UNIVERSITI PUTRA MALAYSIA

ANTIOXIDANT ACTIVITY AND BIOACTIVE COMPOUNDS OF SELECTED TROPICAL PLANTS

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By

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Thesis Submitted to the School of Graduate Studies, Universiti Putra Malaysia, in Fulfilment of the Requirements for the Degree of Master of Science

September 2009

In The Name of ALLAH, The Beneficent, The Merciful

This thesis is dedicated to my beloved Dad, Mom, Family and Friends

Abstract of thesis presented to the Senate of Universiti Putra Malaysia in fulfilment of the requirement for the degree of Master Science

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September 2009

Supervisor: Azizah Abdul Hamid, PhD

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Antioxidant does not only prevent food containing lipid and oils from becoming rancid, but it also provides protection against harmful free radical and has been strongly associated with reduced risk of chronic diseases such as cardiovascular diseases, cancer and diabetes. The antioxidant activity of 21 selected tropical plant extracts was evaluated utilizing four different assays including conjugated diene, scavenging DPPH radical, TBA and chelating effect on ferrous ion. Bioactive compounds (total phenolics, flavonoids, β-carotene, lycopene, α-tocopherol and ascorbic acid) were also determined in the plants extracts. Results of the study showed that *C.caudatus* extract was highly effective in the prevention of conjugated diene formation and scavenging effect on DPPH radicals. On the other hand, *C.asiatica* and *P.tetragonolobus* extracts demonstrated excellent activity in

inhibiting malondialdehyde (MDA) formation and chelating ability on ferrous ion. As expected, increasing the concentration of extracts used increased the antioxidant activity in all plants tested. The antioxidant activities of some of these plants were found to be as good as that of α-tocopherol and BHA (Butylated hydroxyanisole). Results of the study revealed that *L.inermis* extracts consisted of the highest content of phenols and then flavonoids (catechin, epicatechin and naringenin) compared to all the plants tested. On the other hand, excellent concentration of quercetin and ascorbic acid were identified in *V.negundo* extract whilst surprisingly, *P.bleo* extract showed high content of lycopene and α-tocopherol. Highest kaempferol and myricetin was found in *S.grandiflora* and *C.asiatica* extracts whilst luteolin and apigenin were predominantly found in *P.cordifolia* and *K.galanga* extracts. On the other hand, highest β-carotene content was found in *G.procumbens* extract. Excellent correlation (R^2=0.8613) was found between radical scavenging activity and the total content of phenolic compounds. Similar correlation (R^2=0.8430) was seen between radical scavenging activity and flavonoids compounds. The study suggested that phenolic compounds in particular, the flavonoids contribute to the antioxidant activity.

Keywords: Antioxidant activity; tropical plants; bioactive compounds; phenolic compounds; ascorbic acid.

Abstrak thesis yang dikemukakan kepada Senat Universiti Putra Malaysia sebagai memenuhi keperluan untuk ijazah Master Sains

AKTIVITI ANTIOKSIDAN DAN SEBATIAN BIOAKTIF PADA TUMBUHAN TROPIKAL TERPILIH

Oleh

ROHEEYATI ASHIKIN BINTI MUSTAFA

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Antioksidan bukan hanya menghalang makanan yang mengandungi lemak dan minyak menjadi tengik tetapi juga memberi perlindungan yang lengkap daripada bahaya radikal bebas serta mempunyai hubungan yang kukuh dengan menurunkan risiko penyakit kronik termasuklah kardiovaskular, kanser dan diabetes. Aktiviti antioksidan dalam 21 ekstrak tumbuhan tropikal terpilih telah dinilai menggunakan empat kaedah berbeza termasuklah diene berkonjugat, penyaringan bagi radikal DPPH, TBA dan kesan pengikatan pada ion ferrous. Sebatian bioaktif (kandungan fenolik, flavonoid, β-karotin, likopena, α-tocoferol dan asid askorbik) telah dikaji dalam tumbuhan tropikal terpilih. Keputusan kajian menunjukkan bahawa ekstrak *C.caudatus* mempamerkan keberkesanan yang tinggi dalam menghalang pembentukan diene berkonjugat serta penyaringan pada radikal

