ANALYSIS OF PHYTOCHEMICAL COMPOUNDS, ANTIOXIDANT AND ANTIPROLIFERATIVE ACTIVITY OF AQUEOUS EXTRACTS OF *CAPSICUM* FRUITS

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by

ROHANIZAH BINTI ABDUL RAHIM

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In the Name of Allah, the Most Gracious, the Most Merciful

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LIST OF ABBREVIATIONS

ABTS	2,2'-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid)
$AlCl_3$	Alumunium (III) chloride
BHA	Butylated hydroxyanisol
˚C	Degree celsius
CE	Capillary electrophoresis
cm	Centimeter
CO_2	Carbon dioxide
COX-2	Cyclooxygenase-2
CS	Capsaicin synthase
DI	Deionized water
DPPH	1, 1-diphenyl-2-picrylhydrazyl
eV	Electron volt
FBS	Fetal bovine serum
$FeCl_3$	Iron (III) chloride
FRAP	Ferric reducing antioxidant power
g	gram
GCMS	Gas Chromatography Mass Spectrometry
GAE	Gallic acid equivalent
HPLC	High Performance Liquid Chromatography
IC_{50}	Inhibition concentration which is inhibited 50% of cell proliferation
ISO	International Organization for Standardization
K	Potassium
kV	Kilovolt

L	Liter
LC	Liquid chromatography
LCMS	Liquid chromatography mass spectrometry
LDH	Lactate dehydrogenase
LOD	Limit of detection
LOQ	Limit of quantitation
m	Meter
mg	Milligram
MS	Mass spectrometer
MTS	(3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H-tetrazolium
mL	Milliliter
m/z	Mass to charge ratio
N	Normality
Na	Sodium
nm	Nanometer
OH	Hydroxyl
O_2	Oxygen
ORAC	Oxygen radical antioxidant capacity
PBS	Phosphate buffered saline
PDA	Photodiode array detector
PMS	Phenazinemethosulfate
PVDF	Polivinyldeneflouride
QE	Quercetin equivalent
ROS	Reactive oxygen species

rpm	revolutions per minute
RPMI	Roswell Park Memorial Institue
µg	microgram
µl	microlitre
µm	micrometer
UV	Ultraviolet
v/v	volume per volume
w/v	weight per volume
WHO	World Health Organization

LIST OF PUBLICATIONS, CONFERENCES AND PRESENTATIONS

Proceeding

1. **Rohanizah Abdul Rahim** and Ishak Mat. Phytochemical contents of *Capsicum frutescens* (Chilli padi), *Capsicum annum* (Chilli pepper) and *Capsicum annum* (Bell pepper) aqueous extracts. Proceeding at International Conference Bilogical Life Sciences 2012, 23-24 July 2012, Quality Hotel, Singapore.

Oral Presentation

1. **Rohanizah Abdul Rahim**, Nurulhuda Zakaria, Ishak Mat. Antioxidant activity of *Capsicum* variety (Bell pepper, Chilli pepper and Chilli padi) aqueous extracts. Oral Presentation at 1st Annual IMMB Postgraduate Colloquium 2011, 25 -26 November 2011, Carlton Hotel, Shah Alam, Malaysia.

2. **Rohanizah Abdul Rahim** and Ishak Mat. Phytochemical contents of *Capsicum frutescens* (Chilli padi), *Capsicum annum* (Chilli pepper) and *Capsicum annum* (Bell pepper) aqueous extracts. Oral presentations at International Conference Biological Life Sciences 2012, 23-24 July 2012, Quality Hotel, Singapore.

Poster Presentation

1. **Rohanizah Abdul Rahim,** Ishak Mat, Mohd Nizam Mordi, Muhammad Nazri Ismail. Antioxidant and antiproliferative activities of *Capsicum frutescens* aqueous extracts. 1st International Conference on Antioxidants and Degenerative Diseases (ICADD 2015), 3-4 Jun 2015, Istana Hotel, Kuala Lumpur, Malaysia.

2. **Rohanizah Abdul Rahim,** Syazwani Ismail, Ishak Mat. Comparison of capsaicinoid content between *Capsicum frutescens* (chilli padi) and *Capsicum annuum* (chilli pepper and bell pepper). 19th Scientific Meeting from Local to Global: Highlighting The Success of Malaysian Bioscientist , 31 October – 01 November 2012, Universiti Malaya, Kuala Lumpur, Malaysia.

3. **Rohanizah Abdul Rahim**, Nurulhuda Zakaria, Syazwani Ismail, Ishak Mat. Comparative analysis of capsaicinoid compounds in *Capsicum annuum* and *Capsicum frutescens*. Translational Science Seminar, 12-13 December 2011, Park Royal Hotel Batu Feringghi, Malaysia.

ANALISIS KOMPAUN FITOKIMIA, AKTIVITI ANTIOKSIDA DAN ANTIPROLIFERASI EKSTRAK AKUEUS BUAH-BUAHAN CAPSICUM

ABSTRAK

Lada merupakan salah satu sayur-sayuran yang paling popular yang dimakan setiap hari. Terdapat kurangnya kajian dan bukti ke atas ekstrak lada terhadap pertumbuhan sel kanser yang berkait dengan sebatian fitokimia dan aktiviti antioksida untuk kultivar Malaysia. Tujuan kajian ini adalah untuk menganalisis sebatian fitokimia dalam ekstrak lada menggunakan sistem GCMS dan HPLC, untuk menentukan aktiviti antioksida menggunakan ujian DPPH dan untuk menentukan aktiviti antiproliferasi ekstrak lada menggunakan ujian MTS dalam kultivar Malaysia. Keputusan menunjukkan kompaun fitokimia, aktiviti antioksida dan antiproliferasi berbeza di dalam ekstrak *Capsicum frutescens* (cili padi), *Capsicum annuum* (cili lada) dan *Capsicum annuum* (lada loceng). Kandungan kompaun fenol (124.77 ± 1.70 mg/g GA bersamaan) dan aktiviti antioksida (520.68 mg/g ± 14.57 mg/g BHA bersamaan) adalah yang tertinggi dalam ekstrak lada loceng. Kandungan kompaun flavonoid (55.12 ± 0.27 mg/g kuarcetin bersamaan), kapsaicin (1.09 ± 0.03 mg/g) dan dihidrokapsaicin (0.63 ± 0.07 mg/g) dan aktiviti antiproliferatif adalah yang tertinggi dalam ekstrak cili padi. Tiada kaitan didapati antara aktiviti antioksida DPPH dan aktiviti antiproliferasi ekstrak lada dalam kultivar Malaysia. Ekstrak lada tidak toksik ke atas sel Jurkat. Pada kepekatan yang sama iaitu 1mg/mL, ekstrak cili padi mempunyai aktiviti perencatan tertinggi kepada sel Jurkat iaitu 63% dibandingkan ekstrak cili lada iaitu 30%. Sementara ekstrak lada loceng tidak

menunjukkan sebarang aktiviti perencatan terhadap sel Jurkat. Hasil keputusan ini mencadangkan kepentingan kompaun organik meruap dan fenolik kepada aktiviti antioksida ekstrak lada loceng dan kepentingan kompaun flavonoid dan kapsaicinoid kepada aktiviti antiproliferasi ekstrak cili padi dalam kultivar Malaysia. Hasil keputusan daripada aktiviti antioksida dan antiproliferasi dari kultivar Malaysia juga menunjukkan perbezaan daripada kajian sebelum ini dari kultivar lain. Penemuan ini mencadangkan bahawa perbezaan spesis, varieti dan kultivar telah menyumbang kepada perbezaan fitokimia yang terdapat di dalam lada. Kehadiran fitokimia yang berbeza menyumbang kepada perbezaan aktiviti antioksida dan antiproliferasi di dalam ekstrak lada. Hasil keputusan menunjukkan cili padi dari kultivar Malaysia mungkin memberikan kesan yang lebih baik kepada manusia sebagai satu sumber sebatian bioaktif yang berpotensi untuk ejen semulajadi dan kemopencegahan.

