PHYTOCHEMICALS AND ANTIOXIDATIVE PROPERTIES OF *COFFEA LIBERICA* GREEN BEANS AND COMPARISON WITH THAT OF *COFFEA ARABICA* AND *COFFEA ROBUSTA*

by

TEO HUI MIN

Thesis submitted in fulfillment of the requirements
for the degree of
Master of Science

December 2016

ACKNOWLEDGEMENT

First of all, I would like to express my deepest gratitude towards my supervisor, Dr. Lai Choon Sheen, for giving me her guidance, valuable suggestions and continuous encouragement throughout the course of this research project and thesis writing. She never fails to do her best in introducing me to many innovative ideas of conducting research and studying as well as directing me to a right path. Thank you for being such a dedicated mentor. I would also like to extend my gratitude to my co-supervisor, Dr. Yam Wan Sinn for her help and advices throughout my study.

I would like to thank the Ministry of Higher Education (MOHE) (My Master) and the Research Creativity and Management Office (RCMO) of USM (grant: 1001/PKIMIA/811187) for their financial support in the completion of this thesis. I thank Centre for Drug Research (CDR) for providing me with the facilities and a comfortable environment to conduct the experiments.

I have been fortunate enough to have many people who had helped me along the way. Without them, I could not have completed my project smoothly. I thank Mr Zahari and Mr Nizam from the School of Chemical Sciences, USM for helping me to carry out the NMR analysis. Also, I would like to thank Mr Azizo who had assisted me in performing the FTIR analysis. I wish to thank all the support staffs in CDR especially Mr. Hilman, Mr. Asokan and Mr Razak for their assistance in GC-MS and HPLC analysis. I thank Mr. Zamri, Mr Rahim and all other lab and admistrative staffs who had helped me in one way or another. Special thanks to John Dolan from the Separation Science website, who had shared the knowledge of the

chromatography with me. Many thanks to my colleagues, Nelson, Thenmoly, Gaik Ling, Sutha, Yasodha and Jimmy for lending their hands and moral support during my hard time. I greatly appreciate it.

I thank my best friends, Hsin Chi, Gui San, Chian Fang, Hui Teng, Chiew Ling, Si Ning, Yi Ying, Jia Min, Joshua and Seow Lay who shared my ups and downs and are always supportive.

Last but not least, I wish to thank my dearest family members especially my parents, aunties and brothers who have never stopped believing in me, and for their love, support, understanding and encouragement that gave me the strength to complete my studies.

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LIST OF SYMBOLS AND ABBREVIATIONS

%	Percentage
α	Alpha
β	Beta
®	Registered
°C	Degree celcius
$\bar{x}$	Mean
ε	Molar absorptivity
c	Centi
σ	Standard deviation
S	Slope
µL	Microliters
g	Gram
J	Coupling constant
p	Para-substituted
r^2	Coefficient of determination
λ_{max}	Maximum absorption
m	Meter
µm	Micrometer
µg	Microgram
dm^3	Cubic desimeter
cm	Centimeter
mm	Millimeter
nm	Nanometer
mg	Milligram
mM	Millimolar

µM	Micromolar
mg AAE/g	Milligram ascorbic acid equivalent per gram
mg GAE/g	Milligram gallic acid equivalent per gram
mg Glc/g	Milligram glucose equivalent per gram
mL	Mililiters
mL/min	Milliliters per minute
kHz	Kilo hertz
ACN	Acetonitrile
1D	One dimension
2D	Two dimension
A.A	Acetic acid
AR	Analytical grade
ATR	Attenuated Total Reflection
ANOVA	One way analysis of variance
BHT	Butylated hyroxytoulene
CQA	Caffeoylquinic acid
COSY	Homonuclear Correlation spectroscopy
CD_3OD	Deuterated chloroform
$CHCl_3$	Chloroform
$CDCl_3$	Deuterated chloroform
C-5-HT	N^{β}-alkanoyl-5-hydroxytryptamine
DCQA	Disubstituted caffeoylquinic acid/ dicaffeoylquinic acid
DCM	Dichloromethane
D_2O	Deuterium oxide
DAD	Dioade array
DEPT	Distortionless enhancement by polarization transfer
DMSO	Dimethyl sulfoxide

DPPH	2,2-α-diphenyl-1-picrylhydrazil
et al.	and other
ESI	Electrospray ionization
EtOAc	Ethyl acetate
ft	Feet
F.A	Formic acid
FC	Folin-ciocalteu
$FeCl_3$	Ferric (III) chloride
$FeCl_3 \bullet 6H_2O$	Ferric (III) chloride hexahydrate
FRAP	Ferric reducing antioxidant power
FTIR	Fourier Transformed Infrared spectroscopy
g/g	Gram per gram
HSQC	Heteronuclear Single Quantum Correlation spectroscopy
HMBC	Heteromultiple Bond Correlation spectroscopy
HPLC	High Performance Liquid Chromatography
H_2O	Water
HCl	Hydrochloric acid
IC_{50}	Half maximal inhibitory concentration
ICH	International Conference on Harmonisation
i.d.	Internal diameter
i.e.	That is
IM/oa-TOF-MS	Ion mobility/orthogonal acceleration time-of-flight mass spectrometry
LOD	Limit of detection
LOQ	Limit of quantification
lit.	Literature
min	Minute

ms	Mass spectrometry
MeOH	Methanol
MCT	Monocarboxylic acid transporter
MTT	3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide
$NaCO_3$	Sodium carbonate
NIST	National Institute of Standards and Technology
NMR	Nuclear Magnetic Resonance
no.	Number
ppm	Parts per million
PPT	Precipitate
PFP	Penfluorophenyl
PDA	Photodiode array
PTLC	Preparative thin layer chromatography
R_f	Retention factor
R_t	Retention time
RSD	Relative standard deviation
ROS	Reactive oxygen species
RP C18	Reversed phase carbon 18
Semi-prep	Semi preparative
TPTZ	2,4,6-tris(2-prydyl)-s-triazine
TLC	Thin layer chromatography
THBP	2,4,5-trihydroxybutyrophenone
T	Temperature
temp.	Temperature
TFA	Trifluoroacetic acid
UV	Ultraviolet
UV-Vis	Ultraviolet-visible

USDA	United States Department of Agriculture
w/w	Weight per weight
w/v	Weight per volume

FITOKIMIA DAN SIFAT ANTIOKSIDAN BIJI KOPI MENTAH *COFFEA LIBERICA* DAN PERBANDINGAN DENGAN *COFFEA ARABICA* DAN *COFFEA ROBUSTA*

