ANTIOXIDATIVE, ANTIKINASE OR ANTIPHOSPHATASE AND ANTIMICROBIAL ACTIVITIES OF SELECTED VEGETABLES IN SABAH, MALAYSIA

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2014

DECLARATION

I hereby declare that the material in this thesis is my own except for quotations, excerpts, equations, summaries and references, which have been duly acknowledged.

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ABSTRACT

Vegetables offer various health benefits to humans. However, there is a lack of investigation on the biological activities of these vegetables especially vegetables originating from Sabah. Thus, fifty two species of vegetable samples were collected from local markets in Sabah and were screened for their antioxidant activity using DPPH• assay. Five samples with high DPPH• scavenging activity ranging from 84.32% to 94.72% were selected for further study which were *Cosmos caudatus, Eryngium foetidum, Ipomoea batatas, Ipomoea batatas* var. green star shaped leaf and *Manihot esculenta* Crantz. These five samples were subjected to different solvent extractions (air-dried sample) and different cooking methods (raw sample). All of these extracts exhibits antioxidant activities ranging from 3.52% to 92.22% for all of the three antioxidant assays (DPPH•, O_2• and NO• scavenging assays). Boiled water extracts of *I. batatas* var. green star shaped leaf and *M. esculenta* Crantz exhibits high antioxidant activity compared to the other solvent extractions whereas steaming and microwave cooking extracts of *I. batatas* var. green star shaped leaf and *M. esculenta* Crantz, respectively exhibits high antioxidant activity compared to the other cooking methods. Neither of the single solvent extractions or cooking methods extracts of *C. caudatus* and *I. batatas* that exhibits high antioxidant activity. In addition to that, steaming cooking extracts of *E. foetidum* was the only extract with high antioxidant activity. None of these extracts were exhibiting inhibition activity against GSK-3β screening test whereas there were two extracts that exhibits toxic activity against MKK1 and MSG5 screening test. These extracts were raw sample of *I. batatas* and *I. batatas* var. green star shaped leaf. Antikinase or antiphosphatase activities of these extracts were 9.33 mm (glucose media) for MKK1 whereas ranging from 7.67 mm to 8 mm (glucose media) and 8 mm to 8.33 mm (galactose media) for MSG5 screening tests. Among nine microbial strains that were tested, only two bacterial strains were inhibited by almost all of these extract which were *Staphylococcus aureus* and *Pseudomonas aeruginosa*. The antibacterial activities of these extracts were ranging from 6.67 mm to 16 mm. Raw samples of *I. batatas* and *I. batatas* var. green star shaped leaf exhibits the largest inhibition activity against *S. aureus* (10 mm) and *P. aeruginosa* (16 mm), respectively. Proximate analysis test results showed that *E. foetidum* has the highest moisture (87.57%) and protein contents (17.29 mg/mL) whereas *M. esculenta* Crantz has the highest ash content (4.11%) compared to the other vegetable species. Furthermore, *I. batatas* have the highest fat content (1.08%).

