ANTIOXIDANT AND ALPHA GLUCOSIDASE INHIBITORY ACTIVITIES OF EXTRACTS FROM CUCURBIT FRUITS AND IDENTIFICATION OF ACTIVE AND MAJOR CONSTITUENTS FROM PHENOLIC-RICH EXTRACTS OF *Lagenaria siceraria* AND *Sechium edule*

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by

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LIST OF SYMBOLS AND ABBREVIATIONS

ANOVA	analysis of variance
BAW	buthanol : acetic acid : water (4 : 1 : 5)
DMSO	dimethyl sulphoxide
DNA	deoxyribo nucleic acid
DPPH	diphenyl picryl-hydrazyl
EA	ethyl acetate
EDTA	ethylene diamine tetraacetic acid
EC_{50}	effective concentration
GAE	gallic acid equivalent
GC	gas chromatography
HOAc	acetic acid
HPLC	high-performance liquid chromatography
HSD	honestly significant different
H_2O	water
IDDM	insulin-dependent diabetes mellitus
IR	infrared
g	gram
kg	kilogram
LC-MS	liquid chromatography – mass spectrometry
M	molar
MeOH	methanol
mg	milligram
mg/mL	milligram per mililitre
mL	mililitre
mM	milimolar
MS	mass spectrometry
NaCl	sodium chloride
NIDDM	non-insulin-dependent diabetes mellitus
nm	nanometer
NMR	nuclear magnetic resonance spectroscopy
OD	optical density

PBS	phosphate buffered saline
PC	paper chromatography
PDA	photo diode array
PNPG	4-nitrophenyl-α-D-glucopyranoside
R^2	linear regression coefficient
Rf	retention factor
ROS	reactive oxygen species
SD	standard deviation
SPSS	statistical package for the social sciences
TEAC	trolox equivalent antioxidant capacity
TLC	thin layer chromatography
TPC	total phenolic content
μg/mL	microgram per mililitre
μL	microlitre
μM	micromolar
UPLC	ultra performance liquid chromatography
UV	ultraviolet
v/v	volume to volume
w/v	weight to volume
λmax	lambda max

AKTIVITI ANTIOKSIDA DAN PERENCATAN ALFA GLUCOSIDASE BAGI EKSTRAK DARIPADA BUAH-BUAHAN CUCURBIT DAN PENGENALPASTIAN SEBATIAN AKTIF DAN UTAMA DARIPADA EKSTRAK KAYA-FENOL *Lagenaria siceraria* DAN *Sechium edule*

ABSTRAK

Lapan belas sampel buah-buahan daripada famili Cucurbitaceae yang biasa ditemui di Malaysia, telah dipilih dalam kajian ini. Semua ekstrak dinilai untuk aktiviti antioksidan dan jumlah kandungan fenolik. Aktiviti antioksida primer telah dinilai menggunakan asai penyingkiran radikal bebas 1, 1 Difenil-2-pikrilhidrazil (DPPH) dan asai pengurangan kuasa, dan aktiviti antioksidan sekunder telah ditentukan dengan menggunakan asai pengkelatan logam. Buah-buahan yang boleh dimakan mentah, diekstrak secara segar, manakala sayur-sayuran yang perlu dimasak, direbus sebelum pengekstrakan. Peratusan etanol yang berbeza dalam air suling (0, 20, 40, 60, 80 dan 100%) telah digunakan sebagai pelarut pengekstrakan. Ekstrak 80% (i/i) etanol daripada *Lagenaria siceraria* menunjukkan aktiviti DPPH, pengurangan kuasa dan pengkelatan logam tertinggi dengan nilai-nilai EC_{50} 8.85 ± 0.09 mg/mL, 28.33 ± 1.67 mg/mL dan 6.51 ± 0.17 mg/mL masing-masing. Seterusnya, empat pelarut dengan polariti menaik (heksana, kloroform, etil asetat dan metanol) telah digunakan secara berperingkat untuk mengekstrak sampel buah-buahan kering. Ekstrak metanol dan etil asetat *L. siceraria* masing-masing menunjukkan aktiviti DPPH (EC_{50} = 370.01 ± 12.51 μg/mL) dan pengkelatan logam tertinggi (EC_{50} = 203.44 ± 4.01 μg/mL), manakala ekstrak etil asetat *Sechium edule* menunjukkan aktiviti pengurangan kuasa tertinggi (EC_{50} = 409.35 ± 9.25 μg/mL). Ekstrak ini telah difraksinasikan menggunakan kromatograf kertas. Kesemua fraksi kemudiannya diuji untuk aktiviti antioksidan primernya dan fraksi eaLsf3 daripada ekstrak etil

asetat *L. siceraria*, fraksi mLsf3 daripada ekstrak metanol *L. siceraria* dan fraksi eaSef4 daripada ekstrak etil asetat *S. edule* telah menunjukkan aktiviti terbaik. Sebatian aktif dalam fraksi tersebut telah disisihkan dan dikenalpasti masing-masing sebagai asid kafeik, isokuersitrin (kuersetin 3-*O*-glukosida) dan asid galik. Memandangkan kebanyakan sayur-sayuran dalam famili ini terkenal kerana mempunyai khasiat anti diabetik, kesemua ekstrak sampel kering telah diuji untuk aktiviti perencatan α-glucosidase. Ekstrak etil asetat daripada *Momordica charantia* var. *maxima* menunjukkan perencatan tertinggi dengan nilai EC_{50} terendah sebanyak 1.18 ± 0.05 mg/mL. Ini diikuti oleh ekstrak metanol *L. siceraria* dengan tiada perbezaan signifikan dalam nilai EC_{50} (1.25 ± 0.03 mg/mL). Analisis kuantitatif menunjukkan asid kafeik sebagai sebatian utama dalam ekstrak metanol *L. siceraria*, tetapi isokuersetin didapati menjadi penyumbang utama aktiviti antioksidan dan aktiviti α-glucosidase. Asid kafeik dan asid galik (dengan aktiviti perencatan α-glucosidase yang rendah) telah dikenal pasti sebagai sebatian antioksidan utama dan aktif dalam ekstrak etil asetat *L. siceraria* dan *S. edule* masing-masing.

