ANTHOCYANIN PROFILES OF MALE BRACTS OF WILD BANANA SPECIES IN THAILAND

KASIPONG KITDAMRONGSONT

A THESIS SUBMITTED IN PARTIAL FULFILLMENT
OF THE REQUIREMENTS FOR
THE DEGREE OF MASTER OF SCIENCE
(BIOCHEMISTRY)
FACULTY OF GRADUATE STUDIES
MAHIDOL UNIVERSITY
2008

Thesis
entitled

ANTHOCYANIN PROFILES OF MALE BRACTS OF WILD BANANA SPECIES IN THAILAND

……………………………………
Mr. Kasipong Kitdamrongsont
Candidate

……………………………………
Lect. Dr. Jamorn Somana,
M.D., Ph.D. (Plant Molecular Biology)
Advisor

……………………………………
Lect. Dr. Kittisak Yokthongwattana,
Ph.D. (Agricultural & Environmental Chemistry)
Co-Advisor

……………………………………
Prof. Banchong Mahaisavariya,
M.D.
Dean
Faculty of Graduated Studies

……………………………………
Assoc. Prof. Sumalee Tungpradubkul,
Ph.D. (Molecular Biology)
Chair
Master of Science Program in Biochemistry
Faculty of Science

Thesis
entitled

ANTHOCYANIN PROFILES OF MALE BRACTS OF WILD BANANA SPECIES IN THAILAND

was submitted to Faculty of Graduate Studies, Mahidol University
for the degree of Master of Science (Biochemistry)
on
April 23, 2008

…………………………………
Mr. Kasipong Kitdamrongsont
Candidate

…………………………………
Prof. Sakol Panyim
Ph.D. (Biochemistry)
Chair

…………………………………
Lect. Dr. Sasivimon Swangpol,
Ph.D. (Biological Sciences)
Member

…………………………………
Lect. Dr. Jamorn Somana,
M.D., Ph.D. (Plant Molecular Biology)
Member

…………………………………
Lect. Dr. Chotika Yokthongwattana,
Dr. Sc. (Biology)
Member

…………………………………
Lect. Dr. Kittisak Yokthongwattana,
Ph.D. (Agricultural & Environmental Chemistry)
Member

…………………………………
Prof. Banchong Mahaisavariya,
M.D.
Dean
Faculty of Graduated Studies

………………………………….
Prof. Skorn Mongkolsuk,
Ph.D.
Dean
Faculty of Science
Mahidol University

ACKNOWLEDGEMENT

I would like to express my deepest appreciation to my advisor, Dr. Jamorn Somana, for opportunity in this project, his valuable advice, kindness, encouragement, and patience throughout my graduate study, especially for my thesis. I am also grateful to my co-advisor Dr. Kittisak Yokthongwattana, for his valuable suggestions.

My appreciation is also expressed to Dr. Sasivimon Sawangpol for her kindness, valuable suggestions and comments.

I would like to thank Central Instrument Facility officers for their valuable suggestions during the training on the instrument and also for their Happy New Year cake.

I wish to thank all members in *Musa* project and B317 Lab members for their sincerity, cheerfulness, kind support and friendship. Thank also to all my colleagues and staff at Department of Biochemistry, Faculty of Science, Mahidol University.

I would like to thank New Researcher Support Fund from Mahidol University and the Thailand Research Fund grant MRG5080329 for their financial support.

Finally, I wish to express my deepest gratitude to my family for their love, support, understandings, concerns and encouragements throughout my study in the Master degree.

Kasipong Kitdamrongsont

ANTHOCYANIN PROFILES OF MALE BRACTS OF WILD BANANA SPECIES IN THAILAND

KASIPONG KITDAMRONGSONT 4836407 SCBC/M

M.Sc. (BIOCHEMISTRY)

THESIS ADVISORS: JAMORN SOMANA, Ph.D. (PLANT MOLECULAR BIOLOGY), KITTISAK YOKTHONGWATTANA, Ph.D. (AGRICULTURAL & ENVIRONMENTAL CHEMISTRY)

ABSTRACT

Anthocyanins were isolated from male bracts of 67 wild banana (*Musa* spp. and *Ensete* spp.) accessions distributed in Thailand. With high performance liquid chromatography (HPLC), mass spectrometry (MS), and tandem mass spectrometry (MS/MS), six major anthocyanin pigments were identified: delphinidin-3-rutinoside (m/z 611.2), cyanidin-3-rutinoside (m/z 594.7), petunidin-3-rutinoside (m/z 624.8), pelargonidin-3-rutinoside (m/z 579.3), peonidin-3-rutinoside (m/z 608.7) and malvidin-3-rutinoside (m/z 638.8). By type of pigment, wild bananas can be divided into four groups. The first group, comprised of *M. acuminata*, *M. itinerans* and *Musa* sp., contained five types of anthocyanin but not pelargonidin-3-rutinoside. The second group, comprised of *M. acuminata* subsp. *malaccensis*, contained three types of anthocyanin: cyanidin-3-rutinoside, peonidin-3-rutinoside and malvidin-3-rutinoside. The third group comprises all yellow bract color in which no anthocyanin was detected. The forth group, comprises of *M. balbisiana*, *M. velutina*, *M. laterita*, *M. acuminata* subsp. *zebrina* and *E. superbum*, contained cyanidin-3-rutinoside and delphinidin-3-rutinoside. The three remaining groups, which could not be classified according to type of pigment, were 1) *M. coccinea* which contained cyanidin-3-rutinoside and pelargonidin-3-rutinoside 2) *M. ornata* which contained only peonidin-3-rutinoside and 3) *M. acuminata* subsp. *siamea* with yellow plus little purple edges bract color contained delphinidin-3-rutinoside and peonidin-3-rutinoside. Total anthocyanin contents in analyzed bracts ranging from 0-2.23 µmole/g bract. The differences between anthocyanin types and proportions revealed a biochemical diversity of wild bananas, which might be useful for identification of banana species or inferring flavonoid metabolic evolution among each banana group.

KEY WORDS: MALE BRACT/ FLORAL PIGMENT/ ANTHOCYANIN/ MUSACEAE/ PHENOLIC COMPOUNDS

75 pp.

องค์ประกอบของแอนโทไซยานินจากกาบปลีกล้วยป่าในประเทศไทย (ANTHOCYANIN PROFILES OF MALE BRACTS OF WILD BANANA SPECIES IN THAILAND)

กาสิพงษ์ กิจดำรงค์สันต์ 4836407 / SCBC/M

วท.ม. (ชีววเคมี)

คณะกรรมการควบคุมวิทยานิพนธ์: จามร สมณะ, Ph.D. (PLANT MOLECULAR BIOLOGY), กิตติศักดิ์ หยกทองวัฒนา, Ph.D. (AGRICULTURAL & ENVIRONMENTAL CHEMISTRY)

บทคัดย่อ

แอนโทไซยานินที่สกัดจากกาบปลีกล้วยป่า *Musa* spp. และ *Ensete* spp. จำนวน 67 ตัวอย่าง ที่กระจายพันธุ์อยู่ในประเทศไทยได้ผ่านการวิเคราะห์โดยการแยกสารในสถานะของเหลวโดยวิธีโครมาโทกราฟีภายใต้แรงดันสูง (HPLC) การวัดมวลด้วยแมสสเปกโทรมิเตอร์ (MS) และการวัดมวลจากส่วนของโมเลกุลที่ถูกทำให้แตก (MS/MS) พบว่ามีแอนโทไซยานินอยู่ 6 ชนิด คือ delphinidin-3-rutinoside , cyanidin-3-rutinoside, petunidin-3-rutinoside, pelargonidin-3-rutinoside, peonidin-3-rutinoside และ malvidin-3-rutinoside และสามารถใช้จำแนกกล้วยป่า ได้เป็น 4 กลุ่มดังนี้ กลุ่มแรก กล้วยป่า (*M. acuminata*) กล้วยหก (*M. itinerans*) และกล้วยป่า *Musa* sp. มีแอนโทไซยานินเป็นองค์ประกอบ 5 ชนิด ได้แก่ delphinidin-3-rutinoside, cyanidin-3-rutinoside, petunidin-3-rutinoside, peonidin-3-rutinoside และ malvidin-3-rutinoside ยกเว้น pelargonidin-3-rutinoside กลุ่มที่สองกล้วยป่า *M. acuminata* subsp. *malaccensis* ที่มีแอนโทไซยานินเป็นองค์ประกอบ 3 ชนิด ได้แก่ cyanidin-3-rutinoside, peonidin-3-rutinoside และ malvidin-3-rutinoside กลุ่มที่สาม กล้วยป่าทุกตัวที่มีปลีสีเหลืองไม่พบว่ามีแอนโทไซยานินเป็นองค์ประกอบ และ กลุ่มที่สี่ กล้วยตานี (*M. balbisiana*), กล้วยรุ่งอรุณ (*M. velutina*), กล้วยบัวสีส้ม (*M. laterita*) และกล้วยผา (*E. superbum*) มี cyanidin-3-rutinosideและ delphinidin-3-rutinoside เป็นองค์ประกอบ สามกลุ่มสุดท้ายที่เหลือไม่สามารถจัดกลุ่มตามชนิดของแอนโทไซยานินให้เข้ากับกลุ่มอื่นได้คือ 1) กล้วยรัตกัทลี (*M. coccinea*) เป็นกลุ่มเดียวที่มี pelargonidin-3-rutinoside 2) กล้วยบัวสีชมพู (*M. ornata*) มีเพียง peonidin-3-rutinoside และ 3) กล้วยป่าปลีเหลืองที่มีสีม่วงที่ขอบกาบปลีเล็กน้อยมี delphinidin-3-rutinoside และ peonidin-3-rutinoside เป็นองค์ประกอบ ทั้งนี้พบว่า กล้วยที่ใช้วิเคราะห์มีพิสัยปริมาณแอนโทไซยานินทั้งหมดในกาบปลี 0-2.23 ไมโครโมลต่อกรัม ของกาบปลีกล้วยสด ความแตกต่างระหว่างองค์ประกอบของแอนโทไซยานินทั้งชนิดและปริมาณเผยให้เห็นถึงความหลากหลายทางชีวเคมีของกล้วยป่าซึ่งอาจจะใช้ประโยชน์ในการระบุชนิดกล้วยและอธิบายวิวัฒนาการของเมตาโบลิสมในสารกลุ่มฟลาโวนอยด์ในกล้วยแต่ละกลุ่มได้

75 หน้า

CONTENTS

CONTENTS (continued)

LIST OF TABLES

Page

LIST OF FIGURES

Page

LIST OF ABBREVIATIONS

%	=	percentage
^oC	=	degree Celsius
λ max-vis	=	maximum absorption wavelength in visible range
A_{310}	=	Absorption at 310 nanometer
A_{440}	=	Absorption at 440 nanometer
$A_{\lambda\ max\text{-}vis}$	=	Absorption at maximum absorption wavelength in visible range
cm	=	centi (10^{-2}) meter
dH_2O	=	distilled water
ESI	=	electrospray ionization
Fig.	=	Figure
g	=	gram
GPS	=	Global Positioning System
HCl	=	hydrochloric acid
HPLC	=	high performance liquid chromatography
KCl	=	potassium chloride
l	=	litre
m	=	mili ($x10^{-3}$)
M	=	molar (mole per liter)
m/z	=	mass per charge ratio
M^+	=	molecular mass ion
min	=	minute
MS	=	mass spectrometry
MS/MS	=	tandem mass spectrometry
ng/µl	=	nanogram per microlitre
nm	=	nanometer
NMR	=	nuclear magnetic resonance

LIST OF ABBREVIATIONS (continued)

OD	=	optical density
PDA	=	photodiode array
pH	=	power of Hydrogen ion (- log [H+])
rpm	=	round per minute
sp. or spp.	=	species
subsp.	=	subspecies
TFA	=	trifluoroacetic acid
UV	=	ultraviolet
v/v	=	volume per volume
µ	=	micro ($x10^{-6}$)

CHAPTER I
INTRODUCTION

1. Overview on the bananas

Banana (Figure 1) is an important fruit crop of the world. They form an integral component of the farming system in humid agro-ecological zones of the tropics [1]. Bananas are produced for local consumption and trading mostly in the tropical and subtropical areas [2]. Bananas belong to the plant family Musaceae. *Musa* is the largest genus in the family and was classified into five sections based on their chromosome number and morphological characters i.e., Musa x=11, Rhodochlamys x=11, Australimusa x=10, Callimusa x=10 and Ingentimusa x=7 (x is the number of chromosome in haploid genome) [3], [4]. Most cultivated bananas are believed to originate from two wild banana species in Musa section: *Musa acuminata* Colla and *M. balbisiana* Colla [1], [5], [6]. Simmonds [1], [4], [7] proposed that wild and cultivated bananas had their origins in Southeast Asia and were later introduced to other regions in tropical and subtropical parts of the world. In many Southeast Asian locations, male buds of some wild and cultivated bananas are consumed as vegetables, and bract color is a potential source of food colorants [8].

In banana classification [4], male bract color is one of the most discriminating characters among others. Most bananas have the bract colors of red, purple, or violet bract, while cyanic-green or yellow are rare [4]. In *M. acuminata*, the variation in bract colors is correlated with the compositions of the pigment mixtures [4]. Preliminary results by paper chromatography [4], [8], [9] and by acid extraction [4], indicated that glycoconjugated anthocyanin is the major pigments in bract color.

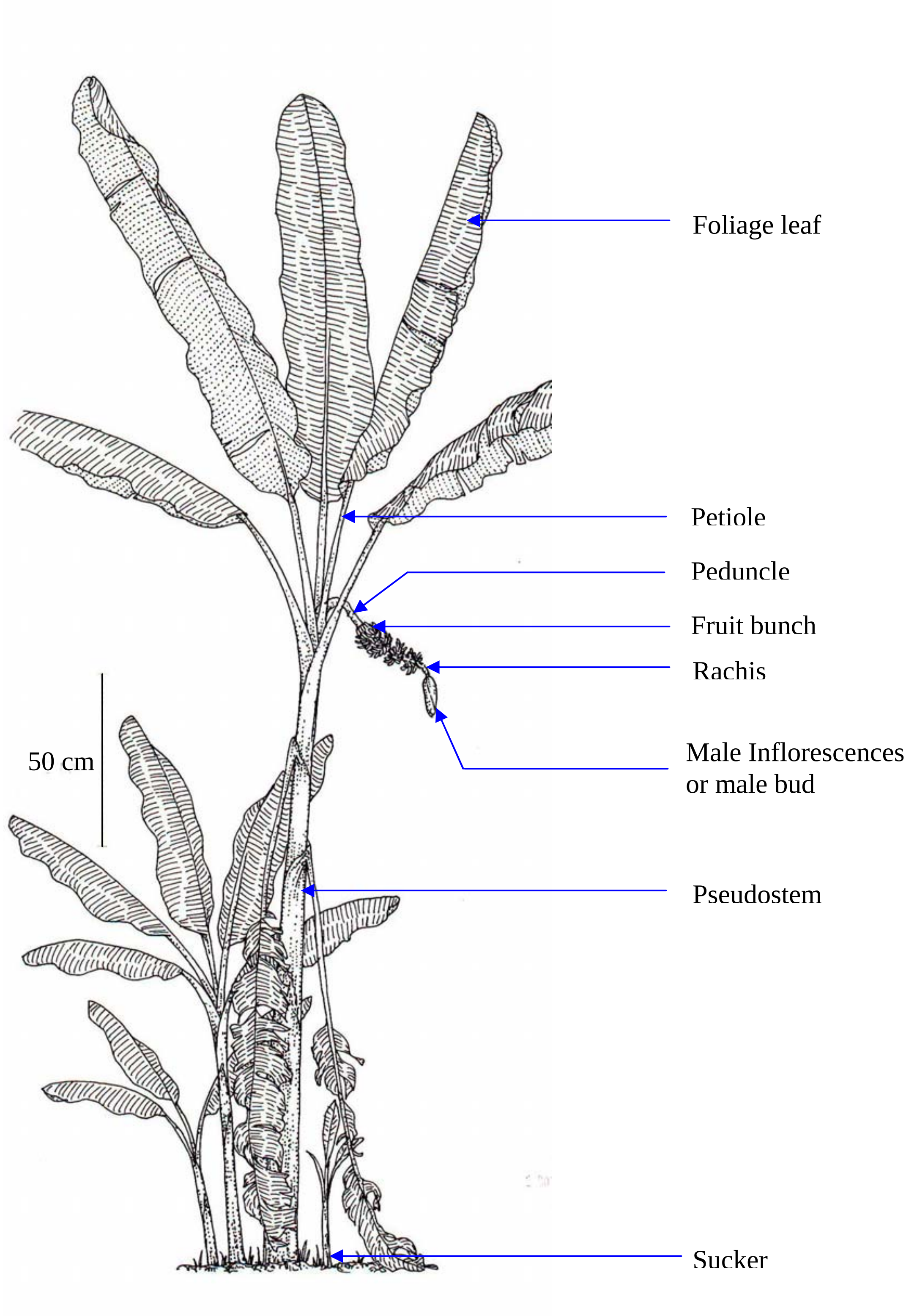


Figure 1 Line drawing of a wild banana plant; *M. acuminata* accession SS&JS 307 (Line drawing by Ms. Potjana Kiatprapai)

2. ANTHOCYANINS

2.1 Introduction to anthocyanin

The word anthocyanin, derived from two words in Greek *anthos* or flower and *kyanos* or blue. Anthicyanins, the secondary metabolite produced by plants are water-soluble pigment which are responsible for colors of plant from purple, blue, pink, orange, and red, of most flowers [10]. Anthocyanin also found in other plant organs, i.e. root and leaves and accumulating in the vacuoles of epidermal or subepidermal cells [11].

Anthocyanins belong to a class of phenolic compounds collectively named flavonoids [11]. They have roles in attracting the insect and birds for plant pollination and seed dispersal, protect plant from light stress, and inhibition of pest infestations and fungal infections [12]. Anthocyanin pigments are glycoside of anthocyanidin. They exist in several forms from six common anthocyanidin aglycones; pelargonidin, cyanidin, peonidin, delphinidin, petunidin and malvidin (Figure 2) [11].

2.2 Structure and properties

Anthocyanins are C_{15} ($C_6C_3C_6$) phenolic compound, composed of two rings; A-ring is hydroxycinnamoyl system and B-ring is benzoyl system and numbering system shown in Fig. 2 [11]. The common attaches of sugar to anthocyanidin is glucose, rhamnose, galactose, xylose which general glycosylation position are 3-, 5-, and the rare position at 7- of hydroxycinnamoyl ring sometime linked to 3'- or 5'- of benzoyl ring [10]. Acyl groups have been found in anthocyanin from many plants. The common acyl group is aromatic acid such as hydroxycinnamic acid, *p*-coumaric acid, caffeic acid and ferulic acid [11]. Aliphatic acyl group are also found in anthocyanin such as malonic acid, acetic acid, malic acid, oxalic acid and succinic acid [10].

Anthocyanin has many forms (Figure 3) with different colors that affect stability of anthocyanin [10]. At pH 1-3 anthocyanin is in flavylium cation form with red color, at pH 4-5 form carbinol pseudo-base with colorless, at pH 6-7 form quinonoidal-base with blue to purple color, then turn to chalcone form with yellow color at pH 7-8 [13].

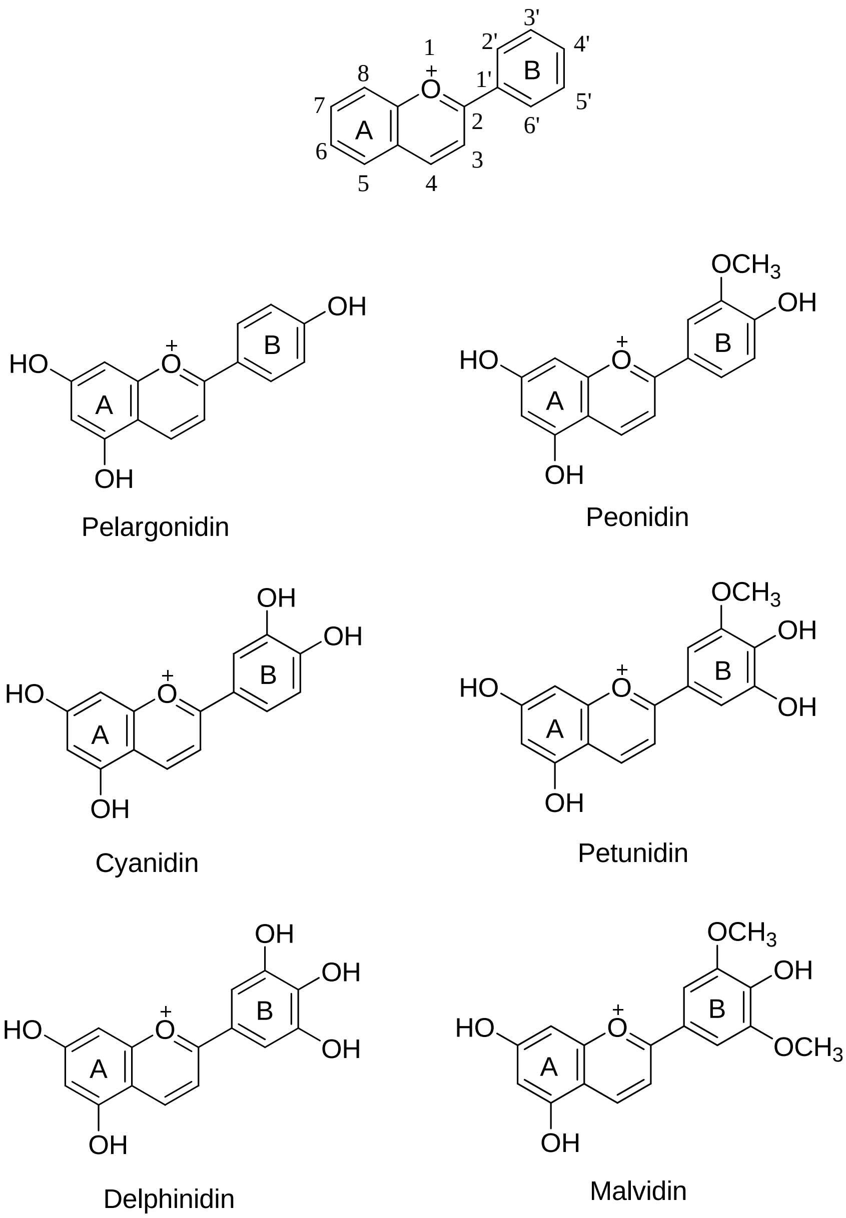


Figure 2 The core molecular and common six anthocyanidin aglycones.