ABSTRAK

AKTIVITI ANTIOKSIDA, ANTIKINASE ATAU ANTIFOSFATASE DAN ANTI MIKROBAKTERIABAGI SAYURAN YANG TERPILIH DI SABAH

Sayur-sayuran menawarkan pelbagai manfaat kesihatan untuk manusia akan tetapi kajian terhadap aktiviti biologi sayur-sayuran ini masih kurang terutamanya sayur-sayuran yang berasal dari Sabah. Oleh itu, sebanyak 52 spesis sayuran yang diperolehi dari pasar tempatan di sabah telah disaring untuk aktiviti antioksida dengan menggunakan ujian DPPH•. Sebanyak lima sampel yang menunjukkan activiti memerangkap DPPH• yang tinggi (84.32% hingga 94.72%) telah dipilih untuk dikaji dengan lebih lanjut iaitu Cosmos caudatus, Eryngium foetidum, Ipomoea batatas, Ipomoea batatas var. green star shaped leaf dan Manihot esculenta Crantz. Kelima-lima sampel ini diekstrak dengan menggunakan larutan pengekstrakkan yang berlainan (sampel kering) dan dimasak dengan pebagai kaedah masakan (sampel segar). Aktiviti antioksida bagi kesemua ekstrak ini ialah dari 3.52% hingga 92.22% untuk kesemua ujian antioksida (ujian memerangkap DPPH•, O_2• dan NO•). Ekstrak air panas bagi sampel I. batatas var. green star shaped leaf dan M. esculenta Crantz menunjukkan aktiviti antioksida yang tinggi berbanding dengan pelarut pengekstrakan yang lain manakala masakan mengukus dan kelombang ketuhar bagi I. batatas var. green star shaped leaf dan M. esculenta Crantz pula masing-masing menunjukkan aktiviti antioksida yang tinggi berbanding dengan kaedah masakan yang lain. Tiada satu pun keadah pengekstrakkan dan kaedah masakan bagi sampel C. caudatus dan I. batatas yang menunjukkan aktiviti antioksida yang tinggi. Tambahan pula, masakan mengukus bagi sampel E. foetidum merupakan satu-satunya kaedah yang menunjukkan aktiviti antioksida yang tinggi. Tiada aktiviti perencatan yang dapat dilihat dalam ujian penyaringan GSK-3β akan tetapi terdapat dua ekstrak yang menunjukkan aktiviti toksik dalam ujian penyaringan MKK1 dan MSG5 iaitu ekstrak bagi sampel segar I. batatas dan I. batatas var. green star shaped leaf. Aktiviti perencatan bagi kedua-dua ekstrak ini adalah 9.33 mm (media glukosa) bagi ujian penyaringan MKK1 manakala dari 7.67 mm hingga 8 mm (media glukosa) dan dari 8 mm hingga 8.33 mm (media galaktosa) bagi ujian penyaringan MSG5. Ujian antimikrobakteria ke atas sembilan jenis mikrobakteria menunjukkan bahawa hanya terdapat dua jenis bakteria sahaja yang berjaya direncat oleh hampir kesemua ekstrak iaitu bakteria Staphylococus aureus and Pseudomonas aeruginosa. Aktiviti antibakteria bagi ekstrak-ekstrak tersebut ialah dari 6.67 mm hingga 16 mm. Ekstrak bagi sampel segar I. batatas dan I. batatas var. green star shaped leaf menunjukkan aktiviti perencatan yang terbesar terhadap bakteria S. aureus (10 mm) and P. aeruginosa (16 mm) masing-masing. Bagi ujian analisis komposisi sayur, E. foetidum mempunyai kandungan kelembapan (87.57%) dan protein (17.29 mg/mL) yang tinggi manakala M. esculenta Crantz pula mempunyai kandungan abu (4.11%) yang tinggi berbanding dengan sampel lain. Tambahn pula, I. batatas mempunyai kandungan lemak (1.08%) yang tinggi.

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LIST OF ABBREVATION

ATCC	American type culture collection
BuOH	Butanol
CE	Catechin equivalent
DNA	Deoxyribonucleic acid
DPPH	1, 1-Diphenyl-2-picrylhydrazyl
DPPH●	1, 1-Diphenyl-2-picrylhydrazyl radical
ET	Electron transfer
FCR	Folin-Ciocalteu reagent
GAE	Gallic acid equivalent
GSK-3β	Glycogen synthase kinase – 3 beta
HAT	Hydrogen atom transfer
HCl	Hydrochloric acid
MAPK	Mitogen activated protein kinase
MAPKs	Mitogen activated protein kinases
MKK1	Mitogen activated protein kinase kinase
MSG5	Mitogen activated protein kinase phosphatase
NADH	Nicotinamide adenine dinucleotide
NBT	Nitroblue tetrazolium
NO	Nitric oxide
NO●	Nitric oxide radical
O_2	Oxygen
O_2●	Superoxide radical
PMS	Phenazine methosulphate
ROS	Reactive oxygen species

CHAPTER 1

INTRODUCTION

The increase of awareness among consumers and mass media publicity coverage of antioxidant's heath benefits have made the "antioxidant" word become increasingly popular in modern society (Van Der Sluis *et al*., 2000; Huang *et al*., 2005). Antioxidants are capable of scavenging free radicals that were shown to be linked to age related illnesses and a large number of other illnesses (Kumpulainen and Salonen, 1999; Pourmorad *et al*., 2006; Ratnam *et al*., 2006; Amol *et al*., 2013). Therefore, the restriction to the use of synthetic antioxidants which have been suspected to promote carcinogenesis has lead to the research of effective antioxidants with less toxicity especially those originating from natural plants that were used in folk medicine and food (Namiki, 1990; Alzoreky and Nakahara, 2001; Ali *et al*., 2008; Kumar *et al*., 2008; Kathirvel and Sujatha, 2012; Amol *et al*., 2013).