ANTIOXIDANT AND ALPHA GLUCOSIDASE INHIBITORY ACTIVITIES OF EXTRACTS FROM CUCURBIT FRUITS AND IDENTIFICATION OF ACTIVE AND MAJOR CONSTITUENTS FROM PHENOLIC-RICH EXTRACTS OF *Lagenaria siceraria* AND *Sechium edule*

ABSTRACT

Eighteen fruit samples from Cucurbitaceae family that are commonly found in Malaysia, were selected in this study. All extracts were evaluated for their antioxidant activities and total phenolic contents. The primary antioxidant activities were evaluated using 1, 1 diphenyl-2-picrylhydrazyl (DPPH) free radical scavenging and reducing power assays, and the secondary antioxidant activity was determined using metal chelating assay. For fruits that are edible in raw form, they were freshly extracted, whereas for the cooking vegetables, they were being boiled prior to extraction. Different percentages of ethanol in distilled water (0, 20, 40, 60, 80 and 100%) were used as the extraction solvents and 80% (v/v) ethanol extract of *Lagenaria siceraria* showed the highest DPPH, reducing power and metal chelating activities with the EC_{50} values of 8.85 ± 0.09 mg/mL, 28.33 ± 1.67 mg/mL, and 6.51 ± 0.17 mg/mL, respectively. Furthermore, four solvents of ascending polarities (hexane, chloroform, ethyl acetate and methanol) were being used to sequentially extract the dried fruit samples. The methanol and ethyl acetate extracts of *L. siceraria* respectively showed the highest DPPH (EC_{50} = 370.01 ± 12.51 and 469.00 ± 2.89 µg/mL) and metal chelating activities (EC_{50} = 249.78 ± 5.03 and 203.44 ± 4.01 µg/mL), while the ethyl acetate extract of *Sechium edule* showed the highest reducing power (EC_{50} = 409.35 ± 9.25 µg/mL). These extracts were subjected for

fractionation process using paper chromatography. All the fractions were then tested for their primary antioxidant activities and fraction eaLsf3 of the ethyl acetate extract of *L. siceraria*, fraction mLsf3 of the methanol extract of *L. siceraria* and fraction eaSef4 of the ethyl acetate extract of *S. edule* showed the best activities. The active compounds in the fractions were isolated and identified as caffeic acid, isoquercitrin (quercetin 3-*O*-glucoside) and gallic acid, respectively. As most of the cooking vegetables are reputed for having anti diabetes property, all extracts of the dried samples were subjected to α-glucosidase inhibitory assay. The ethyl acetate extract of *Momordica charantia* var. *maxima* showed the highest inhibition with the lowest EC_{50} value of 1.18 ± 0.05 mg/mL. This was followed by the methanol extract of *L. siceraria* with no significant difference in the EC_{50} value (1.25 ± 0.03 mg/mL). The quantitative analysis revealed caffeic acid was the major constituent in the methanol extract of *L. siceraria*, whereas isoquercetin was found to be the main contributor to its antioxidant and α-glucosidase activities. Caffeic acid and gallic acid (with low α-glucosidase inhibitory activity) were identified as both the main and active antioxidant constituents in the ethyl acetate extracts of *L. siceraria* and *S. edule*, respectively.

CHAPTER 1

GENERAL INTRODUCTION

1.1 Antioxidant activity

Antioxidant is defined as a molecule that prevents, inhibits or delays the oxidation of other molecules (Anjum *et al*., 2011). Oxidation is a chemical reaction that transfers electrons from a molecule to an oxidizing agent. Oxidation occurs in organisms when continuous production of reactive oxygen species (ROS) as a by-product of normal metabolic process, activated the formation of free radicals. A free radical is defined as an unstable and highly reactive molecule that contains at least one unpaired electron (Lobo *et al.,* 2010). Free radical has both beneficial and toxic effects to human. At low or moderate concentration, some of the free radicals play important roles in physiological processes such as in the production of energy, and for differentiation and growth of cells (Poli *et al*., 2004; Zhang *et al.,* 2011). Overproduction of these free radicals can cause oxidative damage to biomolecules (such as proteins, lipids and DNA/nucleic acids) (Valko *et al.,* 2006), which may lead to several physiological and pathological disorders, such as inflammation, cancer, Alzheimer's, arteriosclerosis, diabetes and cardiovascular diseases (Germano *et al*., 2006; Valko *et al.,* 2007; Uttara *et al*., 2009).

The harmful effects of free radicals are counterbalanced by the antioxidant activity of non-enzymatic antioxidants and enzymatic antioxidants (Balsano and Alisi, 2009; Anjum *et al*., 2011). Most of the non-enzymatic antioxidants are obtained from dietary intake, while living organisms possess various enzymatic antioxidants such as superoxide dismutase (SOD), glutathione peroxidase (GPx), catalase and methionine sulfoxide reductase (Fusco *et al*., 2007). Generally, the protection mechanism of antioxidants works at several different levels within cells in human body by inhibiting the formation of free radicals, converting existing free radicals into less harmful molecules and repairing oxidative damage (Du *et al*., 2009).

There are two mechanisms of action of antioxidants. The first mechanism involves the donation of an electron by primary antioxidant to the free radical present in the system, in order to directly break the chain reaction. The primary antioxidant activity can be measured using colorimetric methods such as 1-diphenyl-2-picrylhydrazyl (DPPH) and ferric reducing antioxidant power (FRAP) assays. The second mechanism is caused by the indirect involvement of secondary antioxidant in preventing the formation of free radicals by removing the free radical initiators. Metal-chelating assay is often used to measure the secondary antioxidant activity. The secondary antioxidants may bind to ferrous (Fe(II)) ion and disrupt the formation of Fe(II)-ferrozine complex (which is intense red-purple in color).

1.2 Fruits and vegetables with antioxidant activity

Fruits and vegetables are rich in phenolic compounds, ascorbic acid (vitamin C), carotenoids (α-carotene, β-carotene, β-cryptoxanthin, lutein, zeaxanthin and lycopene) and other biologically active constituents that have positive influence to health (Liu, 2004; Almeida *et al.,* 2011; Singh and Rao, 2012). Consumption of fruits and vegetables with antioxidant constituents may help to decrease DNA damage, lipid peroxidation, protein degradation, maintain immune function, inhibit malignant transformation or cancer cell proliferation. They are associated in preventing degenerative diseases and could delay the aging process (Liu, 2003; Boyer and Liu, 2004; Russo *et al.,* 2005; Brambilla *et al.,* 2008).