DPPH. Manakala, ekstrak dari *C.asiatica* dan *P.tetragonolobus* pula menunjukkan kecemerlangan aktiviti dalam menghalang pembentukan malondialdehyde (MDA) dan keupayaan mengikat ion ferrous. Seperti yang dijangkakan, peningkatan kepekatan ekstrak yang digunakan akan meningkatkan aktiviti antioksidan dalam tumbuhan kajian. Sebilangan dari tumbuhan ini ditemui setanding dengan α-tocoferol dan BHA (Butylated hydroxyanisole). Keputusan kajian menunjukkan bahawa kandungan tertinggi bagi sebatian fenolik dan flavonoid (katekin, epikatekin dan naringenin) di temui pada ekstrak *L.inermis* berbanding dengan tumbuhan kajian yang lain. Sementara itu, kepekatan tertinggi bagi kuersetin dan asid askorbik telah dikenal pasti dalam ekstrak *V.negundo.* Manakala, ekstrak *P.bleo* pula menunjukkan kandungan tertinggi dalam likopena dan α-tocoferol. Kandungan tertinggi bagi kaempferol dan myricetin telah di temui pada ekstrak *S.grandiflora* dan *C.asiatica* manakala, sebatian luteolin dan apigenin di temui dengan banyaknya dalam ekstrak *P.cordifolia* dan *K.galanga.* Kandungan tertinggi bagi sebatian β-karotin telah ditemui dalam ekstrak *G.procumbens*. Hubung kait yang kukuh (R^2=0.8613) telah ditemui antara aktiviti penyaringan radikal dengan jumlah kandungan sebatian fenolik. Jalinan hubungan (R^2=0.8430) yang sama juga telah di dapati antara aktiviti penyaringan radikal dan sebatian flavonoid. Kajian menunjukkan bahawa kandungan fenolik terutamanya sebatian flavonoid menyumbang kepada aktiviti antioksidan pada tumbuhan kajian.

Kata kunci: Aktiviti antioksidan; tumbuhan tropikal; sebatian bioaktif, sebatian fenolik; asid askorbik

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This thesis was submitted to the Senate of Universiti Putra Malaysia and has been accepted as fulfilment of the requirement for the degree of Master of Science. The members of the Supervisory Committee were as follows:

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DECLARATION

I declare that the thesis is my original work except work for quotations and citations which have been duly acknowledged. I also declare that it has not been previously, and is not currently, submitted for any other degree at Universiti Putra Malaysia or at any other institution.

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ROHEEYATI ASHIKIN MUSTAFA

Date:

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LIST OF ABBREVIATIONS

BHA	Butylated hydroxyanisole
BHT	Butylated hydroxytoulene
DNA	Deoxyribonucleic acid
DPPH	1,1-diphenyl-2-picrylhydrazil
GAE	Gallic acid equivalent
HAT	Hydrogen atom transfer
HCl	Hydrochloric acid
KOH	Potassium hydroxide
LDL	Low density lipoprotein
MARDI	Malaysian Agricultural Research and Development Institute
MDA	Malondialdehyde
mM	Milimolar
PG	Propyl gallate
ppm	Part per million
PUFA	Polyunsaturated fatty acid
RDA	Recommended dietary allowance
SET	Single electron transfer
TBA	Thiobarbituric acid
TBHQ	Tert-butylhydroquinone
TFA	Triflouroacetic acid

CHAPTER I

INTRODUCTION

Lipid oxidation is a highly deteriorative process in foods, as it leads to unacceptable properties of the products and lost in shelf life, palatability, functionality and nutritional quality. Loss of palatability in foods is partly due to the generation of off flavours that arise primarily from the breakdown of unsaturated fatty acid during autoxidation (Jadhav et al., 1996). The high reactivity of the carbon double bonds in unsaturated fatty acids makes these substances primary target for free radical reactions. The chain of autoxidation reactions includes initiation, propagation and termination. Propagation reactions are primarily responsible for the autocatalytic nature of autoxidation. *In vivo*, lipid oxidation may play a role in cancer, atherosclerosis, Alzheimer's and cardiovascular diseases (Wojdylo et al., 2007).