ABSTRAK

Coffea arabica, *Coffea robusta* dan *Coffea liberica* merupakan tiga spesies kopi utama yang ditanam untuk kegunaan komersial. Walaupun *C. liberica* merupakan spesies yang paling jarang diperdagangkan, ia merupakan suatu komoditi yang penting di Malaysia. Penyelidikan terhadap spesies ini adalah sangat terhad terutamanya terhadap biji mentahnya. Projek penyelidikan ini telah dijalankan untuk mengenalpasti juzuk kimia yang bertanggungjawab terhadap aktiviti antioksidan biji mentah *C. liberica* dan juga kandungan fitokimia serta aktiviti antioksidan berbanding dengan kedua-dua spesies yang lebih popular. Dengan menggunakan pendekatan pemencilan sebatian kimia berpandukan bioaktiviti, kafein (**2**), asid kafeik (**3**), dan satu siri asid klorogenik termasuk asid 5-kafeoilkuinik (**1**), asid 3-kafeoilkuinik (**4**), asid 4- kafeoilkuinik (**5**), asid 5- kafeoilkuinik (**6**), asid 4,5-di kafeoilkuinik (**7**), asid 3,4-di kafeoilkuinik (**8**) dan asid 3,5-di kafeoilkuinik (**9**) telah dipencilkan daripada biji mentah *C. liberica*. Aktiviti pemerangkapan radikal bebas bagi sebatian-sebatian ini yang ditentukan melalui cerakin DPPH adalah antara IC_{50} 12.33 – 39.76 μM. Secara amnya, asid kafeoilkuinik dwitukarganti dan asid kafeik menunjukkan aktiviti pemerangkapan radikal DPPH yang lebih tinggi (IC_{50} = 12.33– 22.99 μM) berbanding dengan asid kafeoilkuinik mono-tukarganti (IC_{50} = 29.90 – 39.76 μM) dan asid 5-feruloikuinik (IC_{50} = 84.99 μM). Aktiviti bagi asid kafeoilkuinik mono- dan dwitukarganti serta asid kaffeik adalah setanding dengan aktiviti asid askorbik (IC_{50} = 21.27 μM) dan trolox (IC_{50} = 26.72 μM) manakala

aktiviti bagi asid 5-feruloylquinik adalah setanding dengan aktiviti BHT (IC_{50} = 78.80 μM)**.** Hasil kajian ini menunjukkan bahawa ektrak metanol *C. liberica* mempunyai profil kimia yang serupa dengan kedua-dua spesies *Coffea* yang lain dengan menggunakan kaedah kromatografi cecair berprestasi tinggi dengan pengesan tatasusun diod (HPLC-DAD) yang telah dibangunkan dan disahkan. Kuantiti asid kafeoilkuinik mono-tukarganti dan kafein yang terdapat dalam *C. liberica* adalah di antara kuantiti yang didapati dalam *C. arabica* dan *C. robusta* manakala kuantiti asid kafeoilkuinik dwitukarganti adalah lebih kurang daripada yang terdapat dalam kedua-dua spesies yang lain. Namun begitu, perbezaan dalam kandungan sebatian-sebatian ini tidak menyebabkan banyak perbezaan dalam aktiviti-aktiviti antioksidan antara spesies ini. Ketiga-tiga ekstrak kopi tersebut juga didapati mempunyai keupayaan yang serupa dalam sifat perlindungan mereka ke atas fibroblas kulit manusia diaruh tekanan oksidatif daripada hidrogen peroksida. Kajian ini menunjukkan bahawa biji mentah *C. liberica* mempunyai fitokimia dan potensi antioksidan yang setara dengan *C. arabica* dan *C. robusta*. Dengan itu, spesies ini mungkin berfungsi sebagai satu alternatif yang sesuai kepada *C. arabica* dan *C. robusta* sebagai sumber yang kaya dengan antioksidan.

PHYTOCHEMICALS AND ANTIOXIDATIVE PROPERTIES OF *COFFEA LIBERICA* GREEN BEANS AND COMPARISON WITH THAT OF *COFFEA ARABICA* AND *COFFEA ROBUSTA*

ABSTRACT

Coffea arabica, *Coffea robusta* and *Coffea liberica* are three main coffee species cultivated for commercial consumption. *C. liberica*, being the least traded species among the three, is an important commodity to Malaysia. However, limited research has been carried out on this species, in particular on its green beans. The present work was carried out to identify the chemical constitutents responsible for the antioxidant activity of *C. liberica* green beans, as well as to compare its phytochemical content and antioxidant activities with the other two more popular species. By using an bioactivity-guided approach, caffeine (**2**), caffeic acid (**3**) and a series of chlorogenic acids, namely 5-caffeoylquinic acid (**1**), 3-caffeoylquinic acid (**4**), 4-caffeoylquinic acid (**5**), 5-feruloylquinic acid (**6**), 4,5-dicaffeoylquinic acid (**7**), 3,4-dicaffeoylquinic acid (**8**) and 3,5-dicaffeoylquinic acid (**9**) were isolated from the green beans of *C. liberica*. The free radical scavenging activity of these compounds as determined by the DPPH assay was between IC_{50} 12.33–39.76 μM. Generally, the di-substituted caffeoylquinic acids and caffeic acid exhibited stronger DPPH radical scavenging activity (IC_{50} = 12.33 – 22.99 μM) than the mono-substituted caffeoylquinic acids (IC_{50} = 29.90–39.76 μM) and 5-feruloylquinic acid (IC_{50} = 84.99 μM). The activity of the mono- and di- substituted caffeoylquinic acids as well as caffeic acid was comparable with that of the ascorbic acid (IC_{50} = 21.27 μM) and trolox (IC_{50} = 26.72 μM) while the activity of 5-feruloylquinic acid was comparable with that of BHT (IC_{50} = 78.80 μM). The study revealed that *C. liberica* methanol

extract has a similar chemical profile as those of the other two *Coffea* species by means of high performance liquid chromatography method with diode array detection (HPLC-DAD) that was developed and validated in-house. The quantity of the mono-substituted caffeoylquinic acids and caffeine in *C. liberica* was in between those found in *C. robusta* and *C. arabica*, while the quantity of the di-substituted caffeoylquinic acids was slightly lower than that in the other two species. Nevertheless, the differences in the content of these compounds did not result in much difference in the antioxidant activities among these species. All three coffee extracts demonstrated similar capacity in their protective properties towards human skin fibroblasts against hydrogen peroxide-induced oxidative stress. The present study indicated that *C. liberica* green beans have similar constituents and antioxidative potential as those of *C. arabica* and *C. robusta*. The species may serve as a good alternative to *C. arabica* and *C. robusta* for their rich source of antioxidants.

