UNIVERSITI PUTRA MALAYSIA

IN VIVO AND IN VITRO STUDIES OF ANTI-AGING PROPERTIES OF Dendrobium sabin FLOWER

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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

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

IN VIVO AND *IN VITRO* STUDIES OF ANTI-AGING PROPERTIES OF *Dendrobium sabin* FLOWER

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Chair: Che Norma Binti Mat Taib, PhD

Faculty: Medicine and Health Sciences

Exposure to UV radiation has induced reactive oxygen species (ROS) which enhanced the photo-induced aging of the skin characterized by wrinkles appearance. The antioxidant is one of the effective approaches to avoid from photo-induced aging problem. *Dendrobium sabin* flower was investigated for its antioxidant and anti-aging properties. The oven-dried *Dendrobium sabin* flower was extracted using 100% methanol (w/v), 100% ethanol (w/v) and 100% water (w/v). The 100% methanolic crude extract showed the highest total phenolic content (40.33 ± mg GAE/g extract) and the best antioxidant properties as showed by DPPH, ABTS and FRAP assays. A correlation relationship between antioxidant activity and total phenolic content showed that phenolic compounds were the dominant antioxidant components in this flower extract. The microbial fermentation on *Dendrobium sabin* flower showed a potential in increasing the phenolic content and DPPH-scavenging activity. The TPC of final fermented medium showed the 18% increment while the DPPH of fermented medium increased significantly to approximately 80% at the end of the fermentation. The treatment on brine shrimp and human dermal fibroblast primary cell using 100% methanolic crude extract demonstrated insignificant toxicity level. Topical application of 50 mg/ml of 100% methanolic crude extract showed improvement in epidermis and dermis thickness, which was comparable to the positive control treatment effect. The potential of the antioxidant and anti-aging properties found in *Dendrobium sabin* flower would help in treating undesirable photo-induced aging problems.

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

KAJIAN *IN VIVO* DAN *IN VITRO* KE ATAS CIRI-CIRI ANTI-PENUAAN BUNGA *Dendrobium Sabin*

Oleh

FARAHZIELA BINTI ABU

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Pengerusi: Che Norma Binti Mat Taib, PhD

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Pendedahan kepada sinaran UV mendorong pembentukan spesies oksigen reaktif yang menyebabkan penuaan kulit serta kehadiran kedutan. Antioksida ialah salah satu cara yang berkesan untuk mengelak dari masalah penuaan sebegini. Bunga *Dendrobium sabin* diuji untuk mengenalpasti ciri-ciri antioksida dan anti-penuaan. Bunga *Dendrobium sabin* yang dikeringkan menggunakan ketuhar telah diekstrak menggunakan 100% methanol, 100% etanol, dan 100% air. Ekstrak dari 100% metanol menunjukkan kandungan fenolik yang tertinggi (40.33 ± mg GAE / ekstrak g) serta ciri-ciri antioksida terbaik seperti yang ditunjukkan oleh ujian-ujian DPPH, ABTS dan FRAP. Hubungan kolerasi antara aktiviti antioksida dan kandungan fenolik menunjukkan sebatian fenolik adalah komponen dominan dalam ektrak bunga ini. Penapaian bakteria ke atas bunga *Dendrobium sabin* menunjukkan potensi peningkatan terhadap kandungan fenolik dan kegiatan antioksida ujian DPPH. Jumlah kandungan fenolik meningkat kepada 18% manakala antioksida ujian DPPH meningkat kepada 80% pada akhir proses penapaian bunga tersebut menggunakan bakteria. Rawatan ke atas udang dan sel fibroblast dermis manusia menggunakan ekstrak 100% methanol menunjukkan tahap ketoksikan yang rendah. Sapuan ekstrak 100% methanol dengan kepekatan sebanyak 50 mg/ml secara topikal menunjukkan penambahbaikan terhadap ketebalan epidermis dan dermis yang hampir menyerupai rawatan kontrol positif. Potensi ciri ciri antioksida dan anti-penuaan yang ditemui pada bunga *Dendrobium sabin* akan membantu dalam merawat masalah-penuaan yang tidak menyenangkan.