Vegetables are essential to human due to the ability of providing nutrients and various phytochemicals with different bioactivities (Odufuwa *et al*., 2013). Consumption of vegetables could aid the prevention of aging related diseases and cancer (Alzoreky and Nakahara, 2001; Yen *et al*., 2001; Olorunnisola *et al*., 2012). Various studies were done towards different types of vegetables for their antioxidant activities. The ten most studied vegetables in an antioxidant assay includes ginger, amaranth, eggplant, sweet potato leaf, spinach, leafy chinese cabbage, pak choi (bok choy), onion, Welsh onion and tomato (Easdown and Kalb, 2004).

Cancer is the leading cause of death in worldwide and it could happen due to the mis-regulated of the signal transductions (Lobbezoo *et al*., 2003; Are *et al*., 2013). Antioxidant compounds isolated from plants such as polyphenol and flavonoid are said to be associated with the potential of preventive activity against cancer in the initiation stage (Larson, 1988; Manson, 2003; Kristal, 2002; Thomas, 2008; El-Sayed *et al*., 2013). A study done by Wu *et al*. (2004) revealed that vegetables with anticancer properties such as broccoli and cauliflower were showing modest antioxidant activities *in vitro*. In addition, another different study revealed that *Cinnamomum iners* plant extracts have significant anti-kinase activity against the MKK1 in the MAPK signal transduction pathways as well as strong antioxidant activity (Pang *et al*., 2009).

Vegetables also have been long used in folk medicine to treat infectious disease caused by microbial and these have been supported by the discovery and characterization of antimicrobial phytochemicals from vegetables (Delaquis and Mazza, 1995; Thomas, 2008). Flavonoid is one of the antioxidant compounds that was suggested to posses antibacterial activities (Cook and Samman, 1996; Murakami *et al*., 1996; Thomas, 2008; Ahmad *et al*., 2012). Several studies reported that a few vegetables such as *Anacardium occidentale*, *Melicope ptelefolia* and *Polygonum hyrdopiper* posses both antimicrobial activities as well as antioxidant activities (Furuta *et al*., 1986; Rasadah and Zakaria, 1988; Kudi *et al*., 1999; Rahman *et al*., 2002).

Vegetables especially green leafy vegetables are among the top four common food items eaten daily by Malaysian people (Karim *et al*., 2008). To date, even though vegetables offer various health benefits to humans, there is a lack of investigation on the biological activities of these vegetables especially vegetables originating from Sabah. Therefore, the main objectives of this study were (i) to screen antioxidant activities of combined methanol and acetone extracts of 52 selected vegetables in Sabah using 1,1-diphenyl-2-picryl hydrazyl (DPPH) scavenging assay; (ii) to study the effect of different extraction methods on antioxidant activities; (iii) to determine antioxidant activities of different cooking methods and (iv) to screen antikinase or antiphosphatase activities of the selected vegetables; (v) to screen antimicrobial activities of the selected vegetables; (vi) to evaluate nutrition values of the selected vegetables.

CHAPTER 2

LITERATURE REVIEW

2.1 Vegetables in Sabah

Sabah is one of the 13 states of Malaysia and it is the second largest state in Malaysia after Sarawak. Sabah with the population about 3.21 million is located at the northernmost part of Borneo with a landmass of approximately 7.4 million hectares (Dept. Statistics Malaysia, 2010). Sabah being a part of Borneo is rich with plant biodiversity. The conditions and range of altitude in Sabah allows the production of both tropical and temperate vegetables. Most of the vegetable farming (<2 acres) is dominated by smallholder activity (80%) that produced tropical vegetables in the lowlands (900 feet above sea level) around the coastal and Keningau area while the temperate vegetables are produced in the highlands (3000-5000 feet above sea level) of Kundasang and the largest vegetable growing area is located within the Kundasang-Ranau region (Jipanin *et al.*, 2001).

Sabah Ministry of Agriculture Development and Food Industry reported in the *Second Sabah Agricultural Policy 1999-2010* that most of the vegetable production in Sabah is mainly used for domestic consumption. As years passed, there is an increasing demand of local and regional markets on vegetable in Sabah. This is shown in the statistic reports that have been published by the Department of Agriculture Sabah where from year 2007 to 2009 shows that there is an increase of vegetable production (from 26301.9 tonne to 38467.9 tonne) and import (from 25484 tonne to 28604 tonne) in Sabah. In addition to that, Department of Agriculture Sabah also reported that there is a decrease in the export of vegetable in Sabah which was 5245 tonne in the year of 2007 decrease to 3118 tonne in the year of 2009.