Several comparative studies had revealed the significant antioxidant activity of fruits such as sapodilla (*Manilkara zapota*) (Leong and Shui, 2002; Isabelle *et al*., 2010a), guava (*Psidium guajava*) (Thaipong *et al.,* 2006; Lim *et al*., 2007; Fu *et al.,* 2011), Chinese guava (*Psidium cattleianum*) (Luximon-Ramma *et al*., 2003), camu-camu (*Myrciaria dubia*) (Rufino *et al*., 2010) and chebulic myrobalans (*Terminalia chebula*) (Kubola *et al*., 2011), and vegetables such as coriander (*Coriandrum sativum*) (Isabelle *et al*., 2010b), spinach (*Spinacia oleracea*) (Song *et al*., 2010), ulam raja (*Cosmos caudatus*) (Wong *et al*., 2006), ladies finger (okra) (*Abelmonchus esculentus*) (Sreeramulu and Raghunath, 2010) and Chinese cedar (*Cedrela sinensis*) (Yang *et al*., 2006).

1.3 α-Glucosidase inhibitory activity

α-Glucosidase inhibitors are oral anti-diabetic drugs that prevent the final step in the digestion of carbohydrate to absorbable monosaccharides. Commercial α-glucosidase inhibitors such as acarbose, 1-deoxynojirimycin and miglitol are classified into antihyperglycemic drugs that are often administered to Type 2 diabetic patients prior to a meal for controlling postprandial glucose levels. They are effective to prolong the digestion of carbohydrates and cause the delay in absorption of glucose into the bloodstream (Krentz and Bailey, 2005).

Diabetes is commonly divided into Type 1 and Type 2 diabetes mellitus. Type 1 that is also known as insulin-dependent diabetes mellitus (IDDM) is characterized by the failure of insulin-producing cells in the pancreas to produce insulin that leads to insulin deficiency. Meanwhile, Type 2 is the most frequent form of diabetes that occupying more than 90% of cases worldwide. It is a result from insulin resistance, a condition in which cells fail to use insulin properly and is also known as non-insulin-dependent diabetes mellitus (NIDDM) or adult-onset diabetes.

Although current treatment using oral antihyperglycemic agents has resulted in high initial response rates, they are mostly restricted by dose-limiting side effects, including hypoglycemia, toxicity of liver, weight increase, abdominal pain, flatulence and diarrhea (Marles and Farnsworth, 1994; Fujisawa *et al*., 2005). Therefore, many diabetic patients have utilized herbal medicines as a complementary therapy.

1.4 Fruits and vegetables with α-glucosidase inhibitory activity

The hypoglycemic effects of fruits and vegetables are often being associated with their polyphenolic types and contents. Many studies have revealed a high correlation between α-glucosidase inhibitory activity and total phenolic content (Pandey and Rizvi, 2009). Brindis *et al.* (2013) found that rutin and isoquercitrin are the major contributors to the α-glucosidase inhibitory activity of the aqueous extract of the leaves of annona (*Annona macroprophyllata*). Moreover, the α-glucosidase inhibitory activity mangosteen (*Garcinia mangostana*) was found to be attributed by prenylated xanthones (Ryu *et al.,* 2011). Park *et al.* (2012) had compared the α-glucosidase inhibitory activity of fruits and vegetables that are commonly consumed in Korea and found the highest activity of the ethanol extract of potato (*Solanum tuberosum*), followed by sesame leaf (*Perilla frutescens*) and the juice of lotus root (*Nelumbo nucifera*). Another comparative study on 12 aqueous extracts of dried culinary herbs had identified Vietnamese mint (*Persicaria odorata*) as the most potent α-glucosidase inhibitor (Kee *et al*., 2013).

1.5 Cucurbitaceae Family

The family Cucurbitaceae is one of the most economically important plant families. For examples, the genus *Cucumis* consists of two major commercial crops, which are fruit vegetable [cucumber (*Cucumis sativus*)] and fruit [melon (*Cucumis melo*)]. The species in Cucurbitaceae are mainly distributed throughout tropical and subtropical regions, and the vegetables were amongst the earliest cultivated crops worldwide (Achigan-Dako, 2008). The family Cucurbitaceae is a family of plants consists of about 119 genera and between 850 to 1760 species (Asyaz *et al.,* 2010; Dhiman *et al*., 2012). Most of the species are climbers with tendrils that arise from the base of the petiole, and are rarely shrubs or trees (Bates *et al.,* 1990; Achigan-Dako, 2008; Ibrahim *et al.,* 2010). Many plants in this family are cultivated especially for their fruits (Rahman *et al*., 2008; Lim, 2012).

The plants in Cucurbitaceae are usually monoecious and dioecious plants, with solitary and unisexual flowers. The leaves are mostly exstipulate, alternate arrangement, with simple sub-orbicular to ovate, palmately lobed or palmately compound in shape. The flowers usually have five petals with yellow or whitish in color. Male flowers have a tubular calyx with imbricate or open lobes. The corolla can be either polypetalous or gamopetalous and the stamens (five) are often free or united. Female flowers have a calyx-tube adnate to the ovary which is inferior or very rarely free. The ovary usually has numerous ovules. The fruits are categorized as pepo with parietal placentation. The sizes of the fruits are differed from one another, some are very small with less than 1 cm diameter (for example: *Zehneria scabra*) or very large with more than 50 cm width (for examples: *Cucurbita maxima* and *Lagenaria siceraria*). The seed of Cucurbitaceae also are often flattened and flat oval in shape.

The common species that can be found in Malaysia, were selected in this study, which are white gourd (*Benincasa hispida*) (Plate 1.1), water melon (*Citrullus lanatus*) (Plate 1.2), cucumber (*Cucumis sativus*) (Plate 1.3), Japanese cucumber and apple cucumber (*Cucumis sativus*) (Plate 1.4), winter melon/sun melon (*Cucumis melo* var. *inodorus*) and musk melon/honey dew melon (*Cucumis melo*) (Plate 1.5), shark fin melon (*Cucurbita ficifolia*) (Plate 1.6), pumpkin (*Cucurbita maxima*) (Plate 1.7), zucchini (*Cucurbita pepo*) (Plate 1.8), bottle gourd (*Lagenaria siceraria*) (Plate 1.9), ridged gourd (*Luffa acutangula*) (Plate 1.10), bitter gourd (*Momordica charantia*) (Plate 1.11), chayote (*Sechium edule*) (Plate 1.12) and snake gourd (*Trichosanthes cucumerina*) (Plate 1.13).

