CORRELATION BETWEEN TOTAL PHENOLICS AND MINERAL CONTENT WITH ANTIOXIDANT ACTIVITY AND DETERMINATION OF BIOACTIVE COMPOUNDS IN VARIOUS LOCAL BANANAS (*Musa* sp.)

by

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ABBREVIATIONS

AAS	atomic absorption spectroscopy
AES	atomic emission spectroscopy
ANOVA	analysis of variance
AOAC	Association of Official Analytical Chemists
BAW	solvent mixtures of butanol, acetic acid and water (4:1:5)
BEW	solvent mixture of butanol, ethanol and water (4:1:2.2)
BHA	butylated hydroxyanisole
CC	column chromatography
DPPH	2, 2 - diphenyl - 1 - picrylhydrazyl
DPPH•	2, 2 - diphenyl - 1 - picrylhydrazyl radical
DRI	daily reference intake
ESI-MS	electrospray ionization - mass spectroscopy
FORESTAL	solvent mixtures of concentrated sulphuric acid, acetic acid and water (3:30:10)
FRAP	ferric reducing antioxidant power
FTC	ferric thiocyanate
FT – IR	fourier transform infrared
GAE	gallic acid equivalent
IC_{50}	sample concentration providing 50% inhibition
IOM	Institute of Medicine
LC-MS	liquid chromatography - mass spectroscopy
MARDI	Malaysian Agricultural and Research Development Institute
MDA	malondialdehyde
m/z	mass charge ratio
NMR	nuclear magnetic resonance
OH	hydroxyl
PC	paper chromatography
ppm	part per million
R_f	the distance a compound moves in chromatography relative to the solvent front
RNS	reactive nitrogen species
ROS	reactive oxygen species
TBA	thiobarbituric acid
TLC	thin layer chromatography
TPC	total phenolic content
TPTZ	tripyridyltriazine
UV-Vis	ultraviolet – visible
US - RDA	United State Recommended Daily Allowance

KORELASI ANTARA BAHAN FENOLIK DAN KANDUNGAN MINERAL DENGAN AKTIVITI ANTIOKSIDA DAN PENENTUAN SEBATIAN BIOAKTIF BAGI PELBAGAI PISANG (*Musa* sp.) TEMPATAN

ABSTRAK

Kajian ini dijalankan bagi menilai korelasi antara bahan fenolik dan kandungan mineral dengan aktiviti antioksida di dalam pelbagai ekstrak kulit dan buah pisang, *Musa* sp. tempatan. Di samping itu, pengenalpastian and pencirian komponen aktif turut dijalankan. Ekstrak kajian dihasilkan melalui tiga teknik pengekstrakan yang berbeza. Jumlah kandungan bahan fenolik ditentukan dengan menggunakan kaedah kolorimetrik Folin – Ciocalteu, manakala aktiviti antioksida diukur melalui kaedah penyingkiran radikal bebas 2, 2 – difenil – 1 – pikrilhidrazil (DPPH). Ekstrak – ekstrak yang dikaji menunjukkan julat jumlah kandungan bahan fenolik dan aktiviti antioksida yang luas, masing – masing daripada 12.47 ± 0.12 hingga 175.47 ± 0.31 mg GAE/g ekstrak kering dan 10.12 ± 0.64% hingga 80.04 ± 0.66% pada kepekatan 2000 μg/ml. Secara umum, ekstrak dari kulit pisang menunjukkan jumlah kandungan bahan fenolik dan aktiviti antioksida yang lebih tinggi jika dibandingkan dengan ekstrak daripada buah pisang. Antara semua ekstrak, ekstrak daripada kulit dan buah pisang yang dikeringkan dan diekstrak dengan pelarut berbeza kepolaran menggunakan Soxhlet menunjukkan jumlah kandungan bahan fenolik dan aktiviti antioksida yang paling memuaskan. Korelasi yang signifikasi dan positif wujud antara jumlah kandungan bahan fenolik dan aktiviti antioksida ($r^2 = 0.6073$, $p < 0.0001$). Hal ini membuktikan bahawa bahan fenolik merupakan komponen antioksida yang utama di dalam ekstrak. Sementara itu, nilai R_f dan warna pada plat kromatografi lapisan nipis yang dibangunkan dengan menggunakan pelbagai sistem pelarut, spektrum jisim dan spektrum ultraungu digunakan bagi pengenalpastian dan pencirian komponen bioaktif. Di dalam kajian ini, dua komponen fenolik telah dikenalpasti iaitu 3, 5, 8 – trimetil 6, 8 dihidroksimirisetin dan asid ferulik. Jumlah kandungan bahan fenolik bagi kedua – dua komponen bioaktif ini masing – masing ialah 395.07 ± 0.12 mg GAE/ gram ekstrak kering dan 175.87 ± 0.12 mg GAE/ gram ekstrak kering. Manakala aktiviti antioksida yang direkodkan ialah 86.15 ± 0.05% (3, 5, 8 –

trimetil 6, 8 dihidroksimirisetin) dan 27.42 ± 1.16% (asid ferulik). Kewujudan dua komponen ini di dalam kulit pisang belum pernah dilaporkan lagi di dalam kajian terdahulu. Kandungan mineral utama (K, P, Mg, Ca dan Na) dan mineral sampingan (Fe, Zn, Cu dan Mn) di dalam buah pisang tempatan ditentukan dengan menggunakan spektrofotometer penyerapan atom (AAS) dan spektofotometer penyebaran atom (AES). Kalium adalah elemen mineral yang paling signifikan wujud di dalam kedua – dua bahagian buah dan kulit pisang dengan anggaran nilai sebanyak 295.68 – 463.57 mg/100 g berat segar dan 1071.20 – 1361.56 mg/100 g berat segar, masing - masing. Keseluruhannya, aturan relatif kepekatan mineral utama di dalam buah dan kulit pisang adalah seperti berikut K > P > Mg > Na > Ca, manakala bagi mineral sampingan, aturannya adalah Mn > Fe > Zn > Cu. Selain daripada itu, tiada korelasi yang signifikasi wujud di antara kandungan mineral dan aktiviti antioksida melainkan K dan Mn yang membentuk korelasi sederhana dengan aktiviti antioksida di dalam ekstrak air.

CORRELATIONS BETWEEN TOTAL PHENOLICS AND MINERAL CONTENT WITH ANTIOXIDANT ACTIVITY AND DETERMINATION OF BIOACTIVE COMPOUNDS IN VARIOUS LOCAL BANANAS (*Musa* sp.)