Vegetables can be defined as parts of plants such as leaf, stem, root, flower or fruit that can be consume as raw or after cooking and are rich in vitamins and minerals (Biggs *et al.*, 2003; Gopalakrishnan, 2007; Adewale *et al.*, 2013). Aside from the main food sources such as rice or other carbohydrate sources, vegetables are also one of the important food sources that are essential for humanity. Vegetables not only provides the essential vitamins and minerals for a balanced diet but they also contained phytochemical compounds which are generally non-nutritive but have the potential to affect disease such as stroke, metabolic syndrome and cancer (Faiza *et al.*, 2013; Odufuwa *et al.*, 2013). Since ancient times, vegetables are considered as protective foods as humans have used vegetables not only as food but also to prevent and treat several diseases (Halimathul, 1998; Gopalakrishnan, 2007; Ong, 2008; Kaisoon *et al.*, 2011; Sripriya, 2013).

Almost all vegetables play important roles in traditional systems of medicine and some vegetables such as celery started off solely as a medicinal plant before it became a vegetable (Gopalakrishnan, 2007; Ong, 2008). Nowadays, there is an increased of epidemiological and pharmacological evidence saying that plants contained biologically active components that can scavenger free radical and consumption of fruits and vegetables can aid in the prevention of degenerative processes by lowering the incidence of mortality rate of cancer and cardiovascular disease (Alzoreky and Nakahara, 2001; Yen *et al.*, 2001; Olorunnisola *et al.*, 2012). In addition to that, several chemicals with therapeutic value were also identified from vegetables (Gopalakrishnan, 2007).

Human earliest diets as a hunter and gatherer including gathering a wide range of seeds, fruits, nuts, roots, leaves and hunting any moving thing that can be catch but gradually over millennia, human have learned which plants could be eaten and also learned how to prepare them as well (Biggs *et al.*, 2003). Vegetables are usually processed prior to consumption and cooking is one of the common methods used to process vegetable. Cooking can help in improving the flavor, nutrient retention, bioavailability and the content of chemopreventive compounds that present in the vegetables (Odufuwa *et al.*, 2013). However, preparation of the vegetables can affect both the nutrients and non- nutrients elements of the vegetables especially when thermal treatment is being applied where it can caused the loss of antioxidant compounds in vegetables up to 30% (Faller and Fialho, 2009; Odufuwa *et al.*, 2013).

Cooking can either increase or decrease the vegetables composition depending on the types of the cooking methods that are being used and also depending on the nature of the chemical compounds and types of the vegetables (Ismail *et al.*, 2004; Lombard *et al.*, 2005; Turkmen *et al.*, 2005; Bernhardt and Schlich, 2006; Faller and Fialho, 2009). Among the four different cooking methods that are commonly used in household (boiling, microwave, steaming and stir fry), boiling and steaming cooking methods are the best way in retaining the lipophilic vitamins such as beta carotene and alfa tocopherol but cooking methods with less water are more favorable in preserving the hydrophilic compounds such as ascorbic acid and minerals (Bernhardt and Schlich, 2006).

2.2 Free Radicals and Oxidative Stress

Free radicals can be defined as a highly reactive and unstable chemical species due to the presence of one or more unpaired electrons at their outer orbital and thus cause damage to other molecules by extracting electrons from them in order for it to attain stability (Clarkson, 1996; Ali *et al.*, 2008). Free radicals are continuously produced in the human body as they are an integral part of human metabolism where they are essential for energy supply, detoxification, chemical signaling and immune function (Cao and Prior, 1998; Kitao *et al.*, 2005; Ali *et al.*, 2008; Dai and Mumper, 2010; Banerjee *et al.*, 2014). Besides being produced endogenously, free radicals can also be generated from exogenous factor such as radiation, environmental pollutants, chemical, toxins, deep fried food, diets rich in saturated fatty acids and carbohydrates, spicy food and also from physical stress such as exercise can adversely affect the production of free radical and lead to oxidative stress (Clarkson, 1996; Pourmorad *et al.*, 2006; Céspedes *et al.*, 2008; Niederländer *et al.*, 2008; Dai and Mumper, 2010; Olorunnisola *et al.*, 2012).