Plate 1.1 Leaves, flower and fruit of *Benincasa hispida* (Thunb.) Cogn. (white gourd)

Plate 1.2 Red and yellow fleshes of two varieties of *Citrullus lanatus* L. (water melon)

Plate 1.3 A. Unripe fruit, flower and leaves; B. Ripe fruit of *Cucumis sativus* L. (cucumber)

Plate 1.4 Fruits of other varieties of *Cucumis sativus* L. (A. Japanese cucumber; B. Apple cucumber)

Plate 1.5 A. Bright yellow fruits of winter melon (*Cucumis melo* var. *inodorus*) and white-green fruit of musk melon (*Cucumis melo*); B. Orange flesh and seeds of winter melon and white-green flesh and yellow seeds of musk melon

Plate 1.6 Fruit of *Cucurbita ficifolia* Bouche (shark fin melon)

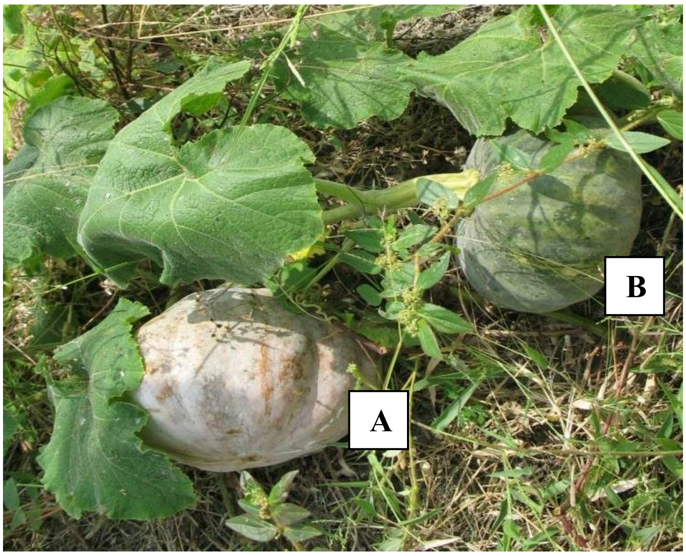

Plate 1.7 A. Mature; B. Immature fruits of *Cucurbita maxima* Duchesne (pumpkin)

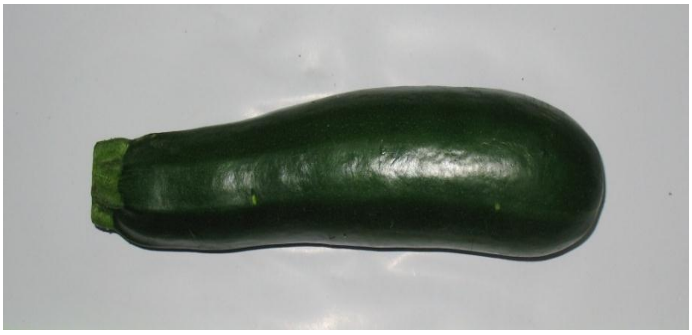

Plate 1.8 Fruit of *Cucurbita pepo* L. (zucchini)

Plate 1.9 Leaves and fruits of *Lagenaria siceraria* (Molina) Standl. (bottle gourd)

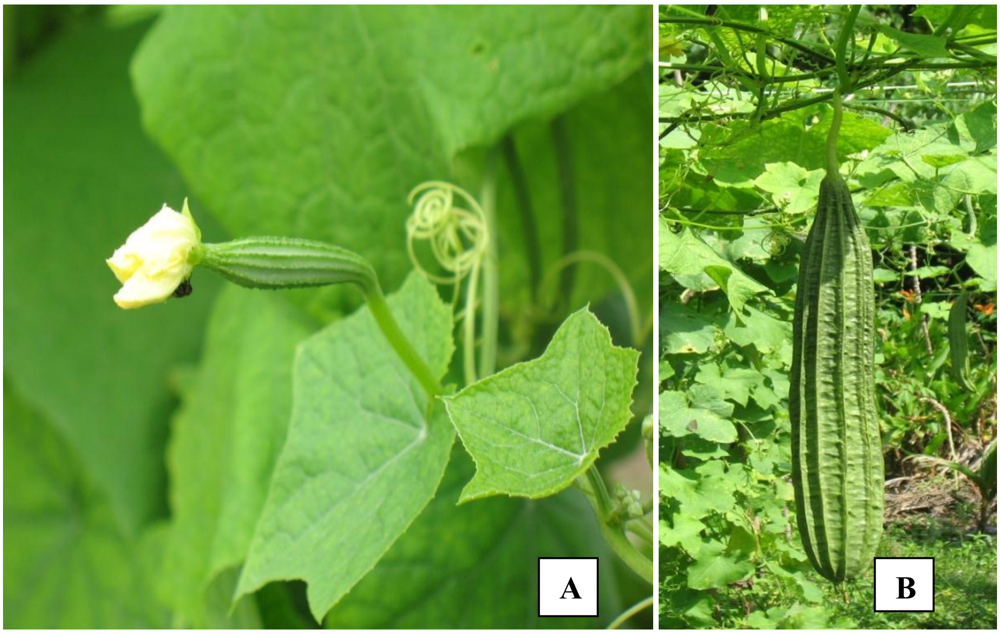

Plate 1.10 A. Flower and immature fruit; B. Mature fruit of *Luffa acutangula* (L.) Roxb. (ridged gourd)