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Thank you.

I certify that a Thesis Examination Committee has met on 21 November 2016 to conduct the final examination of Farahziela binti Abu on her thesis entitled "*In vivo* and *in vitro* studies of anti-aging properties of *Dendrobium sabin* flower" in accordance with the Universities and University Colleges Act 1971 and the Constitution of the Universiti Putra Malaysia [P.U.(A) 106] 15 March 1998. The Committee recommends that the student be awarded the Master of Sciences.

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TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF APPENDICES

LIST OF ABBREVIATIONS

ABTS	2,2-azino-bis(3-ethylbenzthiazoline-6-sulphonic acid)
ACUC	Animal Care and Use Committee
BSLT	Brine Shrimp Lethality Test
C 100	100% methanolic crude extract
C A	Acidified methanolic crude extract
DMSO	Dimethyl sulfoxide
DNA	Deoxyribonucleic acid
DPPH	2,2-dipenyl-1-picrylhydrazyl
DPX	Distyrene Plasticizer Xylene
DS	*Dendrobium sabin*
DSF	*Dendrobium sabin* flower
DSF ECE	*Dendrobium sabin* flower ethanolic crude extract
DSF MCE	*Dendrobium sabin* flower methanolic crude extract
DSF WCE	*Dendrobium sabin* flower water crude extract
EA 100	Ethyl acetate layer partition from 100% methanolic crude extract
EA A	Ethyl acetate layer partition from acidified crude extract
ECM	extracellular matrix
FBS	Fetal Bovine serum
FRAP	Ferric reducing antioxidant power
GAE	Gallic Acid Equivalent
GAGs	Glycoaminoglycans
H 100	Hexane layer partition from 100% methanolic crude extract
H A	Hexane layer partition from acidified methanolic crude extract
Hdf-a	Human dermal fibroblast- adult
HME	human macrophage elastase

HNE	human neutrophile elastase
HPLC	High Performance Liquid Chromatography
MMPs	matrix metalloproteinases
MTT	3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide
OD	Optical density
PBS	Phosphate buffered saline
ROS	reactive oxygen species
rpm	Revolutions per minute
SD	Standard deviation
SDS	Sodium dodecyl sulphate
SOD	superoxide dismutases
SPSS	Statistical Package for the Social Sciences
TPC	Total Phenolic Content
UV	Ultraviolet
W 100	water layer partition from 100% methanolic crude extract
W A	water layer partition from acidifed methanolic crude extract

CHAPTER 1

INTRODUCTION

1.1 Background of Study

Normal cell metabolism is continuously producing dangerous by-product substances known as free radicals. Natural antioxidants produced within the cells are acting energetically to neutralize the harmful effect of free radicals. However, the additional antioxidants substances are still required by the body to fight the worst consequence of free radicals (Halliwell, 1996). Additional antioxidants can possibly be acquired for the body by eating fruits and vegetables that are highly rich with antioxidants. It is also possible to take antioxidant supplements such as vitamin C (L-ascorbic acid), vitamin E (α-tocopherol) and β-carotene to assist in reducing excessive free radicals within the body (Kerscher and Buntrock, 2011).

Aging is caused by few changes in organisms. Human will undergo physiologically, hormonally, mentally and physically alterations with time. There is a proposed theory that relates the free radicals with aging. Harman (1956) proposed that free radicals are the substance that caused such changes to occur with the facts that the free radicals are the main cause of cellular damages (DNA, protein and lipid). Continuous of cellular damages lead to aging process. Therefore, aging process is highly demanding for anti-aging supplements or products with antioxidant base which can eradicate the free radicals. These antioxidant supplements subsequently will slow down the aging process and at the same time increase a healthier life span of human. The interventions of the aging process also include the nutritional regimen, hormonal supplementation, physical modification, pharmological approach and genetic modification (Yu, 1999).