CHAPTER 1

INTRODUCTION

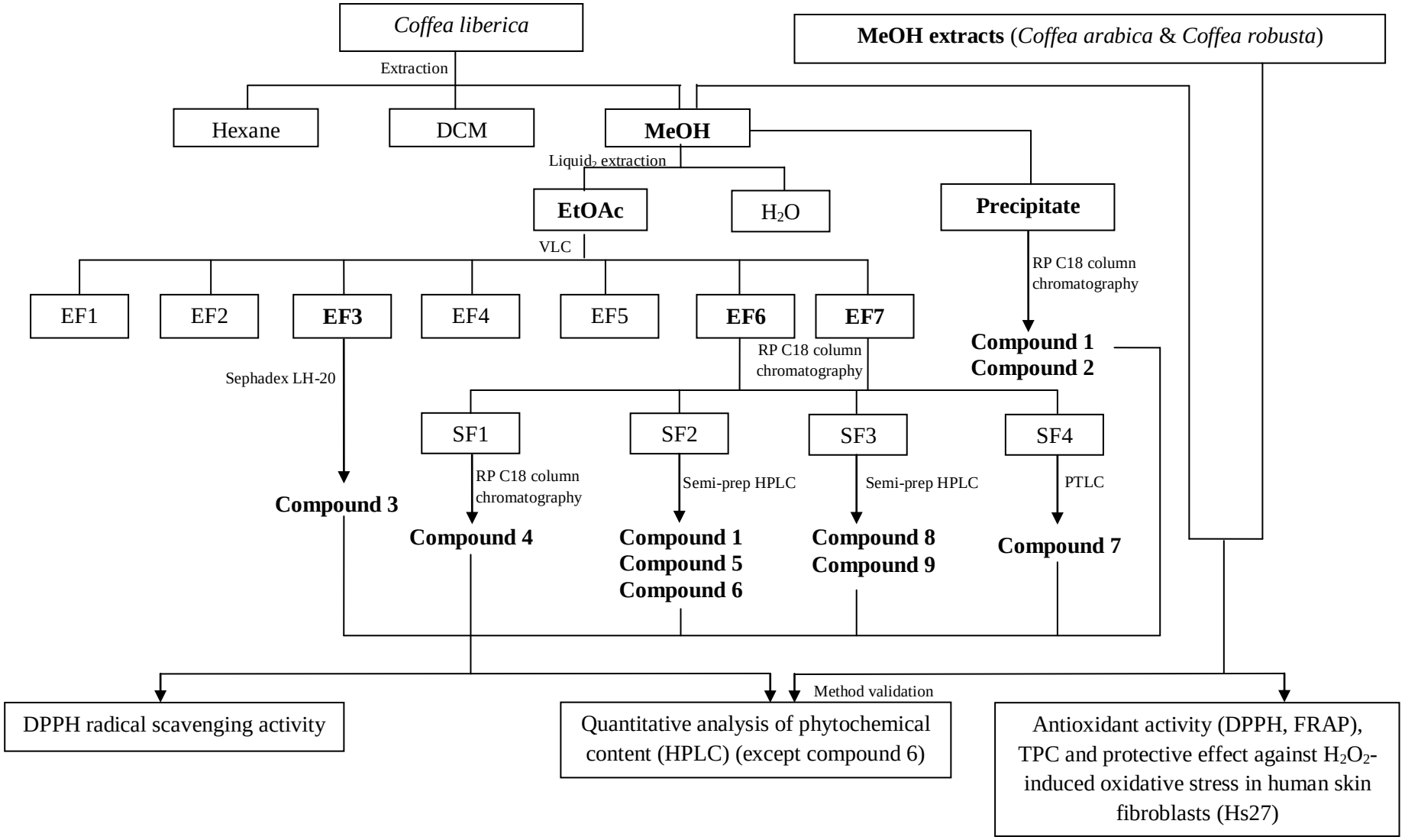
Coffee, being the most consumed beverage in the world, is well known for its antioxidative properties. There are three main coffee species cultivated for commercial consumption, namely *Coffea arabica*, *Coffea robusta* and *Coffea liberica*, among which *C. arabica* and *C. robusta* are more popular. Another species, *C. liberica*, is less traded because its cultivation is limited to small scale in few places; hence, it is commercially less successful. The total production of *C. liberica* only accounts for less than 1% of the world's coffee production (Davis et al., 2006). Even so, this species is rather important to Malaysia as it accounts for approximately 95% of the total coffee production of this country (Wallengren, 2002).

Throughout centuries, coffee is brewed from roasted beans. The roasting process changes the flavour of green coffee beans and brings out its delightful aroma and taste. However, during the roasting process, many natural products present originally in the green coffee beans are lost due to degradation and/or transformation (Perrone et al., 2008; Moon et al., 2009; Moreira et al., 2013). Green coffee beans are rich in chlorogenic acids such as caffeoylquinic acids, feruloylquinic acids, *p*-coumarouylquinic acids, small amount of sinapoylquinic acids and other types of mixed esters (Clifford et al., 2003; Jaiswal et al., 2010). The loss of chlorogenic acids due to roasting directly influences the antioxidant capacity of green coffee and hence the many natural benefits of coffee are lost (Daglia et al., 2000; Castillo et al., 2002;

Somporn et al., 2011; Pino-García et al., 2012). Green coffee beans extract was also found to have better anti-inflammatory activity than that of the roasted beans of *C. arabica* (Moreira et al., 2013). Its consumption may also prevent various chronic diseases such as cancer, cardiovascular disease and diabetes (Kozuma et al., 2005).