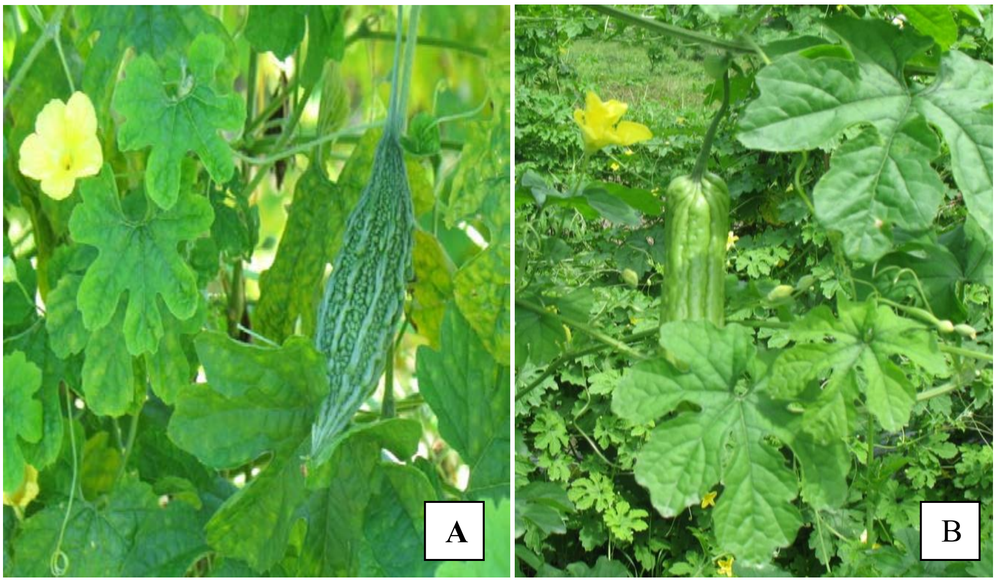

Plate 1.11 Flowers, fruits and leaves of A. *Momordica charantia* var. *minima* L.; B. *Momordica charantia* var. *maxima* L. (bitter gourd)

Plate 1.12 Immature fruit of *Sechium edule* (Jacq.) Sw. (chayote)

Plate 1.13 Leaves and fruits of *Trichosanthe cucumerina* L. (snake gourd)

1.6 Medicinal values of Cucurbits fruits

Cucurbit fruits are consumed for their health benefits such as to purify blood, treat constipation, as a diuretic, good for digestive system and source of energy (Rahman *et al*., 2008). For instances, the pulp of *Benincasa hispida* (white gourd) is used to make sweets, jam and jelly, while the pulp of *Cucurbita maxima* (pumpkin) is used for baking in pie and cake. The fruit of cucumber (*Cucumis sativus*) that is edible in raw form is used as a salad, whereas the fruits of water melon (*Citrullus lanatus*), winter melon (*Cucumis melo* var. *inorus*) and musk melon (*Cucumis melo*) are commonly used as dessert fruits. The fruits are also traditionally used to treat various diseases. Cucurbit fruits are best consumed during hot weather, as they possess cooling properties (Ong, 2004). Table 1.1 shows the medicinal values and local names of cucurbit fruits that were selected for this study.

Table 1.1 Medicinal values of cucurbit fruits that were selected in this study

Scientific name	Local name	Medicinal value
Benincasa hispida	Kundur	The whole fruit is used as a main ingredient in ayurvedic medicine and fruit juice was consumed to relieve hypertension (Yayasree *et al*., 2011). The fresh fruit is also consumed in India and China as an anthelmintic agent, to treat respiratory, urinary and heart diseases, diabetes mellitus and ulcers (Him-Che, 1985; Lim, 2012). In China, the dried peel is applied externally as a poultice for moisturizing, preventing from sun damage, and to treat oedema (Lim, 2012).
Citrullus lanatus	Tembikai	The ripe fresh pulp and its juice are consumed as a febrifuge and a diuretic, and in the treatment of dropsy and renal stones, and to reduce high blood pressure (Hassan *et al*., 2011).
Cucumis sativus	Timun	The fresh fruit and its juice are used externally as a poultice to treat burn and also for softening, moisturizing and whitening the skin (Lim, 2012; Mukherjee *et al.*, 2013).
Cucumis melo var. *inodorus*	Tembikai susu	The fresh pulp and juice are consumed as tonic, laxative, diuretic and diaphoretic (Dhiman *et al.*, 2012).
Cucurbita ficifolia	Labu Asia	The freeze-dried juice is consumed for treating diabetes (Alarcon-Aguilar *et al*., 2002; Andrade-Cetto and Heinrich, 2005; Lim, 2012; Daiz-Flores *et a.*, 2012).
Cucurbita maxima	Labu	The fruit is consumed as a diuretic. In India, the fruit pulp has been applied externally as a poultice to treat carbuncles, inflammation, boils and ulcer (Lim, 2012). It is also used as an ingredient for herbal formulation in Traditional Chinese Medicine (Que *et al*., 2008). The fruit juice is used in Mexico to treat diabetes (Andrade-Cetto and Heinrich, 2005)

Table 1.1 Continued

Scientific name	Local name	Medicinal value
Cucurbita pepo	Zukini	The fruit pulp part is freshly consumed to treat cold and alleviate ache (Wang *et al*., 2008). In Africa, the fruit pulp is applied externally as a poultice to treat burn and inflammations, and is used as a cooling compress to treat headache (Lim, 2012).
Lagenaria siceraria	Labu air	The fresh fruit juice is consumed to alleviate stomach acidity, indigestion and ulcers (Lim, 2012). In India and China, juice of fresh fruit is also traditionally used as cardio-protective, cardio-tonic, diuretic and aphrodisiac agents, to treat stomach-ache and skin rashes due to the cooling effects (Deshpande *et al*., 2007; Deshpande *et al*., 2008; Ghule *et al*., 2009; Lim, 2012; Nadeem *et al.,* 2012). The decoction of the fruit is commonly used in Traditional Chinese Medicine to treat diabetes (Covington, 2001; Gorasiya *et al.,* 2011).
Luffa acutangula	Petola segi	The fresh fruit juice is consumed as a demulcent and has diuretic property. In Kelantan, Malaysia, fresh fruit juice is administered to women after childbirth. Juice of leaves is applied externally as eye drop to treat conjunctivitis. The seed acts as a purgative (Lim, 2012).
Momordica charantia	Peria	The fruit of *Momordica charantia* is applied externally by the Turkish to heal wounds and is consumed to treat of peptic ulcers (Grover and Yadav, 2004). Whole plant of bitter gourd is consumed as anti-diabetic, anthelmintic and antimalarial agents, to treat gout, jaundice, kidney stone, rheumatism and scabies (Covington, 2001; Grover and Yadav, 2004; Pieroni *et al*., 2007).
Sechium edule	Labu jipang	This whole plant is consumed as diuretic, anti-inflammatory, anticoagulant and antihypertensive agents, to treat cardiovascular, kidney stones and arteriosclerosis (Ordonez *et al*., 2009; Andarwulan *et al*., 2012). The raw fruit is freshly consumed in Mexico to treat diabetes (Andrade-Cetto and Heinrich, 2005).
Trichosanthes cucumerina	Petola ular	The fruit has been consumed as anthelmintic and anti-inflammatory. The root is applied externally to cure bronchitis, headache and boils. The aerial parts are used to treat indigestion, bilious fevers, diabetes, boils, sores and skin eruptions such as urticaria, eczema and dermatitis (Arawwawala *et al*., 2010a; Arawwawala *et al*., 2010b; Dhiman *et al*., 2012).