ANALYSIS OF PHYTOCHEMICAL COMPOUNDS, ANTIOXIDANT AND ANTIPROLIFERATIVE ACTIVITY OF AQUEOUS EXTRACTS OF *CAPSICUM* FRUITS

ABSTRACT

Pepper is one of the most popular vegetables taken daily. There is a lack of study and evidence on the effects of pepper extracts against cancer cell proliferation that relates to their phytochemical and antioxidant activities on Malaysia cultivar. The aims of this study are to analyze the phytochemical in pepper extracts using GCMS and HPLC system, to determine the antioxidant activity of pepper extracts using DPPH assay and to determine the antiproliferative activity of pepper extracts using MTS assay in Malaysia cultivar. Results showed that the phytochemical, antioxidant and antiproliferative activities differ between *Capsicum frutescens* (chilli padi), *Capsicum annuum* (chilli pepper) and *Capsicum annuum* (bell pepper) extracts in Malaysia cultivar. The content of phenolic compounds (124.77 ± 1.70 mg/g GA equivalent) and antioxidant activity (520.68 mg/g ± 14.57 mg/g BHA equivalent) were highest in the bell pepper extract. The content of flavonoid (55.12 ± 0.27 mg/g Quercitin equivalent), capsaicin (1.09 ± 0.03 mg/g) and dihydrocapsaicin (0.63 ± 0.07 mg/g) compounds and antiproliferative activities were highest in the chilli padi extract. No relationship was found between DPPH antioxidant and antiproliferative activities of pepper extracts in Malaysia cultivar. Pepper extracts were not toxic to the Jurkat cells. At an equal concentration of 1 mg/mL, chilli padi extract has a higher inhibitory activity to Jurkat cells, which was 63 % than chilli pepper extract which was 30 %. Meanwhile bell pepper extract did not show any inhibitory activity against Jurkat cells. These results suggest the importance of volatile organic and phenolic compounds for antioxidant activity of bell pepper extract and the importance of flavanoid and capsaicinoid compounds for antiproliferative activity of chilli padi extract in Malaysia cultivar. The results on the antioxidant and antiproliferative activities from Malaysia cultivar also showed different activities from previous studies in other cultivar. These findings suggested that different species, varieties and cultivar have contributed to the different phytochemicals present in pepper. The different phytochemicals present contributed to the different antioxidant and antiproliferative activities of pepper extracts. The results indicate that chilli padi from Malaysia cultivar might be give more beneficial effects to human as a potential source of bioactive compounds for natural antioxidant and chemopreventive agents.

CHAPTER 1

INTRODUCTION

1.1 Background

Cancer is a growing health problem around the world. Cancer is among the leading causes of death between non-communicable diseases worldwide. In 2012, there were 14.1 million new cancer cases, 8.2 million cancer deaths and 32.6 million of people living with cancer within 5 years of diagnosis (WHO, 2012). According to National Cancer Registry of Malaysia Report, 21, 773 Malaysians were diagnosed with cancer in 2006 (Omar *et al*., 2006). In 2007, cancer was the third most common cause of death. The 10 most frequent types of cancer among Malaysians were breast, colorectal, lung, nasopharynk, cervix, lymphoma, leukemia, ovary, stomach and liver as shown in Figure 1 (Omar and Tamin, 2011).

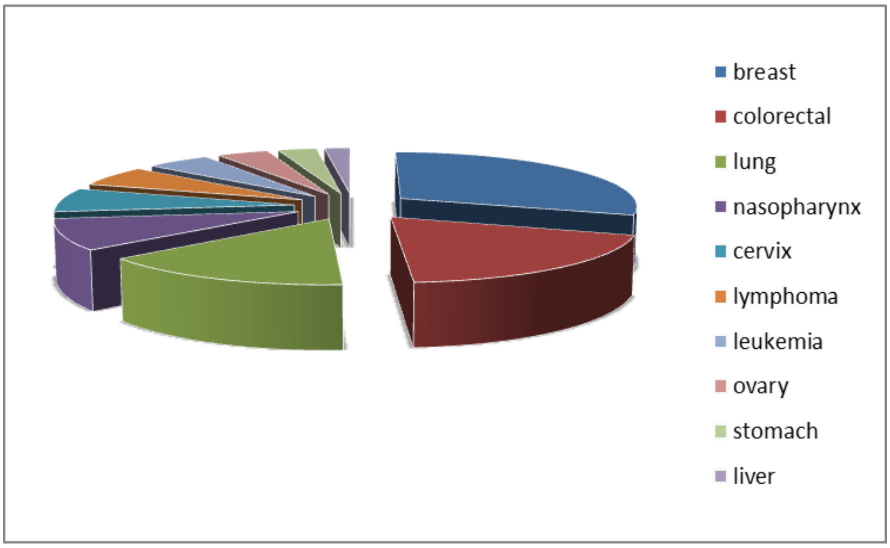


Figure 1.1: The 10 most frequent types of cancer diagnosed among Malaysians in 2007 (Omar and Tamin, 2011).

Chemotherapy and radiation therapy are the most effective way to treat cancer (Ertekin *et al*., 2004). However, these therapies are known to be associated with side effects such as alteration in the immune system, DNA and membrane structure damage (Haboubi *et al.,* 2000) and production of free radicals in human lymphocytes (Belloni *et al*., 2011) and in some cases the patients developed resistance towards chemotherapy or radiotherapy (Materska *et al*., 2015) . Thus, prevention in cancer development and treatment become a serious challenge nowadays. Currently, many studies have been carried out to investigate the potential of natural products as a anticancer agents (Tavakkol-Afshari *et al*., 2008; Domenico *et al*., 2012).

According to WHO, 65 to 80 percent of the world population uses traditional medicines to treat various diseases (Kaur and Arora, 2009). Thus, attention has been focused on the potential of plants for their phytochemical properties that relate to biological, pharmaceutical and pharmacological activity. Natural products are known to be a good source of phytochemicals for the development of anti-cancer drugs (Smith-Warner *et al*., 2000).

Phytochemicals generally act as secondary metabolites in plants. Phytochemicals are natural bioactive compounds produced by the plants that act as a protective agent against external stress and phatogenic attack (Chew *et al*., 2011). It can be divided into several categories such as phenolics, alkaloids, steroids, terpenes, saponins, organosulphur compounds and nitrogen containing compounds (Liu, 2004). Phytochemicals have been found to have anticancer activity through antioxidant reaction by acting as hydrogen donors, reducing agents, metal chelators and singlet oxygen quenchers (Wang *et al*., 2011).