For many years, tremendous amount of research have been done on coffee but the focus was mainly on the roasted beans and their products. There have been renewed interests of late, concerning the beneficial effects of green coffee beans towards human health. However, due to the popularity of *C. arabica* and *C. robusta*, most of the research mainly revolved around these two species but not *C. liberica* (Naidu et al., 2008; Ludwig et al., 2012; Baeza et al., 2014). Phytochemical investigations of the *C. liberica* green beans and their antioxidant activities in comparison with those of *C. arabica* and *C. robusta* are still lacking up to these days. Hence, the present study with the following objectives was carried out:

1. To evaluate the antioxidant activities of the green bean extracts of *C. liberica* through several *in vitro* assays.
2. To isolate the phytochemicals responsible for the antioxidant activities of *C. liberica* using a bioactivity-guided approach.
3. To determine the antioxidant activities of the isolated compounds.
4. To compare the antioxidant activities by using chemical and cell-based models as well as the content of the major phytochemicals of *C. liberica* with those of its more popular counterparts – *C. arabica* and *C. robusta* green beans by HPLC.


Coffea liberica
Extraction
Hexane
DCM
MeOH
Liquid–liquid extraction
EtOAc
H_2O
VLC
EF1
EF2
EF3
EF4
EF5
EF6
EF7
Sephadex LH-20
Compound 3
RP C18 column chromatography
SF1
Compound 4
Semi-prep HPLC
SF2
Compound 1
Compound 5
Compound 6
Semi-prep HPLC
SF3
Compound 8
Compound 9
PTLC
SF4
Compound 7
RP C18 column chromatography
Precipitate
RP C18 column chromatography
Compound 1
Compound 2
MeOH extracts (*Coffea arabica* & *Coffea robusta*)
Method validation
DPPH radical scavenging activity
Quantitative analysis of phytochemical content (HPLC) (except compound 6)
Antioxidant activity (DPPH, FRAP), TPC and protective effect against H_2O_2-induced oxidative stress in human skin fibroblasts (Hs27)

CHAPTER 2

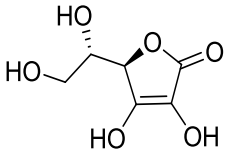
LITERATURE REVIEW

2.1 Antioxidant as chemopreventive and therapeutic agent

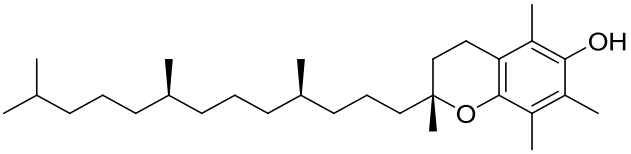
Reactive oxygen species (ROS) such as superoxide anion ($O_2•^-$), hydroxyl (•OH), peroxyl (ROO•), alkoxyl (RO•) and hydroperoxyl ($HO_2•$) radicals are continuously generated as the byproducts of normal cellular metabolism in human body. The body then regulates these free radicals with its natural antioxidant defense system which comprises of the endogenous enzymes such as superoxide dismutase (SOD), catalase and glutathione peroxidase (Halliwell, 1991; Valko et al., 2007). However, air pollution, ultraviolet radiation, unhealthy lifestyle such as smoking and many more can cause an overproduction of ROS. Thus, a depletion of the antioxidant defenses in the endogenous protective system occurs. This phenomenon is known as oxidative stress and can cause the damage in cellular lipids, proteins or DNA which in turn gives rise to various types of chronic diseases such as cancer, diabetes mellitus, cardiovascular diseases, rheumatoid arthritis, ageing and neurodegerative disorder (Halliwell, 1997; Valko et al., 2007; Ziech et al., 2010; Baeza et al., 2014). In order to help reverse the effects caused by the overwhelming presence of ROS, exogenous antioxidants of natural or synthetic origin may be recruited (Wootton-Beard & Ryan, 2011).

2.1.1 Natural antioxidants

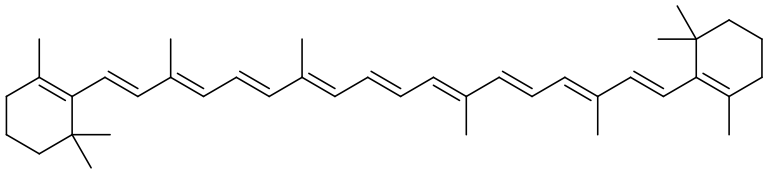
Natural antioxidants may be categorized into hydrophilic or lipophilic, depending on their solubility. They consist of polyphenols, carotenoids, vitamins and minerals which are mostly found in fruits, vegetables and beverages (Bravo, 1998; Wootton-Beard & Ryan, 2011). Vitamin C, E and β-carotene are amongst the most established antioxidants found in dietary sources. Vitamin C, often known as ascorbic acid, is a hydrophilic antioxidant, while vitamin E (mainly presents as α-tocopherol) and β-carotene are lipophilic antioxidants (Niki et al., 1995) (Figure 2.1).



Ascorbic acid (vitamin C)



α-tocopherol (vitamin E)



β-carotene

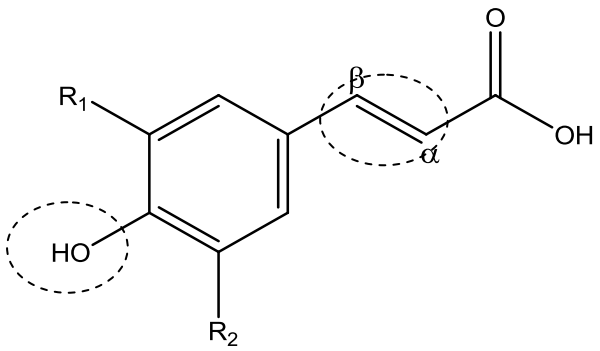
Figure 2.1: Chemical structures of ascorbic acid, α-tocopherol and β-carotene.

Polyphenols, the largest class of dietary antioxidants, are another group of hydrophilic compounds present abundantly in fruits, vegetables and beverages (Ratnam et al., 2006). These types of compounds are synthesized by plant as secondary metabolites to protect themselves against ultraviolet radiation and pathogenic invasion (Manach et al., 2004). Polyphenols can be divided into two groups: non-flavonoid and flavonoid compounds, with different classes and subclasses according to the number of phenol rings and the type of substituents attached to the core structure (Table 2.1). Phenolic compounds present in nature ranges from molecules as simple as phenolic acids, flavonols, flavones, flavanones, flavanols and isoflavones to highly polymerized structures like tannins (Bravo, 1998; Manach et al., 2004).

Table 2.1: The main classes of phenolic compounds (Source: Manach et al., 2004 & Wootton-Beard & Ryan, 2011).