ABSTRACT

This study was designated to evaluate the correlation between total phenolics and mineral content with antioxidant activity in various extracts of pulps and peels of eight local banana cultivars, *Musa* sp. In addition, identification and characterization of the bioactive compounds was also carried out. Samples were extracted using three different extraction procedures. The total phenolic contents were measured by Folin – Ciocalteu colorimetric method, whereas antioxidant activity was estimated by using 2, 2 – diphenyl – 1 – picrylhydrazyl (DPPH) free radical scavenging assay. These extracts exhibited a wide range of total phenolic content and antioxidant activity varying from 12.47 ± 0.12 to 175.47 ± 0.31 mgGAE/g dry extract and 10.12 ± 0.64% to 80.04 ± 0.66%, respectively at 2000 μg/ml concentration. Generally, peel extracts exhibited higher total phenolic content and stronger antioxidant activity than pulp extracts. Among all extracts, the dried banana samples extracted with various solvent polarities using Soxhlet apparatus showed the highest total phenolic content and the most potent antioxidant activity. Significant and positive linear correlation were found between total phenolic content and antioxidant activity (r^2 = 0.6073, p < 0.0001), indicating that phenolics were the major antioxidant constituents in the extracts. Meanwhile, the R_f values and colour on thin layer chromatography plates developed by various solvent systems, mass spectral and ultraviolet spectrum were used to identify and characterize the bioactive compounds. A total two bioactive phenolic compounds were identified, which were 3, 5, 8 – trimetyhl 6, 8 dihydroxymyricetin and ferulic acid. Total phenolic content of both bioactive compounds were 395.07 ± 0.12 mg GAE/ g dry extract and 175.87 ± 0.12 mg GAE/ g dry extract, respectively. Meanwhile, the percentage of antioxidant activity were 86.15 ± 0.05% (3, 5, 8 – trimethyl 6, 8 dihydromyricetin) and 27.42 ± 1.16% (ferulic acid). The presence of these compounds in the banana peels has never been reported. The content of macrominerals (Na, K,

Ca and Mg) and microminerals (Fe, Cu, Zn and Cu) in local bananas has been determined by atomic absorption spectrometer (AAS) and atomic emission spectrometer (AES). Potassium was the most significant mineral element presents in both banana pulps and peels with an estimated value of 295.68 – 463.57 mg/100 g fresh weight and 1071.20 – 1361.56 mg/100 g fresh weight, respectively. Based on the obtained results, bananas are shown to contribute to the recommended daily requirements suggested by US – RDA for K, Mg, Fe and Zn. Overall, the relative order of concentration of macrominerals both in pulps and peels was K > Mg > Na > Ca, while the decrease order of microminerals concentration was Mn > Fe > Zn > Cu. In addition, mineral content was found as not significantly correlate with their antioxidant activity with the exception of K and Mn which form a moderate correlation with antioxidant activity of water extracts.

1 INTRODUCTION

Banana is a tree – like plant of the genus *Musa* in the family Musaceae. It is one of the most popular fruits on the world market. The extensive literature reviews showed that botany, cytology, breeding, horticulture, physiology, biochemistry, nutritional and therapeutic value of banana had been already studied in depth (Stover and Simmonds, 1987). The respected herbalist, Maud Grieve noted in her book published in 1931 that the banana family is more of interest for its nutrient than for its medicinal properties (Carper, 1989). It contains 74% water, 23% carbohydrates, 2.6% fiber, 1% proteins and 0.5% fat (these values vary between different banana cultivars, degree of ripeness and growing conditions).

In recent years, there has been an explosive interest in studying and quantifying antioxidants of fruits due to their health promoting properties (Gil *et al.*, 2002). Experimental evidences prove that antioxidants can protect human body from free radicals and reactive oxygen species (ROS) effects (Policegoudra *et al.*, 2007), thus might retard and prevent various pathophysiological processes associated with oxidative stress such as cancer, neurodegenerative and cardiovascular disease (Rudnicki *et al.*, 2007). According to Kondo *et al.* (2005), the antioxidant activity in fruit is notable since fruits are rich in antioxidants such as phenolic compounds. Fruit polyphenolics consist of a wide range of compounds with antioxidant activity, which are, hydroxybenzoic acid, hydrocinnamic acid, flavonoids (flavones, flavonols, flavanones, flavononols and flavans) and tannins. A large number of studies have demonstrated that phenolic compounds may act as antioxidants by scavenging ROS and chelating free radicals *in vitro* (Rice – Evans *et al,*, 1995). Besides being a valuable supplier of antioxidants, fruits are also widely recognized as the important source of minerals which is essential to maintain peak health. Likewise antioxidants, minerals play a vital role in the proper development and good health of the human body (Chauhan *et al.*, 1991).

Banana pulp had been reported as having various antioxidants such as vitamins (A, B and E), β – carotene (Kanazawa & Sakakibara, 2000) and phenolic compounds like catechin, epicatechin, lignin and tannin (Someya *et al.*, 2002; Macheix *et al.*, 1990). Banana peel also demonstrated the present of various phenolic compounds such as gallocatechin and

anthocyanins like peonidin and malvidine (Harborne, 1967). Banana is also enriched with minerals like potassium, phosphorus, magnesium and calcium (Leterme *et al.*, 2006; Wall, 2006b; Hardisson *et al.*, 2001; Emaga *et al.*, 2006). According to several authors, banana peel recorded stronger antioxidant activity, pooled more quantity of phenolic compounds (Someya *et al.*, 2002), greater range of phenolics composition (Kondo *et al.*, 2005) and higher in minerals content than banana pulp (Emaga *et al.*, 2006).

Most of the reviewed studies were focusing on one type of banana cultivar and *Cavendish* was the most popular cultivar being studied. No comparative study to date is available to compare the level of antioxidant activity, phenolic compounds and minerals on different banana cultivars. A study needs to be done since antioxidant activity, phenolic compounds and mineral content were influenced by the cultivars (Award *et al.*, 2001; Kondo *et al.*, 2005). Additionally, testing samples of a wider range of cultivars from multiple growing environments is needed to estimate the extent of variation in antioxidants, phenolic compounds and mineral content for possible breeding efforts (Emmons and Peterson, 1999). Furthermore, an extensive review of literature found no information on the correlation between antioxidant activity and mineral content. Investigating the role of minerals in antioxidant activity of banana is crucial since banana is enriched with essential minerals like potassium, phosphorus and magnesium. Moreover, understanding the correlation that might exist between these two parameters will help in the future research of banana.

Furthermore, none of the previous studies were ever highlighted on the suitable extraction method for extracting out antioxidants and phenolic compounds from banana tissues. According to Santas *et al.* (2008), the suitable method used for extraction of phenolic compounds from plant materials is important for the accurate quantification of antioxidant activity.

The objectives of the present study were

1. To quantify the phenolic compounds and antioxidant activity of various extracts of local bananas, *Musa* sp.

2. To evaluate the effects of extraction procedures on total phenolics and total antioxidant activity of banana pulp and peel extracts.
3. To identify the bioactive phenolic compounds that play role in the antioxidant activity of the extracts.
4. To assess the concentrations of mineral elements present in banana pulps and peels and compare with those reported in previous studies.
5. To determine the correlation between antioxidant activity of bananas and their total phenolic content and mineral concentrations.

