UNIVERSITI PUTRA MALAYSIA

IMPACT OF ENVIRONMENTAL AND PHYSIOLOGICAL PARAMETERS ON ANTIOXIDANT ACTIVITY IN PREMNA SERRATIFOLIA L.

IRIS CHUA YIEN PING

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IRIS CHUA YIEN PING

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By

IRIS CHUA YIEN PING

Thesis Submitted to the School of Graduate Studies,
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TABLE OF CONTENTS

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

IMPACT OF ENVIRONMENTAL AND PHYSIOLOGICAL PARAMETERS ON ANTIOXIDANT ACTIVITY IN *PREMNA SERRATIFOLIA* L.

By

IRIS CHUA YIEN PING

November 2014

Chair: Patricia King Jie Hung, PhD

Faculty: Agriculture and Food Sciences (Bintulu)

Premna serratifolia L. is believed to have antioxidant properties. However, the scientific research on this species is still lacking. This study was conducted to assess the influence of environmental (light intensity, air temperature, relative humidity) and physiological (photosynthetic rate, transpiration rate and stomata conductance) parameters on the antioxidant content in *P. serratifolia* L.. Fresh leaves at the lower part of *P. serratifolia* L. were collected at 6 am, 9 am, 12 noon, 3 pm and 6 pm on the third consecutive sunny day at the botanical garden, Universiti Putra Malaysia Bintulu Sarawak Campus. Light intensities, air temperature (T_a), relative humidity (RH), photosynthetic rate (A), stomata conductance (g_s) and transpiration rate (E) of *P. serratifolia* L. were measured *in situ* on the day of leaf collection. The antioxidant activities were measured by 1,1-diphenyl-2-picryhydrazyl (DPPH) radical method while total flavonoid (TF) content and total phenolic (TP) were estimated by using aluminium chloride reagent and Folin-Ciocalteu reagent, respectively. The antioxidant compounds present in the leaf extracts of *P. serratifolia* L. with the highest or lowest antioxidant activity (accessed by DPPH antioxidant activity, TP and TF content) was identified by using High Performance Liquid Chromatography (HPLC). Results showed that leaf extracts collected at 9 am had the highest DPPH antioxidant activity and the lowest TF and TP contents. On the other hand, leaf extracts collected at 12 noon had the highest TF and TP contents but the lowest DPPH antioxidant activity. Correlation test showed that DPPH antioxidant activity, TF and TP content were influenced significantly by air temperature. However, other environmental parameters, namely light intensities and physiological parameters were not significantly correlated with DPPH antioxidant activity, TF and TP content in *P. serratifolia* L.. Antioxidant compounds were divided into heat induced and non-heat induced as some antioxidant compounds were found to be significantly correlated with temperature. The identified antioxidant compounds that were possibly heat induced included theaflavins, epigallocatechin, epicatechin and ascorbic acid while the non-heat induced were catechin, quercetin, uric acid and rutin. In conclusion, consideration on environmental parameter, namely air temperature is crucial in order to obtain high antioxidant compounds.

Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia sebagai memenuhi keperluan untuk Ijazah Sarjana Sains

KESAN PARAMETER ALAM DAN FISIOLOGI KE ATAS AKTIVITI ANTIOKSIDAN DALAM *PREMNA SERRATIFOLIA* L.

Oleh

IRIS CHUA YIEN PING

November 2014

Pengerusi: Patricia King Jie Hung, PhD

Fakulti: Sains Pertanian dan Makanan (Bintulu)