Class	Subclass	Examples
Non-flavonoid compounds		
Phenolic acids	Benzoic acids	Gallic acid, protocatechuic acid, *p*-hydroxybenzoic acid
	Hydroxycinnamic acids	*p*-coumaric acid, caffeic acid, ferulic acid, sinapic acid
Tannins	Hydrolyzable tannins	Pentagalloyloylglucose, punicalagins, ellagitannins
Stilbenes		Resveratrol
Lignans		Secoisolariciresinol, matairesinol, lariciresinol, pinoresinol
Flavonoid compounds		
Flavonols		Kampferol, quercetin, myricetin
Flavones		Apigenin, luteolin
Flavanones		Narigenin, hesperetin
Flavanols		Catechins, gallocatechins
Tannins	Condensed tannins	Proanthocyanidins
Isoflavones		Daidzein, genistein, glycitein

Hydroxycinnamic acids (HCA) are ubiquitous constituents in the plant kingdom and well known for their antioxidant activities, particularly the radical scavenging ability. These phytochemicals possess a phenylpropanoic structure with C6-C3 as their basic skeleton (Bravo, 1998; Esteves et al., 2008; Razzaghi-Asl et al., 2013). The *para* hydroxy group on the benzene ring and the α, β unsaturated bond of HCA (highlighted in Figure 2.2) are the requisite factors of the radical scavenging capacity of HCA. The benzene structure which bears the hydroxy group is capable of forming phenoxy radical intermediate that are responsible for free radical chain termination, whereas the α, β unsaturated bond favours the stability of the phenoxy radical by increasing its electron delocalization, at the same time it acts as an additional reaction site for ROS (Graf, 1992; Barone et al., 2009). Caffeic acid, ferulic acid, *p*-coumaric acid and sinapic acid are the most typical examples of HCA (Figure 2.2) (Razzaghi-Asl et al, 2013).

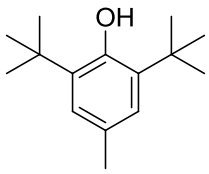


R_1= OH, R_2 = H	caffeic acid
R_1=OCH_3, R_2 =H	ferulic acid
R_1= H, R_2 =H	*p*-coumaric acid
R_1=OCH_3, R_2 = OCH_3	sinapic acid

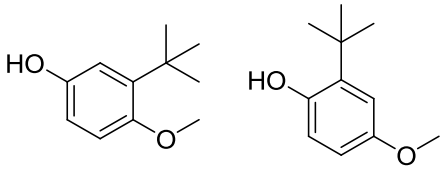
Figure 2.2: The chemical structures of caffeic acid, ferulic acid, *p*-coumaric acid and sinapic acid.

2.1.2 Synthetic antioxidants

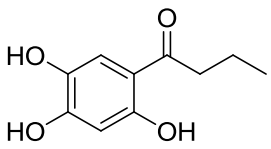
Synthetic antioxidants are used as food additives or preservatives to help prevent food rancidity due to oxidation process. Some of the examples of these antioxidants which are widely used in the food industry include BHT (butylated hydroxytoulene), BHA (butylated hydroxyanisole), THBP (2,4,5-trihydroxybutyrophenone), propyl gallate and octyl gallate (Xiu-Qin et al., 2009; Yehye et al., 2015) (Figure 2.3). However, studies have shown that some of these synthetic antioxidants may have adverse effect on human health (Yehye et al., 2015). Therefore, attention has now been switched to the use of naturally occuring antioxidants as safer substitutes.



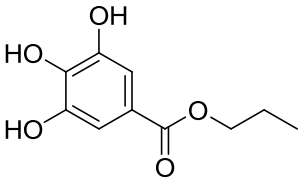
BHT (butylated hydroxytoulene)



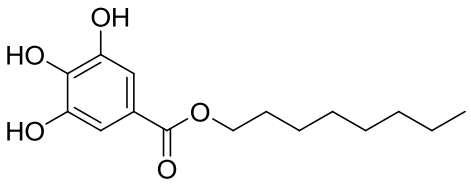
BHA (butylated hydroxyanisole)



THBP (2,4,5-trihydroxybutyrophenone)



Propyl gallate



Octyl gallate

Figure 2.3: Chemical structures of BHT (butylated hydroxytoulene), BHA (butylated hydroxyanisole), THBP (2,4,5-trihydroxybutyrophenone), propyl gallate and octyl gallate.

2.2 Rubiaceae family

Rubiaceae family is one of the largest flowering plant in the plant kingdom with around 600 genera and 13000 species commomly found in the tropical region. Rubiaceae is subdivided into four subfamilies, which are Cinchonoideae, Ixoroideae, Antirheoideae and Rubioideae. Their species vary from small trees, shrubs, herbs to even large woody plant (Bremer, 1996; Davis et al., 2009). Some of the species in this family which are valuable to mankind includes *Cinchona officinalis* (medicinal plant), *Rubia tinctoria* (madder; dye plant), *Neolamarckia chinensis* (timber trees), *Gardenia jasminoides* (ornamental plant) and several *Coffea* species which are by far the most economically important species due to its popularity as a beverage (Kew Royal Botanic Gardens, n.d.).

2.3 Genus of *Coffea*

The genus *Coffea* is native to Africa and is widely distributed across the tropical countries for centuries (Clarke & Macrae, 1985) (Figure 2.4). It is classified under the Ixoroideae subfamily which is divided into two subgenus, *Coffea* subgenus *Coffea* and *Coffea* subgenus *Baracoffea* based on their morphological characteristics (Davis et al., 2006; Bremer & Eriksson, 2009). There are 103 species identified in this genus, of which 95 species are grouped under *Coffea* subgenus *Coffea* including the three main coffee species that are traded in the market, namely, *Coffea arabica*, *Coffea robusta* (*C. canephora*) and *Coffea liberica*, whilst another eight species are under *Coffea* subgenus *Baracoffea* (Davis et al., 2006).