2 LITERATURE REVIEW

2.1 Banana, *Musa* sp.

2.1.1 Taxonomy and classification of banana

Bananas are a large, monocotyledonous herb belong to the Musaceae family of the order Zingiberales. The genus *Musa* is comprised of all edible cultivars that was further divided into four sections, *Eumusa, Rhodochlamys, Australimusa* and *Callimusa*. Among the four sections, *Eumusa* is the largest and most widespread geographically. It has given rise to the great majority of the edible bananas (Palmer, 1971) including the edible bananas which are of primary interest in this study. Besides its edible fruits, *Eumusa* section also produces several minor fibres (e.g. from *Musa basjoo*) and vegetables derived from parts of the plant other than the fruit (Stover & Simmonds, 1987). The section *Australimusa* also yields edible fruits from the Fei'I bananas grown in the Pacific. However its distribution and variability is lesser than the *Eumusa*. It also contains *Musa textilis* (Abaca) which produces the commercial value Manila hemp. The other two sections of *Musa*, *Rhodochlamus* and *Callimusa* are only appreciated for their ornamental properties.

The edible bananas cultivars are mostly derived from two wild species of genus *Musa* (section Eumusa) namely *Musa acuminata* and *Musa balbisiana* (Valmayor *et al.*, 1990). *Musa acuminata* is a diverse species and consists of at least nine subspecies while *Musa balbisiana* is less diverse and no subspecies has been suggested so far. All the edible cultivars originated from these two species belong to various genome groups. They are differed from each other depending on whether the clones are pure *acuminata* and *balbisiana*, diploid or triploid derivative and whether they are diploid, triploid or tetraploid hybrids of two wild species (Valmayor *et al.*, 1990). Hence, a classification system was developed by Simmonds & Shepherd (1955) to classify all the edible banana cultivars systematically. On the basis of 15 vegetative and reproductive morphological characters, the differences between *Musa acuminata* and *Musa balbisiana* (Espino *et al.*, 1992) could clearly be discerned (Table 2.1). It is also necessary to determine the ploidy of a clone before it can be satisfactorily classified. Ploidy and relative contribution of the two species to a given banana cultivar is specified with a shorthand

lettering system (Ploetz, 1992). Haploid contribution of *Musa acuminata* and *Musa balbisiana* are designated as A and B, respectively. Basically, all edible banana cultivars can be classified into six groups which are AA, BB, AAA, AAB, ABB, and ABBB. They are respectively diploid, triploid and tetraploid. However, most of them are triploid.

According to the Malaysian Agricultural Research and Development Institute (MARDI), there are more than 50 Malaysian banana cultivars (Table 2.2). Among the numerous cultivars only eight were selected and studied here. They were chosen based on their high consumption among local people and readily available in the local markets. Characters of all the studied banana cultivars are presented in Table 2.3.

Table 2.1: Characters used in distinguishing banana cultivars (Simmonds & Shepherd, 1955)

Character	***M. acuminate***	***M. balbisiana***
Pseudostem colour	More or less heavily marked with brown or black blotches	Blotches slight or absent
Petiolar canal	Margin erect or spreading, with scarious wings below, not clasping pseudostem	Margin enclosed, not winged below, clasping pseudostem
Peduncle	Usually downy or hairy	Glabrous
Pedicels	Short	Long
Ovules	Two regular rows in each loculus	Four irregular rows in each loculus
Bract shoulder	Usually high (ratio < 0.28)	Usually low (ratio > 0.30)
Bract curling	Bracts reflex and roll back after opening	Bracts lift but do not roll
Bract shape	Lanceolate or narrowly ovate, tapering sharply from the shoulder	Broadly ovate not tapering sharply
Bract apex	Acute	Obtuse
Bract colour	Red, dull purple or yellow outside; pink, dull purple or yellow outside	Distinctive brownish - purple outside; bright crimson inside
Colour fading	Inside bract colour fades to yellow towards the base	Inside bract colour continuous to base
Bract scars	Prominent	Scarcely prominent
Free tepal or male flower	Variably corrugated below tip	Rarely corrugated
Male flower colour	Creamy white	Variably flushed with pink
Stigma colour	Orange or rich yellow	Cream, pale yellow or pale pink

Table 2.2: List of banana accessions in the germplasm collection at MARDI

Acuminata cultivars (AA Genome, seeded)
1. Pisang Kra/ Pisang Kra/ Pisang Kra
2. Pisang Segun
3. Pisang Flava
4. Pisang Sintok
5. Pisang Surong
6. Pisang Rangis
7. *Musa acuminata malacensis* (Lenggeng)

Acuminata cultivars (AA Genome, edible)
1. Pisang Mas/ Pisang Mas Besar/ Pisang Mas Kampong/ Pisang Mas Air/ Pisang Minyak (Kluai Kangsar)
2. Pisang Mas Sagura/ Pisang Perak
3. Pisang Lemak Manis Kelantan/ Pisang Lemak Manis Terengganu/ Pisang Lemak Manis (Raub)/ Pisang Lemak Manis (Lipis)/ Mas Pahang
4. Pisang 40 Hari/ Pisang 40 Hari (Sabah)/ Pisang Boyan/ Pisang Bulin/ Pisang Mas Kertas
5. Pisang Kapas/ Pisang Kapas (Pontian)/ Pisang Kapas (Pisang Aur)/ Pisang Pota (Pisang Aur)/ Pisang Putar (Ulu Terengganu)/ Pisang Lemak Manis Pahang
6. Pisang Berangan/ Pisang Berangan I/ Pisang Berangan II/ Pisang Jelai Berangan/ Pisang Berangan Besi/ Pisang Berangan Buaya/ Lakatan (Philippines)
7. Pisang Nur (Kluai Krai)
8. Pisang Lilin
9. Pisang Jari Buaya/ Pisang Lidah Buaya/ Pisang Rotan
10. Pisang Ekor Kuda/ Pisang Kuda
11. Pisang Masam
12. Pisang Jarum/ Pisang Jarum (Perlis)
13. Pisang Raksa/ Raksa (Pisang Tioman)
14. Pisang Keladi/ Pisang Pinang/ Pisang Ulat
15. Pisang Serindik/ Gu Nin Chio/ Gu Chi Nio

Acuminata cultivars (AAA genome)
1. Pisang Embun/ Pisang Embun (Cameron Highlands)/ Pisang Jelai Bunga (Ulu Kelantan)
2. Pisang Masak Hijau Van/ Pisang Jelai Masak Hijau/ Bungulan (Philippines)
3. Pisang Jelai Buis
4. Pisang Buai/ Pisang Buai (Kelantan)/ Pisang Buruk Bakul/ Pisang Embun Buruk Bakul
5. Pisang Thai/ Pisang Amritsagar/ Pisang Golden Aromatic
6. Pisang Cina (Pisang Aur)/ Pisang Cina (Pisang Pemanggil)
7. Pisang Amping
8. Pisang Serendah/ Pisang Kapal/ Pisang Kalap (Bentong)
9. Pisang Bakar (Taiping)/ Pisang Bakaram (Jerangau)/ Pisang Bakaran (Pisang Pemanggil)/ Pisang Karan (Jeli)/ Pisang Masak Hijau
10. Pisang Tualang (Kluai Krai)
11. Pisang Minyak Laut/ Pisang Mentalun (Sik)/ Pisang Orang
12. Pisang Mundam/ Pisang Mundam (Perak)/ Pisang Raja Udang Hijau

Table 1.2: cont.