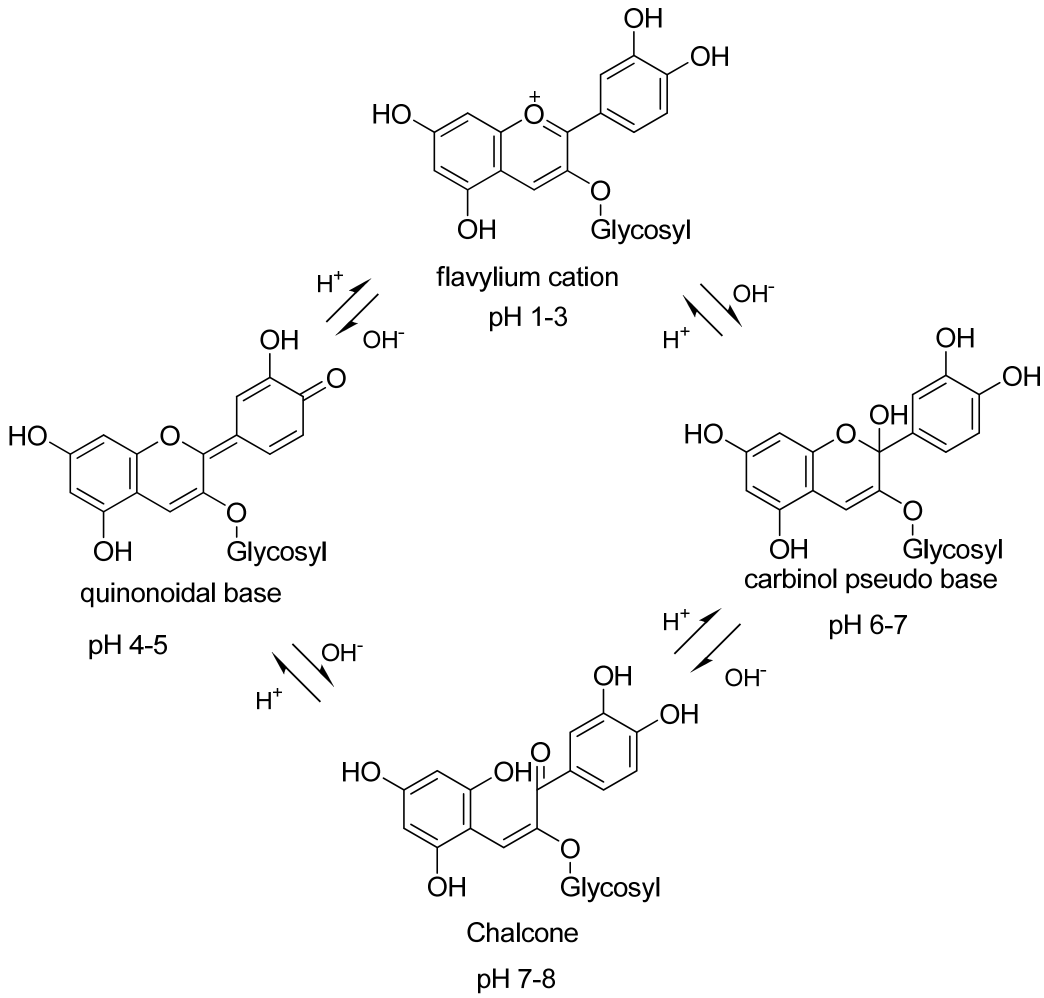


Figure 3 An example of transformation of anthocyanin (cyanidin-3-rutinoside) by pH differences.

2.3 Anthocyanin synthetic pathway

Studies of anthocyanin synthesis in many plants has been reported such as apple skin (*Malus domestica* Borkh.) [14], [15], strawberry (*Fragaria* × *ananassa* Duch.) [10], blue berry (*Vaccinium myrtillus* L.) [16], petunia (*Petunia hybrida* Hort.) [17]. Common biosynthetic pathway is shown in Figure 4. Anthocyanin synthetic pathway is a branch of flavonoid biosynthesis pathway, starting from convention of *p*-coumaroyl CoA and 3 molecules of malonyl CoA to tetrahydroxychalcone which further isomerized to nariginin then a hydroxyl group is added at C3 position of A-ring to form dihydrokaempferol. From dihydrokaempferol, a hydroxyl group is added at C3' position of B-ring to form dihydroquercetin or 2 hydroxyl groups are added at C3', 5' positions of B-ring to form dihydromyricetin. Then 3 types of dihydroflavonols were reduced at C4 position into 3 common leucoanthocyanindins which further loss of hydroxyl group at C4 to form anthocyanidin aglycones; pelargonidin, cyanidin, delphinidin. Glycosylation makes anthocyanidin aglycones to anthocyanins and post modification at B-ring hydroxyl group by methyltransferase to produce 3 additional common anthocyanins; peonidin, petunidin and malvidin.

2.4 Study of anthocyanin in other plants

Individual anthocyanin in many plants has been successfully identified by high performance liquid chromatography (HPLC) [18], [19] and mass spectrometry (MS) i.e., *Musa*× *paradisiaca* L. [8], concord grape (*Vitis labrusca* L.), roselle (*Hibiscus sabdariffa* L.), red cabbage (*Brassica oleracea* L.), red radish (*Raphanus sativus* L.), red fleshed potato (*solanum tuberosum* L.) [20], Tart cherries (*Prunus cerasus* L.), elderberry (*Sambucus nigra* L.), bilberry (*Vaccinium myrtillus* L.), chokeberry (*Aronia melanocarpa* Elliott.) [21], black soybean (*glycine max* (L.) Merr.) [22], strawberry (*Fragaria* × *ananassa* Duch.) [23], grape (*Vitis vinifera* L.), highbush blueberry (*Vaccinium corymbosum* L.), black raspberry (*Rubus occidentalis* L.), red raspberry (*Rubus idaeus* L.) [24], black currant (*Ribes nigrum* L.) [25], and bananas [26]. The anthocyanin profiles of 50 red table grape cultivars (*Vitis vinifera* L.) studied by HPLC showed possibility to distinguish all 50 cultivars from each other [27].

Survey of the wild bananas in Thailand [28], [29] showed diversity of bract colors that somewhat related to their original locations. Since the classification of banana by the color of bract as a character tool with visual bract color or the photographic image is not reliable because the effect of the environment and cameras during the picture taken and image processing, which cause the color in photograph not the same as real color bract. However, information on this character in relation to diversity of native bananas is scarce. The study of anthocyanin components as a chemical marker that cause variation of colors in banana bract along with morphological [28] and molecular [29] analyses would be useful in correlation and validation for classification and distribution pattern of the wild bananas in Thailand.

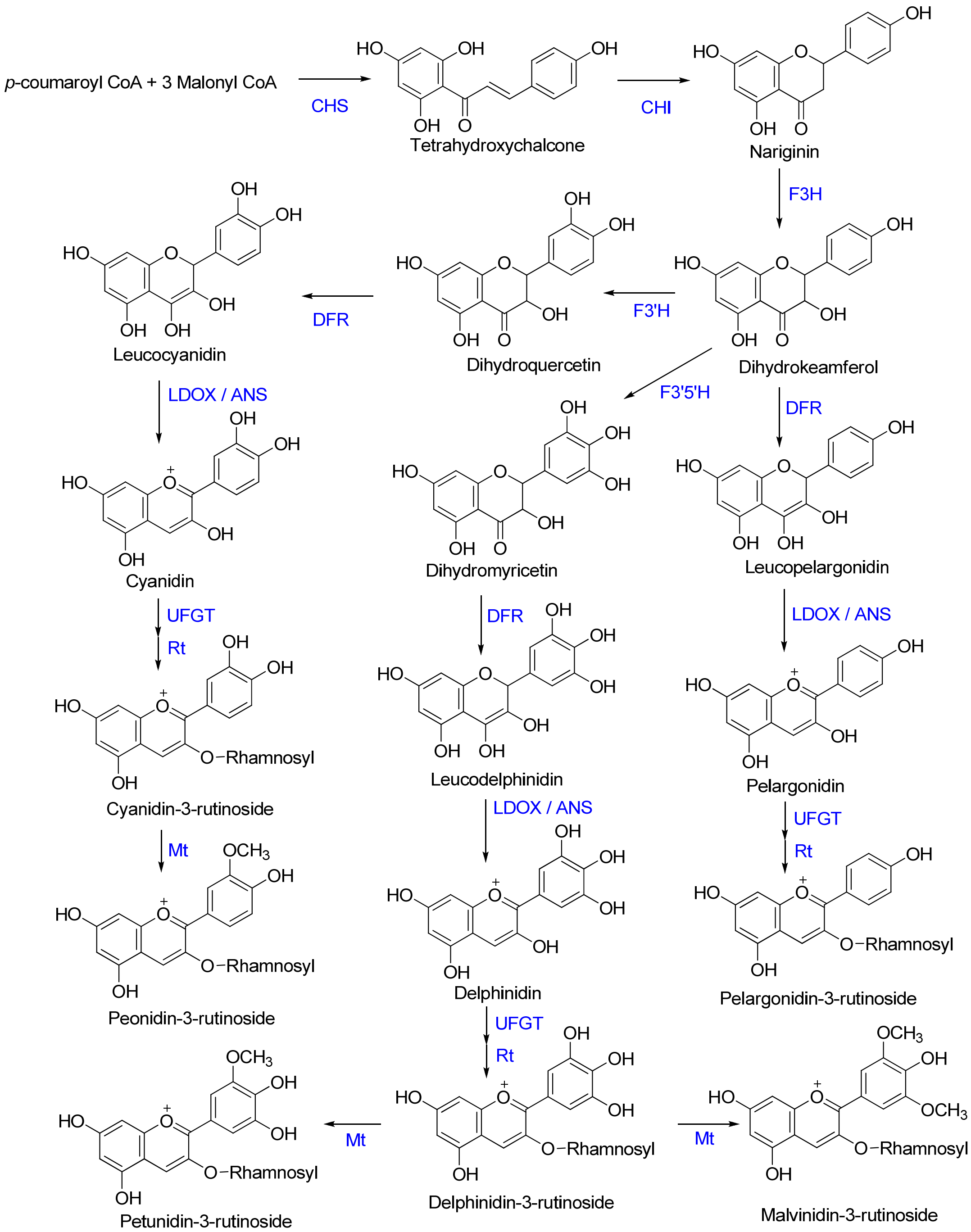


Figure 4 Biosynthesis pathway of anthocyanin (modified from petunia [16]). The enzyme names are CHS, chalcone synthase; CHI, chalcone isomerase; F3H, flavanone-3-hydroxylase; F3’H, flavanone-3’-hydroxylase; F3’5’H, flavanone 3’,5’-hydroxylase; DFR, dihydroflavonol 4-reductase; LDOX/ANS; leucoanthocyanidin dioxygenase/ anthocyanidin synthase; UFGT, UDP glucose:flavonoid 3-O-glucosyltransferase; Rt, UDP rhamnose:anthocyanidin-3-O-glucoside rhamnosyltransferase; Mt, methyltransferase.

OBJECTIVES

1. To investigate types and amount of anthocyanin pigments and relationship with bract color.
2. To investigate anthocyanin patterns and their similarity among wild bananas in Thailand this might be used for banana classification.

CHAPTER II
MATERIALS AND METHODS

1. MATERIALS

1.1 The banana collections

The wild banana accessions in this experiment were collected from many parts of Thailand (Figure 5). To construct our germplasm collection, the suckers were taken out from their original habitats and grown *ex situ* in a private garden at Nakhon Pathom, Thailand. Global position system (GPS) for their native locations and morphological characters were recorded on sites. Specimens were preserved and deposited at Saun Luang Rama IX Herbarium.

1.2 The banana bract collections

The bracts used in this experiment (Figure 6) were taken from an *ex situ* germplasm collection. Male bract which outermost and non-opening (Figure 7) was harvested, and wrapped in paper, kept in sealed plastic bag, immediately brought to the lab and stored at 4°C in refrigerator. Collected bracts were subsequently extracted within one day after harvesting. List of wild banana accessions, their visual bract colors, scientific name, local name and source location are presented in Table 1.

2. METHODS

2.1 Anatomy of the bracts

Observation of anthocyanin in banana bract was preformed under light microscope. Bract was cut and visualized under microscope both cross section and two sides of epidermal layer. Confirm of anthocyanin was done by leasing of anthocyanin in vacuole with mild acidified aqueous methanol [11] (60% methanol containing 0.027% HCl (v/v)).

Figure 5 The original locations of wild bananas used in this study. Numbers indicated accession numbers appeared in Table 1.

M. acuminata subsp. *siamea* 1, with purple, blue bract color, found in the north to lower central and west to east and northeast of Thailand.

M. acuminata subsp. *siamea* 2, with red purple, purple and blue bract color, found in western and north-western part of Thailand

M. acuminata subsp. *malaccensis* 1, the typical form, with red, pink purple or purple brown bract color and sometimes with yellow color streaks commonly found in southern part of Thailand.

M. acuminata subsp. *malaccensis* 2, Kra Isthmus form, with rusty brown, purple brown, pink purple and red purple bract color, found in Ranong and neighboring provinces.

Figure 6 Bract colors of wild bananas in Thailand.

note: Identification based on [28], [29]

M. acuminata subsp. *siamea* with yellow bract color, found in northeastern and eastern of Thailand along with *M. acuminata* subsp. *siamea* 1.

M. acuminata subsp. *truncata*, with deep blue bract color, found only in highland of Yala province.

M. laterita., orange red bract color, found in western part of Thailand.

M. balbisiana, with mostly red and green bract color found rarely as wild, but commonly in cultivation throughout Thailand.

Figure 6 Bract colors of wild bananas in Thailand. (continue)

Musa sp., with purple or red-purple bract color outside and cream to yellow inside, found in northwestern part of Thailand.

E. superbum, with red bract color, found in lime stone mountains in many parts of Thailand.

E. superbum, with yellow bract color found in Kanchanaburi province.

E. glaucum, with only green bract color, found in many parts of Thailand, both in wild and cultivated areas.

Figure 6 Bract colors of wild bananas in Thailand. (continue)

M. itinerans, with red-purple to purple brown bract color, found in the northern part of Thailand.

M. coccinea, probably imported from other countries.

M. velutina, probably imported from other countries.

Figure 6 Bract colors of wild bananas in Thailand. (continue)

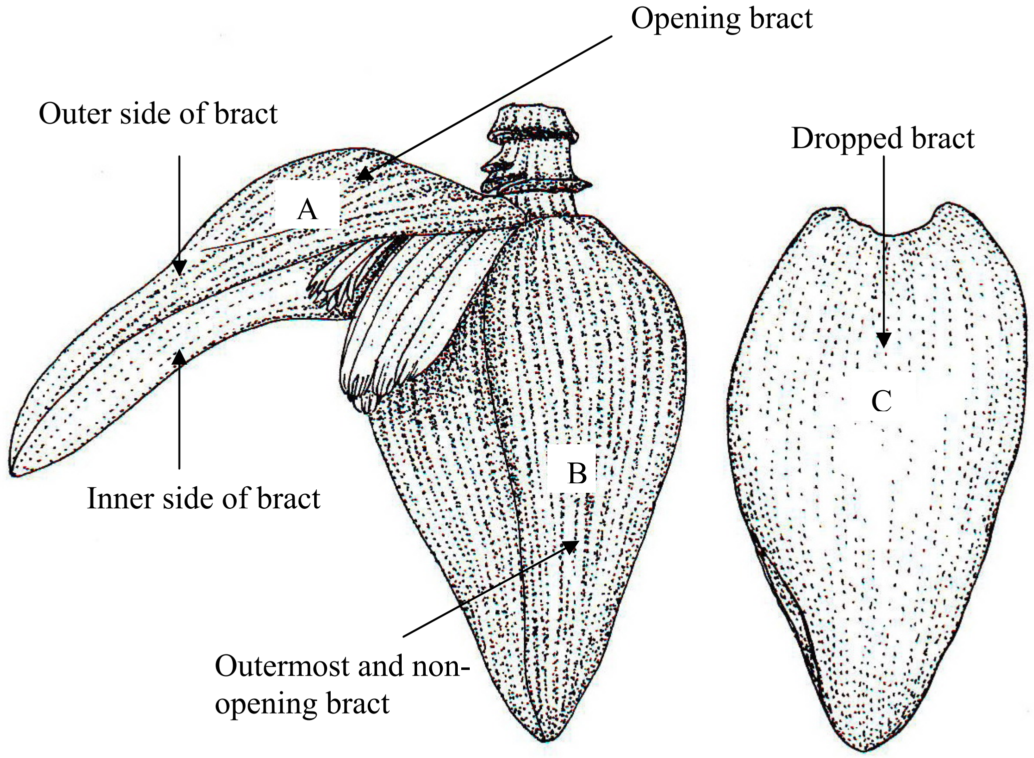


Figure 7 Banana male bud showing bracts. (A) Opening bract, (B) Outermost and non-opening bract and (C) Dropped bract. (Line drawing by Ms. Potjana Kiatprapai)

2.2 Anthocyanin extractions

To extract anthocyanin content in banana bracts [11], one gram of the fresh bracts were cut into small pieces and ground with 60% aqueous methanol containing 0.027% HCl (v/v) [30]. The slurry was transferred into 15 ml capped tube, adjusted volume to 15ml with 60% aqueous methanol containing 0.027% HCl (v/v), and kept in dark at 4°C overnight. After centrifugation at 4000 g, the supernatant were collected, adjusted to final volume of 14 ml and stored at -20°C in freezer until analysis.

2.3 Measurement of total anthocyanin in bract

Total anthocyanin in the extracts were measured with pH-differential method as described by Gusti & Wrolsted [31], [32]. Total anthocyanin content was calculated as equivalent mole of cyanidin-3-rutinoside. Spectral absorption of the extract sample were measured with UV-Visible spectrophotometer (UV-201PC; Shimadzu, Japan). The extract was divided into two parts, the first part was 5 times diluted with 2.5 mM KCl, pH 1.0, the second part was 5 times diluted with 0.4 M Sodium acetate, pH 4.5, then both parts were left to develop color at room temperature for 15 minutes. Then the spectra were measured absorption at 700 nm and at maximum absorption wavelength in visible range ($\lambda_{max\text{-}vis}$), was fixed at 530 nm. In the scanning mode of absorption, the absorption was recorded from 400 nm to 720 nm. The absorption (optical density unit; OD) at 700 nm and at 530 nm was extracted from ASCII files of each sample. Total anthocyanin was calculated in equivalent mole/g fresh weight to cyanidin-3-rutinoside (ε=32298 ($OD.M^{-1}.cm^{-1}$), MW=595.53) as following equations.

1. $A_{total} = (A_{530} - A_{700})_{pH1.0} - (A_{530} - A_{700})_{pH4.5}$

2. $[\text{Anthocyanin}]\ (\text{mole/l}) = \dfrac{A_{total} \times \text{Dilution Factor}}{\varepsilon = 32298 \times 1}$

3. $\text{Anthocyanin}\ (\text{mmole/g}_{bract}) = \dfrac{[\text{Anthocyanin}](\text{mmole/ml}) \times \text{Extract Volume (ml)}}{\text{Bract weight (g)}}$

Table 1. List of wild bananas used in this experiment.

No.	Accession no. SS & JS	Visual Bract Colors [1]	Scientific names	Accession names	Source location
1	2	RD	*Musa itinerans*	Hok Phetchabun	Phetchabun
2	3	PB	*M. balbisiana*	Tani Sawankhalok	Sukhothai
3	6	BL	*M. acuminata* subsp. *malaccensis* 2	Pa Khao Lak	Phangnga
4	7	BP tip YW	*M. acuminata* subsp. *siamea* 2	Pa Dong Phaya Yen	Nakhon Ratchasima
5	8	BL	*M. acuminata* subsp. *siamea* 1	Pa Kroeng Krawia	Kanchanaburi
6	9	PB	*M. acuminata* subsp. *malaccensis* 2	Pa Ranong 1	Ranong
7	19	PP tip YW	*M. acuminata* subsp. *siamea* 2	Pa Huai Rai	Uttaradit
8	27	YW	*M. acuminata* subsp. *siamea* (yellow bract)	Pa Pli Luang Pak Chong	Kamphaengphet
9	45	Light PP	*M. ornata*	Bua Si Chomphu	Kamphaengphet
10	57	OR	*M. laterita*	Bua Si Som	Kamphaengphet
11	91	BL	*M. acuminata* subsp. *siamea* 1	Pa Nam Nao 2	Phetchabun
12	96	BL	*M. acuminata* subsp. *siamea* 1	Pa Pak Tom	Loei
13	100	PB streak GY	*M. itinerans*	Hok Khao Kho	Phetchabun
14	106	RP	*M. acuminata* subsp. *malaccensis* 2	Pa Pang Wan	Chumphon
15	108	RP	*M. acuminata* subsp. *malaccensis* 2	Pa Hat Prapat	Ranong
16	109	OR streak YW	*M. acuminata* subsp. *malaccensis* 1	Pa Wat Tham Sua	Krabi
17	113	RD	*M. acuminata* subsp. *malaccensis* 1	Pa Chang Klang	Nakhon Si Thammarat

Table 1. List of wild bananas used in this experiment (continue)

No.	Accession no. SS & JS	Visual Bract Colors [1]	Scientific names	Accession names	Source location
18	114	PP streak LG tip YW	*M. acuminata* subsp. *malaccensis* 1	Pa Namtok Phromlok	Nakhon Si Thammarat
19	115	PP	*M. acuminata* subsp. *malaccensis* 1	Pa Si Chon	Nakhon Si Thammarat
20	116	PB streak YW,RP	*M. acuminata* subsp. *malaccensis* 1	Pa Ta U-tae	Surat Thani
21	125	RP	*Musa* sp.	Pa Huai Nam Dang	Chiang Mai
22	126	BL tipYW	*M. acuminata* subsp. *siamea* 1	Pa Muang Pam 1	Mae Hong Son
23	127	PL	*Musa* sp.	Pa Muang Pam 2	Mae Hong Son
24	132	BL+PL tip YW	*M. acuminata* subsp. *siamea* 2	Pa Khanong Phra	Nakhon Ratchasima
25	135	GR	*M. balbisiana*	Tani Huai Mae Phriang	Phetchaburi
26	139	RD streak OR,YW	*M. acuminata* subsp. *malaccensis* 2	Pa Tap Kut	Phangnga
27	142	PB	*M. acuminata* subsp. *malaccensis* 2	Pa Khlong Nakha	Ranong
28	145	PB	*M. acuminata* subsp. *malaccensis* 2	Pa Na Sak	Chumphon
29	146	PL	*M. acuminata* subsp. *malaccensis* 2	Pa Hong Charoen	Chumphon
30	147	PL	*M. acuminata* subsp. *siamea* 1	Pa Dan Singkhon	Prachuap Khirikhan
31	155	BL+PL	*M. acuminata* subsp. *zebrina*	Thahan Phran	Nakhon Ratchasima
32	168	pale PL	*M. velutina*	Rung Arun	Nakhon Ratchasima
33	172	RP	*M. acuminata* subsp. *siamea* 2	Pa Ban Pa Kluai	Nakhon Ratchasima
34	173	GY+YW	*M. acuminata* subsp. *siamea* (yellow bract)	Pa Pli Luang Ban Pa Kluai	Nakhon Ratchasima

Table 1. List of wild bananas used in this experiment (continue).