1.7 Antioxidant compounds from Cucurbit fruits

Fruits and vegetables contain various phytochemicals and vitamins with antioxidant activities (Grubben *et al.*, 1994; Ong, 2004). Phenolic compounds represent a group of phytochemicals that is commonly found in fruits and vegetables, and exhibit biological activities such as protection against oxidative stress and degenerative diseases (Han *et al.*, 2007). A comprehensive literature review of chemical constituents found in the selected fruits is shown in Table 1.2.

Table 1.2 Antioxidant compounds and their contents in cucurbit fruits

Scientific name	Phenolic compound or/and content	Other compound or/and content
Benincasa hispida	- three flavonoids: astilbin, catechin and naringenin (Du *et al*., 2005). - total phenolic content in fresh juice: 169.1 mg GAE/g fresh weight (Huang *et al*., 2004). - total phenolic content in acetone: water: acetic acid (70:29.5:0.5) extract: 0.17 mg GAE/g fresh weight (Isabelle *et al*., 2010a).	- terpenes and sterols (Shetty *et al*., 2008). - vitamin A: 20 mg/100 g of edible portion; thiamine (vitamin B1): 0.06 mg/100 g of edible portion; vitamin C: 1 mg/100 g of edible portion (Rahman *et al*., 2008).
Citrullus lanatus	- 23 flavonoid derivatives such as kaempferol hexoside-rhamnoside and isorhamnetin 3-*O*-rutinoside (Abu-Reidah *et al*., 2013). - 11 phenolic acids such as protocatechuic and vanillic acids (Abu-Reidah *et al*., 2013). - 21 cinnamic acids such as *p*-coumaric and ferulic acids (Abu-Reidah *et al*., 2013).	- vitamin A: 599 mg/100 g of edible portion; thiamine (vitamin B1): 0.05 mg/100 g of edible portion; vitamin C: 6 mg/100 g of edible portion (Rahman *et al*., 2008).
Cucumis sativus	- three flavonols: kaempferol, quercetin and isoramnetin (Krauze-Baranowska and Cisowski, 2001). - four flavones: isovitexin, saponarin (Dhiman *et al.,* 2012), apigenin 7-*O*-(6-*O*-*p*-coumaroyglucoside) and vitexin (Mukherjee *et al.,* 2013). - four cinnamic acids: *p*-coumaric, caffeic, ferulic and chlorogenic acids (Lim, 2012). - total phenolic content in 60% methanolic extract containing 0.1% hydrochloric acid: 31.46 mg GAE/100 g fresh weight (Sreeramulu and Raghunath, 2010).	- total carotenoids: 48 μg/100 g fresh weight; β-carotene: 0 μg/100 g fresh weight (Kandlakunta *et al*., 2008). - vitamin A: 40 mg/100 g of edible portion; thiamine (vitamin B1): 0.03 mg/100 g of edible portion; vitamin C: 7 mg/100 g of edible portion (Rahman *et al*., 2008).

Table 1.2 Continued

Scientific name	Phenolic compound or/and content	Other compound or/and content
Cucumis melo	- a phenolic acid: benzyl *O*-β-D-glucopyranoside, (E)-4-hydroxycinnamyl alcohol 4-*O*-(2-*O*-β-D-apiofuranosyl)-β-D-glucopyranoside, 3-*O*-dibenzoylmultiflor-triol and 3-*O*-*p*-amino-benzoyl-*O*-benzoylmultiflor-triol (Marino *et al.,* 2009).	- vitamin A: 190 mg/100 g of edible portion; thiamine (vitamin B1): 0.06 mg/100 g of edible portion; vitamin C: 35 mg/100 g of edible portion (Rahman *et al.*, 2008). Vitamin C: 13.1 ± 5.8 to 43.2 ± 5.7 mg/100 g of edible portion (Laur and Tian, 2011).
Cucurbita ficifolia	-	- cucurbitacin (a steroid), ascorbic acid and thiamine (vitamin B1) (Roman-Ramos *et al.,* 2012). - D-chiro-inositol myo-inositol and fagopyritols (Xia and Wang, 2006; Roman-Ramos *et al.,* 2012).
Cucurbita maxima	- a phenolic acid: syringic acid (Dragovic-Uzelac *et al.,* 2005). - total phenolic content in 60% methanolic extract containing 0.1% hydrochloric acid: 46.43 mg GAE/100 g fresh weight (Sreeramulu and Raghunath, 2010). - total phenolic content in methanolic extract: 12 mg GAE/g extract (Attarde *et al*., 2010). - total phenolic content in 50% ethanolic extract: 13.3 mg GAE/g extract (Gacche *et al*., 2010).	- total carotenoids: 2120 μg/100 g fresh weight; β-carotene: 1180 μg/100 g fresh weight (Kandlakunta *et al*., 2008). - vitamin A: 1.84 g/100 g of edible portion; thiamine (vitamin B1): 0.06 mg/100 g of edible portion; vitamin C: 2 mg/100 g of edible portion (Rahman *et al*., 2008). - a water-soluble polysaccharide (Nara *et al*., 2009).
Cucurbita pepo	- five flavonols: quercetin 3-*O*-rutinoside, quercetin 3-*O*-glucoside, isorhamnetin, kaempferol rutinoside and kaempferol 3-*O*-glycoside (Iswaldi *et al*., 2013). - two phenolic acids: protocatechuic and vanillic acids (Mattila and Helstrom, 2007; Iswaldi *et al*., 2013). - four cinnamic acids: *p*-coumaric, ferulic, caffeic and chlorogenic acids (Mattila and Helstrom, 2007; Iswaldi *et al*., 2013).	- vitamin A: 1.7 g/100 g of edible portion; thiamine (vitamin B1): 0.07 mg/100 g of edible portion; vitamin C: 20 mg/100 g of edible portion (Rahman *et al*., 2008).