Acuminata cultivars (AAA genome)
13. Pisang Ayam Man/ Pisang Tioman/ Pisang Apau (Pisang Tioman)/ Pisang Buloh/ Pisang Raga I (Gua Musang)
14. Pisang Raja Udang Merah
15. Pisang Pelimbing/ Pisang Palembang Selangor
16. Pisang Susu (Taiping)
Acuminata x Balbisiana hybrids (AAB Genome)
1. Pisang Raja (Raub)/ Pisang Raja Pahang (Pisang Tinggi)/ Pisang Raja (Pisang Pemanggil)/ Pisang Kelat Raja/ Pisang Raja Talun/ Pisang Raja Kepek
2. Pisang Rastali
3. Pisang Pulot
4. Pisang Keling/ Pisang Ceylon
5. Pisang Tanduk (Kluang)/ Pisang Lang/ Pisang Gading
6. Pisang Kelat Air
7. Pisang Kelat Jambi/ Pisang Beraksa
8. Pisang Seribu/ Pisang Belalai Gajah
9. Pisang Kapor
10. Pisang Nangka (Raub)/ Pisang Nangka (Klang)/ Pisang Nangka (Pisang Pontian)/ Pisang Nangka (Pisang Tioman)
11. Pisang Raja Talong
12. Pisang Laknao/ Pisang Raga II (Gua Musang)
Acuminata x Balbisiana hybrids (ABB Genome)
1. Pisang Abu Perak
2. Pisang Kelat Legor (Kelantan)/ Pisang Awak Legor/ Pisang Awak Legor (Sik)/ Pisang Wak Biji (Sik)/ Pisang Awak Betul Besar/ Pisang Kelat Siam/ Pisang Kelat Siam I/ Pisang Kelat Siam II/ Pisang Kelat/ Pisang Siam (Pisang Pemanggil)
3. Pisang Awak Betul/ Pisang Abu Betul
4. Pisang Kebatu (Taiping)/ Pisang Kebatu (Kelantan)/ Pisang Tematu atau Nipah (Pisang Tioman)
5. Pisang Abu Keling/ Pisang Kari (Jerangau)
6. Pisang Sematu (Raub)/ Pisang Chematu (Raub)/ Sebatu
Balbisiana cultivars (BBB Genome)
1. Pisang Nipah/ Pisang Abu Nipah/ Pisang Nipah (Pontian)
2. Binendito (Philippines)
3. Sabang Puti (Philippines)
Acuminata x Balbisiana hybrids (ABBB Genome)
1. Pisang Abu Siam (Selangor)
2. Pisang Benggala Barat
Balbisiana cultivars (BB Genome)
1. Pisang Gala

Table 2.3: Characteristics of the eight local banana cultivars which were used in this study (Valmayor *et al.*, 1990)

Characters	**Pisang Mas**	**Pisang Kapas**	**Pisang Berangan**	**Pisang Rastali**	**Pisang Raja**	**Pisang Nangka**	**Pisang Awak**	**Pisang Nipah**
Genome	AA	AA	AA	AAB	AAB	AAB	ABB	BBB
Bunch	Bunch weight is 8-12 kg with 5-9 hands and 14-18 fingers per hand	Bunch weight is 8-12 kg with 5-9 hands and 14-18 fingers per hand	Bunch weight is 12-20 kg with 8-12 hands and 12-20 fingers per hand	Bunch weight is 10-14 kg with 5-9 hands and 12-16 fingers per hands	Bunch weight is 12-16 kg with 6-9 hands and 14-16 fingers per hand	Bunch weight is 12-14 kg with 6-8 hands and 14-24 fingers per hand	Bunch weight is 18-22 kg with 8-12 hands and 10-16 fingers per hand	Bunch weight is 14-22 kg with 10-16 hands and 12-20 fingers per hand
Fruit	Small, 8-12 cm in length and 3-4 cm in diameter	Small, 8-12 cm in length and 3-4 cm in diameter	Medium to large, 12-18 cm in length and 3-4 cm in diameter.	Small to medium, 15 cm in length and 3-4 cm in diameter. Easily detaches from the hand	Medium and angular, 15-20 cm in length and 3.5-4.5 cm in diameter	Medium to large, 18-24 cm in length and 3.5-5 cm in diameter	Small to medium, 10-15 cm in length and 3-4 cm in diameter	Short, stout and angular. 10-15 cm in length and 3.5-4.5 cm in diameter

Table 2.3: Cont.

Characters	Pisang Mas	Pisang Kapas	Pisang Berangan	Pisang Rastali	Pisang Raja	Pisang Nangka	Pisang Awak	Pisang Nipah
Skin	Thin, golden yellow in colour when ripe	Thin, turns yellow when ripe.	Thick, leaves some rags on the pulp when peeled off. Yellow upon ripening	Very thin, has many dark brown to black blotches when fully ripe. Turn golden yellow when ripening	Thick and coarse. Orange-yellow in colour when ripe	Thick and remains light green when ripe	Thick, turns yellow when ripe	Thick and turns yellow when ripe
Flesh/ Pulp	Firm, light orange in colour, aromatic and very sweet	Firm, soft, light yellow in colour.	Firm, light orange in colour, aromatic, dry but sweet with excellent flavour	Soft, white in colour, slightly subacid in taste and distinctive in flavour	Creamy orange, very sweet but coarse in texture and well developed core	Creamy white, fine textured, starchy and subacid in taste	White, firm and sticky	Creamy – white, finely textured and well developed core
Plate number	2.1	2.2	2.3	2.4	2.5	2.6	2.7	2.8