Reactive oxygen species (ROS) are one of the most reactive free radicals. There are six major ROS that have been suggested through experimental evidence that can either directly or indirectly cause oxidative damage in the human body

(Huang *et al.*, 2005). These species are superoxide anion ($O_2^{\bullet -}$), hydrogen peroxide (H_2O_2), peroxyl radical ($ROO^{\bullet}$), hydroxyl radical ($HO^{\bullet}$), singlet oxygen ($^1O_2$), and peroxynitrite ($ONOO^-$) (Huang *et al.*, 2005). ROS are molecules that unstable and highly reactive thus they can cause damage to the cells by alteration of physiologically critical molecules and this is including the direct attack of hydroxyl radical to the DNA molecule that could cause cancer promoting mutations or cell death (Laguerre *et al.*, 2007; Kumar *et al.*, 2008; Dai and Mumper, 2010). In addition to that, ROS are also capable of attacking other cellular components such as the side chains of all amino acids residues of protein especially cysteine and methionine residues (Stadtman, 2004; Dai and Mumper, 2010) and also capable of attacking polyunsaturated fatty acid residues of phospholipids (Siems *et al.*, 1995; Dai and Mumper, 2010).

Even though free radicals are continuously produced in living organism, interestingly the body of living organism has developed a complex antioxidant defense system against the oxidative damage induced by free radicals to counteract reactive species and to reduce their damage (Cao and Prior, 1998; Ali *et al.*, 2008; Reihani and Azhar, 2012; Amol *et al.*, 2013). This antioxidant system includes enzymes such as superoxide dismutase; macromolecules such as albumin; an array of small molecules such as ascorbic acid and some hormones such as estrogen (Prior *et al.*, 2005). Although human body is equipped with natural antioxidant defense system but as the amount of free radicals started to build up in human body due to the exogenous factors, the balance between the antioxidants and the free radicals started to crumble and thus causing the oxidative stress in human body (Cao and Prior, 1998; Ou *et al.*, 2002; Laguerre *et al.*, 2007; Dai and Mumper, 2010; Olorunnisola *et al.*, 2012). Oxidative stress that are caused by free radicals have been claimed to play an important roles in affecting human health by causing severe disease such as atherosclerosis, hypertension, ischaemic diseases, diabetes mellitus, cardiovascular diseases, cancer, osteoporosis and degenerative diseases such as Alzhemier and parkinsonism (Shirwaikar *et al.*, 2006; Kubola and Siriamornpun, 2008; Ozsoy *et al.*, 2009; Olorunnisola *et al.*, 2012; Amol *et al.*, 2013).

2.3 Antioxidants in Tackling Free Radicals

2.3.1 Types of antioxidants

There are at least four general sources of antioxidants within biological system which are enzymes, large molecules (albumin, ceruloplasma, ferritin, and other proteins), small molecules [ascorbic acid, glutathione, uric acid, tocopherol, carotenoids and (poly)phenols] and some hormones (estrogen, antiotensin, melatonin) (Prior *et al.*, 2005). But basically, antioxidant can be divided into two main groups which are enzymatic antioxidant and nonenzymatic antioxidant. Enzymatic antioxidant can be divided further into two groups which are primary enzyme and secondary enzyme whereas the nonenzymatic antioxidant can be divided further into seven groups which are minerals, carotenoids, vitamins, organosulfur compounds, antioxidant cofactors, low molecular weight antioxidant and polyphenols as shown in Figure 2.1 (Ratnam *et al.*, 2006).

Enzymatic antioxidants especially superoxide dismutases, gluthation peroxidases, catalases and thioredoxin are recognized as being highly efficient in reactive oxygen species detoxification (Laguerre *et al.*, 2007) whereas the nonenzymatic antioxidants are important in scavenging peroxyl radical ($ROO^{\bullet}$), hydroxyl radical ($HO^{\bullet}$), singlet oxygen ($^{1}O_2$) and peroxynitrite anion ($ONOO^{-}$) because so far there is no enzymatic antioxidant that can scavenge $ROO^{\bullet}$, $HO^{\bullet}$, $^{1}O_2$ and $ONOO^{-}$ (Huang *et al.*, 2005). Nonenzymatic antioxidants such as ascorbic acid, carotenoids and phenolic compounds (flavonoids, phenolic acids and alcohols, tocopherol, tocotrienols) are commonly found in both edible and non edible plants (Kähkönen *et al.*, 1999; Laguerre *et al.*, 2007).