Antioxidants have been added in food for years to prevent the process of oxidation and are widely used today for food preservation. It can affect different stages of an oxidative sequence. Antioxidant can be specific or has multiple sites of action either as reducing agent, free radical scavenger, quenching of singlet oxygen and potential complexing of pro-oxidant metal. A number of specific reactions can be used to monitor the different molecular mechanism of antioxidants (Chen et al., 2007).

A number of synthetic antioxidants such as BHA (Butylated hydroxyanisole), BHT (Butylated hydroxytoulene), TBHQ (tert-butylhydroquinone) and propyl gallate have been added in foodstuffs during processing to suppress lipid peroxidation and consequently to improve food quality and stability. Since consumers are concerned about long term safety and negative perception of the used of synthetic antioxidants

in lipid containing food, there is an interest in identifying alternative natural and safe sources of antioxidant especially from plant origin. Studies have shown that many fruits, vegetables, spice, grains and herbs exhibited strong antioxidant capacities which are attributed to the presence of active compounds or phytochemicals in the plants (Thaipong et al., 2006).

Plants contain a variety of active compound with unique chemical properties. Phenolic compounds (flavonoids, phenolic acids and tannins), nitrogen-containing compounds (alkaloids, chlorophyll derivatives, amino acids, peptides and amines), carotenoids, tocopherols or ascorbic acids and its derivatives play important role in antioxidant properties (Velioglu et al., 1998). Phenolic compounds become very interesting to the food industry because of their capability to retard the oxidative degradation of lipid. This bioactivity of phenolics may be related to their abilities to act as efficient free radical scavenging, reducing agents, hydrogen donor, singlet oxygen and metal chelator (Rice- Evans et al., 1996).

Flavonoids have been proven to display a wide range of pharmacological and biochemical action such as antimicrobial, antithrombotic, antimutagenic and anticarcinogeni activities (Turkoglu et al., 2007). In food systems, flavonoids can act as free radical scavengers and terminate the radical chain reactions that occur during the oxidation of triglycerides. Therefore, they present antioxidative efficiency in oils, fats and emulsions (Roedig-Penman and Gordon, 1998).

Ascorbic acid shows antioxidant potential by acting as chain breaking scavenger for peroxy radicals and also to act synergistically with tocopherol by donating a

hydrogen atom to the tocopherol-derived phenolate radical, thus regenerating its activity. Tocopherol is one of the best quenchers for singlet oxygen and chain-breaking antioxidant. Singlet oxygen is powerfully quenched by carotenoids, especially β- carotene.

Malaysia has an amazing diversity of plant species. Some of them have long been used as traditional medicine. Medicinal plant parts (leaves, roots, barks, flowers, stems and fruits) commonly rich in phenolic compound have multiple biological effects including antioxidant activity. The plants, *Andrographis paniculata, Boesenbergia rotunda, Centella asiatica, Cosmos caudatus, Curcuma xanthorrhiza, Gynura procumbens, Justicia gendarussa, Kaempferia galanga, Lawsonia inermis, Melicope lunu, Morinda citrifolia, Murraya koenigii, Pereskio bleo, Piper betel, Piper longum, Pluchea indica, Premna cordifolia, Psophocarpus tetragonolobus, Sesbania grandiflora, Talinum triangulare* and *Vitex negundo* are local herbs in Malaysia, well known for their medicinal properties. However, scientific information on these plants were lacking and some were not available or reported.

The herbs were examined as potential sources of bioactive compounds within the framework of this research project. Therefore, the objectives of this study are:

1. To evaluate the antioxidant activity of selected tropical plants utilizing different methods including formation of conjugated diene, free radical scavenger, formation of malondialdehyde (MDA) and chelating activity on ferrous ions.

2. To identify and determine the bioactive compounds including phenolic compounds, flavonoids (flavanol, flavones, flavonol and flavonones groups), β-carotene, lycopene, α-tocopherol and ascorbic acids in the plants.
3. To evaluate the relationship between bioactive compounds and antioxidant activity in the plants.