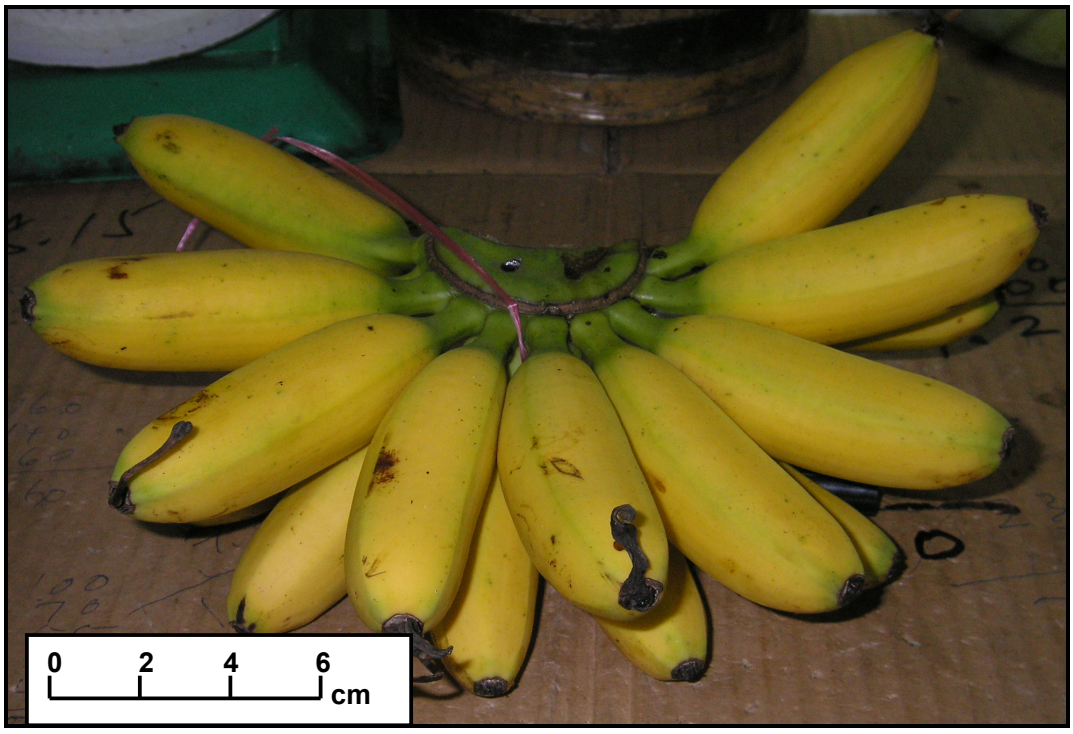

Plate 2.1: Pisang Mas

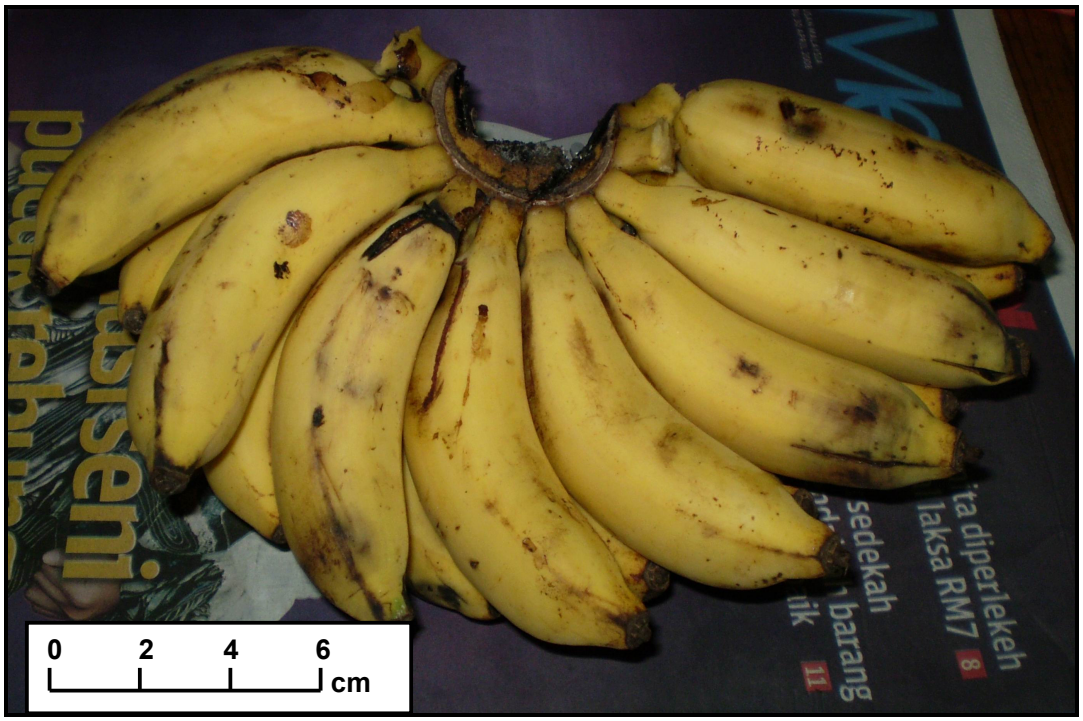

Plate 2.2: Pisang Kapas

Plate 2.3: Pisang Berangan

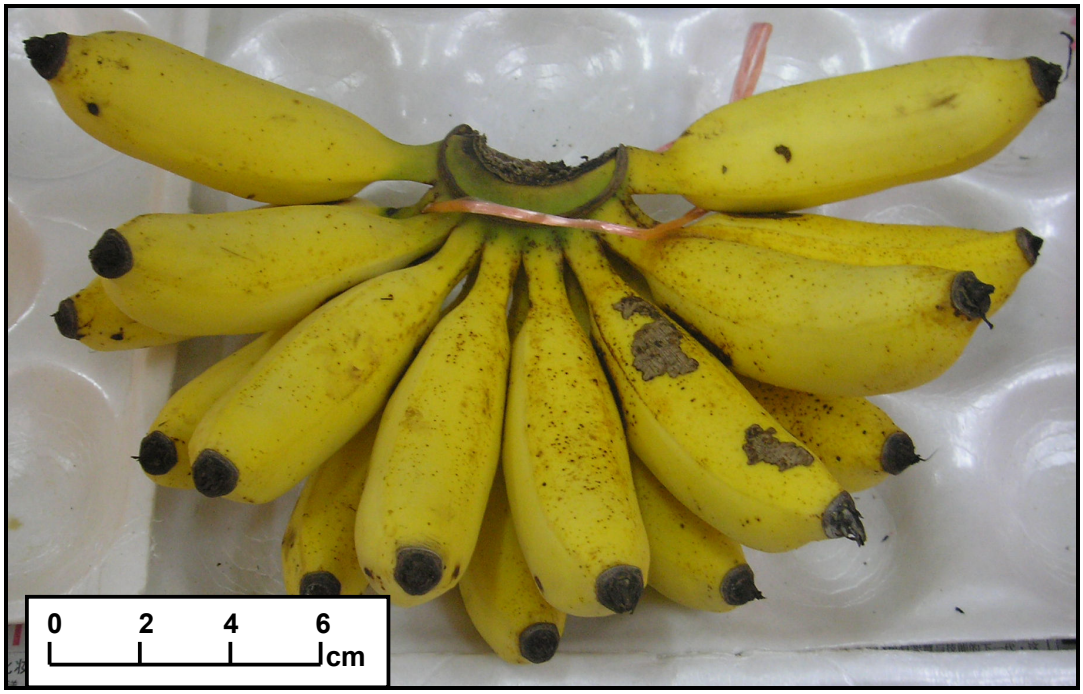

Plate 2.4: Pisang Rastali

Plate 2.5: Pisang Raja

Plate 2.6: Pisang Nangka

Plate 2.7: Pisang Awak

Plate 2.8: Pisang Nipah

2.1.2 Anatomy and morphology of banana

Bananas are treelike perennial herbs 2 – 9 meters in height (Espino *et al.*, 1992). They are vegetatively propagated from the rhizome. The entire above ground portion of the plant consists of only pseudostem until the flower occurs. The pseudostem is formed from the overlapping and tightly packed leaf sheath (Palmer, 1971). The leaves originated from a meristematic region located at the apex of the rhizome, at about the soil surface. Each leaf is 150 – 400 cm long, 70 - 100 cm wide and supported in a petiole 30 – 90 cm in length. It has pinnated vein and prominent midrib. Upon emerging from the pseudostem, it is tighly roll as cylinder, unfurl 6 – 8 days after emergence. Interestingly, the next leaves emerge through the centre of the previous leaf sheath.

At the time of flowering about nine months after planting, the true stem and associated growing point (apical meristem) rise within the rhizome. As they elongate, the floral apex is forced up the inside of the pseudostem and eventually emerge out the top of the pseudostem (Palmer, 1971).

The inflorescence comprises of many group of flowers which are arranged on the stem in nodul clusters in a radial fashion. Each flower cluster is borne on a prominent peduncle known as crown and is covered by a bract. Each cluster produces an approximately 12 – 20 flowers. The bract drops off in a few days leaving the female flowers to develop into mature fruit in the next 90 -150 days.

The fruits are attached to the peduncle by pedicles. The sum of fruits in the inflorescence is known as the bunch, individual cluster of fruits is known as hand, and individual fruit is called finger (Ploetz, 1992). Each hand has a crown to which 10 to 20 fingers are attached. Morphology of the developing banana fruits, both seeded and pathenorcarpic varieties was already studied by Ram *et al.* (1962). Peel cells consist of an outer cuticle and epidermis, several layers of hypodermal parenchyma and parenchyma cells interspersed with latex vessels, vascular bundles, and air spaces. In addition, the hydrodermal cells and the innermost pulp – initiating cells tend to be smaller and more tightly packed than the other cells. Scattered starch grains are visible. Pulp cells consist of a large numbers of starch grains in mature, pre-climateric tissue. During ripening, the pulp cells become progressively depleted of

starch and individual cells can be revealed in details. This study also reveals the significance contribution of the peel to the overall metabolism of the banana fruit. Large proportion of peel tissue makes up about 80%, 40% and 33% of the fresh weight of juvenile, mature and fully ripe fruit, respectively. According to Espino *et al.* (1992), as the fruit grow, the pulp/skin ratio will rise steadily.

2.1.3 Nutritional and therapeutic values of banana