Table 1.2 Continued

Scientific name	Phenolic compound or/and content	Other compound or/and content
Lagenaria siceraria	- four flavone *C*-glycosides: isovitexin, isoorientin, saponarin, and saponarin 4'-*O*-glucoside (Krauze-Baranowska and Cisowski, 1995). - two flavonols: isoquercitrin (quercetin-3-*O*-β-D-glucose) and kaempferol (Gangwal *et al.*, 2010). - four cinnamic acids: (E)-4-hydroxymethyl-phenyl-6-*O*-caffeoyl-β-D-glucopyranoside, 1-(2-hydroxy-4-hydroxymethyl)-phenyl-6-*O*-caffeoyl- β–D-gluco-pyranoside, caffeic, and 3,4-dimethoxy cinnamic acids (Mohan *et al.*, 2012). - two phenolic acids: gallic and protocatechuic acids (Mohan *et al.*, 2012).	- vitamin A: 60 mg/100 g of edible portion; thiamine (vitamin B1): 0.03 mg/100 g of edible portion; vitamin C: 6 mg/100 g of edible portion (Rahman *et al.*, 2008). - sitosterol, campesterol and oleanolic acid (Gangwal *et al.*, 2010). - a water-soluble polysaccharide (Ghosh *et al.*, 2009).
Luffa acutangula	- total phenolic and flavanoid content of pulp ethanol/water extracts 5022 ± 21 ppm and 668 ± 14 ppm (Padmashree *et al.*, 2012). - total phenolic content in 60% methanolic extract containing 0.1% hydrochloric acid: 27.04 mg GAE/100 g fresh weight (Sreeramulu and Raghunath, 2010).	- total carotenoids: 991 μg/100 g fresh weight; β-carotene: 324 μg/100 g fresh weight (Kandlakunta *et al.*, 2008). - vitamin A: 56 mg/100 g of edible portion; thiamine (vitamin B1): 0.07 mg/100 g of edible portion; vitamin C: 5 mg/100 g of edible portion (Rahman *et al.*, 2008).
Momordica charantia	- four phenolic acids: gallic, protocatechuic, vanillic and gentisic acids (Horax *et al.*, 2005; Horax *et al.*, 2010). - three cinnamic acids: chlorogenic, *p*-coumaric and caffeic acids (Kubola and Siriamornpun, 2008; Horax *et al.*, 2010). - two flavan-3-ols: catechin and epicatechin (Horax *et al.*, 2005; Kubola and Siriamornpun, 2008; Horax *et al.*, 2010).	- total carotenoids: 967 μg/100 g fresh weight; β-carotene: 84 μg/100 g fresh weight (Kandlakunta *et al.*, 2008). - vitamin A: 210 mg/100 g of edible portion; thiamine (vitamin B1): 0.07 mg/100 g of edible portion; vitamin C: 88 mg/100 g of edible portion (Rahman *et al.*, 2008).

Table 1.2 Continued

Scientific name	Phenolic compound or/and content	Other compound or/and content
	- total phenolic content in aqueous extract: 68.8 mg GAE/ g extract; total phenolic content in 95% ethanol extract: 51.6 mg GAE/g extract (Wu and Ng, 2008). - total phenolic contents in aqueous, 20, 40, 60, 80 and 95% ethanol extracts of dried immature, mature and ripe pulps were ranging from 4.3 to 15.7 mg GAE/g extract (Horax *et al.*, 2010). - total phenolic content in 60% methanolic extract containing 0.1% hydrochloric acid: 139.67 mg GAE/100 g fresh weight (Sreeramulu and Raghunath, 2010).	- momordicoside K (a triterpenoid glycoside) (Lin *et al.*, 2011).
Sechium edule	- trace amount of vicenin 2, vitexin and luteolin 7-*O*-rutinoside (Siciliano *et al.,* 2004). - total phenolic content in acetone: methanol: water (7:7:6) extract: 66.10 mg GAE/g dry weight (Chanwitheesuk *et al.*, 2005).	- total carotenoids: 97 μg/100 g fresh weight; β-carotene: 2 μg/100 g fresh weight (Kandlakunta *et al.,* 2008). - vitamin C: 17.0 mg/g dry weight; vitamin E: 0.02 mg/g dry weight (Chanwitheesuk *et al.*, 2005).
Trichosanthes cucumerina	- total phenolic content in aqueous extract: 71.90 mg GAE/100 g fresh weight (Adebooye, 2008).	- α-carotene: 10.7 mg/100 g fresh weight; ascorbic acid: 23.2 mg/100 g fresh weight, β-carotene: 2.80 mg/100 g fresh weight (Adebooye, 2008).

1.8 Problem statements

The antioxidant activity in the fruit pulps of the selected Cucurbitaceae (excluding *Sechium edule*) were mostly investigated on an individual basis (Huang *et al*., 2004; Adebooye, 2008; Kubola and Siriamornpun, 2008; Wu and Ng, 2008; Erasto and Mbwambo, 2009; Nara *et al.*, 2009; Attarde *et al*., 2010; Horax *et al*., 2010; Reddy *et al.,* 2011; Mohan *et al*., 2012; Dhiman *et al*., 2012). Moreover, the antioxidant activity of *Sechium edule* was determined only from the aerial part extracts (Ordonez *et al*., 2006; Andarwulan *et al.*, 2010). Earlier comparative studies mostly used one polar solvent (such as 80% methanol) for extraction and reported lower antioxidant activity of cucurbit fruits as compared to other fruits and vegetables (Ansari *et al.,* 2005; Chanwitheesuk *et al*., 2005; Yang *et al.,* 2006; Stangeland *et al*., 2009; Gacche *et al*., 2010; Isabelle *et al.,* 2010b; Raghu *et al*., 2010; Sreeramulu and Raghunath, 2010). However, this information is still inadequate to categorize them as vegetables with low antioxidant activity.

Thus, in Chapter 2 of this thesis, different percentages of ethanol in distilled water were used as the extraction solvents. These two solvents are considered to be "green solvents" and are acceptable for food application (Horax *et al.,* 2010). For vegetables and fruits that can be eaten raw, they were freshly extracted, whereas for the cooking vegetables, they were being boiled prior to extraction. However these two solvents are polar solvents and only can extract polar compounds. Thus, to optimize the recovery of various hydrophilic and lipophilic antioxidants from these vegetables, sequential extraction using Soxhlet extractor was used to extract the dried samples (in Chapter 3).

