a blood cholesterol-lowering effect in human (30). Kaempferol and quercetin showed an activity in anti-inflammation and antivenom effects (31). Quercetin-3-*O*-rutinoside also helped in strengthening capillaries and stopping bleeding (32). Apigenin has been reported to inhibit many types of cancer cell lines by promotion of cell cycle arrest and apoptosis (33). Finally, myricetin was linked to reduced risk of neurodegenerative diseases such as Alzheimer's disease (34).

The result partially corresponded to taxonomic classification of the *Musa* species. All species contain a myricetin-3-*O*-rutinoside (Table 3), which may be used as a food marker for banana. Sap from all *M. acuminata* subspecies, *M. laterita*, and *M. ornata* contains dopamine and *N*-acetylserotonin. The phytochemical profile in *M. ornata* sap is similar to that in *M. acuminata* subsp. *siamea* 1 but differs in proportions among each compound (Table 3). *M. balbisiana* has unique phenolic compounds among other bananas, which were caffeoylquinic acid and myricetin glycoside (unknown glycoconjugated). *M. acuminata* with yellow bracts, but having no anthocyanin in the bracts (35), has a distinct phenolic pattern compared to other *M. acuminata*. It has myricetin-3-*O*-rutinoside and apigenin glycoside I as in cultivated bananas, which suggested the ability to synthesize flavones and flavonols but unable only for anthocyanins (Figure 1). *M. acuminata* accessions from the same subspecies also have diversity in phytochemical profiles. This phenomenon possibly caused variation among subpopulations in the subspecies from different geographical distributions, which can be detected by differences not only in flavonoid metabolic profiles but also in their morphologies as between *M. acuminata* subsp. *malaccensis* typical and Kra Isthmus forms. The other reason might be due to variation only in flavonoid metabolism but cannot be distinguished by morphologies except for the color from anthocyanin as in *M. acuminata siamea* types 1 and 2 and the yellow bract. Cultivated bananas also have their own phytochemical profiles, which are the same among them regardless of different genome designations. However, these were different from wild bananas in Thailand and might be the dominant influence of the A genome of *M. acuminata* parental side from other regions outside Thailand (35, 36).

In summary, the combination of HPLC-ESI-MS allowed the characterization of phytochemical compositions in banana sap as the representative fluid of the whole plant. This study demonstrated the occurrence of dopamine, *N*-acetylserotonin, hydroxycinnamic acids, two apigenin glycosides, two naringenin glycosides, and four flavonol glycosides. Most of the glycosides identified were conjugated to the sugar; glucose and rhamnose. These findings have impact on further research such as quality control for food authenticity and potential sources for polyphenols and aromatic amines. The results of this study also suggested the possibility of developing a marker system for classification of banana clones and assessment of the physiological status and roles of each functional component in the banana sap. However, further investigations such as metabolic regulation and production of phenolic and aromatic amino compounds in each banana group and individual compounds for their in vivo antioxidant activity and mechanism, as well as biological properties, are necessary before the use of banana extracts in pharmaceutical and food products can be positively recommended.

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