No.	Accession no. SS & JS	Visual Bract Colors [1]	Scientific names	Accession names	Source location
35	174	PL	*M. acuminata* subsp. *siamea* 1	Pa Pang Sida	Srakeao
36	177	OG	*M. acuminata* subsp. *siamea* (yellow bract)	Pa Pli Luang Khlong Tani	Chanthaburi
37	178	RP	*M. balbisiana*	Tani Khlong Tani	Chanthaburi
38	181	PL tip YW	*M. acuminata* subsp. *siamea* 1	Pa Namtok Krating	Chanthaburi
39	182	PL tip YW	*M. acuminata* subsp. *siamea* 1	Pa Khao Chamao	Rayong
40	183	DG	*M. balbisiana*	Tani Thong Suk College	Bangkok
41	206	BL	*M. acuminata* subsp. *truncata*	Pa Umong (Tunnel) Piyamit	Yala
42	223	OR	*M. coccinea*	Rattakatthali	Prachinburi
43	224	GY+YW	*M. acuminata* subsp. *siamea* (yellow bract)	Pa Pli Luang Ban Sai Thong	Nakhon Ratchasima
44	228	RP	*M. acuminata* subsp. *siamea* 2	Pa Doi Musoe	Tak
45	233	BL tip YW	*M. acuminata* subsp. *siamea* 2	Pa QSBG 1	Chiang Mai
46	238	PL	*M. acuminata* subsp. *siamea* 1	Pa Kang Som Maeo	Ratchaburi
47	242	GY	*Ensete superbum* (yellow bract)	Pha Pli Luang	Kanchanaburi
48	243	OR	*M. laterita*	Bua Si Som Tha Khanun	Kanchanaburi
49	245	BL	*M. acuminata* subsp. *siamea* 1	Pa Tha Khanun	Kanchanaburi
50	246	PP	*M. laterita*	Bua Si Som Dan Chedi Sam -	Kanchanaburi
51	247	BL	*M. acuminata* subsp. *siamea* 1	Pa Dan Chedi Sam Ong	Kanchanaburi

Table 1. List of wild bananas used in this experiment (continue).

No.	Accession no. SS & JS	Visual Bract Colors [1]	Scientific names	Accession names	Source location
52	274	PB	*M. acuminata* subsp. *malaccensis* 1	Pa Doi Musoe 2	Tak
53	281	RD	*M. acuminata* subsp. *malaccensis* 1	Pa Salawin	Mae Hong Son
54	283	BL+PL	*M. acuminata* subsp. *siamea* 2	Pa Doi Pha Hom Pok	Chiang Mai
55	286	BL	*M. acuminata* subsp. *siamea* 2	Pa Doi Tung	Chiang Rai
56	289	BL+PL	*M. acuminata* subsp. *siamea* 2	Pa Doi Phu Nang	Phayao
57	290	PP+BL	*M. acuminata* subsp. *siamea* 2	Pa Nanthaburi	Nan
58	291	BL+PL	*M. acuminata* subsp. *siamea* 2	Pa Du Phong	Nan
59	293	PP	*M. acuminata* subsp. *siamea* 2	Pa Thung Lang	Phrae
60	306	RP	*M. itinerans*	Hok Chong Yen	Kamphaengphet
61	307	BP, PL tip BY+YW	*M. acuminata* subsp. *siamea* (yellow bract)	Pa Pli Luang Chong Yen	Kamphaengphet
62	310	PL	*M. acuminata* subsp. *siamea* 2	Pa Phop Phra 2	Tak
63	312	BL	*M. acuminata* subsp. *siamea* 1	Pa Mogro 1	Tak
64	313	RP	*M. itinerans*	Hok Mogro	Tak
65	314	RP	*Musa* sp.	Pa Mogro 2	Tak
66	317	MG	*E. glaucum* (yellow bract)	Nuan Mae Klong	Tak
67	319	RP	*E. superbum*	Pha Mogro	Tak

[1], See color chart in APPENDIX A for color abbreviation.

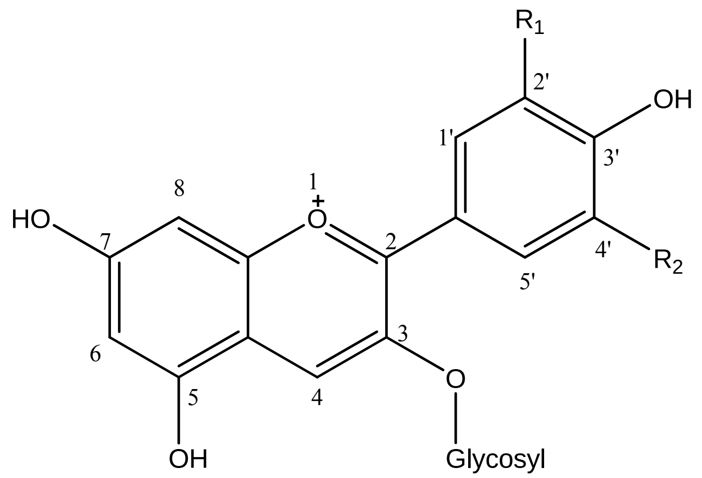
2.4 Analysis of anthocyanin components using High Performance Liquid Chromatography (HPLC)

To analyze the anthocyanin components in wild bananas, the extracted samples were filtered through 0.45 μm nylon membrane (Millex, Millipore). The 20 μl of each sample was loaded into the column by auto injector. All samples were analyzed with reverse-phases high performance liquid Chromatography (HPLC model 717 plus Auto sampler, pump model 600 and detectors model 2996 photodiode array (PDA); Waters). The column used was a reverse-phase column (Nova pak C-18; Waters) diameter 3.9 mm and lengths 300 mm with protected guard (Symmetry C-18 type; Waters). Solvent A was acetronitrile containing 0.1% trifluoroacetic acid (TFA) (v/v). Solvent B was 3A grade H_2O containing 0.1% TFA (v/v). The program (condition) used was a linear gradient from 15% to 95% solvent A for 45 minutes and return to 15% solvent A in 2 minutes, then equilibrate at 15% solvent A for 13 minutes before the next sample analysis. The flow rate used was 0.5 ml/minute. The PDA data of anthocyanin were simultaneously recorded from 260 nm to 650 nm with program "Millennium 2000" and were online detected at 530 nm and 280 nm during analysis. Standard cyanidin-3-rutinoside was used to confirm anthocyanin peak both by individual injection and by spike in samples.

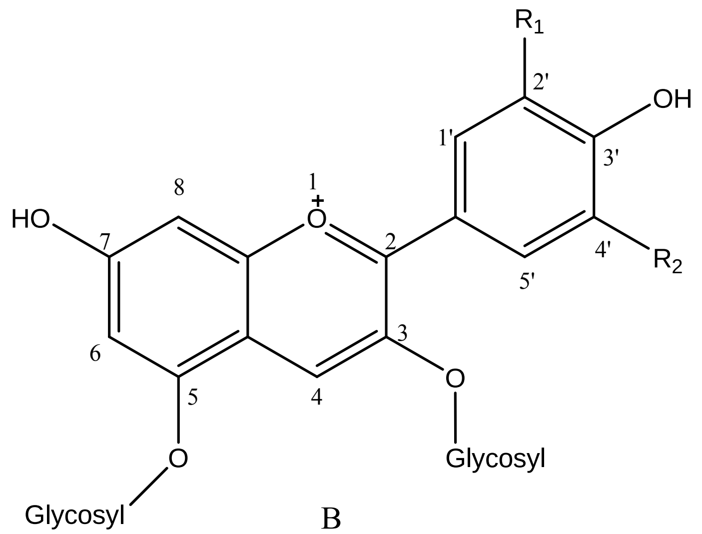
For further identification of each anthocyanin components in extract by mass spectrometer, 60 μl of samples (Selected samples; SS&JS 96, 106, 178, 206, 319) were used in HPLC analysis, then anthocyanin peaks were simultaneously fractionated with fraction collector. The collector was set to collect samples every minute for 30 minutes, flow rate at 0.5 ml/min; 500 μl per fraction. Then the fractions were kept at -20 °C until analysis with mass spectrometry.

The data from HPLC give information of acylation and glycosylation position in anthocyanin structure [11] [31] [32]. Acylation of anthocyanin information is interpreted from the ratio of absorption at 310 nm (A_{310}) to Absorption at maximum absorption in visible region ($A_{\lambda\ max\text{-}vis}$); $A_{310}/A_{\lambda\ max\text{-}vis}$. If percent ratio of $A_{310}/A_{\lambda\ max\text{-}vis}$ is less than 50 %, interpretation would be no acylation, from 50 % to 70 % should have single acylation and if it more than 80 %, it should have two acylations. While glycosylation position information is from the ratio of absorption at 440 nm (A_{440}) to absorption at maximum absorption in visible region ($A_{\lambda\ max\text{-}vis}$); $A_{440}/A_{\lambda\ max\text{-}vis}$.

If percent ratio A $_{440}$/ A $_{\lambda\ max\text{-}vis}$ around 30 %, it is interpreted that anthocyanin should have glycosylation at position 3 of anthocyanidin aglycone, and if it less than 20 %, anthocyanin should have glycosylation at position 3 and 5 of anthocyanidin aglycone (Figure 8).



A



B

Figure 8 Glycosylation positions of anthocyanidin. (A) 3-glycosylation, (B) 3, 5-diglycosylation.

2.5 Identification of anthocyanin using Mass Spectrometry (MS)

The anthocyanin fractions were directly infused via auto injector with the flow rate 150 µl/hour into mass spectrometer (Esquire 3000 plus; Bruker) equipped with electro spray ionization (ESI) source. Then the anthocyanin fraction (single peak) were analyzed in MS in positive ion mode both molecular ion mass (M^+) and their tandem mass spectrogram (MS/MS). (see parameters setup in Appendix B)

The molecular mass and their tandem mass spectrograms of anthocyanin obtained were matched to molecular mass and tandem mass spectrogram of anthocyanin in others plants from previously reported. Standard cyanidin-3-rutinoside chloride was used as a positive anthocyanin (M^+; 595 m/z and MS/MS; 449, 287 m/z).

Mass spectrometry data particularly molecular ion mass (M^+) will give overall expectation of anthocyanin molecular mass ion and tandem mass spectrograms will tell type of anthocyanidin aglycone and their putatively glycoside during fraction of molecular ion mass into daughter ions; fragmented ions, (Figure 9).

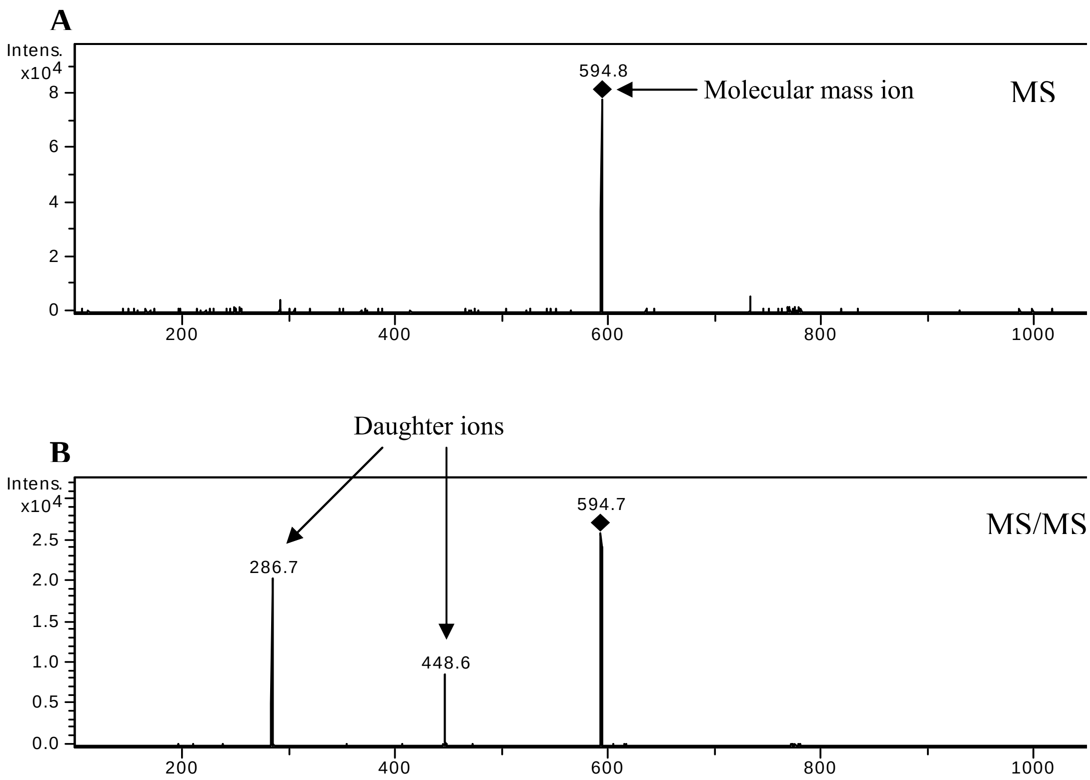


Figure 9 Mass spectra of standard cyanidin-3-rutinoside chloride (mw 630.98), A is mass spectra of cyanidin-3-rutinoside (m/z 594.8), B is tandem mass spectra of cyanidin-3-rutinoside; fragmented ion are m/z 448 (M^+-162) and m/z 286.7 (cyanidin aglycone)

2.6 Cluster analysis of the wild bananas using anthocyanin profiles

Combine types of anthocyanin with ratios of area under the anthocyanin peaks from HPLC (as following equation; an example is cyanidin-3-rutinoside peak from SS&JS 106). Cluster analysis was then performed based on similarity of interval data (SIMINT; Computes a variety of similarity and dissimilarity of interval data) which are anthocyanin types and ratios of area under each anthocyanin peak as input data. Using NTSYSpc 2.1 (Rohlf, 2000) with Sequential, Agglomerative, Hierarchical, and Nested (SAHN) clustering method and Unweighted Pair-Group Method, Arithmetic average (UPGMA), and dendrogram was generated.

$$\text{Ratio area under peak} = \frac{\text{area peak of cyanidin - 3 - rutinoside in SS \& JS 106}}{\text{total areas of all anthocyanin peaks in SS \& JS 106}}$$

CHAPTER III
RESULTS

1. Distribution of anthocyanin in bananas bract

Anthocyanin pigments of wild bananas were found in vacuoles of epidermal cells on both outer and inner sides of the bracts. Almost all of epidermal cells except stomata apparatus on the outer side of the bract uniformly contained red vacuoles, while less than 50% of epidermal cells on the inner side stored anthocyanin with various color of vacuoles (Figure 10).

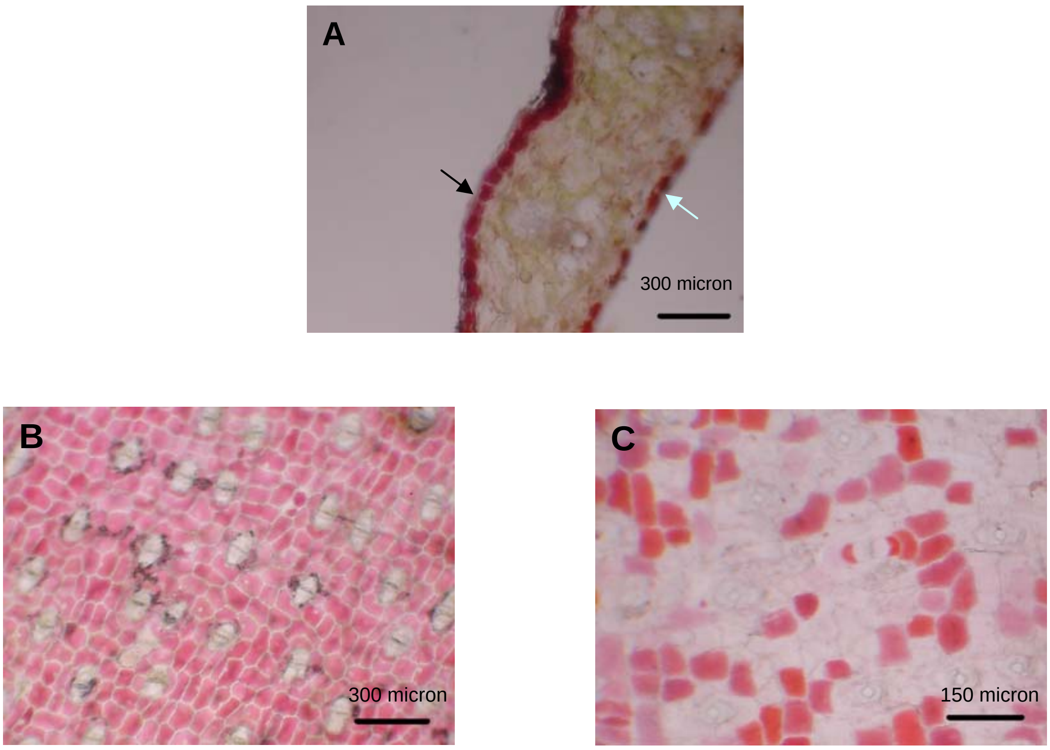


Figure 10 Anatomy of bract tissue and cells. (A) cross section of the bract, black arrow indicated outer side, white arrow indicated inner side; (B) epidermal cells on the outer side of bract and (C) on the inner side of bract.

2. Anthocyanin absorption spectra of wild banana bracts in visible region.

The absorption spectrum of anthocyanin in visible region (400 nm – 700 nm) was shown in Figure11 A and B. All sample were normalized in equal bract fresh weight In Figure11 A, all samples of; *Musa acuminata* subsp. *siamea* 1, *siamea* 2, *malaccensis* 1, *malaccensis* 2, *truncata*, *zebrina* and *Musa* sp. showed very similar absorption spectrum with the maximum absorption ranging from 500 nm to 550 nm in the same type of solvent (0.25 mM KCl pH 1.0). *M. acuminata* subsp. *siamea* in yellow bract color, there is no absorption in these visible regions. The rests of wild bananas; *M. laterita*, *M. balbisiana*, *M. itinerans*, *M. coccinea*, *E. superbum* and also showed absorption the same as *M. acuminata* in Figure 11 A. *M. coccinea* had the maximum absorption around 500 nm which differ from others wild bananas. *E. glaucum* with yellow bract color and *M. ornata* with light pink purple bract color were no absorption in visible region like in yellow bract color of *M. acuminata*.