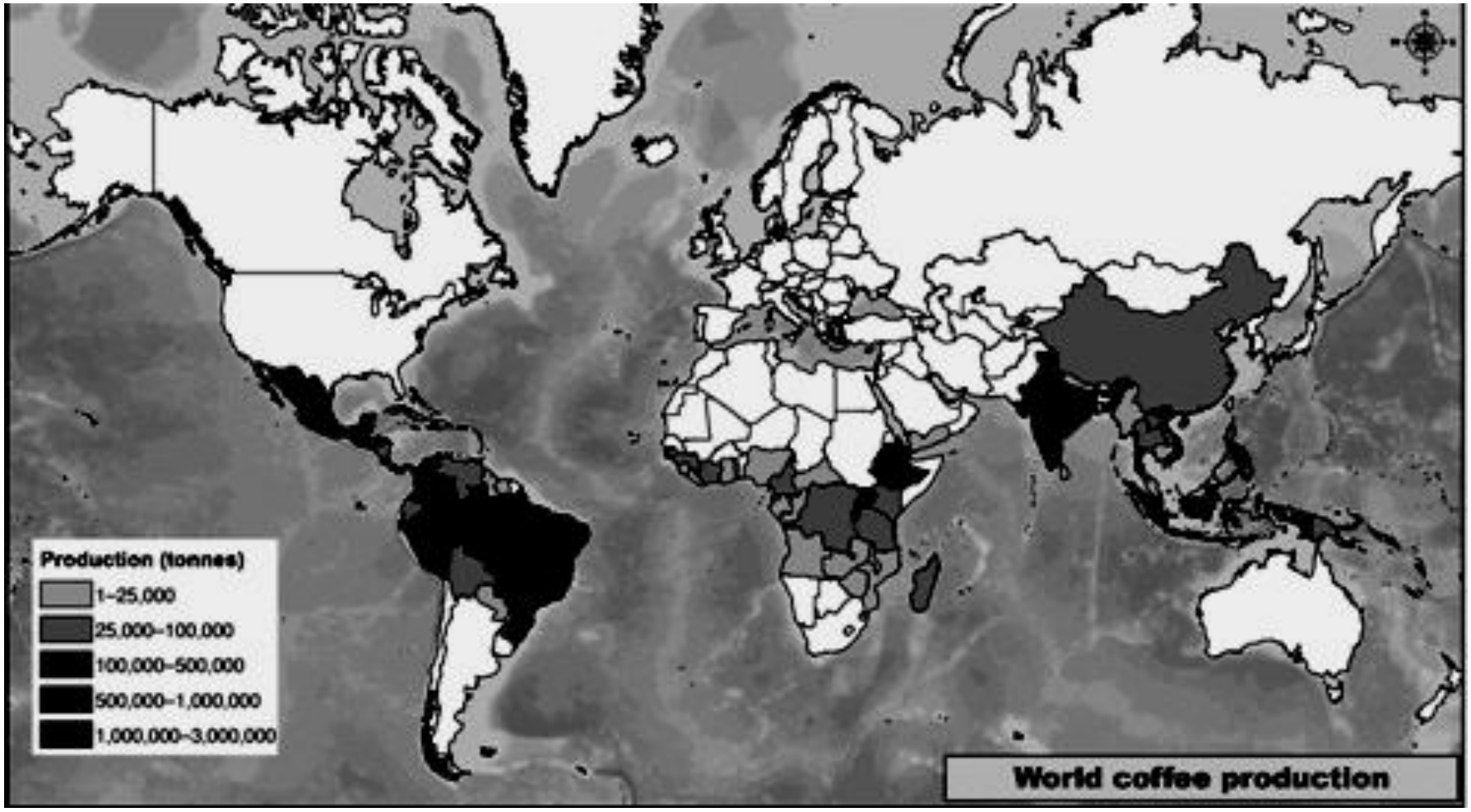


Figure 2.4: World coffee production (Source: Jha et al., 2014).

The spread of coffee cultivation is mainly based on the economical importance of a species and geographical aspects such as adaptation to soils, climate and altitude (Wellman, 1961). Among the three coffee species sold for coffee beverages, *C. arabica* and *C. robusta* are more widely traded, while *C. liberica* is less sought after (Davis et al., 2006; USDA, 2014). The main producers for *C. arabica* are countries like Brazil, Central America and Africa, where their contribution of coffee production was 41.4%, 15.4% and 10.9%, respectively. *C. robusta* is mainly produced in Vietnam, Brazil, Indonesia and Africa accounting for 42.1%, 25.3%, 11.0% and 10.4%, respectively (USDA, 2014). On the other hand, *C. liberica* is mainly produced in Malaysia, some places in the Philipines and parts of Africa such as Liberia (Wellman, 1961; Davis et al., 2006).

C. canephora is more known as *C. robusta* due to its resistance towards *Hemileia* rust (Wellman, 1961; Davis et al., 2006). There are few interesting differences among the trees of these three coffee species (Table 2.2). *C. arabica* is self-pollinating, has little blossoms and small flowers while the flowers of *C. robusta* and *C. liberica* are often bigger and rely on cross-pollination by means of pollination agents like wind and insects. Under cultivation, *C. liberica* is grown as the tallest tree and *C. arabica* being the shortest. Unlike *C. robusta* and *C. liberica*, *C. arabica* has the smallest leaves. The leaves of *C. liberica* are leathery and the largest among three species. Apart from that, the cherries of *C. liberica* are large and thick-skinned with big seeds/beans. For *C. arabica*, the cherries and beans are often medium-sized while the cherries and beans of *C. robusta* are the smallest. The beans of *C. liberica* and *C. arabica* are oval in shape except for *C. robusta*, which are round. Among the three species, *C. arabica* is more vunerable to rust, disease and harsh climates (Wellman, 1961). *C. liberica* is well adapted to various elevations,

soils, climates and sun expose cultivations. Therefore, it is probably more suited to grow at lowland and is also the most tolerant species towards heat amongst the three.

Table 2.2: Characteristic differences between *Coffea arabica*, *Coffea robusta* and *Coffea liberica* (Wellman, 1961; Clarke & Macrae, 1985; Davis et al. 2006; Ismail et al., 2014).

Characteristic	Species		
	C. arabica	*C. robusta*	*C. liberica*
Pollination	Self-fertile	Self-sterile	Self-sterile
Tree height in wild	26-33 ft	6.5-16 ft	18-36 ft
Relative leaf size	Smallest; dark green	Largest; light green	Medium; dark green, leathery
Relative flower size	Small	Medium	Large
Cherries/fruits	Medium; red	Small; red	Large; red to red-brownish
Bean size (no. of beans per pound)	1200	1600	800
Bean shape	Oval	Round	Oval
Optimum altitude for growth	2500-5000 ft	600-2400 ft	Sea level-1800 ft
Ecological niche	Humid, evergreen forest	Humid, evergreeen forest, sometimes in seasonally dry humid forest, rarely in gallery forest	Humid, evergreen forest, or seasonally dry, evergreen forest, sometimes in seasonally dry mixed evergreen-deciduous forest, gallery forest

2.4 Coffee as a source of medicine and beverage

In the early days, coffee was used as medicine in places like Arabia, Europe and America. Coffee infusion is believed to soothe the mind, relieve pains in the head, lethargy and cough. Besides, it is also effective in treating rheumatism, gout and intermittent fever. Before roasted coffee beans were introduced, dried young coffee leaves, cherry pulp or whole cherry were infused like tea in order to make a refreshing drink. The coffee leaf is also used traditionally for preparing drinks in some parts of Malaysia, Jawa and Sumatra (Wellman, 1961). This refreshing and stimulating effect of coffee was most probably due to the presence of caffeine.