Previous studies showed that phytochemicals in plants such as mangosteen and black plum contribute to the biological activities. *Gastinia mangostana* (mangosteen) reported to have antioxidant and antiproliferative activities (Moongkarndi *et al*., 2004) and *Eugenia jambolana* Lam. (black plum) reported to have antiviral, anti-diabetic, antihypertensive, anti-allergic and anticancer activities (Baliga *et al*., 2011). Therefore, these phytochemicals may have beneficial effects against various diseases such as cancer, coronary, diabetes and infectious diseases (Dillard and German, 2000).

Pepper is one of the most important spice crops worldwide and it is cultivated all over the world. In Malaysia, pepper is one of the most popular vegetables taken daily. The most popular pepper, which is planted in Malaysia are chilli pepper and chilli padi while the least popular is bell pepper (Vegetables and Crops Statistics, 2012). Normally, chilli pepper has been used mostly as an ingredient in any food for spicy taste while chilli padi has been used mostly as a main source for preparation of "sambal belacan" and bell pepper has been used mostly for preparation of salad.

Pepper is a good source of phytochemicals such as flavonoids, phenolic acids, carotenoids, vitamins (Morales-Soto *et al*., 2013) and capsaicinoids (Jayaprakasha *et al.*, 2012). Previous *in vitro* studies showed that the pure capsaicin, which is an active compound in pepper, inhibited the proliferation of leukemic cells (Ito *et al*., 2004), prostate cancer PC-3 cells (Sanchez *et al*., 2007) and esophagus carcinoma cells (Wu *et al*., 2006). According to *Duo et al.* (2011), crude pepper extracts inhibited the growth of breast cancer and Jurkat-T cell lines (Dou *et al*., 2011). In contrast with a study by Archer and Jones (2002), they showed that the consumption of pepper can cause a carcinogenic effect on the stomach. Thus, it is important to study the biological effects of pepper extracts to consumer since pepper is widely consumed over the world.

Different pepper types and cultivar have different phytochemical contents, antioxidant activity (Deepa *et al.*, 2007; Sun *et al.*, 2007; Ghasemnezhad *et al*., 2011) and antiproliferative activity (Duo *et al*., 2011).

Therefore, this study focuses on the phytochemical analysis and relates the phytochemicals present in pepper extracts to the antioxidant and antiproliferative activities of pepper extracts which were cultivated in Malaysia. Preliminary cytotoxicity screening analysis using MTS assay was done to test the antiproliferative activity of pepper extracts. In this study, pepper extracts were tested for the antiproliferative activity against Jurkat cells. Due to the limitation of this study to get the normal peripheral bloods, L929 cell line was used to assess the cytotoxicity of pepper extracts. L929 cells are one of the cell lines that have been used to assess the *in vitro* cytotoxicity of drug or compounds for the determination of the biological response of mammalian cells *in vitro* as suggested by the International Organization for Standardization (ISO 10993-5, Biological Evaluation of Medical Devices, Part 5: Test for *in vitro* cytotoxicity) (ISO, 2009).

The findings of this study may provide preliminary data on the antioxidant and antiproliferative activities of pepper extracts which were cultivated in Malaysia. It also provides the preliminary data on the phytochemical compounds which may have given an effect to the antioxidant and antiproliferative activities of pepper extracts for the development of natural antioxidant and chemoprevention agents.

1.2 Problem Statements

Many studies related to antioxidant activity and composition analysis on pepper have been performed (Deepa *et al.*, 2007; Sun et al., 2007; Ghasemnezhad *et al*. 2011). All researchers used bell pepper in different genotype and colour in determination of the composition analysis and antioxidant activity of *Capsicum annuum* (bell pepper). Study by Duo *et al*. (2011) used ten different varieties which are *Capsicum annuum* (bell pepper, pimento pepper, poblano pepper, anaheim pepper, hungarian wax pepper, jalapeno pepper, serrano pepper, cayenne pepper), *Capsicum frutescens* (thai pepper) and *Capsicum chinense* (habanero pepper) for determination of tumor cell growth inhibition. However, in the present study, bell pepper, chilli pepper and chilli padi in Malaysia cultivar were used. Moreover there are lack of studies on the effects of *Capsicum annuum* (chilli pepper and bell pepper) and *Capsicum frutescens* (chilli padi) extracts against cancer cell proliferation that relate to their phytochemicals, antioxidant and antiproliferative activities which were cultivated in Malaysia.

Therefore the study aims to determine the phytochemical properties, antioxidant activities and antiproliferative activities in chilli padi, chilli pepper and bell pepper extracts that are cultivated in Malaysia using Jurkat and L929 cell lines.

1.3 Hypothesis

Different pepper species (*Capsicum annuum* and *Capsicum frutescens*) and different pepper varieties of *Capsicum annuum* (chilli pepper and bell pepper) may have different phytochemical compositions that may contribute to different antioxidant and antiproliferative activities.

1.4 Research Objectives

The objectives of the present work are:

i. To analyze the phytochemicals in chilli padi, chilli pepper and bell pepper aqueous extracts.
ii. To determine the antioxidant activity of chilli padi, chilli pepper and bell pepper aqueous extracts.
iii. To determine the effect of chilli padi, chilli pepper and bell pepper aqueous extracts using Jurkat and L929 cell lines.
iv. To compare the phytochemicals, antioxidant and antiproliferative activities between chilli padi, chilli pepper and bell pepper aqueous extracts.

v. To determine the relationship between antioxidant measure by DPPH and antiproliferative activities of pepper aqueous extracts.

CHAPTER 2

LITERATURE REVIEW

2.1 Botanical, taxonomic description and distribution of *Capsicum annuum* (bell pepper and chilli pepper) and *Capsicum frutescens* (chilli padi)

Pepper is a common vegetable or fruit consumed worldwide. It was originated from America (Giufrida *et al.*, 2010). Christopher Columbus has introduced pepper to Europe, Africa and Asia (Meghvansi *et al*., 2010). Pepper belongs to the family *Solanaceae* and genus *Capsicum* that has more than 200 species cultivated in various locations over the world (Junior *et al*., 2011). Five major species of *Capsicum* which are *Capsicum frutescens, Capsicum annuum, Capsicum baccatum, Capsicum chinense* and *Capsicum pubescens.* These *Capsicum* species can be distinguished and described based on many factors such as trade, colour, aroma and pungency level (Maillard, Giampaoli and Richard, 1997, Meghvansi *et al*., 2010).

Capsicum annuum is the most widely cultivated pepper all around the world. *Capsicum annuum* has sweet and hot varieties and it includes bell pepper, chilli pepper, cayenne, African sweet pepper and Italian sweet pepper. *Capsicum frutescens* is a hot species which includes African birdseye, tabasco, balanco chilli, siling labuyo and bolivian rainbow. These varieties depend on the regions where they are cultivated (Bailey *et al.* 1941; Chowdhury *et al.* 1996; Cichewicza and Thorpe, 1996; Hedrick 1919, Van Wyk, 2005).