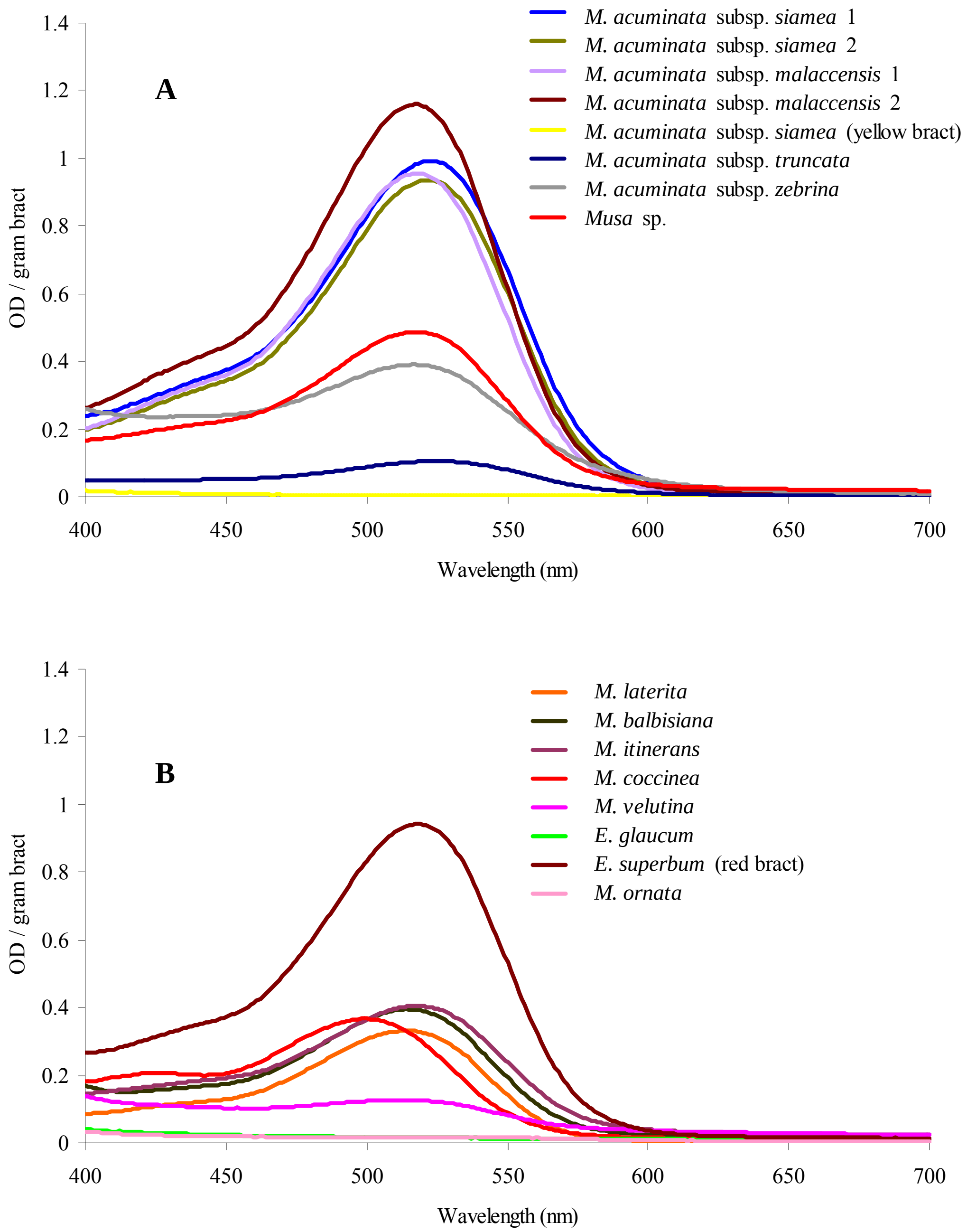


Figure 11 The absorption spectra of anthocyanin in crude extracts.

3. High Performance Liquid Chromatography (HPLC) chromatograms of wild bananas.

As the HPLC was programmed for increasing the ratio of non polar solvent over time, the most polar anthocyanin was eluted first and the least polar one was eluted last. From anthocyanin structures, the elution series of anthocyanin in wild bananas were delphinidin-3-rutinoside, cyanidin-3-rutinoside, petunidin-3-rutinoside, pelargonidin-3-rutinoside, peonidin-3-rutinoside and malvidin-3-rutinoside, respectively. there are corresponding to degree of hydroxylation and methylation of anthocyanins.

The sample chromatograms of anthocyanin in crude extract of wild bananas bract acquire at 530 nm (detected wavelength) from HPLC is shown in Figure12 A to P. All wild bananas possess six peaks (no.1-6) of anthocyanin which different types and quantities. *M. acuminata* subsp. *siamea* 1 and subsp. *siamea* 2, possessed the same five types of anthocyanin (peak no. 1, 2, 3, 5 and 6) while subsp. *malaccensis* 1 and subsp. *malaccensis* 2 possessed the same four types of anthocyanin (peak no. 1, 2, 5 and 6), *M. acuminata* subsp. *truncata* possessed only one anthocyanin peak (peak no.6), *M. acuminata*, with yellow bract did not show any anthocyanin peak, *M. acuminata* subsp. *zebrina* possessed one type of anthocyanin (peak no. 2) which differ from *M. acuminata* subsp. *truncata*, and *Musa* sp. possessed three anthocyanin peaks (peak no. 2, 5 and 6). In *M. balbisiana*, *M. laterita*, *M. velutina* and *E. superbum* possessed two peaks of anthocyanin (peak no. 1 and 2). *M. itinerans*, possessed five peaks the same as in *M. acuminata* subsp. *siamea* 1 and 2 but in different the amount of each anthocyanin type. *M. ornata* possessed one trace peak of anthocyanin (peak no. 5) this peak was detected in very low absorption value. *M. coccinea* possessesd two anthocyanin peaks (peak no. 2 and 4) one of this (peak no.4) not found in other banana samples in this experiment.

The character of anthocyanin peaks; retention time and maximum absorption wavelength from 260 nm to 650 nm together with percentage ratio of absorption value at 310 nm and 440 nm per maximum absorption at visible region (% A_{310} /$A_{vis\text{-}max}$ and % A_{440}/$A_{vis\text{-}max}$, respectively) from HPLC chromatograms are shown in APPENDIX C. The ratio of % A_{310}/$A_{vis\text{-}max}$ and % A_{440}/$A_{vis\text{-}max}$ roughly imply about acylation on anthocyanin and glycosylation on anthocyanidin aglycone, respectively. From the experiment, % $A_{310}/A_{vis-max}$ were below 50% (see discussion and APPENDIX C), this indicated that anthocyanin in wild bananas should have no acylation.

The value of % $A_{440}/A_{vis-max}$ of all peaks were around 30% indicating that all anthocyanins should have glycosylation at position 3 of anthocyanidin aglycone, not at positions 3 and 5 of anthocyanidin aglycone.

The retention time of anthocyanin peaks from each sample were slightly shifted by minor variations during the experiment but the elution series of anthocyanins were still in ordered. To guarantee that all wild bananas possessed six types of anthocyanin but had only shifted of retention time, an experiment was established by mixing all crude extracts and was run in HPLC. The result is still shown in well ordered for six peaks of anthocyanin in the chromatogram (see Appendix D).

However, in yellow bract colors and *M. ornata*, another detected wavelength was established during analysis with HPLC (at 370 nm for monitored flavonoid compounds; absorption of hydroxycinnamoyl system). The chromatograms detected at 370 nm shown in figure 13 A to O, wild bananas shown many peaks of flavonoid (square boxes). In yellow bract color and possessed flavonoid peaks in the same retention time that anthocyanin was eluted and after 20 min which present in all wild bananas in these experiments. However, there are other flavonoid detected in *M. ornata*, *M. itinerans*, *M. acuminata* subsp. *zebrina*, *M. balbisiana*, *M. coccinea* and *M. velutina*.

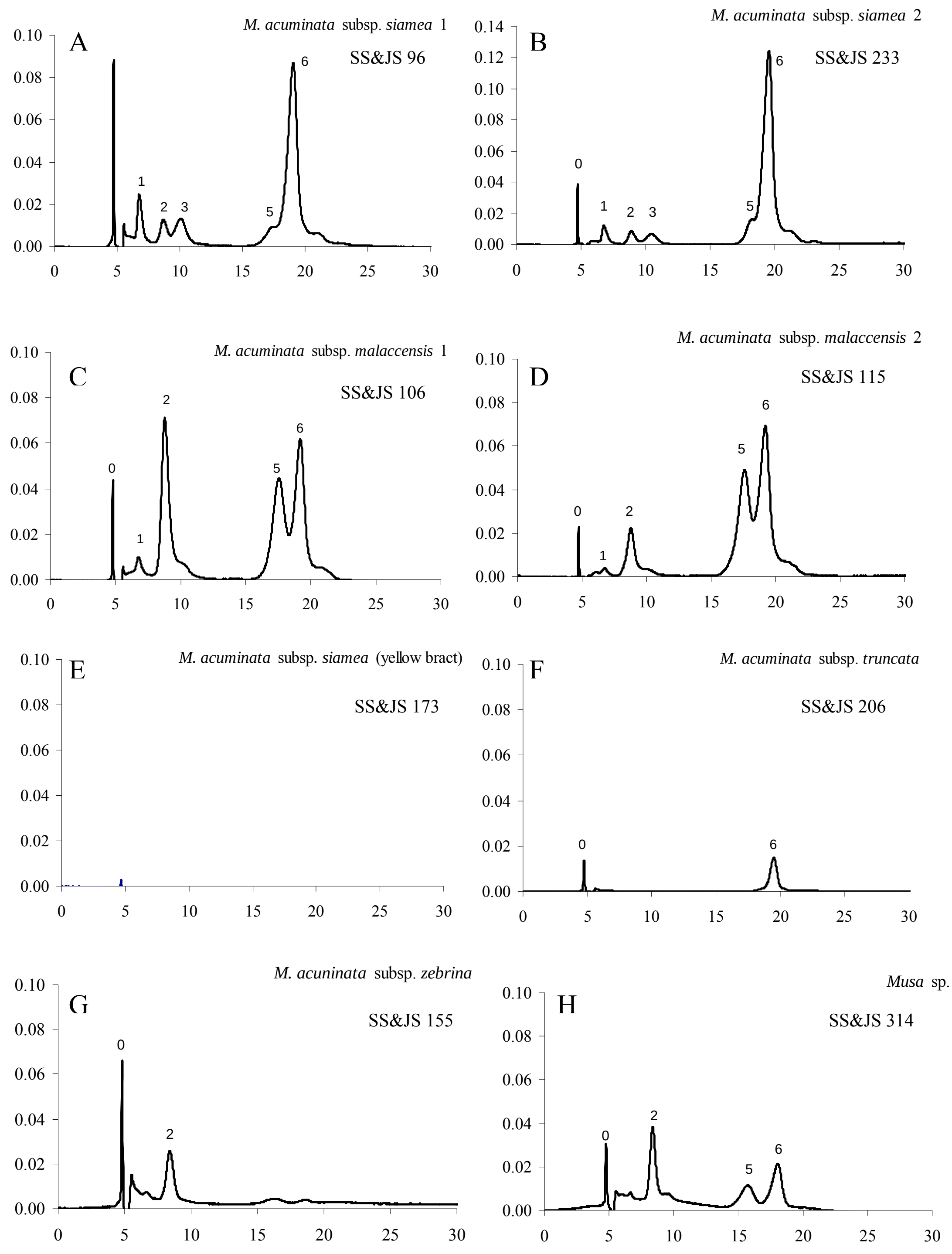


Figure 12 HPLC chromatograms of wild bananas detected at 530 nm. A, *M. acuminata* subsp. *siamea* 1; B , *M. acuminata* subsp. *siamea* 2; C, *M. acuminata* subsp. *malaccensis* 1; D, *M. acuminata* subsp. *malaccensis* 2; E, *M. acuminata* subsp. *siamea* with yellow bract; F, *M. acuminata* subsp. *truncata*; G *M. acuminata* subsp. *zebrina*., and H, *Musa* sp. Peak no.0 in all chromatograms is unbounded compounds.

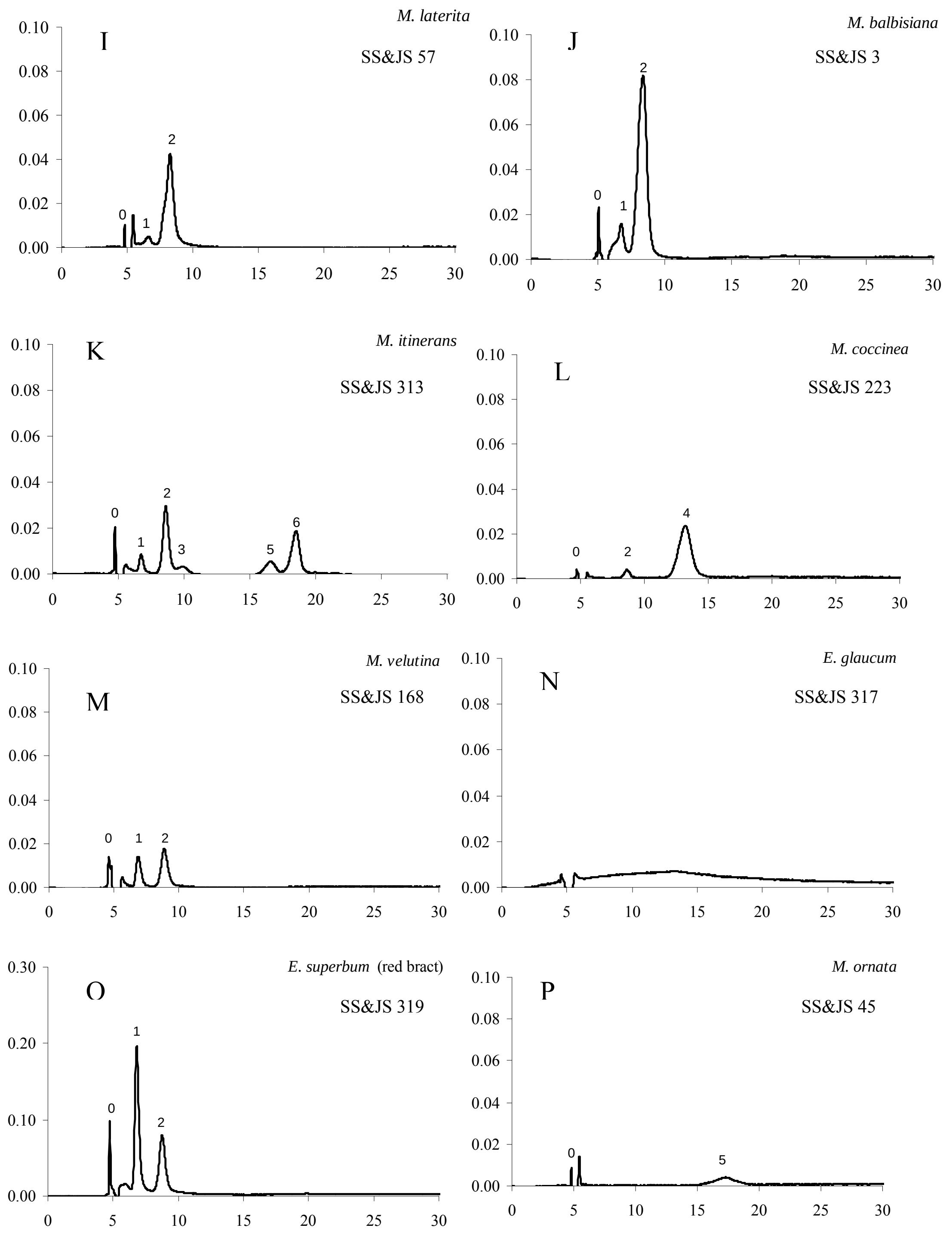


Figure 12 HPLC chromatograms of wild bananas detected at 530 nm. I, *M. laterita*; J, *M. balbisiana*; K, *M. itinerans*; L, *M. coccinea*; M, *M. velutina*; N, *E. glaucum*; O, *E. superbum* and P, *M. ornata*. Peak no.0 in all chromatograms is unbounded compounds. (continue)

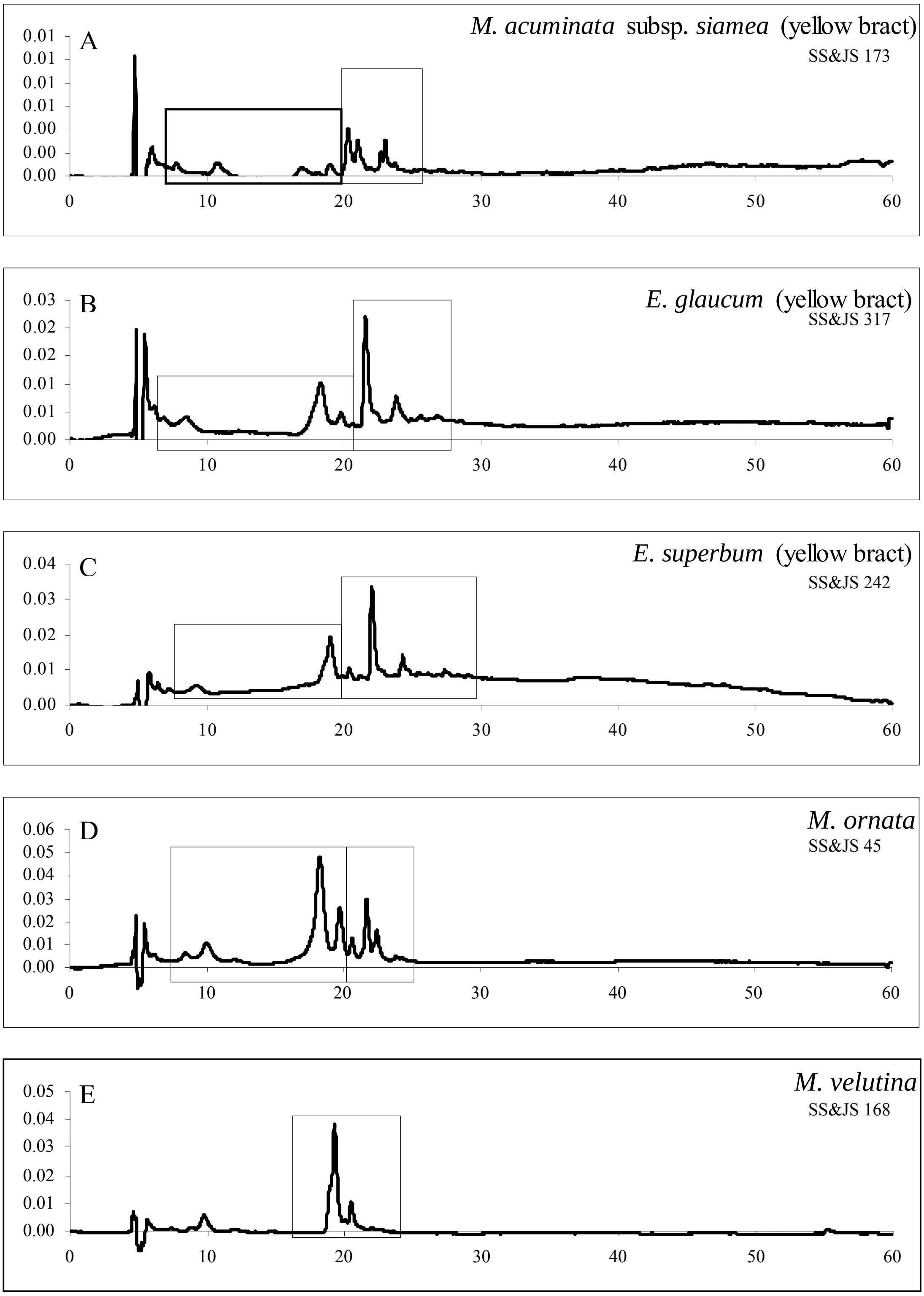


Figure 13 HPLC chromatograms of wild bananas at 370 nm. A, *M. acuminata* subsp. *siamea* with yellow bract; B, *E. glaucum*; C, *E. superbum* with yellow bract; D, *M. ornata* and E, *M. velutina*.

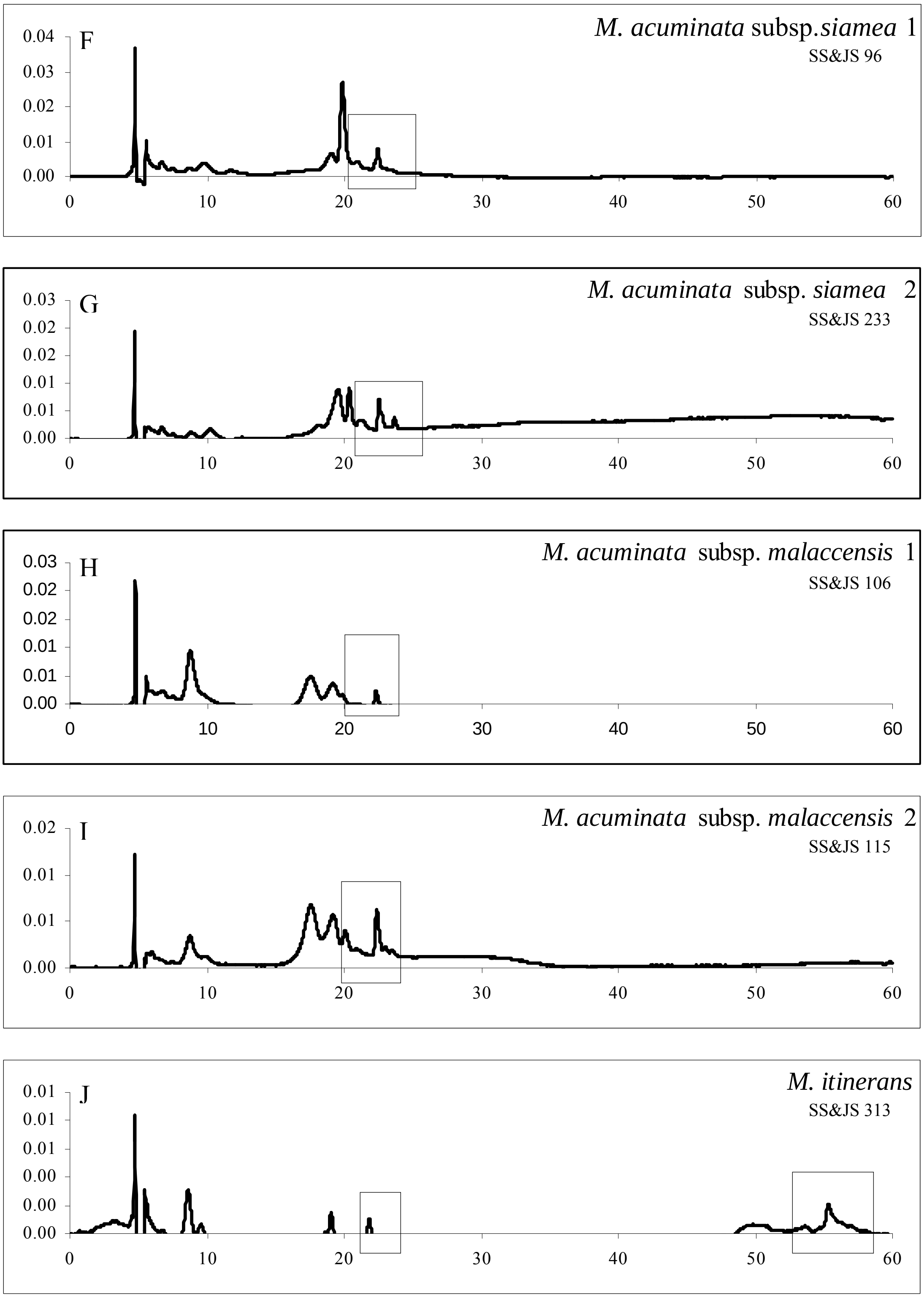


Figure 13 HPLC chromatograms of wild bananas at 370 nm. F, *M. acuminata* subsp. *siamea* 1; G, *M. acuminata* subsp. *siamea* 2; H, *M. acuminata* subsp. *malaccensis* 1; I, *M. acuminata* subsp. *malaccensis* 2 and J, *M. itinerans*.(continue)