Premna serratifolia L. dipercayai mempunyai ciri-ciri antioksidan. Namun, penyelidikan saintifik pada masa ini tentang spesies ini masih kekurangan. Kajian ini dijalankan untuk menilai pengaruh parameter alam (keamatan cahaya, suhu udara dan kelembapan relatif) dan fisiologi (kadar fotosintesis, stomata kealiran dan kadar transpirasi) ke atas kandungan antioksidan *P. serratifolia* L.. Daun segar *P. serratifolia* L. yang berada di bahagian bawah dikumpul pada 6 pagi, 9 pagi, 12 tengahari, 3 petang dan 6 petang pada hari ketiga berturut-turut menerima cahaya penuh di taman botani, Universiti Putra Malaysia Kampus Bintulu Sarawak. Keamatan cahaya, suhu udara (T_a), kelembapan relatif (*RH*), kadar fotosintesis (*A*), stomata kealiran (g_s) dan kadar transpirasi (*E*) bagi *P. serratifolia* L. diukur secara *in situ* semasa pengumpulan daun. Aktiviti antioksidan ditentukan oleh kaedah *cerakinan 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical* manakala jumlah kandungan flavonoid (*TF*) dan phenolik (*TP*) dikuantitikan masing-masing dengan reagen aluminium klorida dan *Folin-Ciocalteu*. Kuantifikasi antioksidan bagi sampel daun yang mempunyai kandungan antioksidan, *TF* dan *TP* tertinggi atau terendah dilakukan dengan menggunakan Kromatografi Cecair Prestasi Tinggi (*HPLC*). Keputusan menunjukkan ekstrak daun yang dikutip pada pukul 9 pagi mempunyai aktiviti antioksidan DPPH yang tertinggi tetapi kandungan *TF* dan *TP* yang terendah. Ekstrak daun yang dikutip pada pukul 12 tengahari mempunyai kandungan *TF* dan *TP* yang tertinggi tetapi aktiviti antioksidan yang terendah. Ujian korelasi menunjukkan aktiviti antioksidan *DPPH*, kandungan *TF* dan *TP* dipengaruhi dengan ketara oleh T_a. Walau bagaimanapun, parameter alam sekitar seperti keamatan cahaya dan parameter fisiologi yang lain tidak mempuyai hubungan yang ketara dengan aktiviti antioksidan DPPH, kandungan *TF* dan *TP*. Antioksidan dibahagikan kepada antioksidan haba teraruh dan bukan haba teraruh memandangkan antioksidan yang dikenal pasti didapati berkolerasi dengan suhu. Antioksidan yang dikategorikan sebagai haba teraruh termasuk theaflavins, epigallocatechin, epicatechin dan asid askorbik manakala antioksidan bukan haba teraruh ialah catechin, quercetin, asid uric dan rutin. Kesimpulannya, pertimbangan ke atas parameter alam, iaitu suhu udara adalah sangat penting untuk mendapatkan kandungan antioksidan yang tinggi.

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

Patricia King Jie Hung, PhD
Senior Lecturer
Faculty of Agriculture and Food Sciences
Universiti Putra Malaysia
(Chairman)

Ong Kian Huat, PhD
Senior Lecturer
Faculty of Agriculture and Food Sciences
Univerisiti Putra Malaysia
(Member)

Yiu Pang Hung, PhD
Associate Professor
Faculty of Agriculture and Food Sciences
Universiti Putra Malaysia
(Member)

_____
BUJANG KIM HUAT, PhD
Professor and Dean
School of Graduate Studies
Universiti Putra Malaysia

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Signature: _____
Name of
Chairman of
Supervisory
Committee: Dr. Patricia King Jie Hung

Signature: _____
Name of
Member of
Supervisory
Committee: Dr. Ong Kian Huat

Signature: _____
Name of
Member of
Supervisory
Committee: Assoc. Prof. Dr. Yiu Pang Hung

LIST OF TABLES

LIST OF FIGURES

LIST OF ABBREVIATIONS

ROS	Reactive Oxygen Species
ATP	Adenosine triphosphate
Q^-	Semiquinone
G	Guanine
T	Thymine
AsA	Ascorbate
GSH	Gluthathione
GSSG	Gluthathione disulfide
$^1O_2$	Singlet oxygen
^{3}Chl*	Triplet sensitizer
Chl*	Excited chlorophyll
CAT	Catalase
APX	Ascorbate peroxidase
MDHA	Monodehydroascorbate
SOD	Superoxide dismutase
MDHAR	Monodehydroascorbate reductase
FAD	Flavin adenine dinucleotide
DHAR	Dehydroascorbate reductase
GR	Glutathione reductase
GPX	Guaiacol peroxidase
kDa	kilodalton
IAA	Indoleacetic acid
TNF-α	Tumor necrosis factor alpha
DPPH	1,1-diphenyl-2-picryl-hydrazil
BHA	butylated hydroxyanisole
TP	total phenolic
TF	total flavonoid
HPLC	High Performance Liquid Chromatography
T_a	air temperature
RH	relative humidity
A	photosynthetic rate
E	transpiration rate
g_s	stomata conductance
NADPH	nicotinamide adenine dinucleotide phosphate-oxidase

CHAPTER 1

INTRODUCTION

1.1 Introduction

The reactive oxygen species (ROS) have been the companions which are not welcomed by aerobic life ever since the molecular oxygen (O_2) is being introduced into our atmosphere by O_2-evolving photosynthetic organisms ~2.7 billion years ago (Mittler *et al.*, 2004). In contrast to O_2, free radicals such as superoxide anion (O_2^-), hydroxyl radical (HO•), singlet oxygen ($^1O_2$) and hydrogen peroxide (H_2O_2) are highly reactive and toxic. These free radicals can result in the oxidative destruction of cells.