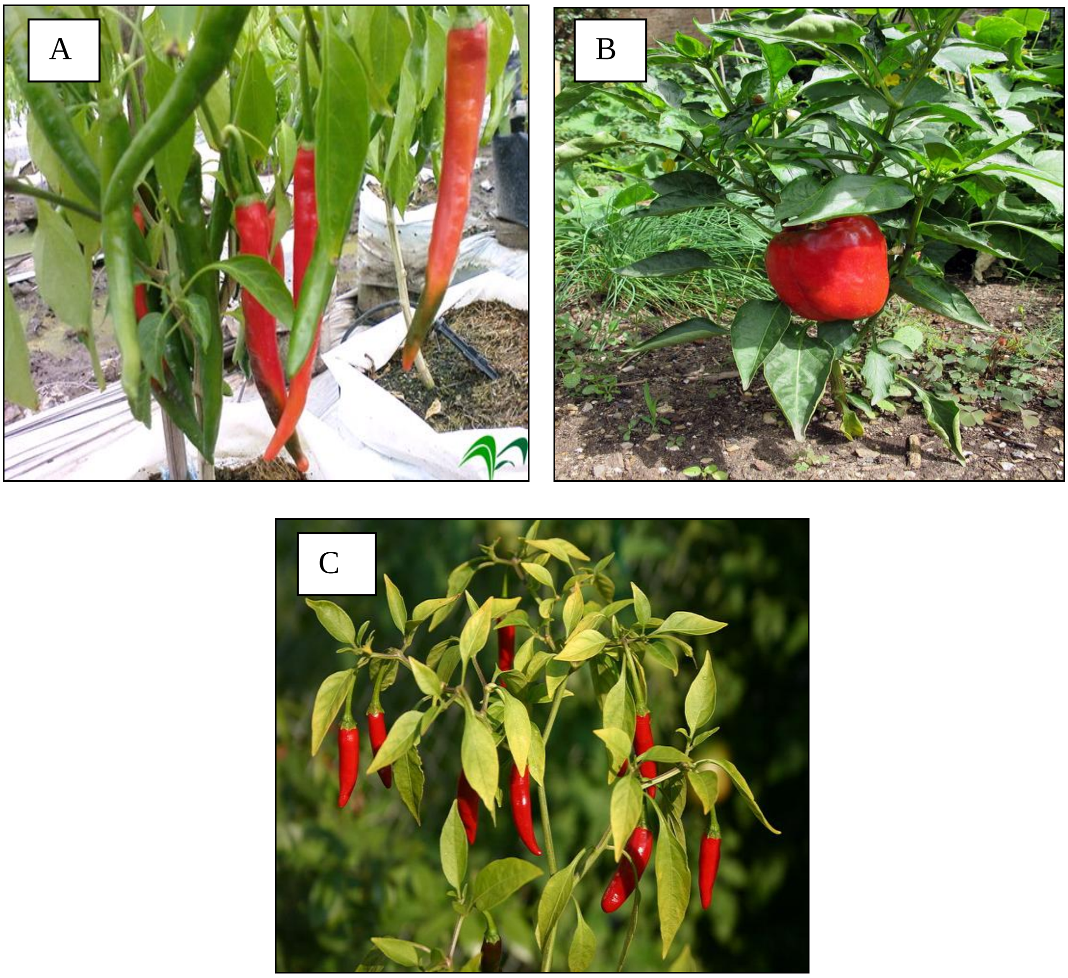


Figure 2.1: Images of (A) chilli pepper (B) bell pepper (C) chilli padi

Chilli pepper (Figure 2.1 (A)) is a species of *Capsicum annuum.* It can grow up from 60-70 cm height. The stem is erect upwards, with branches and green. The length of the fruit is from 7-10 cm with 10-15 g weight and pointy at the end. The fruit's colour is green and it turns into red when it ripens. It also has tap roots and fibrous system. The colour of its flowers is white from the leaves armpit.

Bell pepper (Figure 2.1(B)) is a species of *Capsicum annuum*. Bell pepper plants are short bushes. It has woody stems and it also grows bright colored fruits. It can grow up to 1 m and it is grown in moderate regions for one growing season. Bell pepper grows in climate which is warm and moist, at temperatures between 18 and

30°C. It grows in a deep, well-draine soil which has a pH between 6.0 and 6.8. The leaves are elliptical in shape and has smooth edged. It also has white or purple bell-shaped flowers which are 2.5 cm in diameter. Bell pepper is a fruit which has 3-5 lobes. About 3-6 weeks after flowering, the fruits are produced each season. The fruits are green in colour and they turn to yellow and red when ripen. Their flesh are mild, sweet flavour,crisp, and juicy. The fruits vary from 3.5- 5.5 inches long and from 2.5- 4 inches wide. The seeds are cling to the central core (placenta). Bell pepper is propagated from seed and seedling (Bailey *et al.* 1941, Chowdhury *et al.* 1996, Cichewicza and Thorpe 1996, Hedrick 1919, Van Wyk, 2005)

Chilli padi (Figure 2.1(C)) is a species of *Capsicum frutescens*. It is a hot variety and can grow up to 1 m. The flowers are hermaphrodite which have both male and female organs. The fruits are around 4 cm long, are initially pale yellowish-green and turn yellow and orange before ripening to bright red. Fruits are pointy and erect. Fruit flesh often soft. Fruits have fleshy mesocarp and a stone like stiffened endocarp. It is suitable in acid, neutral and basic soils and can grow in very acid and very alkaline soils (Bailey,1941; Bentley and Trimer, 1880; Liogier, 1995; Bosland and Votava 2000).

2.2 Phytochemicals present in pepper

Phytochemicals are natural bioactive compounds in plants that act as a defense system and are also known as secondary metabolites. The defense system protects plant cells from environmental hazards such as pollution, stress, drought, UV exposure and pathogenic attack (Sermakkani and Thangapandian, 2012). Most phytochemicals are produced from three metabolic pathways which are shikimate pathway, cinnamic pathway and isoprenoid pathway (Lampe, 2003).

According to Lampe (2003), more than 25000 terpenoids, 12000 alkaloids and 8000 phenolics were identified in the plants. However there are also other phytochemicals which are still unknown and needs to be identified and quantified for the evaluation of their health benefit. Phytochemicals was suggested to contribute to the important biological activities such as antioxidant (Farag *et al.*, 2004; Colombo, 2010), anticancer (Aggarwal *et al.*, 2006), antimicrobial (Farag *et al.*, 2004), antiproliferative (Samarghandian, Boskabady and Davoodi, 2010; Kim *et al*., 2012) and antimicrobial (Farag *et al*., 2004).

Some of the important phytochemicals present in pepper extracts are phenolic and volatile organic compounds which have been identified previously are discussed below.

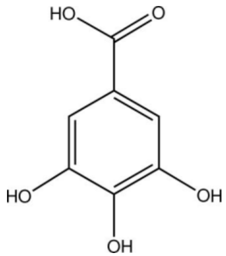
2.2.1 Phenolic compounds present in pepper

Phenolic compounds are a group of secondary metabolites which are widely distributed in plants (Wahyuni *et al*., 2013). Phenolic compounds are synthesized by plants due to the plant adaptation to biotic and abiotic stress conditions such as infection, wound, water stress, cold stress and high visible light (Materska and

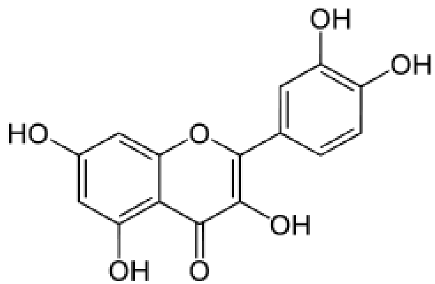
Perucka, 2005). Phenolic compounds are derived from phenylalanine and tyrosine pathways (Shahidi and Naczk, 2004).