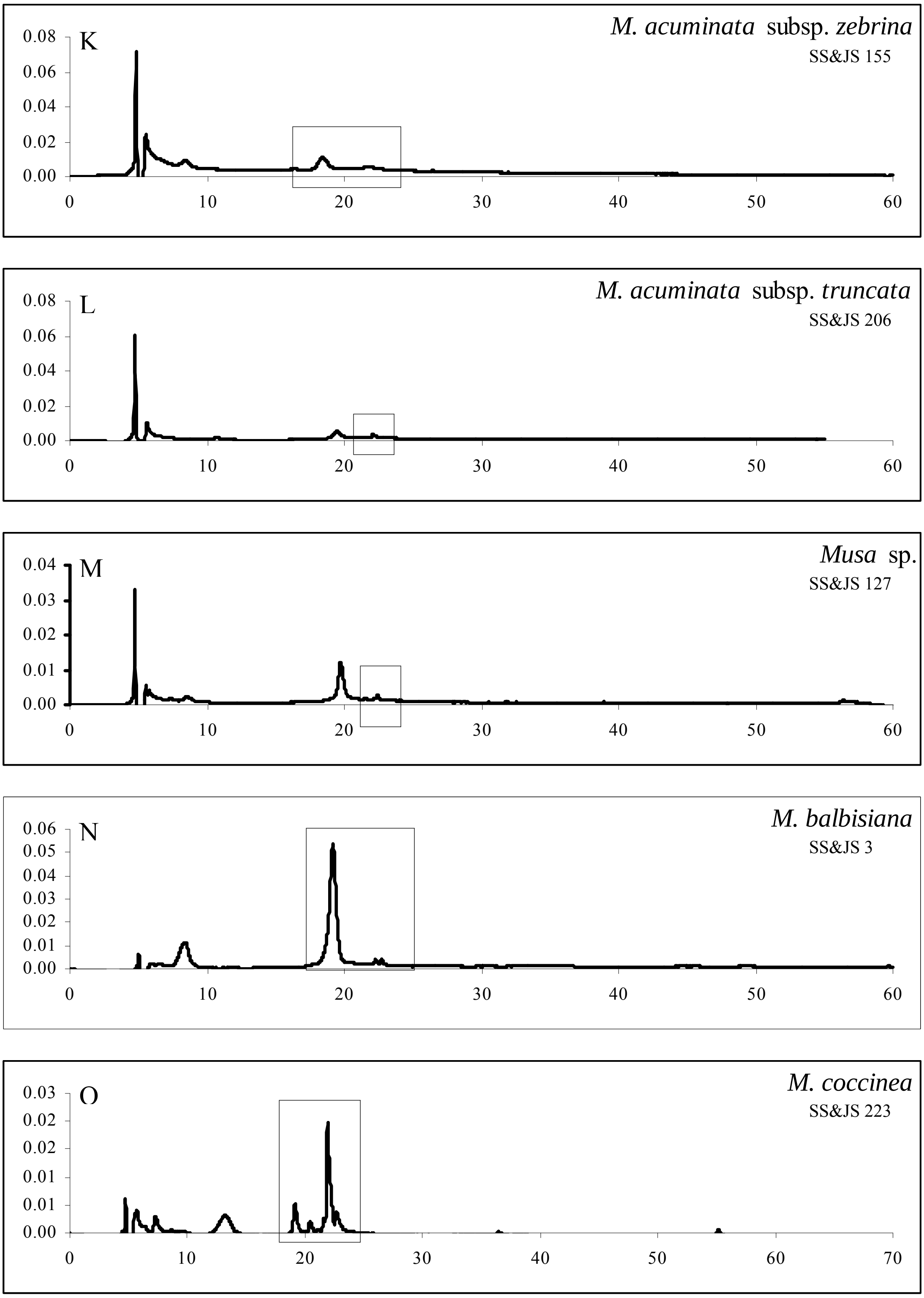


Figure 13 HPLC chromatograms of wild bananas at 370 nm. K, *M. acuminata* subsp. *zebrina*; L, *M. acuminata* subsp. *truncata*; M, *Musa* sp.; N, *M. balbisiana* and O, *M. coccinea.* (continue)

4. Mass Spectrometry of anthocyanin from wild bananas.

Six types of anthocyanin detected by HPLC method were analyzed by mass spectrometer as described in material and methods. The molecular ion (m/z) of selected anthocyanin peaks from wild bananas; SS&JS 319 (peak no.1), 206 (peak no.6), 178 (peak no.2), 223 (peak no. 4), 183 (peak no.1 and 2), 96 (peak no. 1, 2, 3 and 6) and 106 (peak no. 2, 5 and 6) were shown in Table 2. Molecular mass ions of six anthocyanin peaks were 611.2, 595.8, 624.9, 579.4, 608.7 and 638.8 m/z, respectively. During tandem mass spectrometry (MS/MS) analysis, molecular ions produced fragment ions at 464.8, 448.6, 478.6, 433.3, 462.6, and 492.7 m/z indicating that all molecular ions lost 146 m/z, corresponding to a rhamnosyl group and further loss of a glucosyl group (162 m/z) to yield the common anthocyanidin aglycone cations at 302.7 (delphinidin, Dp), 286.7 (cyanidin, Cy), 316.6 (petunidin, Pt), 271.3 (pelargonidin, Pg), 300.7 (peonidin, Pn) and 330.7 (malvidin, Mv), respectively (Figure 15 and 16 for example). The fragmentation pattern of molecular ions matched with those of previous studies in *Musa x paradisiaca* [8] and blackcurrants [25] (see APPENDIX E). This confirmed that the rutinosyl group attaches to the anthocyanidin core, and the fragmentation pattern from anthocyanin in peak no. 2 from wild banana SS&JS 106 was identical to standard cyanidin-3-rutinoside chloride (Figure 9).

Combine the data from HPLC and mass spectrometry, then anthocyanin in wild bananas bract was assigned as following: peak no.1; delphinidin-3-rutinoside, peak no.2; cyanidin-3-rutinoside, peak no.3; petunidin-3-rutinoside, peak no.4; pelargonidin-3-rutinoside, peak no.5; peonidin-3-rutinoside and peak no.6; malvidin-3-rutinoside, as shown in Figure 17. From these assigned anthocyanin, *M. acuminata* subsp. *siamea* 1, 2 and *M. itinerans* possessed 3-rutinoside of delphinidin, cyanidin, petunidin, peonidin and malvidin. *M. acuminata* subsp. *malaccensis* 1 and 2 possessed 3-rutinoside of delphinidin, cyanidin, peonidin and malvidin (some sample possessed petunidin). *Musa* species possessed 3-rutinoside of cyanidin, peonidin and malvidin. *M. acuminata* subsp. *zebrina* possessed one type of anthocyanin which is cyanidin-3-rutinoside, while in *M. acuminata* subsp. *truncata* possessed malvidin-3-rutinoside.

M. laterita, *M. balbisiana*, *M. velutina* and *E. superbum* possessed the same two anthocyanin types which are delpinidin-3-rutinoside and cyanidin-3-rutinoside. *M. coccinea* possessed cyanidin-3-rutinoside and pelargonidin-3-rutinoside.

With total anthocyanin experiment the amount of each anthocyanin contents was calculated as following equation i.e. cyanidin-3-rutinoside in peak no.2 (for each wild banana).

$$\text{Amount of anthocyanin } (\mu\text{mole/g}) = \frac{\text{Total anthocyanin} \times \text{area under peak no. 2}}{\text{Sum of area under the peak of all peaks}}$$

The amount of anthocyanin in fresh tissue from 67 samples of wild banana samples were ranging from 0-1.12 µmole/g for delphinidin-3-rutinoside, 0-1.17 µmole/g for cyanidin-3-rutinoside, 0-0.25 µmole/g for petunidin-3-rutinoside, 0.34 µmole/g for pelargonidin-3-rutinoside, 0-1.02 µmole/g for peonidin-3-rutinoside and 0-1.25 µmole/g for malvidin-3-rutinoside respectively. Total anthocyanin contents from each 67 samples were ranging from 0-2.23 µmole/g (Table 3).

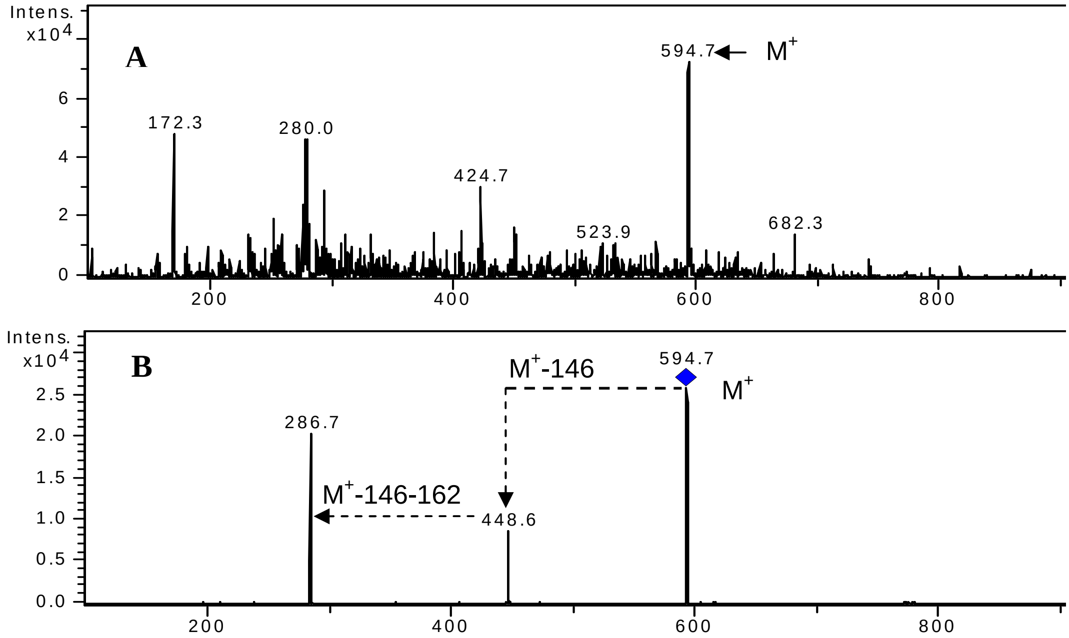


Figure 14 The mass spectra of anthocyanin peak no.2 from SS&JS 106. A, mass spectra of molecular mass ion at 594.7 m/z (black arrow); B, mass spectra of daughter ions at 448.6 m/z and 286.7 m/z (black dash arrow) obtained from tandem mass spectrometry.

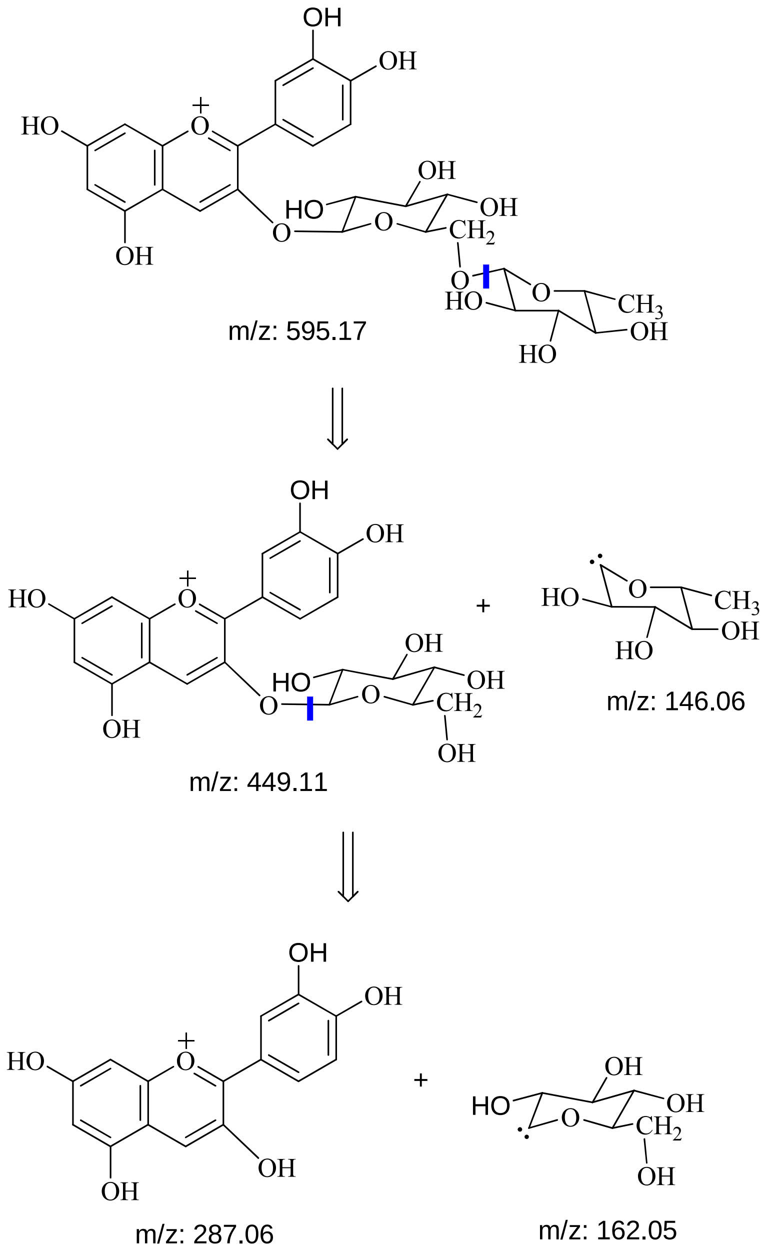


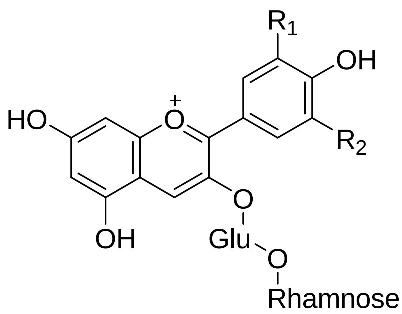
Figure 15 An example of fragmentation from molecular mass ion of anthocyanin peak no. 2 from SS&JS 106. (m/z value in fig. are calculated by ChemBioDraw)

Table 2 Molecular mass ion and tandam mass ion of anthocyanin peaks from selected wild bananas.

Accession no.	Peak no.	$[M]^+$ m/z	Fragments [1] m/z	Identity [2]
96	1	611.2	464.8, 302.9	Dp-3-rutinoside
	2	595.8	448.7, 286.7	Cy-3-rutinoside
	3	624.9	478.7, 316.7	Pt-3-rutinoside
	6	638.8	492.7, 330.6	Mv-3-rutinoside
106	2	594.8	448.6, 286.7	Cy-3-rutinoside
	5	608.7	462.6, 300.7	Pn-3-rutinoside
	6	638.8	492.7, 330.7	Mv-3-rutinoside
178	2	594.6	448.5, 286.6	Cy-3-rutinoside
183	1	610.8	464.8, 302.7	Dp-3-rutinoside
	2	594.7	448.6, 286.6	Cy-3-rutinoside
206	6	638.8	492.7, 330.6	Mv-3-rutinoside
223	4	597.4	433.3, 271.2	Pg-3-rutinoside
319	1	611.2	465.0, 303.0	Dp-3-rutinoside

[1], $[M^+$-rhamnose] and $[M^+$-rhamnose-glucose]

[2], Proposed anthocyanin pigment, identified by combination of HPLC data, Mass spectra and previous reports in other plants (see APPENDIX E).



1. Dp-3-rutinoside; R_1=OH, R_2=OH
2. Cy-3-rutinoside; R_1=OH, R_2=H
3. Pt-3-rutinoside; R_1=OCH_3, R_2=OH
4. Pg-3-rutinoside: R_1=H, R_2,=H
5. Pn-3-rutinoside; R_1=OCH_3, R_2=H
6. Mv-3-rutinoside; R_1=OCH_3, R_2=OCH_3

Figure 16 Structure of anthocyanin in wild bananas of Thailand. Dp, delphinidin; Cy, cyanidin; Pt, petunidin; Pg, pelargonidin; Pn, peonidin; Mv, malvidin.

Table 3 Anthocyanin contents from bracts of wild bananas in Thailand.

No.	Accession no.	Anthocyanin content (µmole/g fresh bract)[1]						
		1[2]	2	3	4	5	6	total
1	2	0.01	0.04	0	0	0.02	0.06	0.13
2	3	0.16	0.76	0	0	0	0	0.92
3	6	0.02	0.21	0.04	0	0.39	0.92	1.58
4	7	0.10	0.06	0.07	0	0.06	0.93	1.22
5	8	0.03	0.08	0.03	0	0.22	0.47	0.83
6	9	0.03	0.19	0	0	0.12	0.48	0.83
7	19	0.02	0.05	0.01	0	0.04	0.22	0.35
8	27	0	0	0	0	0	0	0
9	45	0	0	0	0	trace[3]	0	trace
10	57	0.11	0.33	0	0	0	0	0.44
11	91	0.38	1.17	0	0	0	0	1.55
12	96	0.20	0.14	0.20	0	0.12	1.18	1.85
13	100	0.23	0.36	0	0	0.07	0.14	0.79
14	106	0.02	0.57	0.06	0	0.62	0.74	2.01
15	108	0.04	0.15	0	0	0.36	0.72	1.27
16	109	0	0.33	0	0	0.53	0.24	1.09
17	113	0	0.21	0	0	0.46	0.26	0.93
18	114	0	0.49	0	0	1.02	0.07	1.58
19	115	0.04	0.19	0	0	0.70	0.87	1.80
20	116	0.07	0.36	0	0	0.49	0.84	1.76
21	125	0.01	0.19	0	0	0.47	0.32	0.98
22	126	0.07	0.10	0	0	0.13	0.79	1.09
23	127	0	0.12	0	0	0.10	0.16	0.38
24	132	0.07	0.14	0.07	0	0.19	1.16	1.63
25	135	0.11	0.55	0	0	0	0	0.66
26	139	0.04	0.45	0	0	0.44	0.26	1.20
27	142	0.18	0.34	0.13	0	0.20	0.88	1.73
28	145	0.19	0.64	0.12	0	0.47	0.80	2.23
29	146	0.39	0.36	0.25	0	0.12	1.07	2.19
30	147	0.09	0.03	0.10	0	0.08	1.06	1.36
31	155	0	0.51	0	0	0	0	0.51
32	168	0.05	0.09	0	0	0	0	0.14
33	172	0.12	0.32	0	0	0.42	0.73	1.59
34	173	0	0	0	0	0	0	0
35	174	0.13	0.16	0.14	0	0.10	0.95	1.48
36	177	0	0	0	0	trace	trace	trace
37	178	0.02	0.11	0.00	0	0	0	0.13
38	181	0.23	0.09	0.12	0	0	0.66	1.10
39	182	0.06	0.11	0.06	0	0.06	0.81	1.11

Table 3 Anthocyanin contents from bracts of wild bananas in Thailand (continued)

No.	Accession no.	Anthocyanin content (µmole/g fresh bract)[1]						
		1[2]	2	3	4	5	6	total
40	183	0.06	0.50	0	0	0	0	0.56
41	206	0	0	0	0	0	0.63	0.63
42	223	0	0.02	0	0.34	0	0	0.36
43	224	0	0	0	0	0	0	0.00
44	228	0.02	0.06	0.03	0	0.13	0.48	0.72
45	233	0.12	0.08	0.08	0	0.10	1.25	1.64
46	238	0.02	0.05	0.03	0	0.15	1.22	1.47
47	242	0	0	0	0	0	0	0
48	243	0	0.58	0	0	0	0	0.58
49	245	0.01	0.07	0	0	0.02	0.40	0.49
50	246	0.09	0.13	0.07	0	0.13	0.26	0.67
51	247	0.25	0.33	0.20	0	0.29	0.56	1.62
52	274	0	0.19	0	0	0.27	0.49	0.96
53	281	0.01	0.05	0	0	0.07	0.07	0.20
54	283	0.04	0.12	0.02	0	0.20	0.37	0.76
55	286	0.01	0.04	0.01	0	0.03	0.29	0.38
56	289	0.02	0.03	0	0	0.06	0.20	0.31
57	290	0.02	0.03	0.01	0	0.02	0.26	0.34
58	291	0.01	0.02	0	0	0.02	0.09	0.13
59	293	0.06	0.12	0.07	0	0.16	0.56	0.96
60	306	0.01	0.05	0	0	0.01	0.25	0.32
61	307	trace	0	0	0	trace	0	trace
62	310	0.04	0.06	0.05	0	0.10	1.05	1.31
63	312	0.03	0.07	0.04	0	0.09	0.63	0.87
64	313	0.04	0.27	0.04	0	0.09	0.23	0.68
65	314	0	0.31	0	0	0.21	0.34	0.86
66	318	0	0	0	0	0	0	0
67	319	1.12	0.63	0	0	0	0	1.75

[1], As equivalent moles to cyanidin-3-rutinoside [31]

[2], Peak no., corresponding to delphinidin-3-rutinoside, cyanidin-3-rutinoside, petunidin-3-rutinoside, pelargonidin-3-rutinoside, peonidin-3-rutinoside and malvidin-3-rutinoside respectively.

[3], < 0.001 µmole/g bract

5. Relationship of anthocyanin between wild bananas

The relationship between wild bananas based on types of anthocyanin and their ratio of area under the peak of each anthocyanin were analyzed with NTSYSpc 2.1 program to generate the dendrogram (Figure18) of wild bananas in the experiment.