Banana has earned the status as a high nutritive fruit. It has a unique combination of energy value, tissue – building elements, proteins, vitamins and minerals. Apart from being a nutritious food, banana fruit is already proven as possessing many curative properties because of its various kinds of vitamins, minerals, fibres and carbohydrates. Table 2.4 and 2.5 show the nutritional values and vitamin content of banana.

Banana first emerged in the medical literature as a cure for ulcer in the early 1930s (Carper, 1989). Sanyal *et al.* (1965) reported the ability of banana in reducing gastric ulcers and Mitchell *et al.* (1968) proved that bananas are useful for person with peptic ulcer. The ability of banana in reducing or curing the ulcers is due to its soft texture, smoothness and high in fiber content. Fiber helps to restore normal bowel function without the ill effect of a laxative. In overacidity cases, banana helps to neutralize the gastric juices and reduces the irritation by coating the lining of the stomach.

Likes fiber, potassium, sodium and iron also make an important contribution in the curative properties of bananas. Potassium which is a vital mineral for controlling body's fluid balance, present in substantial amount in banana fruit. Hence, banana is recommended for patient with low potassium level. Being high in potassium yet low in salt, banana can help in reducing the risk of fatal stroke by as much as 40% and lowering the blood pressure. Potassium is also required for normalizing the heart rhythm and transfer of oxygen to the brain (Margen, 2002). Meanwhile, banana also is beneficial for the anemic patient because of its iron content.

Iron can stimulate the production of hemoglobin in the blood which is essential in cases of anemia.

As an excellent source of B vitamins, banana can help in calming the nervous system. Moreover, its vitamin B6 also performs a role in regulating blood glucose level which is related to the mood condition. Therefore its role in normalizing blood glucose level can also help to avoid morning sickness and hangover. Also, vitamin C, A as well as B6 and B12 found in banana might help the body recovers from the effect of nicotine withdrawal.

Because of its low lipid level but high in energy value, banana is recommended for obese and geriatric patient (Gasster, 1963). Besides, banana is also valuable in the treatment of kidney disorder such as uraemia and nephritis due to its low protein and salt content. Interestingly, banana has a type of protein called tryptophan even though its protein level is low. Trytophan can stabilize depression state when the body converts it into serotonin. Data regarding nutritional value of banana clearly shows banana is among the healthiest fruit and is natural remedy for many illnesses.

Apart of the fruit, several other parts of the banana plant possess medicinal properties. The young unfolded leaves are used against chest pains and as a cool dressing for an inflamed or blistered skin. On the other hand, the sap exuding from the base of the cut trunk is used for urethral injection against gonorrhoea, dysentery and diarrhea, to stop the loss of hair and to stimulate hair growth. Not only that, the juice of the root is febrifuge and restorative and in powder form, the juice is used in anaemia cases and general weakness and malnutrition (Espino *et al.*, 1992).