Phenolic compounds are class of chemical compounds consisting of hydroxyl group (-OH) that is bonded to an aromatic hydrocarbon (Khoddami, Wilkes and Roberts, 2013). According to Vaquero *et al.* (2007), phenolic compounds are compounds with a phenol type structure that range from simple phenols to complex which include non-flavonoids, flavonoids and polyphenols.

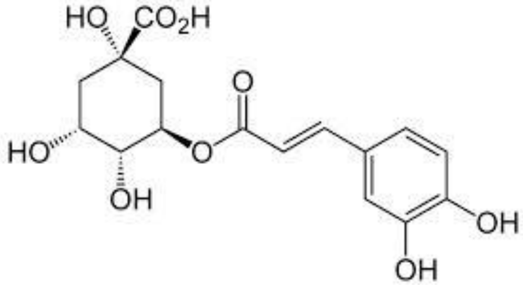
There are more than 8000 phenolic structures found in plants and the structures are based on their carbon skeleton. The structures are classified into simple phenols such as phenolic acids and polyphenols such as flavonoids and tannins (Dai and Mumper, 2010). A study by several researchers in pepper (*Capsicum annuum, Capsicum chinense and Capsicum frutescens*) showed the presence of phenolic compounds including myricetin, quercetin, kaempferol, luteolin, apigenin, trans-p-ferulic acid, trans-p-sinapic acid, trans-pferuoyl-β-d-glucopyranoside and trans-p-sinapoyl-β-d-glucopyranosi phenylpropanoid glycosides (Jayapraksha *et al*., 2012; Wahyuni *et al*., 2013). Structures of some phenolic compounds found in pepper extracts are illustrated in Figure 2.2.



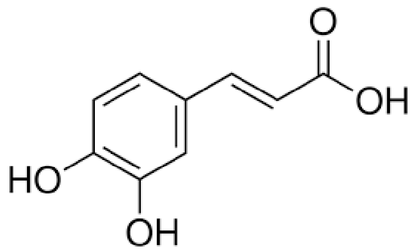
Gallic acid



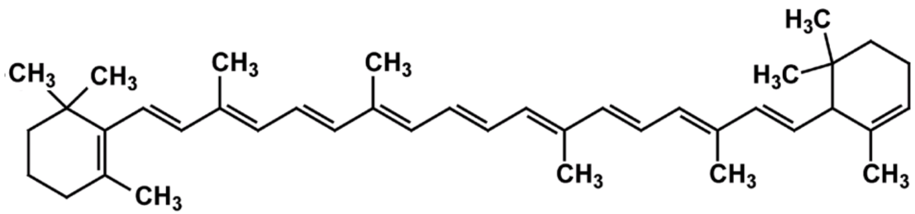
Quercetin



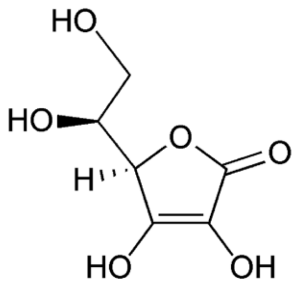
Chlorogenic acid



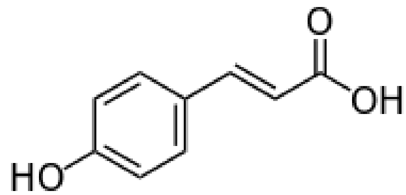
Caffeic acid



B-carotene



Ascorbic acid



Coumaric acid

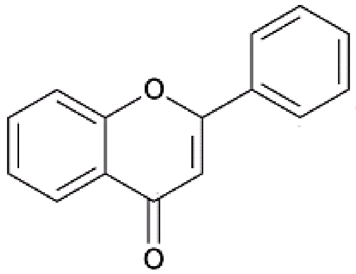
Figure 2.2: Structures of some phenolic compounds present in pepper (Bombardelli *et al*., 1995; Kanadaswami *et al*., 2005; Antonious *et al*., 2006; Zhao *et al.*, 2011).

2.2.1 (a) Flavonoid compounds present in pepper

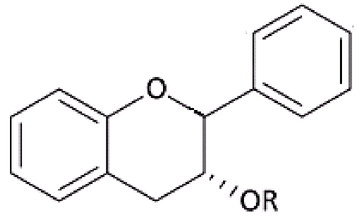
Flavonoid compounds are secondary product of phenylpropanoids and present in all terrestrial vascular plants (Korkina, 2006). According to Khoddami *et al.* (2013), flavonoid compounds are the most common phenolic compounds and widely distributed in plants.

Flavonoid compounds are defined as a substance, which contains a common phenylchromanone structure (C6-C3-C6), with one or more hydroxyl substituents (Birt, Hendrich and Wang, 2001). Flavonoid compounds can be divided into several classes including flavones, flavonols, flavanols, flavones, anthocyanins, aurones, calchones, anthocyanidins and dihydroflavonols (Cook and Samman, 1996; Fine, 2000). Basic structures of flavonoid classes in plants are shown in Figure 2.3.

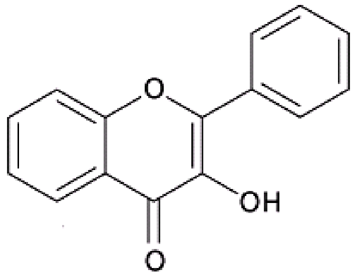
According to Verhoeyen *et al.* (2002), more than 4000 flavonoid compounds have been found and most of them are conjugated to sugar molecules and located in the upper epidermal layers of leaves. The structure of flavonoid compounds depends on the process of hydroxylation, prenylation, alkalinization and glycosylation reactions that alter the basic molecule of flavonoids (Khoddami, Wilkes and Roberts, 2013). Figure 2.4 showed the biosynthetic pathway of flavonoid compounds.



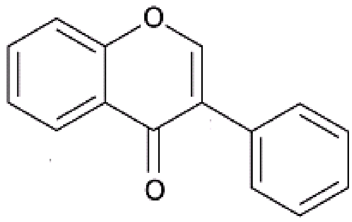
Flavones
(2-phenylchromen-4-one)



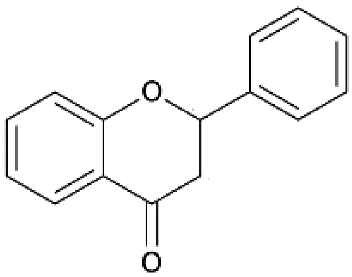
Flavanols
(2-phenyl-3,4-dihydro-2H-chromen-3-ol)



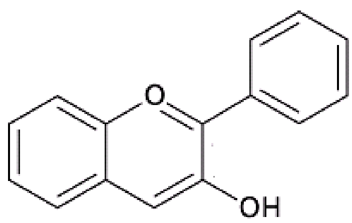
Flavonols
(3-hydroxy-2-phenylchromen-4-one)



Isoflavones
(2,3-dihydro-3-phenylchromen-4-one)



Flavanones
(2,3-dihydro-2-phenylchromen-4-one)



Anthocyanidins
(2-phenylchromenylium)

Figure 2.3: Basic structures of flavonoid classes in plants (Majumdar and Srirangam, 2010).