The dendrogram shows the dissimilarity coefficient of wild bananas in the experiment, less dissimilarity value means that they were more similar to the others. Dendrogram shows possible of 4 groups of wild bananas (AI to AIV) and other out-group wild bananas (B-1 to B-3).

The first group is AI, this groups possessed 4 to 5 type of anthocyanins which are delphinidin-3-rutinoside, cyanidin-3-rutinoside, petunidin-3-rutinoside, peonidin-3-drutinoside and malvidin-3-rutinoside. AI group can be divided into 2 sub groups which are AI-1 and AI-2. Wild bananas in AI-1 group mostly are *M. acuminata* subsp. *malaccensis* 1 and 2, *Musa* sp., *M. itinerans* (SS&JS 2 and 313) and *M. laterita* (SS&JS 246), however some *M. acuminata* subsp. *siamea* 1 and 2 also found in this AI-1 group. Wild bananas in AI-2 group mostly are *M. acuminata* subsp. *siamea* 1 and 2, one accession of *M. itinerans* (SS&JS 306), *M. acuminata* subsp. *malaccensis* 2 (SS&JS 9), *M. acuminata* subsp. *siamea*; yellow bract with streak of pink color (SS&JS 177) and *M. acuminata* subsp. *truncata* (SS&JS 206). Similarity within these 2 sub groups possibly influence by ratio of area under the anthocyanin peak from HPLC (Table 4), in AI-1 by cyanidin-3-rutinoside (0.1-0.4), peonidin-3-rutinoside (0.1-0.4) and malvidin-3-rutinoside (0.35-0.6), wild bananas AI-2 by Delphinidin-3-rutinoside (0.01-0.06), cyanidin-3-rutinoside (0.05-0.12) and malvidin-3-rutinoside (0.6-0.8).

The second group is AII, this group possessed 3 to 4 type (mostly 3 types) of anthocyanins which are delphinidin-3-rutinoside, cyanidin-3-rutinoside, peonidin-3-rutinoside and malvidin-3-rutinoside. Wild bananas in this group mostly are *M. acuminata* subsp. *malaccensis* 1 and one of subsp. *malaccensis* 2, *Musa* sp. is also found in this group. Anthocyanins that possibly influence this group are cyanidin-3-rutinoside (0.2-0.4), peonidin-3-rutinoside (0.35-0.65) and malvidin-3-rutinoside (0.2-0.3).

The third group is AIII, this group does not possess any anthocyanin pigment. Therefor all wild bananas with yellow bract color (*M. acuminata* subsp. *siamea*, *E. glaucum* and *E. superbum*) are in this group.

The forth group is AIV, this group possesses 1 to 2 types of anthocyanin which are delphinidin-3-rutinoside and cyanidin-3-rutinoside. Wild bananas in this group are *M. balbisiana*, *M. laterita*, *M. velutina*, *M. acuminata* subsp. *zebrina*, *E. superbum* with red bract color and one of *M. acuminata* subsp. *siamea* 1 (SS&JS 91). Ratios of area under the peak of both anthocyanin; 0.1-0.6 for delphinidin-3-rutonoside and 0.35-0.9 for cyanidin-3-rutinoside, are major factor of classification for this AIV group.

The last out-group are present in figure 18 as B-1 to B-3. B-1 is *M. acuminata* subsp. *siamea* with yellow bract color and streak with purple color (SS&JS 307). B-2 is *M. ornata* (SS&JS 45) and B-3 is *M. coccinea* (SS&JS 223).

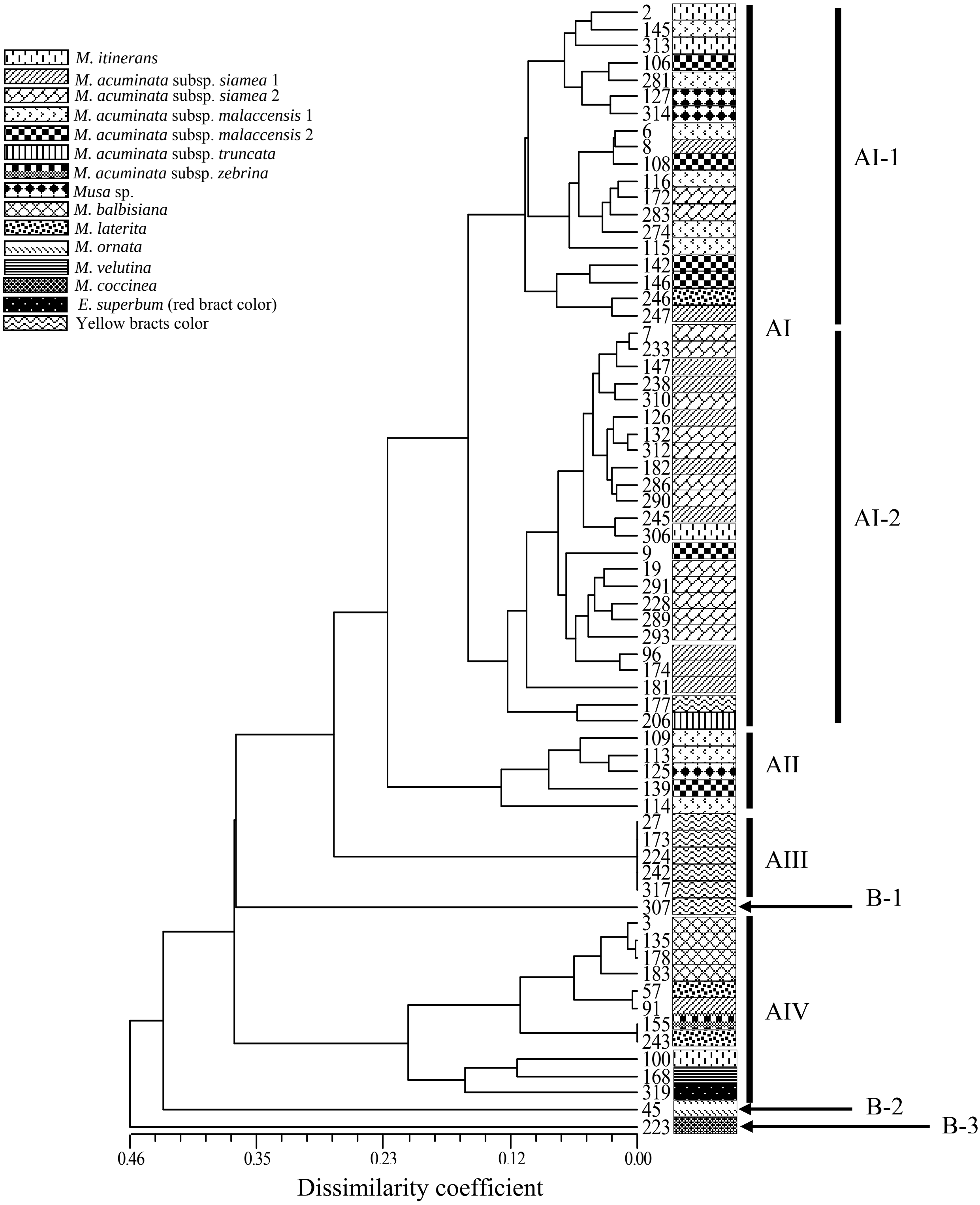


Figure 17 Dendrogram show similarity of wild bananas using types and ratio of area under each peak from HPLC.

Table 4 Ratios of area peak of each anthocyanin from HPLC

Group	Accession no. (SS&JS)	Anthocyanin [1] 1	2	3	4	5	6	Bract colors [2]
AI-1	2	0.08	0.32	0	0	0.17	0.43	RD
	145	0.09	0.29	0.05	0	0.21	0.36	PB
	313	0.07	0.40	0.06	0	0.14	0.34	RP
	106	0.01	0.28	0.03	0	0.31	0.37	RP
	281	0.05	0.26	0	0	0.34	0.35	RD
	127	0	0.31	0	0	0.26	0.43	PL
	314	0	0.36	0	0	0.24	0.39	RP
	6	0.01	0.13	0.03	0	0.25	0.58	BL
	8	0.03	0.10	0.04	0	0.26	0.57	BL
	108	0.03	0.12	0	0	0.28	0.57	RP
	116	0.04	0.20	0	0	0.28	0.48	PB streak YW, RP
	172	0.07	0.20	0	0	0.26	0.46	RP
	283	0.05	0.16	0.03	0	0.27	0.49	BL+PL
	274	0	0.20	0	0	0.29	0.51	PB
	115	0.02	0.11	0	0	0.39	0.48	PP
	142	0.11	0.19	0.08	0	0.11	0.51	PB
	146	0.18	0.17	0.11	0	0.05	0.49	PL
	246	0.13	0.19	0.10	0	0.20	0.38	PP
	247	0.15	0.20	0.12	0	0.18	0.34	BL
AI-2	7	0.08	0.05	0.06	0	0.05	0.76	BP tip YW
	233	0.07	0.05	0.05	0	0.06	0.76	BL tip YW
	147	0.07	0.02	0.08	0	0.06	0.78	PL
	238	0.01	0.03	0.02	0	0.10	0.83	PL
	310	0.03	0.05	0.04	0	0.08	0.80	PL
	126	0.06	0.09	0.00	0	0.12	0.73	BL tip YW
	132	0.04	0.08	0.05	0	0.12	0.71	BL+PL tip YW
	312	0.04	0.09	0.05	0	0.10	0.73	BL
	182	0.06	0.10	0.05	0	0.06	0.73	PL tip YW
	286	0.04	0.10	0.02	0	0.09	0.76	BL
	290	0.06	0.10	0.02	0	0.05	0.77	BL+PL
	245	0.02	0.14	0	0	0.03	0.81	BL
	306	0.04	0.16	0	0	0.03	0.77	RP
	9	0.04	0.23	0	0	0.15	0.58	PB
	19	0.06	0.16	0.04	0	0.11	0.63	PP tip YW
	291	0.07	0.13	0	0	0.13	0.68	BL+PL
	228	0.03	0.08	0.04	0	0.18	0.67	RP
	289	0.05	0.10	0.01	0	0.19	0.65	BL+PL
	293	0.06	0.12	0.07	0	0.16	0.58	PP
	96	0.11	0.08	0.11	0	0.07	0.64	BL
	174	0.09	0.11	0.10	0	0.07	0.64	PL
	181	0.21	0.09	0.11	0	0	0.60	PL tip YW
	177	0	0	0	0	0.09	0.91	OG
	206	0	0	0	0	0	1.00	Deep BL

Table 4 Ratios of area peak of each anthocyanin from HPLC (continue)

Group	Accession no. (SS&JS)	Anthocyanin [1] 1	2	3	4	5	6	Bract colors [2]
AII	109	0	0.30	0	0	0.48	0.22	OR streak YW
	113	0	0.22	0	0	0.50	0.28	RD
	125	0.01	0.19	0	0	0.47	0.32	RP
	139	0.03	0.38	0	0	0.37	0.22	RD streak OR, YW
	114	0	0.31	0	0	0.65	0.04	PP streak LG tip YW
AIII	27	0	0	0	0	0	0	YW
	173	0	0	0	0	0	0	GY+YW
	224	0	0	0	0	0	0	GY+YW
	317	0	0	0	0	0	0	MG
AIV	3	0.18	0.82	0	0	0	0	PB
	135	0.16	0.84	0	0	0	0	GR
	178	0.16	0.84	0	0	0	0	RP
	183	0.11	0.89	0	0	0	0	DG
	57	0.25	0.75	0	0	0	0	OR
	91	0.25	0.75	0	0	0	0	BL
	155	0	1.00	0	0	0	0	BL+PL
	243	0	1.00	0	0	0	0	OR
	100	0.29	0.45	0	0	0.08	0.17	PB streak YW
	168	0.38	0.62	0	0	0	0	pale PL
	319	0.64	0.36	0	0	0	0	RP
B-1	307	0.70	0	0	0	0.30	0	BP, PL tip BY+YW
B-2	45	0	0	0	0	1.00	0	Light PP
B-3	223	0	0.06	0	0.94	0	0	OR

[1], 1 to 6 corresponding to delphinidin-3-rutinoside, cyanidin-3-rutinoside, petunidin-3-rutinoside, pelargonidin-3-rutinoside, peonidin-3-rutinoside and malvidin-3-rutinoside, respectively.

[2], see color chart in APPENDIX A for Abbreviations.

CHAPTER IV
DISCUSSION

1. Anthocyanin in the banana bracts

In this study, found anthocyanin are stored only in epidermis cells, however anthocyanin can be found in other cells i.e. leaf mesophyll cells[11]. Under light microscope, it could not identify about the storage form of anthocyanin in bract. Previous studied by electron microscope report many storage forms of anthocyanin in vacuole i.e. free anthocyanin, anthocyanic vacuolar inclusions (AVIs; membraneless proteinaceous matrix) [33-35]. Moreover in AVIs can be found many forms in lisianthus (*Eustoma grandiflorum* Salisb.) such as vesicle-like, rod-like and irregular shape [36]. However recently reported that anthocyanin which stored in AVIs form are diglycoside and acylated anthocyanin [34]. Identified anthocyanin in wild bananas bract are one glycoside, thus it can imply that storage form might not be in AVIs form. However, to study storage form of anthocyanin in bract compare to other plants need ultra structure analysis.

The different color of vacuole in epidermis cells on both outer and inner side of bract probably come from cellular concentration of each anthocyanin in each vacuole of each epidermal cell or from pH in vacuole which affected structural transformations of anthocyanin [10]. In addition, combination of metal complex i.e. magnesium ion together with intra- and intermolecular copigments i.e. flavone cause bluing of the color [10, 35, 37, 38], however presence of some flavonoids increase the stability of anthocyanin [35]. From HPLC data at 370 nm which used for monitor other flavonoids (not anthocyanin), many peaks were detected and appeared in all wild bananas. These might imply the interaction between anthocyanin and other colorless flavonoids, however it needs further study before confirm their interaction. Moreover, study of flavonoid in bract might reveal accumulation of flavonoid associate in anthocyanin biosynthetic pathway and imply happening in yellow bract color and also in *M. ornata* with light pink purple bract color that could not be strongly detected anthocyanin in this study.

The store of anthocyanin in epidermis cells on both outer and inner side of bract might be to protect bract from stress during exposure to sun light [35] and might play role in attracts pollinator for pollination process by assist pollinator to locate the flower, However, banana inflorescences also have many other factors to invite pollinators such as nectar, smell, and pollen [39], [40].

2. Relationship of anthocyanin in wild bananas

Anthocyanins in wild bananas were identified as 3-rutinosode of delphinidin, cyanidin, pelargonidin, petunidin, peonidin and malvidin which corresponds to previous studies in banana [8, 9, 26]. These six types can divide based on the substitution at positions 3’ and 5’ of anthocyanidin aglycone into 2 groups. The first is methylated anthocyanin; peonidin, petunidin and malvidin. The second is non-methylated anthocyanin [9].

From dendrogram based on type of anthocyanin and ratio of area under each anthocyanin peak (HPLC). AI and AII groups which composed of *M. acuminata* subsp. *siamea* 1 and 2, subsp. *malaccensis* 1 and 2, *Musa* sp. and *M. itinerans* contain 3 to 5 anthocyanin types which both methylated and non-methylated anthocyanin, these may imply that methylation of anthocyanidin aglycone occur in AI and AII group. AI-1 and AI-2 might be able to synthesis anthocyanin from dihydromyricetin more than from dihydroquercetin (see Figure 18 and Table 4), but inversely in AII that might be able to synthesis anthocyanin via dihydroquercetin more than dihydromyricetin. However, wild banana in AI and AII groups are in Musa section. *M. laterita* SS&JS 246 is grouped into AI-1, this *M. laterita* gives different anthocyanin profile from the rest of *M. laterita* which possess 2 types of anthocyanin. However, this SS&JS 246 found next to SS&JS 247 which is *M. acuminata* subsp. *siamea* 1. In AI-2 *M. acuminata* subsp. *truncata* SS&JS 206 is in this group but is has only malvidin-3-rutinoside, this may imply that it might be able to synthesis anthocyanin only via dihydromyricetin. However *M. acuminata* subsp. *siamea* with yellow bract color SS&JS 177 is grouped in the AI-2 group from some detected ratio of malvidin-3-rutinoside peak.

In AIII group, yellow bract color group which is not detected any anthocyanin. Imply for this group is they might have problem in anthocyanin synthetic pathway probably the step of turning leucoanthocyanidin to anthocyanidin or any steps prior to this step [33].

In AIV group which compose of *M. balbisiana* (Musa section), *M. acuminata* subsp. *zebrina* (Musa section), *M. laterita* (Rhodochlamys section), *M. velutina* (Rhodochlamys section) and *E. superbum* with red bract color, possessed 2 types of anthocyanin which are non-methylated anthocyanin. Thus may imply that AIV group might be able to synthesis anthocyanin via both dihydroquercetin and dihydromyricetin only or this group is unable to do methylation of anthocyanin. *M. acuminata* subsp. *siamea* 1 SS&JS 91 and *M. itinerans* SS&JS 100 are in this AIV group, however SS&JS 100 also possessed methylated anthocyanin but in very low of ratio.

The remaining groups, can not grouping may result from they possessed different of anthocyanin profile; B-3, *M. coccinea* SS&JS 223 (Callimusa section) possessed cyanidin-3-rutinoside and pelargonidin-3-rutinoside which is unique in this study. Thus imply for SS&JS 223 that it might be able to synthesis anthocyanin mainly via dihyderkeamferol and a little via dihydroquercetin and unable to do methylation. B-2, *M. ornata* SS&JS 45 (Rhodochlamys section) potentially possessed peonidin-3-rutinoside [9] this may imply that it prefer synthesis via dihydroquercetin and has methylation system. B-1, *M. acuminata* subsp. *siamea* with yellow bract SS&JS 307 possessed delphinidin-3-rutinoside and malvidin-3-rutinoside, may imply that it might be able to synthesis via dihydromyricetin and also presence of methylation system, however amount of anthocyanin are very low.

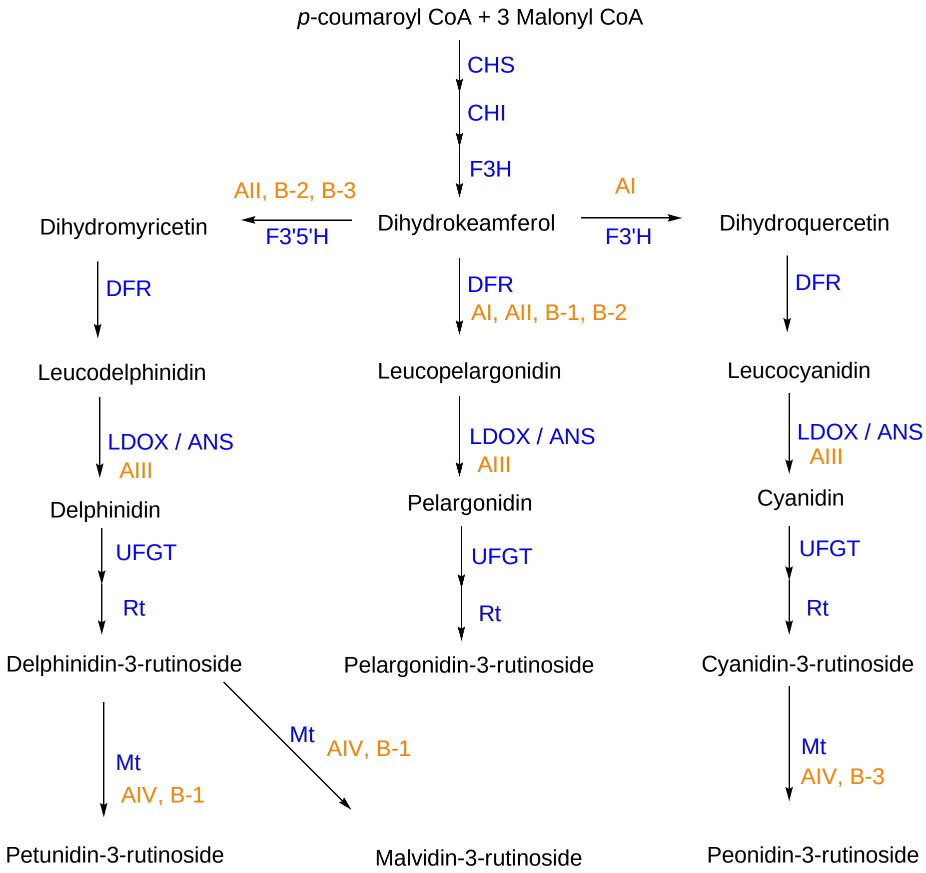


Figure 18 Possible limiting steps in anthocyanin synthesis pathway in each wild banana group (Name of each group in orange color at limiting step) which cause anthocyanin phenotypes in studied wild bananas

3. Characterization of anthocyanin in wild banana

The detection of anthocyanin using photodiode array detector (PDA) in HPLC sometime might not give satisfying result of anthocyanin characters such as maximum absorption wavelength, absorption value at a desirable wavelength. During the PDA measurement of absorption, it could not refer to the baseline absorption value of the solvent at that time. Therefore the instrument may record baseline value both in positive and negative values. Thus absorption value at wavelength 310 nm, 440 nm and at lambda maximum in visible region will affect value of % $A_{310}/A_{vis\text{-}max}$ and % $A_{440}/A_{vis\text{-}max}$. These values of many accessions are negative or over 100% which made the interpretation of acylation or glycosylation are difficult. However these two values are just used for roughly estimate acylation and glycosylation of anthocyanin. These calculated formulae came from statistical analysis of results from previous studies [32], so it can only for remind but not confirm structure of anthocyanin. It should be coupled the data from others methods such as mass spectrometer, NMR and report from other plants to confirm true structure of anthocyanin.

CHAPTER V
CONCLUSION

Analyses 67 samples of wild banana bracts collected from various parts of Thailand by high performance liquid chromatography (HPLC), mass spectrometry (MS) and tandem mass spectrometry (MS/MS) showed that wild banana bract possessed 3-rutinoside conjugation of six common anthocyanidin aglycones; delphinidin, cyanidin, petunidin, pelargonidin, peonidin and malvidin, respectively.