Table 2.4: Nutritional values of banana (Margen, 2002)

Nutrient	Banana/ 1 fruit
Calories	109
Protein (g)	1
Carbohydrates (g)	28
Dietary fiber (g)	2.8
Total fat (g)	0.6
Saturated fat (g)	0.2
Monounsaturated fat (g)	0.1
Polyunsaturated fat (g)	0.1
Cholesterol (mg)	0
Potassium (mg)	467
Sodium (mg)	1
Calcium (mg)	9.2
Magnesium (mg)	44.1

Table 2.5: Vitamin content of banana 'Gros michel' and Cavendish (US RDA, 1963)

Nutrient	'Gros Michel'	Cavendish
	(% US RDA/100g)	
Vitamin A	3.8	5.1
Ascorbic Acid	13.3	20
Vitamin B	25	-
Thiamine	3.3	2.6
Riboflavin	3.8	5.3
Niasin	4.7	4

2.2 The influence of sample preparation in phenolic compounds extraction

Sample preparation is one of the most important factors that contribute to the successive extraction of bioactive compounds in plant materials. Selective extraction methods should be practiced since active compounds in plants that exhibit biological activities are usually present in low concentrations (Kartal *et al.,* 2007). Several authors have reported the influence of sample preparation in the evaluation of total phenolic contents in their study. According to Romero *et al.* (2004), the quality and quantity of phenolic compounds in table olives (from olive tree *Olea europaea*) depend upon the processing method. Likewise, Yu *et al.* (2005) reported that the skin removal methods such as blanching, direct peeling and roasting and extraction solvents had significant effects on total extractable phenolics of peanuts. Meanwhile, Naczk & Shahidi (2004) explained in details that extraction of phenolic compounds in plant materials is influenced by many factors including the extraction method employed, types of solvent polarity used, storage time and conditions as well as their chemical nature, presence of interfering substances and sample particle size. However, it is frequently overlooked by many researchers and poorly discussed in any journals or paperworks even though approximately 30% of analytical error stems from the sample preparation step (Majors, 1995; Majors, 1999).

Sample preparation may consist of multiple steps such as sample processing, sample homogenization, extraction, filtration, preconcentration, hydrolysis and derivatization (Luthria, 2006). With the aim to extract as much as bioactive compounds from the plant materials along with eliminating potential interferences and to optimize the sensitivity of the analytical procedure by increasing the concentration of the analyte in the assay mixture, optimizing each step involved in the sample preparation is crucial for the production of the reproducible and accurate result.

One of the essential steps is the sample processing prior to the extraction. Ideally, fresh plant tissue should be used for phytochemical analysis. Alternatively, plant materials may be dried before their extraction. However, precaution must be taken during drying operation to avoid many chemical changes in the plant materials (Harborne, 1998).

Choosing an appropriate extraction technique also can influence the total extractable phenolic. To date, many extraction techniques have been applied in the research field such as sonication, stirring, percolation, and wrist shaking, as well as the new, automated and high-throughput extraction technologies like soxhlet, microwave, superficial fluid extraction, pressurized liquid extraction and Ankum batch extraction (Luthria, 2006) which enable researchers to efficiently optimize sample preparation. Mukhopadhyay *et al.* (2006) had conducted a systematic investigation to optimize the extraction of total phenolic from black cohosh, *Cimicifuga racemose* by using a pressurized liquid extraction apparatus. They had identified several operative parameters such as solvent composition, solid-to-solvent ratio, particle size and number of extraction particle as the main variables that influence extraction efficiency (Nazck & Shahidi, 2004). Likewise, Naczk & Shahidi (2004) demonstrated that changing ratio of sample – to – solvent from 1:5 to 1:10 (weight/volume) increased the extraction of condensed tannins from commercial canola meals. Moreover, Deshpande *et al*. (1986), also demonstrated that yield of tannin recovery from dry beans, *Phaseolus lunatus* was strongly influenced by variations in the sample particle size.

The recovery of bioactive compounds from plant material is also influenced by type of solvent polarity. Kartal *et al.* (2007) reported that the polar solvents that was a mixture of methanol and water at the ratio of 1:1 is the most suitable extracting solvent for *Ferula orientalis* in terms of higher extract yield, as well as effectiveness in tested assay. Othman *et al.* (2007) also reported that total phenolic compounds and antioxidant activity of cocoa beans (*Theobroma cacao*) was significantly affected by the extracting solvent. The water extract of cocoa beans showed higher value of antioxidant activity based on β – carotene bleaching assay, while the ethanolic extract of cocoa beans showed the highest free radical scavenging and ferric reducing activities. Luthria (2006) reported that when different proportion of methanol and water in a mixture were varied, the composition of the phenolic profiles were changed significantly. In contrast, Chen *et al.* (1992), Chevolleau *et al.* (1992) and Kramer (1985) demonstrated that extraction with non – polar solvents, such as hexane and petroleum ether provided better antioxidant properties than the polar solvents for extraction of rosemary, leaves

of some Mediterranean plants and clove, respectively. All these findings showed that activity may be varied when different solvents are employed and the efficiency of extraction solvent varies among plants.

As a conclusion, studies on extraction procedures are related with plant species and depended on the texture and water content of the plant material as well as the type of substances that is being isolated (Harborne, 1998). Here, extracting phenolic compounds is the primary interest. Since thousand of phenolic compounds with diverse structural configurations have been isolated from different natural resources, it is challenging and practically impossible to develop a single uniform extraction process for the optimum extraction of all phenolic compounds from every possible matrix (Luthria, 2006). Hence, the best solution can be taken by optimizing the sample preparation in order to obtain an accurate and precise result. Furthermore, an intensive review of literatures found no information on methods of extraction for optimal recovery of phenolics from banana pulps and peels.

2.3 Phenolic compounds study

Phenolic compounds belong to the category of natural antioxidants and are the most abundant antioxidants in our diet (Boskou *et al.*, 2006). The term 'phenolic' refers to any compounds with a phenol type structure (Vaquero *et al.*, 2007). They are a very diversified group of phytochemicals derived from phenylalanine and tyrosine (Shahidi & Nazck, 2004). Phenylalanine and tyrosine are produced in plants via the shikimate pathway which is a common precursor for most phenolic compounds in higher plants (Strack, 1997). Singleton & Rossi (1965) described three classes of phenolics in terms of chemicals that range from relatively simple to complex which are non – flavonoids (hydroxybenzoic acid and hydrocinnamic acid), flavonoids (flavones, flavonols, flavanones, flavononols and flavans) and tannins (Figure 2.1).

Phenolic compounds are naturally and commonly found in both edible and inedible plants. The phenolic content and composition in plants are depended on genetic and environmental factors, as well as post – harvest processing and storage conditions (Tepe *et al.*,

2006; Luthria *et al.*, 2006). Additionally, plants vary in content and structure of phenolic compounds such as number of phenolic rings, aromatic substitution, glycosylation and conjugation with other phenolic compounds or organic acid will vary in their antioxidant properties. In plant, they may act as phytoalexins, attractants for pollinators, antifeedants, contributors to the plant pigmentation, protective agents against UV light and antioxidants (Nazck & Shahidi, 2004). Hence, several authors had reported that antioxidant activity of plant materials was well correlated with the content of phenolic compounds (Velioglu *et al.*, 1998; Turkoglu *et al.*, 2007; Katalinic *et al.*, 2004).