2.5 Green beans

A coffee fruit consists of the skin, pulp, mucilage, pachment, silverskin and the bean (Figure 2.5). Green coffee beans are produced by removal of pulp including mucilage and pachment of the ripe coffee berries. After depulping the berries, the green beans (with or whithout the silverskin) are sundried, separated and graded accordingly (Sivaram, 1980; Esquivel & Jiménez, 2012). A picture of the green coffee bean of *C. arabica*, *C. robusta* and *C. liberica*, respectively, is shown in Figure 2.6.

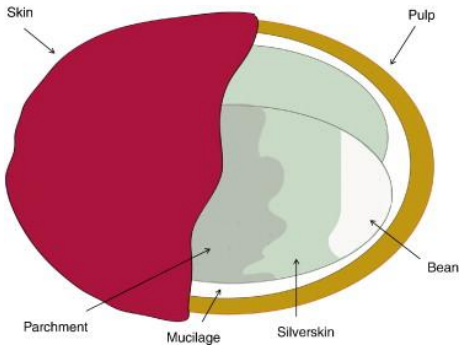


Figure 2.5: The structure of coffee fruit (Source: Esquivel & Jiménez, 2012).

Figure 2.6: The dried green beans of *Coffea arabica*, *Coffea robusta* and *Coffea liberica*.

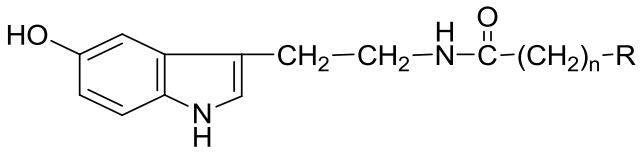
2.5.1 Phytochemicals in green coffee beans

The phytochemicals of coffee have been studied for more than a century. There are tremendous amount of compounds present in coffee beans and more than a thousand of compounds have been identified in roasted beans to date (Hoffman & Gerber, 2012). Green coffee beans are rich in polyphenols particularly 5-caffeoylquinic acid, caffeine other than lipids, volatile compounds and carbohydrates (Redgwell & Fischer, 2006; Speer & Kölling-Speer, 2006; Alonso-Salces et al., 2009; Wagemaker et al., 2011). Most of the studies on the phytochemicals were done on *C. arabica* and *C. robusta* but there is scant information about the phytoconstituents of *C. liberica* (Fischer et al., 2001; Oosterveld et al., 2003; Moon et al., 2009; Perrone et al., 2012). In this section, only important and major components present in green coffee bean especially in *C. arabica* and *C. robusta* were discussed.

2.5.1(a) Lipids

The lipid fraction of coffee beans consists of a minute amount of coffee wax extracted from the outer layer of the beans and coffee oils. Three of the N^{β}-alkanoyl-5-hydroxytryptamine (C-5-HT), N^{β}-arachidoyl-5-hydroxytryptamine, N^{β}-behenoyl-5-hydroxytryptamine and N^{β}-lignoceroyl-5-hydroxytryptamine were found as the predominent components present in the coffee wax (Figure 2.7) (Folstar et al., 1979). The coffee oils is constituted of free fatty acids, triglycerols, sterols, diterpenes and other lipid components found in the endosperm (Speer & Kölling-Speer, 2006) (Figure 2.8). Among the fatty acids present are palmitic (C16:0), stearic (C18:0), oleic (C18:1), linoleic (C18:2), linolenic (C18:3) and arachidic ((C20:0) acids in the form of free fatty acids, with palmitic and linoleic acids being the most predominat

free fatty acids present in the green beans of 10 coffee species (Martin et al., 2001; Wagemaker et al., 2011). However, most of the fatty acids present in nature are either esterified with glycerol or diterpenes to form triacylglycerols or diterpene esters whilst small amounts are esterified with sterol as sterol esters (Nikolova-Damyanova et al., 1998). The diterpenes, cafestol and kahweol are the predominant unsaponifiable matter of coffee oil which is seldom found in free form and present as diterpene esters (Scharnhop & Winterhalter, 2009). The main sterols in green coffee are β-sitosterol, followed by stigmasterol and then campesterol (Carrera et al., 1998).



R = CH_2OH; n = 18	N^{β}-arachidoyl-5-hydroxytryptamine
R = CH_2OH; n = 20	N^{β}-behenoyl-5-hydroxytryptamine
R = CH_3; n = 22	N^{β}-lignoceroyl-5-hydroxytryptamine

Figure 2.7: Chemical structures of N^{β}-arachidoyl-5-hydroxytryptamine, N^{β}-behenoyl-5-hydroxytryptamine and N^{β}-lignoceroyl-5-hydroxytryptamine.