Pelargonidin-3-rutinoside presented only in *M. coccinea*. Different bract colors in *M. acuminata* subsp. *siamea* 1 and 2, subsp. *malaccensis* 1 and 2 possessed the same anthocyanin types with different proportions. *M. acuminata* subsp. *truncata* had only malvidin-3-rutinoside which was different from that in other *M. acuminata* subspecies. All *M. acuminata* and *M. itinerans* possessed non-methylated and methylated anthocyanins pigments.

M. velutina, *M. laterita*, *M. balbisiana* and *E. superbum* revealed two types of anthocyanin which are non-methylated group. This wild banana might be a diverted group in terms of anthocyanin synthetic pathway from *M. acuminata.*

Lack of anthocyanin in wild bananas with yellow bract color indicated deficiency of anthocyanin. This might be due to some steps of gene expression or gene regulation in anthocyanin biosynthesis could be inhibited. To study what causes the yellow bract phenotype in bananas, they should be studied others flavonoid profiles in these yellow bract compare with the normal ones, or searching into the critical metabolic or regulatory step in biosynthetic pathway by some experiment such as gene complementation analysis.

Wild banana in Thailand based on type of anthocyanin and proportions of each anthocyanin can be clearly divided into 4 groups

1. AI-1 and AI-2 possessed 4 to 5 type of anthocyanin which are non-methylated and methylated anthocyanin

2. AII possessed 3-4 type of anthocyanin which are non-methylated and methylated anthocyanin
3. AIII yellow bract color which does not possess anthocyanin
4. AIV possessed 2 type of anthocyanin which are non-methylated anthocyanin

The remaining groups are B-1, B-2 and B-3 which could not classified into previous groups (AI to AIV group) according to their unique patterns of anthocyanin types and proportions.

REFERENCES

1. Crouch JH, Vuylsteke D, Ortiz R. Perspectives on the application of biotechnology to assist the genetic enhancement of plantain and banana (*Musa spp.*) Electronic Journal of Biotechnology [online] 1998:11-22.

2. Price NS. CHAPTER1: The origin and development of banana and plantain . In: Gowen S, ed. *BANANAS AND PLANTAINS*. London: Chapman & Hall 1995:1-13.

3. Jeff Daniells CJ, Deborah Karamura, Kodjo Tomekpe. Musaloque a catalogue of *Musa* germplasm Diversity in the genus *Musa*: IPGRI, INIBAP, CTA and CIRAD-FLHOR 2001.

4. Simmonds NW. The Evolution of Bananas. London: Longmans 1962.

5. Cheesman E, E. Classification of The Bananas. Kew Bull. 1947;2:79-117.

6. Sasivimon Swangpol, Hugo Volkaert, Rachel C. Sotto, Seelanan T. Utility of Selected Non-coding Chloroplast DNA Sequences for Lineage Assessment of Musa Interspecific Hybrids. Journal of Biochemistry and Molecular Biology. 2007;40(4):577.

7. Simmonds NW, Shepherd K. The taxonomy and origins of cultivated bananas. J Linn Soc Bot 1955;55:302-12.

8. Pazmino-Duran EA, Giusti MM, Wrolstad RE, A. GMB. Anthocyanins from banana bracts (*Musa x paradisiaca*) as potential food colorants. Food Chemistry 2001;73:327-32.

9. Simmonds NW. Anthocyanins in bananas. Annals of Botany. 1954c;18 (72): 471-82.

10. Andersen ØM. Anthocyanins. *ENCYCLOPEDIA OF LIFE SCIENCES*: John Wiley & Sons 2002:1-8.

11. Strack D, Wray V. Anthocyanins. In: Dey PM, Harborne JB, eds. *Method in Plant Biochemistry*. San diego: Academic Press 1989:325-59.

12. Ali A, Strommer J. A simple extraction and chromatographic system for the simultaneous analysis of anthocyanins and stilbenes of Vitis species. Journal of agricultural and food chemistry. 2003 Dec 3;51(25):7246-51.

13. Lapidot T, Harel S, Akiri B, Granit R, Kanner J. PH-dependent forms of red wine anthocyanins as antioxidants. Journal of agricultural and food chemistry. 1999 Jan;47(1):67-70.

14. Kim S-H, Lee J-R, Hong S-T, Yoo Y-K, An G, Kim S-R. Molecular cloining and analysis of anthocyanin biosynthesis genes preferntially expressed in apple skin. Plant Science. 2003;165:403-13.

15. Ubi B, E., Honda C, Bessho H, Kondo S, Wada M, Kobayashi S, et al. Expression analysis of anthocyanin biosynthetic genes in apple skin: Effect of UV-B and temperature. Plant Science. 2006;170:571-8.

16. Jaakola L, Ma¨a¨tta K, Pirttila AM, To¨ rro¨nen R, Ka¨renlampi S, Hohtola A. Expression of Genes Involved in Anthocyanin Biosynthesis in Relation to Anthocyanin, Proanthocyanidin, and Flavonol Levels during Bilberry Fruit Development[1]. Plant Physiology. 2002;130:729-39.

17. Holton T, A., Cornish E, C. Genetics and Biochemistry of Anthocyanin Biosynthesis. The Plant Cell. 1995;7:1071-83.

18. Chandra A, Rana J, Li Y. Separation, identification, quantification, and method validation of anthocyanins in botanical supplement raw materials by HPLC and HPLC-MS. Journal of agricultural and food chemistry. 2001 Aug;49(8):3515-21.

19. Zhang Z, Kou X, Fugal K, McLaughlin J. Comparison of HPLC methods for determination of anthocyanins and anthocyanidins in bilberry extracts. Journal of agricultural and food chemistry. 2004 Feb 25;52(4):688-91.

20. Giusti MM, Rodriguez-Saona LE, Griffin D, Wrolstad RE. Electrospray and tandem mass spectroscopy as tools for anthocyanin characterization. Journal of agricultural and food chemistry. 1999 Nov;47(11):4657-64.

21. Sinia de Pascual-Teresa, Celestino Santos-Buelga, Rivas-Gonzalo JC. LC-MS analysis of anthocyanins from purple corn cob. J Sci Food Agric. 2002;82:1003-6.

22. Choung MG, Baek IY, Kang ST, Han WY, Shin DC, Moon HP, et al. Isolation and determination of anthocyanins in seed coats of black soybean (*Glycine max* (L.) Merr.). Journal of agricultural and food chemistry. 2001 Dec;49(12):5848-51.
23. Da Silva FL, Escribano-Bailon MT, Alonso JJP, Rivas-Gonzalo JC, Santos-Buelga C. Anthocyanin pigments in strawberry. LWT - Food Science and Technology. 2007;40:374-82.
24. Tian Q, Giusti MM, Stoner GD, Schwartz SJ. Screening for anthocyanins using high-performance liquid chromatography coupled to electrospray ionization tamdam mass spectrometry with precursor-ion analysis, product-ion analysis, common-neutral-loss analysis, and selected reaction monitoring. journal of Chromatography A. 2005;1091:72-82.
25. Slimestad R, Solheim H. Anthocyanins from black currants (*Ribes nigrum* L.). Journal of agricultural and food chemistry. 2002 May 22;50(11):3228-31.
26. Horry JP, Jay M. Distribution of Anthocyanins in Wild and Cultivated Banana Varieties. Phytochemistry. 1988;27(8):2667-72.
27. F. Pomar, M. Novo, A. Masa. Varietal differences among the anthocyanin profiles of 50 red table grape cultivars studied by high performance liquid chromatography journal of Chromatography A. 2005;1094:34-41.
28. Athawongsa K. Morphological Diversity of Wild Banana (*Musa acuminata* Colla.) in Thailand. M.Sc. Thesis. Bangkok: Mahidol University; 2008.
29. Wongniam S. Genetic Diversity of Thai Banana (*Musa*) Cultivars Using AFLPs. M.Sc. Thesis. Bangkok: Mahidol University; 2008.
30. Ju ZY, Howard LR. Effects of solvent and temperature on pressurized liquid extraction of anthocyanins and total phenolics from dried red grape skin. Journal of agricultural and food chemistry. 2003 Aug 27;51(18):5207-13.
31. Giusti MM, Wrolstad RE. Unit F1.2: Anthocyanins. Characterization and measurement with UV-Visible spectroscopy. In: Wrolstad RE, ed. *Handbook of Analytical Chemistry*. New Jersey: John Wiley & Sons 2005:19-31.
32. Giusti MM, Rodriguez-Saona LE, Wrolstad RE. Molar absorptivity and Color Characteristics of Acylated and Non-Acylated pelargonidin-Base Anthocyanins. Journal of agricultural and food chemistry. 1999;47:4631-7.

33. Grotewold E. The Genetics and Biochemistry of Floral Pigments. Annu Rev Plant Biol. 2006;57:761-80.
34. Markham KR, Gould KS, Winefield SS, Mitchell KA, Bloor SJ, Boase MR. Anthocyanin vacuolar inclusions - their nature and significance in flower colouration. Phytochemistry. 2000;55:327-36.
35. Stintzing FC, Carle R. Functional properties of anthocyanins and betalains in plants, food and in human nutrition. Trends in Food Science & Technology. 2004;15:19-38.
36. Zhang H, Wang L, Delores S, Bennett R, Devies K. New insight into structures and formation of anthocyanic vacuolar inclusions in flower petals. BMC Plant Biology. 2006;6(29).
37. Kondo T, Ueda M, Isobe M, Goto T. A New Molecular Mechanism of Blue Color Development with Protocyanin, a Supramolecular pigment from cornflower, *Centaurea cyanus*. Tetrahedron Letters. 1998;39:8307-10.
38. Tanaka M, Fujimori T, Uchida I, Yamaguchi S, Takeda K. A malonylated anthocyanin and flavonols in blue *Meconopsis* flowers. Phytochemistry. 2001;56:373-76.
39. Schemske DW, H. D. Bradshaw J. Pollinator preference and the evolution of floral traits in monkeyflowers (*Mimulus*). PNAS. 1999;96(21):11901-15.
40. Kudo G, Ishii HS, Hirabayashi Y, Ida TY. A test of the effect of floral color change on pollination effectiveness using artificial inflorescences visited by bumblebees. Oecologia. 2007;154:119-28.
41. IPGRI-INIBAP/CIRAD. Descriptor for banana (*Musa* spp.): International Plant Genetic Resources Institute, Rome, Italy/International Network for the Improvement of Banana and Plantain, Montpellier, France/Centre de Cooperation Internationale en Recherche Agronomique pour le Development, Montpellier, France 1996.
42. Tian Q, Giusti MM, Stoner GD, Schwartz SJ. Characterization of new anthocyanin in black raspberries (*Rubus occidentalis*) by liquid chromatography electrospray ionization tandem mass spectrometry. Food Chemistry. 2006;94:465-8.

APPENDIX

APPENDIX A

Color chart for bananas

COLOUR CHART A	COLOUR CHART B
CREAM; CR R 253 G 221 B 211 C 0 M 15 Y 10 K 0	WHITE; WH R 255 G 254 B 242 C 0 M 0 Y 5 K 0
YELLOW; YW R 255 G 223 B 93 C 0 M 10 Y 75 K 0	CREAM; CR R 240 G 247 B 232 C 5 M 0 Y 10 K 0
WATERY GREEN; WG R 240 G 247 B 232 C 5 M 0 Y 10 K 0	IVORY; IV R 255 G 218 B 162 C 0 M 15 Y 40 K 0
GREEN YELLOW; GY R 185 G 168 B 129 C 30 M 30 Y 55 K 0	YELLOW; YW R 255 G 245 B 109 C 0 M 0 Y 70 K 0
LIGHT GREEN; LG R 207 G 218 B 169 C 20 M 5 Y 40 K 0	BRIGHT YELLOW; BY R 245 G 208 B 15 C 5 M 15 Y 100 K 0
MEDIUM GREEN; MG R 148 G 182 B 146 C 45 M 15 Y 50 K 0	ORANGE; OG R 247 G 148 B 28 C 0 M 50 Y 100 K 0
GREEN; GR R 205 G 157 B 129 C 20 M 40 Y 50 K 0	ORANGE RED; OR R 230 G 89 B 72 C 5 M 80 Y 75 K 0
DARK GREEN; DG R 97 G 100 B 83 C 75 M 65 Y 80 K 0	RED; RD R 218 G 30 B 66 C 10 M 100 Y 75 K 0
WHITE; WH R 255 G 254 B 242 C 0 M 0 Y 5 K 0	RED PURPLE; RP R 148 G 43 B 101 C 50 M 100 Y 45 K 0
ORANGE RED; OR R 230 G 89 B 72 C 5 M 80 Y 75 K 0	PINK; PK R 220 G 100 B 124 C 10 M 75 Y 35 K 0
RED; RD R 218 G 30 B 66 C 10 M 100 Y 75 K 0	RUSTY BROWN; RB R 165 G 94 B 98 C 40 M 75 Y 60 K 0
PINK PURPLE; PP R 240 G 90 B 120 C 0 M 80 Y 35 K 0	BEIGE PINK; BP R 239 G 205 B 176 C 5 M 20 Y 30 K 0
PURPLE BROWN; PB R 84 G 120 B 138 C 75 M 50 Y 40 K 0	SILVERY; SV R 169 G 191 B 179 C 35 M 15 Y 30 K 0
RED PURPLE; RP R 165 G 64 B 110 C 40 M 90 Y 40 K 0	LIGHT GREEN; LG R 207 G 218 B 169 C 20 M 5 Y 40 K 0
PURPLE; PL R 130 G 53 B 127 C 60 M 95 Y 20 K 0	GREEN; GR R 141 G 142 B 130 C 50 M 40 Y 50 K 0
BLUE; BL R 92 G 66 B 104 C 80 M 90 Y 50 K 0	DARK GREEN; DG R 97 G 100 B 83 C 75 M 65 Y 80 K 0

Note: Defined by Color chart from “Descriptor for Bananas” [41]

APPENDIX B

Mass spectrometer parameters setup

The mass spectrometer parameters in tune panel were set as following:

Source:	capillary	4000 V
	end plate	– 500 V
	neubulizer	10.0 psi
	Dry Gas	7.0 l/min
	Dry temperature	250° C
Expert parameter:	Skimmer	40 V
	Cap ext	121 V
	Oct 1 DC	10.00 V
	Oct 2 DC	1.01 V
	Trap Drive	45
	Oct RF	100.00 V
	Lens 1	-10.0 V
	Lens 2	-35.0 V
Polarity:	Positive	
Trap:	ICC, Target	30000
	Max. Accu Time	200 ms
	Scan	50 to 3000 m/z
	Average	8

APPENDIX C

Table 5 Characteristics of anthocyanin of wild bananas in Thailand

Accession no.	Peak No. [a]	RT (min)	Maximum absorption wavelength (nm)	% A $_{310}$ / $A_{vis-max}$ [b]	% A $_{440}$ / $A_{vis-max}$ [c]	Amount of anthocyanin (μmole/g)[d]
2	1	6.6	276.9, 525.4	39	26	0.01
	2	8.8	281.6, 370.7,518.2	14	29	0.04
	5	17.3	281.6, 525.4	10	29	0.02
	6	18.8	276.9, 348.3, 532.7	15	25	0.06
3	1	6.7	279.2, 530.3	37	27	0.16
	2	8.4	281.6, 520.6	21	30	0.76
6	1	10.6	284.0, 348.3, 527.9	-	-	0.02
	2	14.8	218.6, 331.5, 518.2	-	27	0.21
	3	17.3	284.0, 530.3	-	-	0.04
	5	20.0	281.6, 331.5, 520.6	8	30	0.39
	6	20.6	274.2, 350.6, 530.3	13	25	0.92
7	1	6.7	274.5, 326.8, 527.9	33	23	0.10
	2	8.6	276.9, 355.4, 518.2	22	19	0.06
	3	9.9	272.1, 329.2, 527.9	21	10	0.07
	5	17.0	272.1, 326.8, 523.0	-	11	0.06
	6	18.9	276.9, 345.9, 527.9	11	24	0.93
8	1	10.6	276.9, 525.4	20	27	0.03
	2	13.6	279.2, 525.4	3	26	0.08
	3	14.6	281.6, 333.9, 525.4	5	29	0.03
	5	17.2	279.2, 535.2	-	20	0.22
	6	19.6	279.2, 525.4	-	23	0.47
9	1	6.7	276.6, 326.8, 527.9	-	23	0.03
	2	8.6	279.2, 520.6	23	27	0.19
	5	17.3	272.1, 520.6	-	18	0.12
	6	19.0	274.5, 348.3, 532.7	12	24	0.48
19	1	6.7	279.2, 523.0	-	25	0.02
	2	8.7	281.6, 520.6	23	22	0.05
	3	10.1	274.5, 523.0	23	-	0.01
	5	17.4	281.6, 341.1, 520.6	-	12	0.04
	6	18.9	276.9, 345.9, 530.3	11	22	0.22
27	Not detected					
45	5	16.8	265.0, 353.0, 523.0	44	37	trace
57	1	6.6	281.6, 525.4	53	31	0.11
	2	8.3	281.6, 520.5	12	30	0.33

Table 5 Characteristics of anthocyanin of wild bananas in Thailand. (continue)						
Accession no.	Peak No.[a]	RT (min)	Maximum absorption wavelength (nm)	% A_{310} / $A_{vis\text{-}max}$ [b]	% A_{440} / $A_{vis\text{-}max}$ [c]	Amount of anthocyanin (μmole/g)[d]
91	1	6.8	279.2, 527.9	27	28	0.38
	2	8.5	281.6, 518.2	15	30	1.17
96	1	6.8	279.2, 326.8, 527.9	69	28	0.20
	2	8.7	274.5, 518.2	23	30	0.14
	3	10.1	272.1, 338.7, 527.9	32	24	0.20
	5	17.4	272.1, 326.8, 518.2	18	28	0.12
	6	19.0	276.9, 348.3, 530.3	14	26	1.18
100	1	6.6	279.2, 341.1, 525.4	19	25	0.23
	2	8.7	281.6, 520.6	12	28	0.36
	5	17.0	281.6, 525.4	51	41	0.07
	6	18.6	274.5, 341.1, 525.4	44	31	0.14
106	1	6.8	281.6, 324.4, 523.0	-	28	0.02
	2	8.8	281.6, 520.6	13	30	0.57
	3	9.9	274.5, 525.4	27	21	0.06
	5	17.6	279.2, 329.2, 525.4	8	30	0.62
	6	19.2	276.9, 345.9, 532.7	12	25	0.74
108	1	6.8	276.9, 326.8, 527.9	-	31	0.04
	2	8.9	279.2, 518.2	19	30	0.15
	5	17.8	276.9, 329.2, 518.2	12	30	0.36
	6	19.4	276.9, 345.9, 527.9	14	26	0.72
109	2	9.0	281.6, 518.2	14	30	0.33
	5	18.1	281.6, 370.7, 518.2	12	31	0.53
	6	19.5	276.9, 341.1, 530.1	22	27	0.24
113	2	8.7	281.6, 329.2, 520.6	11	28	0.21
	5	17.6	281.6, 520.6	7	29	0.46
	6	19.2	276.9, 348.3, 532.7	11	23	0.26
114	1	6.4	281.6, 525.4	86	34	trace
	2	8.5	281.6, 520.6	12	30	0.49
	5	17.3	281.6 329.2, 520.6	9	31	1.02
	6	19.0	281.6, 341.1, 525.4	11	22	0.07
115	1	6.7	279.2, 370.7, 523.0	51	25	0.04
	2	8.8	281.6, 523.0	16	29	0.19
	5	17.6	279.2, 523.0	11	31	0.70
	6	19.2	279.2, 348.3, 527.9	14	26	0.87

Table 5 Characteristics of anthocyanin of wild bananas in Thailand. (continue)

Accession no.	Peak No.[a]	RT (min)	Maximum absorption wavelength (nm)	% A_{310} / $A_{vis\text{-}max}$[b]	% A_{440} / $A_{vis\text{-}max}$[c]	Amount of anthocyanin (µmole/g)[d]
116	1	6.8	279.2, 326.8, 525.4	-	25	0.07
	2	8.7	279.2, 518.2	15	30	0.36
	5	17.4	279.2, 518.2	10	30	0.49
	6	19.0	276.9, 345.9, 530.3	14	26	0.84
125	1	6.7	274.5, 532.7	56	-	0.01
	2	8.8	279.2, 518.2	-	24	0.19
	5	17.8	279.2, 331.9, 518.2	-	25	0.47
	6	19.3	276.9, 348.3, 532.7	-	18	0.32
126	1	6.8	286.4, 527.9	-	26	0.07
	2	8.8	281.6, 370.7, 520.6	19	25	0.09
	3	10.3	274.5, 329.2, 527.9	42	13	0.07
	5	17.9	281.6, 329.2, 523.0	8	24	0.12
	6	19.3	279.2, 350.6, 527.9	13	25	0.75
127	2	8.5	274.5, 518.2	24	32	0.12
	5	17.2	272.1, 520.6	20	27	0.10
	6	19.0	272.1, 329.2, 532.7	23	27	0.16
132	1	6.7	274.5, 326.8, 530.3	38	24	0.07
	2	8.6	279.2, 520.6	18	27	0.14
	3	9.9	272.1, 338.7,527.9	24	18	0.07
	5	17.1	274.5,326.8, 520.6	15	27	0.19
	6	18.9	276.9, 348.3, 530.3	13	26	1.16
135	1	6.6	276.8, 523.0	86	63	0.11
	2	8.8	279.2, 520.6	19	36	0.55
139	1	6.7	274.5, 525.4	55	25	0.04
	2	8.7	281.6, 520.6	14	30	0.45
	5	17.6	279.2, 520.6	17	33	0.44
	6	19.1	274.5, 343.5, 525.4	27	30	0.26
142	1	6.8	279.2, 326.8, 527.9	72	27	0.18
	2	8.9	279.2, 520.6	19	30	0.34
	3	10.4	272.1, 338.7, 527.9	25	24	0.13
	5	18.0	276.9, 331.5, 518.2	8	28	0.20
	6	19.4	276.9, 350.6, 527.9	13	25	0.88