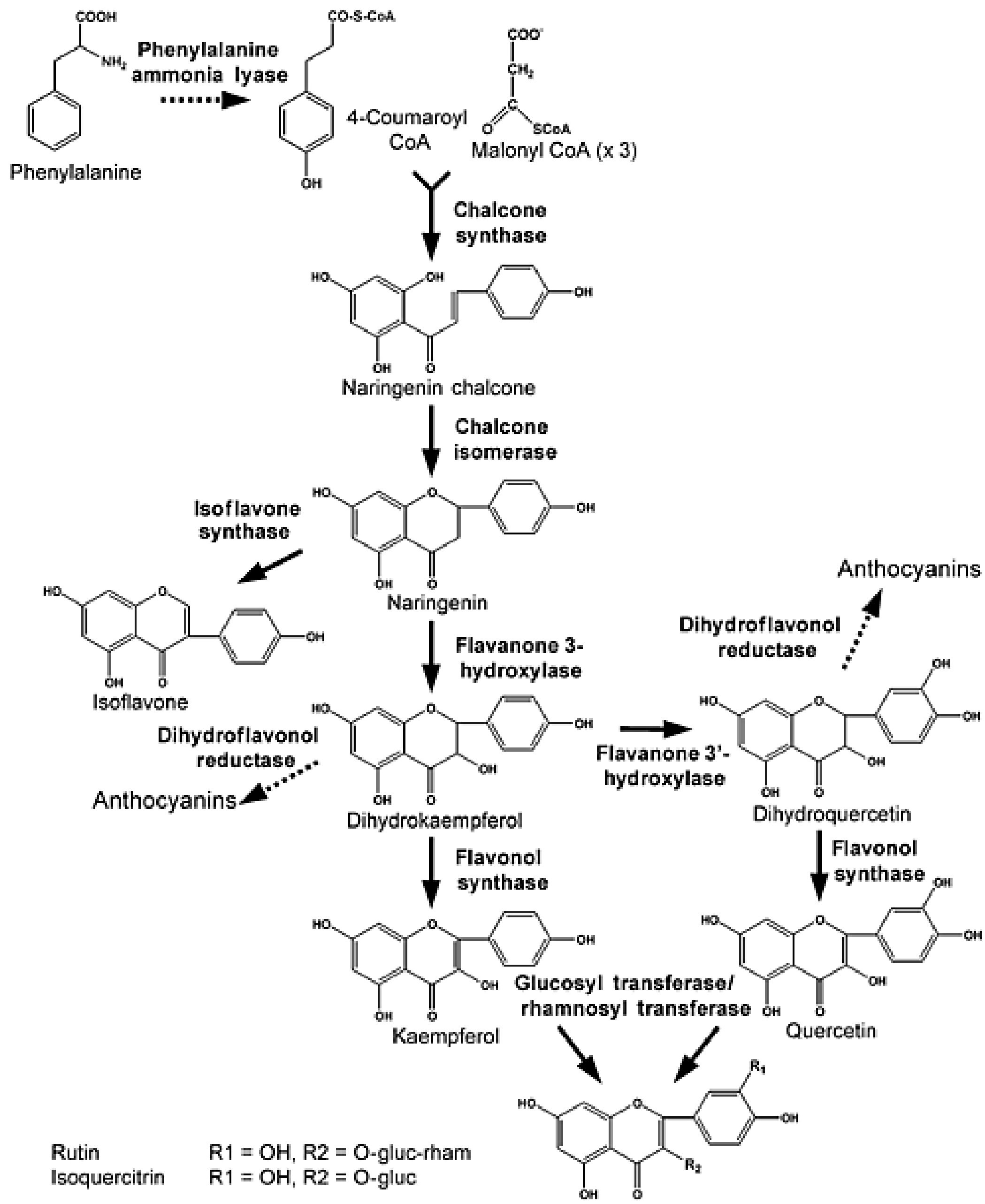
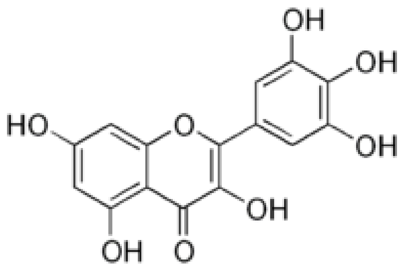
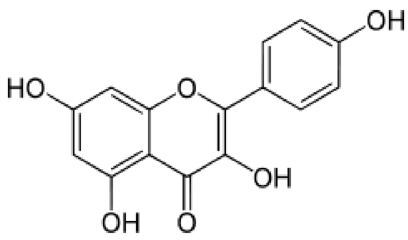


Figure 2.4: Flavonoid biosynthethic pathway (Muir, 2001)

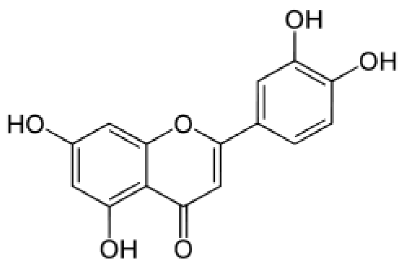
Study by Bae *et al*. (2012) showed the presence of flavonoid compounds such as myricetin, quercetin, luteolin, kaempferol and apigenin in *Capsicum annuum* (paprika) and *Capsicum chinense* (habanero) and the presence of quercetin, luteolin, kaempferol and apigenin in *Capsicum annuum* (cayenne, jalapeno and serrano). The structure for some flavonoid compounds present in pepper are shown in Figure 2.5.



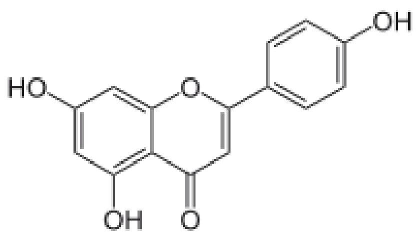
Myricetin



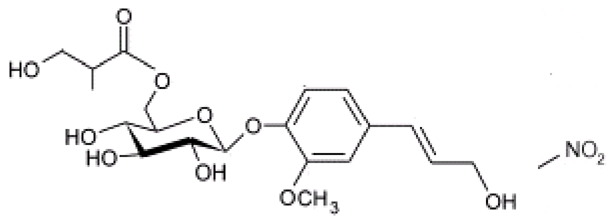
Kaempferol



Luteolin



Apigenin



trans-p-Ferulylalcohol-4-*O*-(6-(2-methyl-3-hydroxypropionyl)glucopyranoside

Figure 2.5: Structures of some flavonoid compounds present in pepper (Materska *et al*., 2003; Jayapraksha *et al*., 2012).

2.2.1(b) Capsaicinoid compounds present in pepper

Capsaicinoid compounds are phenolic compounds derivative of a phenylpropanoid (Estrada *et al*., 2000; Morales-soto *et al.,* 2013). Capsaicinoid compounds are responsible for spiciness of pepper (Perucka and Oleszek, 2000).

Capsaicinoid compounds including capsaicin are biosynthesized by capsaicin synthase (CS) through the condensation of vanillylamine, a phenylpropanoid pathway intermediate and fatty acid in placental tissues of *Capsicum* fruits (Prasad *et al*., 2006). Figure 2.6 shows the biosynthethic pathway of capsaicin compounds.