According to Rice – Evans *et al.* (1995), a polyphenols can be defined as antioxidants when it satisfies two basic conditions. First, it can delay, retard or prevent the autoxidation or free radical – mediated oxidation when present in low concentration relative to the substrate to be oxidized. Second, the resulting radical formed after scavenging must be stable through intramolecular hydrogen bonding on further oxidation. The antioxidant activities of phenolics are related to a number of different mechanism such as interrupting the propagation of the free radical autoxidation chain by contributing a hydrogen atom from phenolic group (which relates to its reduction potential), oxidation of low – density lipoproteins (Andrikopoulus *et al.*, 2002), direct reaction with radical to form less reactive products by stabilizing or delocalizing the unpaired electron and metal ion chelation (Tepe *et al.*, 2006). Furthermore, phenolics combines the three ideal structural chemistry found important for efficient radical-scavenging and resonance stabilization of the phenoxyl radical of the one – electron oxidized compound: (i) the *o* – dihydroxyl (catechol) structure of the B ring; (ii) the 2,3 – double bond in conjunction with a 4 – oxo function; (iii) the additional presence of both 3 – and 5 –hydroxyl groups (Bors *et al.*, 1990).

The flavonoid class is the most prominent and the most important plant antioxidant. Flavonoids have been proven to display a wide range of pharmacological and biochemical actions such as antimicrobial, antithrombotic, antimutagenic, anti – inflammatory, antiallergic, antihypertensive and anticarcinogenic activities by few researchers (Cook & Samman, 1996; Kandaswami & Middleton, 1996; Sahu & Gray, 1997). Epidemiological studies have indicated

that high consumption of phenolic compounds especially flavonoids, might play a role in the prevention of various pathophysiological processes associated with oxidative stress, such as cancer, neurodegenerative, cardiovascular disease and mutagenesis (Manach *et al.*, 2004).

Due to the importance of phenolic compounds as contributors of beneficial health effects, their isolation and structural identification in plant tissues or other biological systems should be made (de Lourdes Mata Bilbao *et al.*, 2007). For qualitative identification of phenolic compounds, liquid chromatography (LC) coupling with electrospray ionization mass spectrometric (ESI – MS) and ultraviolet – visible (UV – Vis) spectrometric were considered as a powerful tool for the identification and quantification of phenolics in plant extracts (Niessen & Tinke, 1995). This approach has been used successfully in the identification of many isoflavonoids and their related glucoside and glucoside malonates in red and white clover (Klejdus *et al.*, 2001). LC/MS has been widely used to identify all kind of flavonoids in different plant samples (Lai *et al.*, 2007). It provides the molecular mass of the different constituents and able to identify unstable compounds in solution, such as acylated flavonoids (Cuyckens & Claeys, 2004).

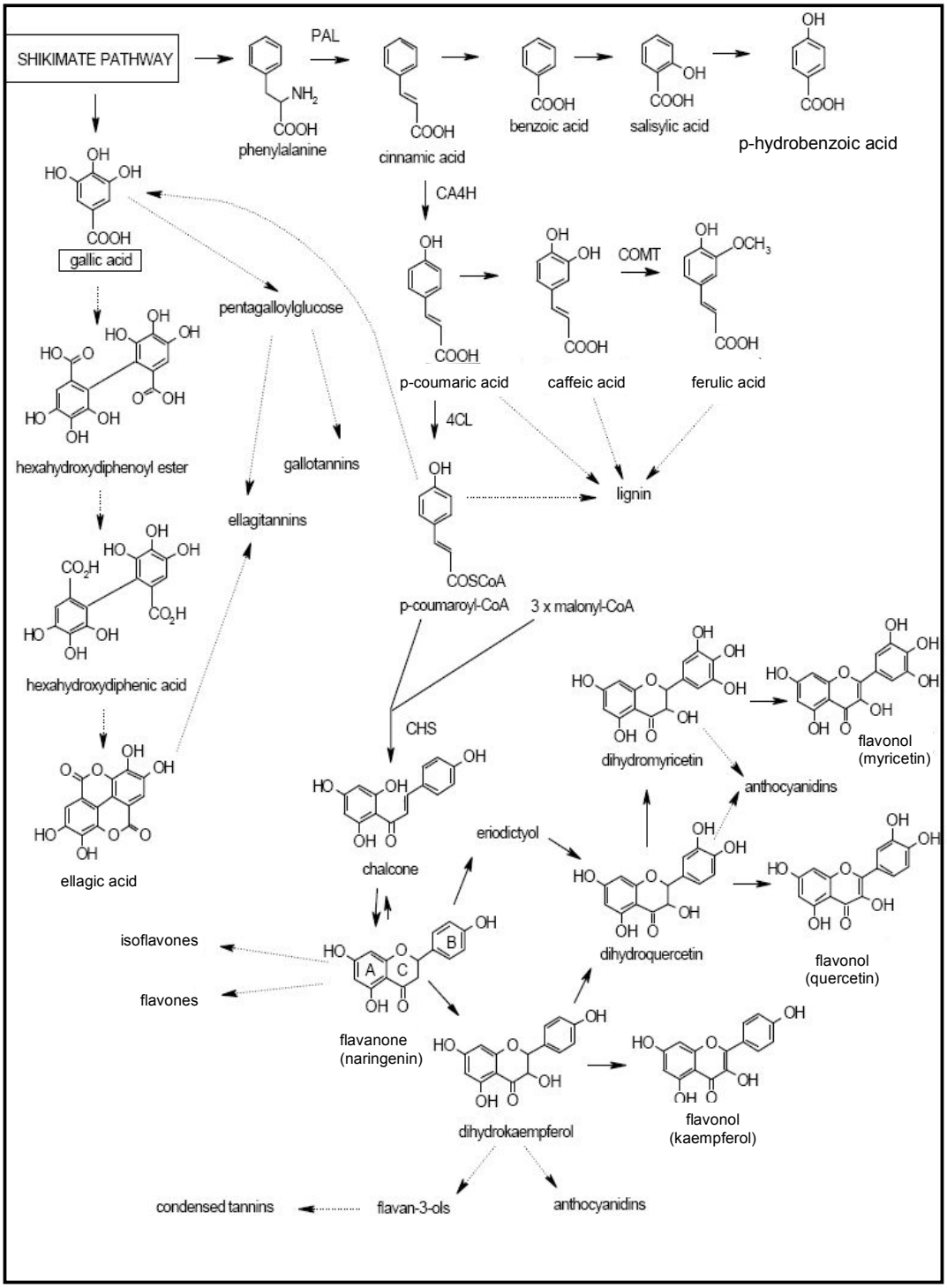

Figure 2.1: Biosynthesis of hydrobenzoic acids, hydrocinnamic acids, and flavonoids. Solid arrows represent well – characterized reactions catalysed by single enzymes. Dashed lines represent transformations that require multiple enzymes that are less characterized or vary among plant species. Enzyme: CA4H, cinnamic acid 4 – hydroxylase; CHS, chalcone synthase; 4CL, 4 – coumarate: coenzyme A ligase; PAL, phenylalanine ammonialyse (Hakkinen, 2000).