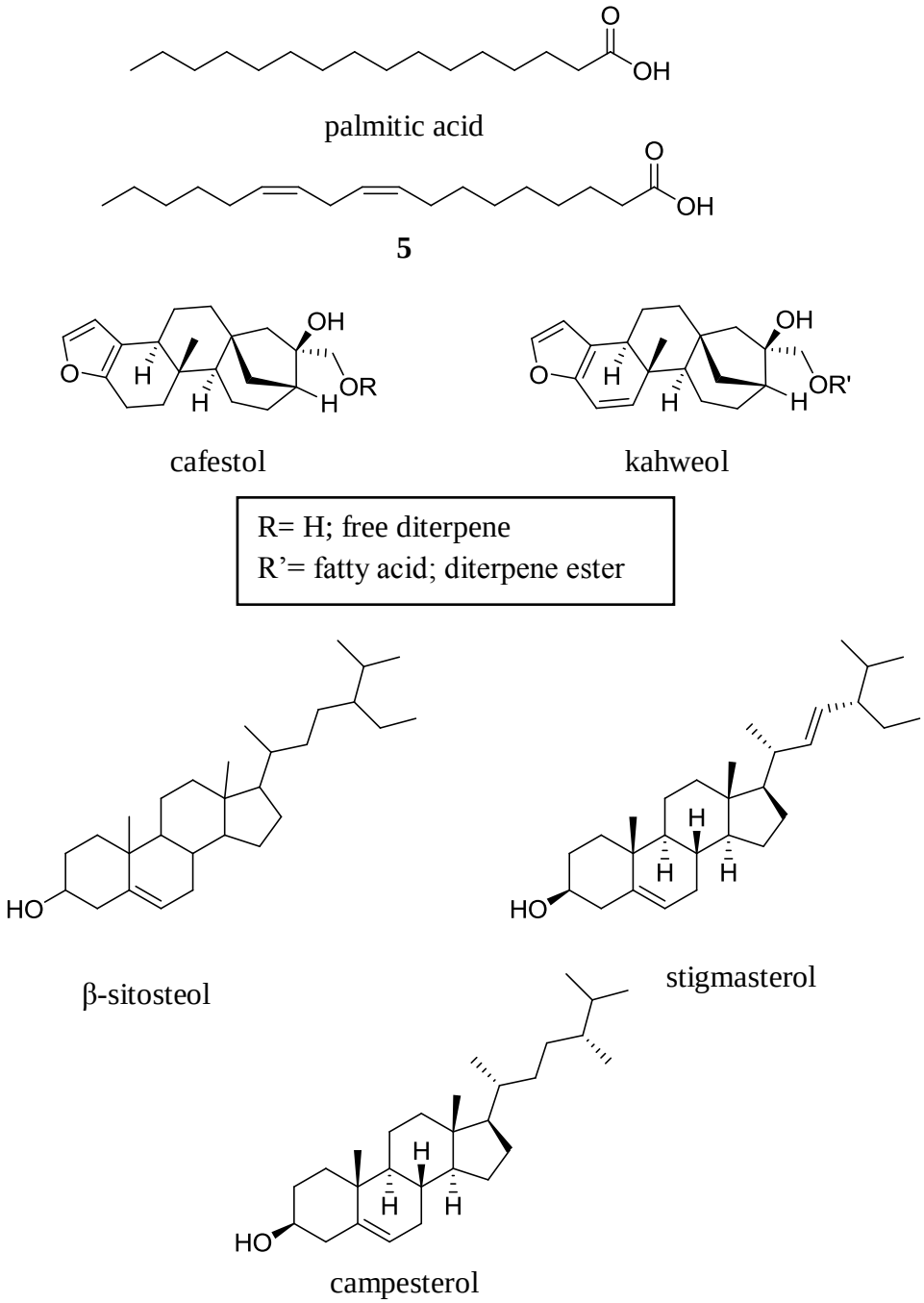
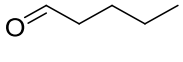


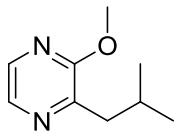
Figure 2.8: Chemical structures of major free fatty acids, diterpenes and sterols.

2.5.1(b) Volatile compounds

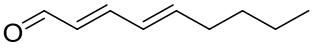
Approximately 300 or more volatile compounds were identified in green coffee. These compounds mainly consisted of hydrocarbons, alcohols, pyrazines, ketone, furans, aldehydes, phenols, esters and some sulfur compounds (Flament & Bessière-Thomas, 2002). Among these volatile components, there are a few major compounds which give rise to the odour of green coffee beans (Figure 2.9). Pentanal or known as veleraldehyde was identified in green beans and is believed in giving penetrating odour to the green coffee beans (Zlatkis & Sivetz, 1960; Flament & Bessiere-Thomas, 2002). The 'green' smell of green coffee was possibly caused by the presence of compounds like 2-isobutyl-3-methoxypyrazine and two pairs of unsaturated aldehydes, (*E,E*)-2,4- and (*2E,4Z*)-2,4-nonadienal; (*E,E*)-2,4- and (*2E,4Z*)-2,4-decadienal. These two pairs of unsaturated aldehyde were identified in the Colombian green coffee (Boosfeld & Vitzthum, 1995). Other than these, *p*-vinylguaiacol was also found to be a major compound present in green coffee that gives its strong, spicy, clove-like odour (Flament & Bessiere-Thomas, 2002; Saw et al., 2015).



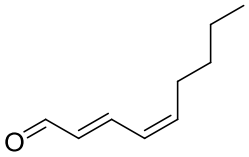
Pentanal



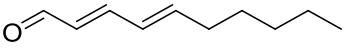
2-isobutyl-3-methoxypyrazine



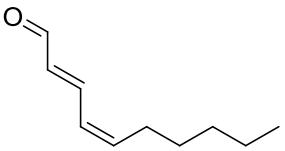
(*E*,*E*)-2,4-nonadienal



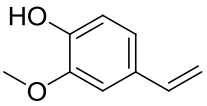
(*2E*,*4Z*)-2,4-nonadienal



(*E*,*E*)-2,4-decadienal



(2*E*,4*Z*)-2,4-decadienal

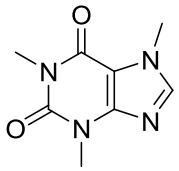


p-vinylguaiacol

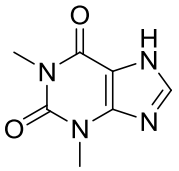
Figure 2.9: Chemical structures of major volatile compounds.

2.5.1(c) Alkaloids

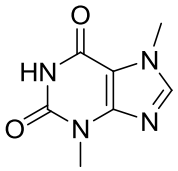
Caffeine (1,3,7-trimethylxanthine), theophylline (1,3-dimethylxanthine), theobromine (3,7-dimethylxanthine) and trigonelline are the alkaloids found in green coffee beans (Clifford & Kazi, 1987; Alonso-Salces et al., 2009) (Figure 2.10). The content of caffeine, a major alkaloid in coffee beans is higher in *C. robusta* than *C. arabica*. The content of caffeine also varies depending on its geographical origin (Table 2.3). Theophylline is only present in *C. robusta* and is often used as a chemical marker for this species. Trigonelline is the second most abundant alkaloid after caffeine, while only trace amount of theobromine is observed in green coffee beans (Clifford & Kazi, 1987; Ky et al., 2001; Alonso-Salces et al., 2009).



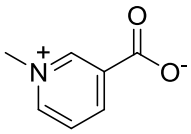
caffeine



theophylline



theobromine



trigonelline

Figure 2.10: Chemical structures of caffeine (1,3,7-trimethylxanthine), theophylline (1,3-dimethylxanthine), theobromine (3,7-dimethylxanthine) and trigonelline.