Capsaicinoid compounds are divided into major and minor capsaicinoids. The major capsaicinoid compounds are capsaicin ((E)-N-[(4-hydroxy-3-methoxyphenyl) methyl]-8-methyl-nonenamide) and dihydrocapsaicin (8-methyl-N-vanillylnonanamide), which contributed 90% of spiciness in pepper (Kobata *et al*., 1998). The minor capsaicinoid compounds are nordihydrocapsaicin, homocapsaicin I, homocapsaicin II, homohydrocapsaicin I, homodihydrocapsaicin II, decanoyl vanillylamide, nonanoyl vanillylamide, octanoyl vanillylamide and non-3-phenyl vanillylamide (Maillard, Giampaoli and Richard, 1997).

Barbero *et al.*(2014) have determined the individual and total capsaicinoid contents in different ripening of cayenne pepper. According to them, capsaicin and dihydrocapsaicin had contributed between 79% to 90%, nordihydrocapsaicin contributed between 6% to 14% and homodihydrocapsaicin and homocapsaicin contributed between 2% to 4% of total capsaicinoids. The contents of capsaicinoids depend on the ripening stage of pepper (Barbero *et al.*, 2014).

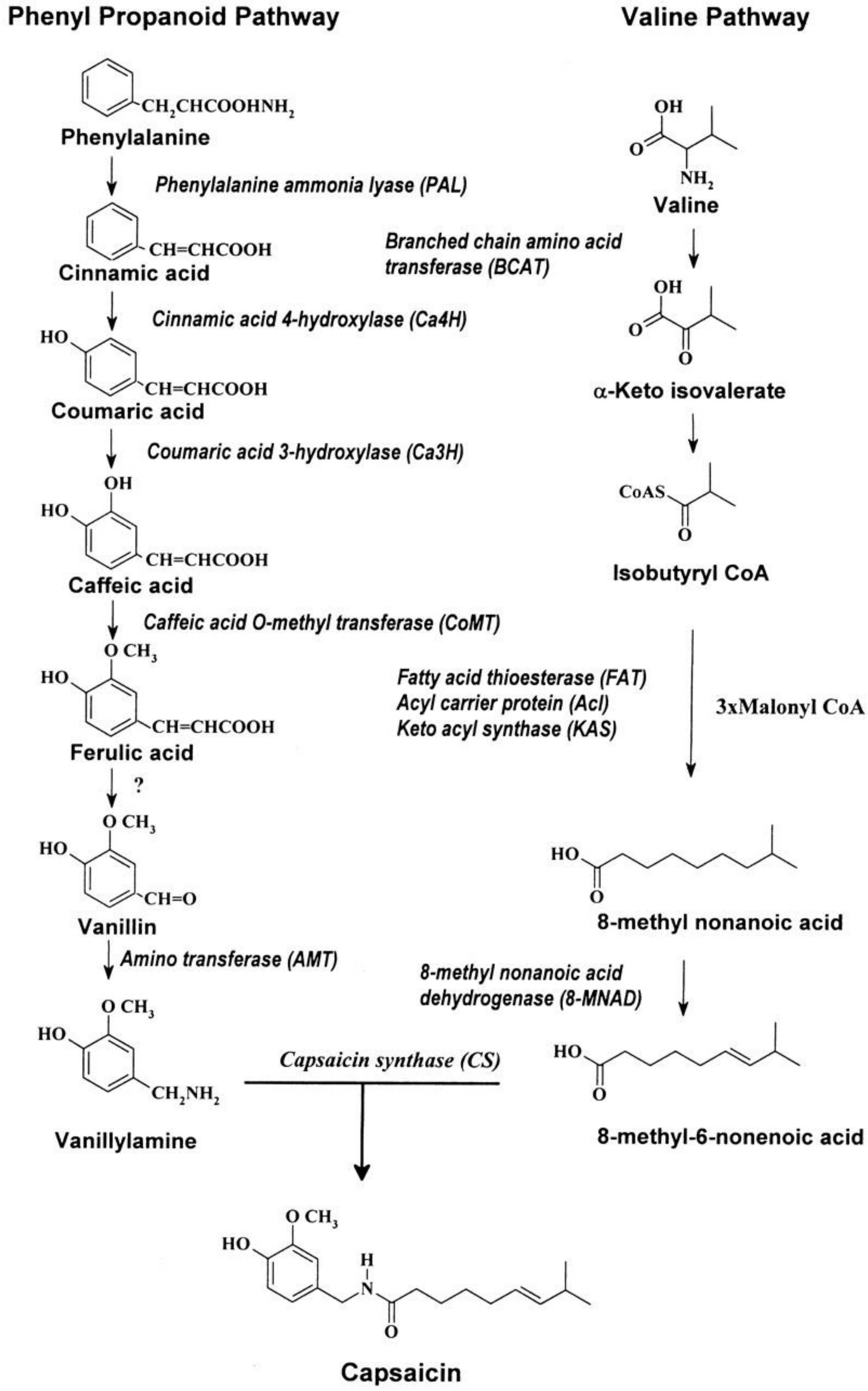


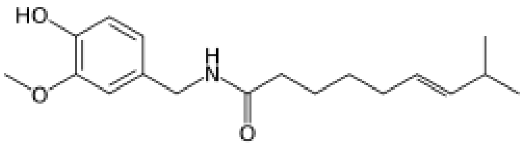
Figure 2.6: Capsaicin biosynthethic pathway (Prasad *et al.*, 2006)

Several studies have reported the presence of capsaicinoid compounds in pepper as shown in Table 2.1.

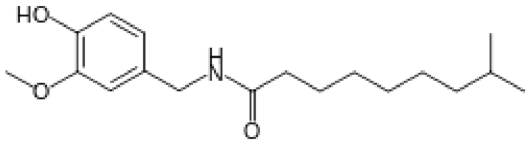
Table 2.1: Capsaicinoid compounds in pepper

Pepper	Phytochemical constituents group	References
Capsicum annuum	Nordihydrocapsaicin, capsaicin, dihydrocapsaicin, homocapsaicin I, homocapsaicin II, homodihydrocapsaicin I, homodihydrocapsaicin II	Kozukue *et al.* (2005); Choi *et al.* (2006) ; Barbero *et al.* (2014)
Capsicum chinense	Capsaicin, dihydrocapsaicin, homocapsaicin, homodihydrocapsaicin, nordihydrocapsaicin	Davis *et al.* (2007)
Capsicum frutescens	Capsaicin, dihydrocapsaicin, homocapsaicin, homodihydrocapsaicin, nordihydrocapsaicin, nornorcapsaicin, norcapsaicin, nornordihydrocapsaicin, N-vanillyl-nonamide	Schweiggert (2006)

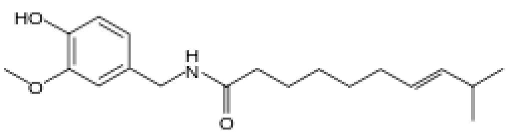
The chemical structures of some capsaicinoid compounds are given in Figure 2.7.