The decoctions of dried pulps of many Cucurbit fruits are traditionally used to treat diabetes (Table 1.1) and phenolic compounds in plants are often being associated as α-glucosidase inhibitors. Therefore, in Chapter 4 of the thesis, the α-glucosidase inhibitory activities of the sequential extracts were determined. In Chapter 5, antioxidant activity guided fractionations of antioxidant-rich extracts of *L. siceraria* and *S. edule* were conducted. Some phenolic compounds were identified from the extracts and in Chapter 6, the compounds were quantified from the sequential extracts of *L. siceraria* and *S. edule*. Figure 1.1 shows a flow chart of this study.

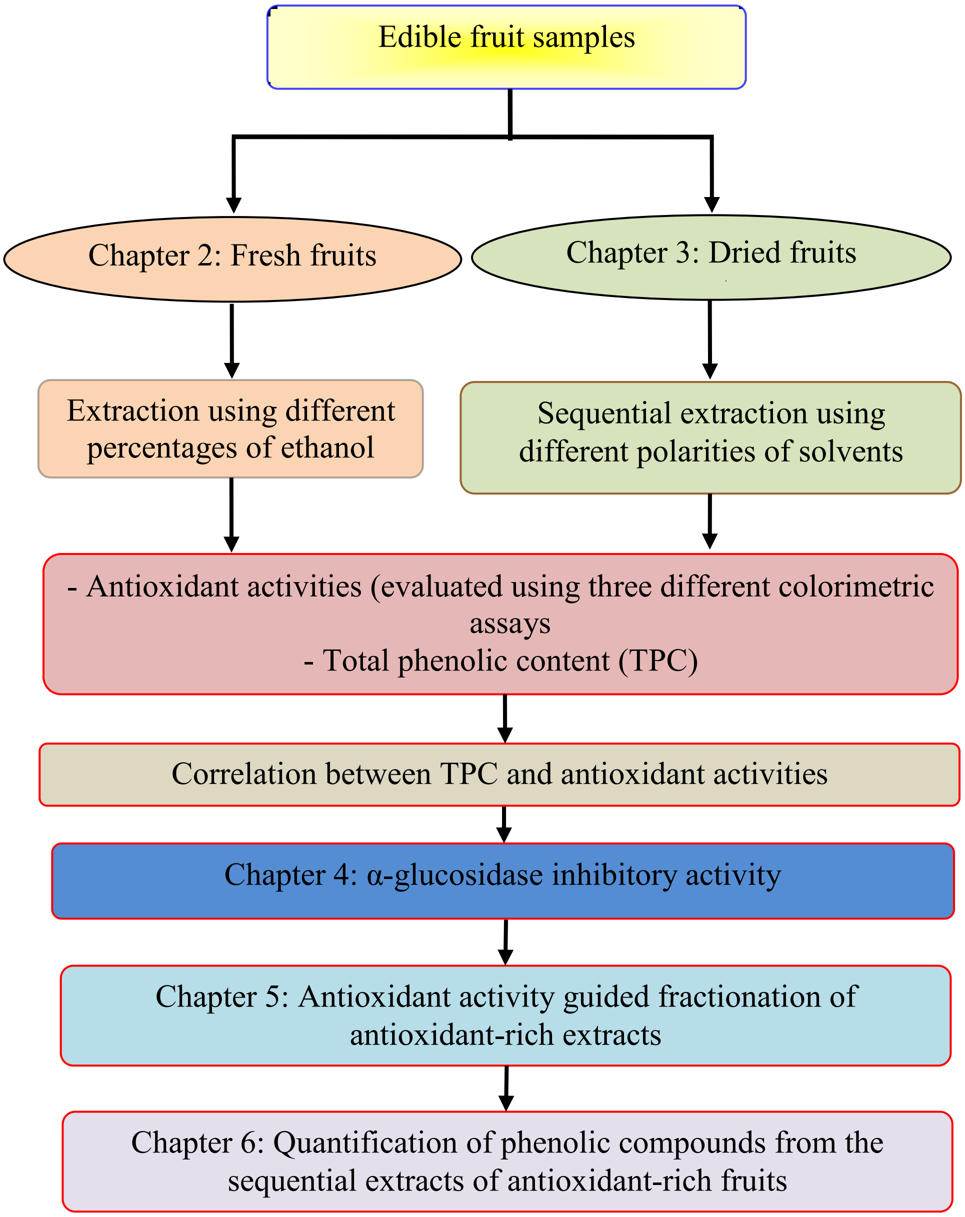

Figure 1.1 Flow chart of this study

1.9 Objectives of the study

The objectives of this study were:

1. To extract antioxidant compounds from the edible fruit parts (fresh or boiled) of eighteen Cucurbitaceae species using different percentages of ethanol in water,

and dried fruit parts by sequential extraction using four different polarities of solvents.

2. To determine the antioxidant activities (using three different colorimetric assays) in correlation to total phenolic content of the different extract preparations.
3. To screen for the α-glucosidase inhibitory activity of the sequential extract preparations.
4. To identify the phenolic compounds with antioxidant activities in the antioxidant-rich extracts.
5. To quantify the phenolic compounds from the sequential extracts of antioxidant-rich fruits.

CHAPTER 2

ANTIOXIDANT ACTIVITIES OF EXTRACTS OBTAINED FROM WATER-ETHANOL EXTRACTION OF FRESH EDIBLE FRUIT PARTS

2.1 Introduction

2.1.1 Edible parts of cucurbit fruits

The fruits from Cucurbitaceae are categorized into three edible groups, which are vegetables that are only edible after being cooked (cooking vegetables), vegetables that can be consumed in raw form (raw vegetables), and fruits. They are mostly harvested at their commercial maturity stages that are unripe for most vegetables excluding *Cucumis sativus* (Cucumber), which is consumed at unripe and ripe stages. Meanwhile, those that belong to fruit group are mostly consumed when they are ripe. Those that belong to cooking vegetables are usually added into soups and curries, and also prepared by boiling, steaming and stir-frying. Raw or boiled vegetables are usually dipped in shrimp and chili paste or peanut sauce before eating to enhance flavors. They are also sliced into tiny pieces and mixed with an assortment of ingredients as mixed salad preparation.

2.1.2 Antioxidant studies of fresh samples of Cucurbitaceae

Various antioxidant studies of fresh fruit samples from this family had been conducted. For examples, Huang *et al.* (2004) found lower radical scavenging activity of the fresh juice extract of *Benincasa hispida* as compared with water, 50%