Table 5 Characteristics of anthocyanin of wild bananas in Thailand. (continue)

Accession no.	Peak No.[a]	RT (min)	Maximum absorption wavelength (nm)	% A_{310} / $A_{vis\text{-}max}$[b]	% A_{440} / $A_{vis\text{-}max}$[c]	Amount of anthocyanin (μmole/g)[d]
145	1	6.8	279.2, 326.8, 527.9	68	30	0.19
	2	8.8	281.6, 326.8, 520.6	16	31	0.64
	3	10.0	272.1, 338.7, 525.4	40	33	0.12
	5	17.7	279.2, 326.8, 518.2	14	32	0.47
	6	19.3	276.9, 348.3, 530.3	15	26	0.80
146	1	6.7	279.2, 530.3	29	27	0.39
	2	8.4	281.6, 518.2	19	28	0.36
	5	16.2	279.2, 530.3	14	28	0.12
	6	18.4	276.9, 530.3	15	26	1.07
147	1	11.5	276.9, 345.9, 527.9	16	25	0.09
	2	16.3	281.6, 520.6	10	24	0.03
	3	18.4	279.2, 345.9, 523.0	16	25	0.10
	5	20.6	281.6, 331.5, 523.0	15	30	0.08
	6	21.2	279.2, 348.3, 530.3	14	25	1.06
155	2	8.4	281.6, 520.6	43	38	0.51
168	1	6.9	279.2, 375.5, 530.3	59	24	0.05
	2	8.9	281.6, 518.2	15	28	0.09
172	1	6.8	281.6, 527.9	40	26	0.12
	2	8.4	281.6, 370.7, 518.2	20	30	0.32
	5	16.3	281.6, 518.2	37	37	0.42
	6	18.6	274.6, 341.1, 527.9	29	31	0.73
173	Not detected					
174	1	6.7	274.5, 326.8, 530.3	27	26	0.13
	2	8.5	276.9, 326.8, 518.2	14	28	0.16
	3	9.8	272.1, 341.1, 530.3	29	23	0.14
	5	17.0	274.5, 326.8, 518.2	-	18	0.10
	6	18.8	276.9, 350.6, 530.3	10	24	0.95
177	5	18.3	271.1, 518.2	-	-	trace
	6	19.6	272.1, 532.7	53	16	trace
178	1	6.9	279.2, 523.0	-	37	0.02
	2	9.1	281.6, 518.2	20	31	0.11

Table 5 Characteristics of anthocyanin of wild bananas in Thailand. (continue)						
Accession no.	Peak No.[a]	RT (min)	Maximum absorption wavelength (nm)	% A_{310} / $A_{vis-max}$[b]	% A_{440} / $A_{vis-max}$[c]	Amount of anthocyanin (µmole/g)[d]
181	1	6.8	276.9, 341.1, 530.3	21	26	0.23
	2	8.6	281.6, 518.2	33	26	0.09
	3	9.7	274.5, 341.1, 530.3	25	23	0.12
	6	18.8	279.2, 348.3, 532.7	19	28	0.66
182	1	6.7	272.1, 525.4	33	27	0.06
	2	8.6	274.5, 518.2	13	27	0.11
	3	9.9	272.1, 341.1, 525.4	37	17	0.06
	5	17.3	271.1, 525.4	9	23	0.06
	6	19.1	276.9, 350.6, 530.3	15	26	0.81
183	1	6.5	281.6, 525.4	-	63	0.06
	2	8.5	281.6, 370.7, 518.2	48	42	0.50
206	6	19.5	279.2, 248.3, 532.7	17	26	0.63
223	1	8.6	271.6, 513.3	88	29	0.02
	4	13.2	281.6, 503.5	10	44	0.34
224	Not detected					
228	1	6.9	274.0, 326.8, 532.7	-	25	0.02
	2	9.1	281.6, 370.7, 523.0	20	26	0.06
	3	10.8	272.1, 341.1, 532.7	55	12	0.03
	5	18.2	281.6, 370.7, 520.6	14	30	0.13
	6	19.6	279.2, 348.3, 532.7	14	25	0.48
233	1	6.8	281.6, 326.8, 530.3	91	25	0.12
	2	8.9	281.6, 370.7, 518.2	13	23	0.08
	3	10.4	272.1, 341.1, 527.9	21	15	0.08
	5	18.2	281.6, 370.7, 520.6	14	28	0.10
	6	19.6	276.9, 348.3, 530.3	13	26	1.25
238	1	6.8	281.6, 530.3	98	27	0.02
	2	8.5	281.6, 525.4	31	25	0.05
	3	9.6	272.1, 530.3	78	33	0.03
	5	16.6	281.6, 341.1, 525.4	8	23	0.15
	6	18.7	279.2, 353.0, 530.3	11	25	1.22
242	Not detected					
243	2	243	2	243	2	243

Table 5 Characteristics of anthocyanin of wild bananas in Thailand. (continue)

Accession no.	Peak No.[a]	RT (min)	Maximum absorption wavelength (nm)	% A_{310} / $A_{vis\text{-}max}$[b]	% A_{440} / $A_{vis\text{-}max}$[c]	Amount of anthocyanin (μmole/g)[d]
245	1	6.8	272.1, 520.6	-	36	0.01
	2	8.4	265.0, 520.6	38	25	0.07
	5	16.4	281.6, 520.6	-	6	0.02
	6	18.7	274.5, 343.5, 532.7	10	21	0.40
246	1	11.1	276.9, 348.3, 525.4	19	26	0.09
	2	15.6	281.6, 333.9, 520.6	12	28	0.13
	3	17.9	276.9, 350.6, 527.9	9	25	0.07
	5	20.3	279.2, 333.9, 520.6	12	30	0.13
	6	20.9	276.9, 350.6, 530.3	19	27	0.26
247	1	5.5	281.6, 348.3, 527.9	-	24	0.25
	2	9.5	284.0, 520.6	-	27	0.33
	3	12.3	532.7	-	13	0.20
	5	18.7	281.6, 520.6	-	28	0.29
	6	20.0	279.2, 350.6, 530.3	7	25	0.56
274	2	8.7	281.6, 525.4	-	-	0.19
	5	17.3	295.8, 525.4	-	-	0.27
	6	18.9	284.0, 373.1, 532.7	-	-	0.49
281	1	6.8	279.2, 527.9	-	-	0.01
	2	9.0	281.6, 520.6	17	24	0.05
	5	17.9	279.2, 368.3, 520.6	1	22	0.07
	6	19.2	276.9, 350.6, 530.3	13	18	0.07
283	1	6.8	281.6, 368.3, 525.4	-	29	0.04
	2	9.0	281.6, 368.3, 518.2	26	29	0.12
	3	10.5	269.8, 353.0, 525.4	78	11	0.02
	5	17.8	279.2, 368.3, 518.2	9	26	0.20
	6	19.1	279.2, 350.6, 527.9	13	23	0.37
286	1	6.8	284.0, 326.8, 525.4	-	25	0.01
	2	9.0	279.2, 368.3, 518.2	-	-	0.04
	3	10.5	269.8, 525.4	39	-	0.01
	5	17.8	279.2, 368.3, 525.4	49	30	0.03
	6	19.1	276.9, 345.9, 527.9	20	22	0.29
289	1	6.8	281.6, 530.1	-	-	0.02
	2	8.9	279.2, 518.2	33	27	0.03
	5	17.7	515.7	-	79	0.06
	6	19.1	274.5, 527.9	81	51	0.20

Table 5 Characteristics of anthocyanin of wild bananas in Thailand. (continue)

Accession no.	Peak No.[a]	RT (min)	Maximum absorption wavelength (nm)	% A_{310} / $A_{vis\text{-}max}$[b]	% A_{440} / $A_{vis\text{-}max}$[c]	Amount of anthocyanin (µmole/g)[d]
290	1	6.7	281.6, 527.9	-	68	0.02
	2	8.9	279.2, 518.2	-	53	0.03
	3	10.3	265.0, 518.2	-	71	0.01
	5	17.5	281.6, 525.4	-	-	0.02
	6	19.0	279.2, 355.4, 532.7	-	-	0.26
291	1	6.9	281.6, 525.4	-	-	0.01
	2	9.1	281.6, 333.9, 525.4	-	-	0.02
	5	17.7	286.6, 525.4	-	-	0.02
	6	19.1	279.2, 363.6, 532.7	-	-	0.09
293	1	6.9	279.2, 530.3	-	28	0.06
	2	9.2	281.6, 518.2	27	28	0.12
	3	10.7	274.5, 333.9, 525.4	45	17	0.07
	5	18.1	279.2, 333.9, 520.6	8	27	0.16
	6	19.4	276.9, 348.3, 530.3	14	25	0.56
306	1	6.8	279.2, 525.4	67	23	0.01
	2	8.6	281.6, 518.2	25	28	0.05
	5	16.1	281.6, 525.4	31	28	0.01
	6	18.2	276.9, 350.6, 525.4	19	27	0.25
307	2	8.6	281.6, 520.6	65	42	trace
	5	16.4	272.1, 353.0, 520.6	83	51	trace
310	1	6.8	279.2, 532.7	28	21	0.04
	2	8.6	281.6, 333.9, 520.6	-	22	0.06
	3	9.9	279.2, 350.6, 530.3	-	9	0.05
	5	16.7	279.2, 373.1, 520.6	-	12	0.10
	6	18.5	279.2, 350.6, 527.9	7	24	1.05
312	1	6.8	279.2, 527.9	69	36	0.03
	2	8.6	281.6, 373.1, 515.7	30	36	0.07
	3	9.9	272.1, 336.3, 527.9	40	31	0.04
	5	16.5	281.6, 515.7	11	29	0.09
	6	18.4	279.2, 350.6, 527.9	14	26	0.63

Table 5 Characteristics of anthocyanin of wild bananas in Thailand. (continue)

Accession no.	Peak No.[a]	RT (min)	Maximum absorption wavelength (nm)	% A_{310} / $A_{vis\text{-}max}$ [b]	% A_{440} / $A_{vis\text{-}max}$ [c]	Amount of anthocyanin (µmole/g)[d]
313	1	6.7	279.2, 336.3, 523.0	21	24	0.04
	2	8.6	281.6, 518.2	8	29	0.27
	3	9.9	281.6, 355.4, 535.2	-	4	0.04
	5	16.6	281.6, 518.2	-	9	0.09
	6	18.5	276.9, 350.6, 532.7	1	20	0.23
314	2	8.4	281.6, 518.2	38	41	0.31
	5	15.7	281.6, 520.6	22	34	0.21
	6	18.0	279.2, 350.6, 525.4	12	25	0.34
318	Not detected					
319	1	6.8	279.2, 345.9, 527.9	13	27	1.12
	2	8.8	281.6, 329.2, 520.6	15	32	0.63

[a], From HPLC

[b], [c], - is negative value or value over 100% due to limitation of the instrument as describe in Chapter V ‘Instrument limitation’

[d], From area under peak of HPLC and total anthocyanin content as equivalent mole to cyanidin-3-rutinoside

APPENDIX D

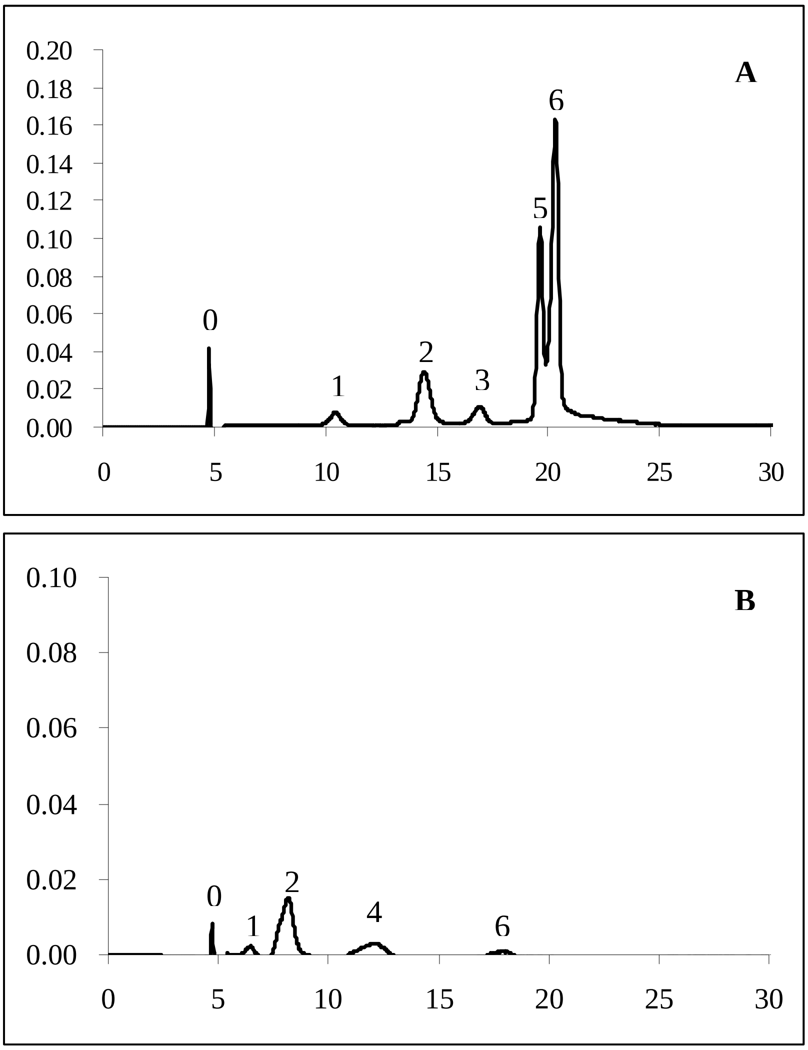


Figure 19 HPLC chromatograms at 530 nm of the mixed wild bananas bract extract. A; mixed of *M. acuminata*. B; mixed of *M. coccinea*, *M. balbisiana*, *M. itinerans*. Peak no.0 is unbound compounds, no. 1-6 rutinoside of delphinidin, cyanidin, petunidin, pelargonidin, peonidin and malvidin, respectively.

APPENDIX E

Table 6 Molecular mass ions and their daughter ions of anthocyanin in other plants from previous studies.

Anthocyanin	Molecular mass ion (m/z)	Daughter ions (m/z)
Black Currant (*Ribes nigrum* L.) [25]		
Delphinidin-3-O-glucoside	456	303
Delphinidin-3-O-rutinoside	611	465, 303
Cyanidin-3-O-glucoside	449	287
Cyanidin-3-O-rutinoside	595	449, 287
Petunidin-3-O-glucoside	479	317
Petunidin-3-O-rutinoside	625	479, 317
Cyanidin-3-O-arabinoside	419	287
Pelargonidin-3-O-glucoside	433	271
Pelargonidin-3-O-rutinoside	579	271
Peonidin-3-O-glucoside	463	301
Peonidin-3-O-rutinoside	609	463, 301
Malvidin-3-O-glucoside	493	331
Malvidin-3-O-rutinoside	639	493, 331
Delphinidin-3-O-(6" coumaroylglucoside)	611	303
Cyanidin-3-O-(6" coumaroylglucoside)	595	287
Black Rapsberry (*Rubus occidentalis*) [42]		
Cyanidin-3-O-glucoside	449	287
Cyanidin-3-O-sambubioside	581	287
Cyanidin-3-O-xylosylrutinoside	727	581, 287
Cyanidin-3-O-rutinoside	595	449, 287
Pelargonidin-3-O-rutinoside	579	271
Cyanidin-3-O-glucoside	449	287
Musa X paradisiaca (cavendish banana) [8]		
Cyanidin-3-rhamnose-7-glucoside	694.8	
Delphinidin-3-O-rutinoside	611.2	
Cyanidin-3-O-rutinoside	595.2	
Petunidin-3-O-rutinoside	625.2	
Pelargonidin-3-O-rutinoside	579.2	
Peonidin-3-O-rutinoside	609.2	
Malvidin-3-O-rutinoside	639.2	
Purple Corn (*Zea mays*) [21]		
Unknown	899	737, 575, 449
Cyanidin-3-O-glucoside	449	287
Pelargonidin-3-O-glucoside	433	271
Peonidin-3-O-glucoside	463	301
Cyanidin-3-(6"malonlyglucuside)	535	449, 287
Pelargonidin-3-(6"malonlyglucuside)	519	433, 271
Peonidin-3-(6"malonlyglucuside)	549	463, 301
Cyanidin-3-(6"ethylmalonlyglucuside)	563	449, 287
Pelargonidin-3-(6"ethylmalonlyglucuside)	547	433, 271
Peonidin-3-(6"ethylmalonlyglucuside)	577	463, 301

Table 6 Molecular mass ions and their daughter ions of anthocyanin in other plants from previous studies. (continue)

Anthocyanin	Molecular mass ion (m/z)	Daughter ions (m/z)
Concord grape (*Vitis labrusca*) [20]		
Peonidin-3-O-glucoside	462.8	301.2
Delphinidin-3-O-glucoside	456.2	303.2
Cyanidin-3-glucoside + *p*-coumaric	595.2	287.2
Delphinidin-3-glucoside + *p*-coumaric	610.8	303.2
Petunidin-3-glucoside + *p*-coumaric	624.8	317.2
Delphinidin-3-glu-5-glucoside + *p*-coumaric	773.2	610.2, 464.8, 303.2
Petunidin-3-glu-5-glucoside + *p*-coumaric	787.2	624.8, 478.8, 317.2
Malvidin-3-glu-5-glucoside + *p*-coumaric	801.2	639.2, 492.8, 331.2
Roselle (*Hibiscus sabdariffa* L.) [20]		
Delphinidin-3-xylosylglucoside	597.2	303.2
Cyanidin-3-xylosylglucoside	581.2	287.2
Red Cabbage (*Brassica oleracea*) [20]		
Cyanidin-3-glu-5-glucoside + sinapic	979.6	817.6, 449.2, 287.2
Cyanidin-3-glu-5-glucoside + ferulic	115.2	993.6, 449.2, 287.2
Cyanidin-3-glu-5-glucoside + 2X sinapic	1185.6	1023.6, 449.2, 287.2
Red Radish (*Raphanus sativus*) [20]		
Pelargonidin-3-sophoroside-5-glucoside	757.2	595.2, 433.2, 271.2
Pelargonidin-3-sopho-5-glu + *p*-coumaric	903.2	741.2, 433.2, 271.2
Pelargonidin-3-sopho-5-glu + ferulic	933.2	771.2, 443.2, 271.2
Pelargonidin-3-sopho-5-glu + *p*-coumaric/ malonic	989.6	741.2, 518.8, 271.2
Pelargonidin-3-sopho-5-glu + ferulic/malonic	1019.2	771.2, 518.8, 271.2
Red_fleshed Potato (*Solanun tuberosum*) [20]		
Pelargonidin-3-rutinoside-5-glucoside	741.4	578.8, 433.2, 271.2
Pelargonidin-3-rutinoside	579.2	433.2, 271.2
Pelargonidin-3-rut-5-glu + *p*-coumaric	887.2	725.2, 433.2, 271.2
Pelargonidin-3-rut-5-glu + ferulic	919.6	755.2, 433.2, 271.2
Pelargonidin-3-rut + *p*-coumaric	725.4	433.2, 271.2
Red Raspberry (*Rubus idaeus*) [24]		
Cyanidin-3-Sophoroside	611	287
Cyanidin-3-glucoside	449	287
Cyanidin-3-(2G-glucoslyrutinoside)	757	611, 287
Pelargonidin-3-Sophoroside	595	271
Cyanidin-3-rutinoside	595	449, 287
Pelargonidin-3-glucoside	433	271
Pelargonidin-3-(2G-glucoslyrutinoside)	741	595, 271

Table 6 Molecular mass ions and their daughter ions of anthocyanin in other plants from previous studies. (continue)

Anthocyanin	Molecular mass ion (m/z)	Daughter ions (m/z)
Highbush blueberry (*Vaccinium corymbosum*) [24]		
Delphinidin-3-galactoside	465	303
Delphinidin-3-glucoside	465	303
Cyanidin-3-galactoside	449	287
Delphinidin-3-arabinoside	435	303
Cyanidin-3-glucoside	449	287
Petunidin-3-galactoside	473	317
Cyanidin-3-arabinoside	419	287
Petunidin-3-glucoside	473	317
Peonidin-3-galactoside	463	301
Petunidin-3-arabinoside	449	317
Peonidin-3-glucoside	463	301
Malvidin-3-galactoside	493	331
Peonidin-3-arabinoside	433	301
Malvidin-3-glucoside	493	331
Malvidin-3-arabinoside	463	331
Grape (*Vitis vinifera*) [24]		
Delphinidin-3,5-diglucoside	627	465, 303
Cyanidin-3,5-diglucoside	611	449, 287
Delphinidin-3-glucoside	465	303
Petunidin-3,5-diglucoside	641	479, 317
Pelargonidin-3,5-diglucoside	595	433, 271
Cyanidin-3-glucoside	449	287
Peonidin-3,5-diglucoside	625	463,301
Petunidin-3-glucoside	479	317
Malvidin-3,5-diglucoside	655	493, 331
Pelargonidin-3-glucoside	433	271
Peonidin-3-glucoside	463	301
Malvidin-3-glucoside	493	331
Delphinidin-3-coumaroylglucoside	611	303
Petunidin-3-coumaroylglucoside	625	317
Malvidin-3-coumaroylglucoside	639	331

BIOGRAPHY

NAME	Mr. Kasipong Kitdamrongsont
DATE OF BIRTH	4 JUNE 1982
PLACE OF BIRTH	Bangkok, Thailand
INSITITUTION ATTENDED	Kasetsart University, Thailand, 2002-2005: Bachelor of Science (Biochemistry) Mahidol University, Thailand, 2005-2008: Master of Science (Biochemistry)
HOME ADDRESS	81 m.1, Wichadwattana Rd., Chahom, Lampang, Thailand, 52120 Mobile phone: 08-9448-4034 E-mail: alcolases@hotmail.com