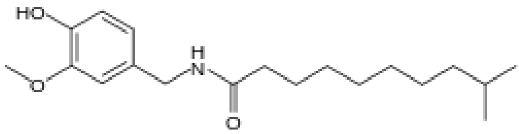
Capsaicin



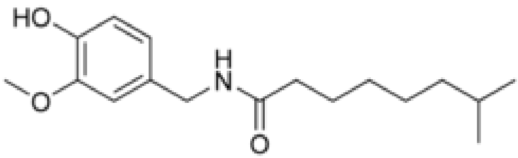
Dihydrocapsaicin



Homocapsaicin



Homodihyrocapsaicin



Nordihydrocapsaicin

Figure 2.7: Structures of some capsaicinoid compounds present in pepper (Wahyuni *et. al*, 2013)

2.2.2 Volatile organic compounds present in pepper by GC-MS

More than 125 volatile organic compounds have been identified in pepper extracts (Nijssen *et al*., 1996) using GCMS system. Previous studies have showed some volatile organic compounds that have been identified in the pepper extracts (Table 2.2).

Table 2.2: List of volatile organic compounds identified in pepper using GC-MS system

Pepper	Phytochemicals	References
Capsicum annuum	Alkanes (cyclotetradecane, tetradecane, cyclopentadecane), terpenoids (valencene, cyclosativene), esters (hexyl-2-methylpropanoate, hexyl-2-methylpropanoate)	Moreno *et al.* (2012)
	Alkanes (1-hexadecane, heptadecane, octadecane),esters (1-heptadecanol acetate, methyl 3-hydroxycholest-5-en-26-oate), alcohols (ergost-5-en-3-ol,β sitosterol), furans (3,6-dimethyl-2,3,3a,4,5,7a-hexahydrobenzofuran, 5-isopropyl-3,3-dimethyl-2-methylene-2,3-dihydrofuran)	Wesołowska, Dorota and Grzeszczuk (2011)
Capsicum chinense	Alkanes (1-hexadecane, 1-pentadecane, 1-nonadecane), aldehydes (hexanal, tetradecanal), esters (isohexyl benzoate, isohexyloctanoate), alcohols (1-hexadecanol, 1-octadecanol)	Pino *et al.* (2011)
	Alkanes (6-methyl-1-octene, tetradecane), alcohols (1-hexanol, 1-decanol, 1-octanol), aldehydes (pentanal, hexanal, dodecanal), esters (hexyl ethanoate, heptyl acetate), ether (2-penthyl furan, monoterpenes), sesquiterpenes (zonarene, valencene)	Junior *et al.* (2012)
	Alkanes (2-methyl-tetradecane, 2-methyl-1-tetradecane, 1-hexadecane, 1-pentacosane), esters (hexylisobutanoate, hexyl-2-methylbutanoate), terpenes (limonene, germacrene, β-chamigrene),	Cuevas-Glory *et al.* (2012)
Capsicum baccatum	Alkanes (Pentane, isooctane, tetradecane), alcohols (5-hexenol, 1-hexanol), aldehydes (2-methyl propanal, butanal, 3-methyl butanal), ketones (1-pentene-3-one), esters (Methyl isopentanoate, Hexyl isovalerate), monoterpenes (Camphene, Limonene, Eucalyptol) sesquiterpenes (Humulene, Cadinene), pyrazine (2-methoxy-3-isobutyl pyrazine).	Junior *et al.* (2012)
Capsicum frutescens	Alkanes (undecane, hexadecane, pentadecane), alcohols (2-propanol, 1-pentanol, 2-decanol), aldehydes (butanal, pentanal, hexanal), ketones (2-nonanone, (E)-5-methyloct-5-en-2-one, esters (hexyl acetate, hexyl pentanoate, ethyl hexanoate), monoterpenes (δ-3-carene,(E)-β-ocemene, sesquiterpenes (cadinadiene, β-ionone)	Junior *et al.* (2012)
Capsicum pubescens	Alcohols (1-pentanol, 1-hexanol, 2-penten-1-ol), monoterpenes (α-pinene, ρ-cymol, limonene), sesquiterpenes (cyprene)	Kollmannsberger *et al.* (2010)

2.3 Traditional uses of pepper

Pepper has been domesticated for over 7000 years (Prasad *et al*., 2006). Pepper has been used in traditional medicine against ulcers, rheumatism, alopecia, toothache (Szallasi and Blumberg, 1999), antimicrobial, anti-convulsive and sedative properties (Junior *et al*., 2012). In Africa, people used pepper as antiseptics for wound healing treatment (Dasgupta and Fowler 1997). People from South America used pepper and honey as a remedy for cough (Dasgupta and Fowler 1997).

In India, pepper was used for the relieve of toothache, muscle pain, dog bite, ascites, snake bite, antipruritic, counter irritant, waist paint, mump in animals, postnatal care, inducing immunity and to tone up body muscles after heavy workouts (Meghvansi *et al*., 2010). The dried fruits of pepper are used in Bangladesh for preparation of fermenting media for their traditional liquor (Meghvansi *et al*., 2010). People from Italy used pepper as cardiotonic, anti-fever, revulsive, anti-hypertensive, anti-rheumatic, anti-fever and mosquito repellant (Meghvansi *et al*., 2010). In Crocker range Sabah, Malaysia, pepper was used to treat discomforts and common ailments associated with pregnancy (Meghvansi *et al*., 2010).

2.4 Medicinal properties of pepper

Pepper are mainly consumed and used worldwide as food, coloring, flavouring agents and medicine because of its pungent aroma and color (Prasad *et al*., 2006; Junior *et al*., 2011).

Previous study found that pepper extracts and capsaicin have potentials as preventive agents for treatment of immune diseases such as allergies by modulating T-cell immune responses (Takano *et al*., 2007). A recent ethno medicinal survey reported that pepper is used to alleviate waist pain in Orissa, India (Meghvansi *et al*., 2010). Ethanol extracts of *Capsicum* fruits with different pungency level is also contributed to the antimicrobial activities (Soetarno *et al.*, 1997). Flavonoid compounds can modulate lipid peroxidation involved in atherogenesis, thrombosis and carcinogenesis (Zhishen *et al.,* 1999). Other than that, flavonoids are capable of modulating the activity of enzymes and affect the behaviour of many cell systems, suggesting that it may possess significant antihepatotoxic, antiallergic, anti-inflammatory, antiosteoporotic and even antitumor activities (Carlo *et al*., 1999). Capsanthin and capsorubin which are unique to red pepper belonging to flavones group showed antioxidative and anti-tumor activities (Kim *et al*., 2010).

Capsaicin has been proven to have hyperlipidemia, antioxidant, anti-inflammatory, antilithogenic, anticancer, antiarthritic and analgesic properties (Prasad *et al*., 2006; Suresh and Sirinivasan, 2007), antibacterial and antidiabetic activities (Edris, 2007). Szallasi and Blumberg (1999) showed that capsaicin have pharmacological effects by the activation of cellular targets from vanilloid receptors and also was found to have immunomodulatory effects. Capsaicin is also found to have chemoprotective effects through modulation of metabolism of carcinogens or mutagens and their interactions with target DNA (Surh and Lee, 1996). Topical capsaicin was used to treat post-hepatic neuralgia, diabetic neuropathy, osteoarthritis, ointment, rheumatoid arthritis and to examine the function of sensory neurons (Erin *et al*., 2009; Meghvansi *et al*., 2010).