About 1% of O_2 consumed by plants is estimated to be diverted to produce ROS in organelles with a highly oxidizing metabolic activity or with an intense rate of electron flow, namely chloroplasts, mitochondria and microbodies (Sharma *et al.*, 2012). The Mehler reaction and the antenna pigments are the primary sources of ROS production in chloroplasts (Mittler *et al.*, 2004). Lipid catabolism produces H_2O_2 as a side-product of fatty acid oxidation in microbodies. Over-reduction of the electron transport chain in mitochondria is the main source in producing O_2^- under specific stress conditions. This ROS production is likely to occur mostly in complexes I and III of the mitochondrial electron transport chain (de Carvalho, 2008). Other additional sources of ROS production in plant cells include the detoxifying reactions that are catalyzed by cytochromes in both the endoplasmic reticulum and cytoplasm (Mittler *et al.*, 2004).

Posmyk *et al.* (2005) found out that antioxidants in plants have the potential in protecting cellular damage induced especially due to the formation of free radical and ROS. Many of these phytochemicals varies in their content with varying plants, time of herbal part collection and others. According to Hemm *et al.* (2004), environment factors such as light intensity and CO_2 concentration, leaf maturity, plant age have influence on synthesis of flavonoids and phenolics in plants. Plant phenolics show marked qualitative and quantitative variation at different genetic levels (Joubert *et al.*, 2008) and between different physiological and developmental stages (Bunning *et al.*, 2010). Besides that, they vary in response to environmental factors, namely light intensity and availability of nutrient as well (Kotilainen *et al.*, 2010, Larbat *et al.*, 2012).

Light is known to adjust plant growth and development. Besides, it also regulates both primary and secondary metabolites biosynthesis (Ghasemzadeh *et al*., 2010b). Previous studies by Graham (1998) showed that changes in light intensity were capable in changing the production of flavonoids and phenolic in herbs. Karimi *et al.* (2013) reported that the existence of interspecific differences to micro-environment

may influence accumulation of plant and distribution of total phenolics and flavonoid compounds.

Therefore, it would be interesting to examine the effect of light intensities, other environmental and physiological parameters on antioxidant activity. This study was conducted to identify the antioxidant compounds in *Premna serratifolia* L. which are capable in protecting cellular damage caused by free radical and ROS formation. Besides, the relationships between the light intensities, temperature, relative humidity, photosynthetic rate, transpiration rate, stomata conductance and antioxidant activity of *P. serratifolia* were determined.

1.2 Research Objectives

The objectives of this study are:

i. To quantify and identify the antioxidant present in *P. serratifolia* L.; and

ii. To investigate the effect of time collection and influence of light intensities, temperature, relative humidity, photosynthetic rate, transpiration rate and stomata conductance on the antioxidant activity of *P. serratifolia* L..

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BIODATA OF STUDENT

Iris Chua Yien Ping was born to Chua King Ming and Khoo Bee King in Sibu, Sarawak. She is the third among the four siblings. She started her education at S.R.B. Sacred Heart Chinese in 1993. During her primary years of schooling, she was very active and took part in *Pertandingan Reka Cipta Model Sains dan Kemahiran Hidup untuk Sekolah-Sekolah Rendah Daerah Sibu*. She continued her secondary study at Methodist Secondary School from 2000 to 2006. She gained 8A for her PMR (*Penilaian Menengah Rendah*) in 2002, 8A and 3B for SPM (*Sijil Pelajaran Malaysia*) in 2004, and successfully completed STPM (*Sijil Tinggi Persekolahan Malaysia*) with 1A and 3B. During her secondary education, she took part in several marching competitions organized by *Ibu Pejabat PBSMM, Cabang Sibu*. Apart from that, she took part in Malaysian National Chemistry Quiz in 2004 and 2006. She was vice secretary of Red Crescent and Sixth Form Society during her secondary study. Besides that, she was the committee member of *Kelab Pembimbing Rakan Sebaya*, an officer of *Kem Persatuan-Persatuan Beruniform* and a prefect in form 6. She received her B.Sc. in Agricultural Science from Universiti Malaysia Sabah in 2011. She involved in the stage decoration of UMS 12nd Convocation and underwent her internship at *Rimbunan Sawit Berhad*. She has had experience as a school teacher. She started her Master of Science at Universiti Putra Malaysia Bintulu Sarawak Campus in 2011. The topic of her M.Sc. research is "Impact of Environmental and Physiological Parameters on Antioxidant Activity in *Premna serratifolia* L." under the supervision of Dr. Patricia King Jie Hung. This research is basically to determine the influence of environmental and physiological parameters on the antioxidant activities in *Premna serratifolia* L..

LIST OF PUBLICATION

Chua, I.Y.P., King, P.J.H., Ong, K.H., Sarbini, S.R. and Yiu, P.H. Influence of Light Intensity and Temperature on Antioxidant Activity in *Premna serratifolia* L. *Journal of Soil Science and Plant Nutrition*. Accepted.