Despite having massive variety of size, colour and design, orchid has a huge number of varieties. *Dendrobium sabin* (DS) is one of 1200 Orchid species from *Dendrobium* genome which has not been explored yet. There is very limited study on DS previously. The identification number given by Herbarium Centre, Universiti Putra Malaysia for DS flower was SK 1966/11. DS is one of the excellent models of successful *Dendrobium* breeding (Thammasiri, 1997). Previous study has shown that there are correlations between antioxidant supplementation and longevity (Yu, 1999). Few preliminary observations on this DS flower show that it can live longer as compared to any other *Dendrobium* species. Thus, there is a positive possibility that relates the life span of DS with antioxidant compounds enclosed in it as well as having potential as

anti-aging. Thus, the main purpose of this study is to investigate the anti-aging properties of DS flower.

1.2 Problem Statement

It has been known for years that much exposure to sun has increased UV radiation effect towards skin, the first layer of skin in human body. Much exposure generates huge number of radicals which degrade the collagen and elastin fibre hence increase the wrinkles production and contribute to aging process. All these will require an investigation to seek for antioxidant to combat the free radicals and subsequently delay and reduce the aging process.

There are also varieties of cosmetic product nowadays which promote the ability of delaying aging process. However, there are few concerns from the consumers who are against chemical based in cosmetic. The enormous number of chemical used in cosmetic product nowadays might contribute to cancer diseases. Hence, this study has decided to take an initiative to study the possible bioactive compounds from the natural products, whilst use them as the basic ingredients in the cosmetics. It is our hope that the cosmetic companies can minimise the usage of chemical compounds in cosmetic products and therefore reduce the cost of chemical used.

1.3 Objectives

Main objective

1. To investigate the antioxidant and anti-aging properties of *Dendrobium sabin* (DS) flower extract.

Specific objectives

1. To determine the total phenolic content in DS flower in crude and partition extract.
2. To evaluate the antioxidant capacity of DS flower crude and partition extract by using:
    a) 1,1-Diphenyl-2-picrylhydrazyl (DPPH) radical scavenging activity assay.
    b) (2,20-azino-bis(3-ethylbenzthiazoline- 6-sulfonic) acid) (ABTS) radical cation-scavenging assay.
    c) Ferric Reduction Antioxidant Power (FRAP) assay.
3. To evaluate the antioxidant activity in DS flower by using microbial fermentation.

4. To determine the toxicity level of DS flower using human dermal fibroblast-adult cell culture and brine shrimp lethality test.
5. To investigate the anti-wrinkle properties of DS flower extract *in-vivo* by using rat's dorsal skin

1.4 Hypothesis

1. *Dendorbium sabin* (DS) flower crude and partition extract will show significant total phenolic content and high antioxidant capacity.
2. Microbial fermentation will assists in increasing the antioxidant capacity of DS flower.
3. DS flower crude extract will shows insignificant toxicity towards human dermal fibroblast-adult cell and brine shrimp.
4. DS flower crude extract will shows anti-wrinkle properties on dermis and epidermis of rat's dorsal skin

1.5 Significance of Study

The phenolic compounds which possess antioxidant capacity are present abundantly in this plant. The first part of this research will focus on determining the antioxidant capacity in the DS flower. With these antioxidant properties findings, DS flower can be further developed for anti-aging purposes, which can then be commercialized as cosmetic products. Thus, this research is conducted to contribute and transform the outcomes into commercial cosmetic products which can be promoted for human use.

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BIODATA OF STUDENT

My name is Farahziela binti Abu, was born on 3rd June 1987 at Hospital Daerah Kluang, Johor. I was studying at Sekolah Kebangsaan Relong, Kuala Lipis for primary school. After achieving 5As in UPSR, I got an offer to Sekolah Menengah Sains Sultan Haji Ahmad Shah (SEMSAS), Kuantan Pahang. With 10A's result during my SPM in 2004, I continued my study to International Education Centre (INTEC) Shah Alam in 2005 as my preparation for continuing degree in BSc majoring in Biochemistry at Otago University of New Zealand. After degree graduation in December 2009, I further my master level in Universiti Putra Malaysia, majoring in Pharmachology and Toxicology. During my fourth semester (April 2013), I've got an offer to government service as Scientific Officer (Biochemist) C41 at Hospital Kuala Lumpur and thus I changed my study mode from full time to part time student. Even though I am working, I pushed myself to come to UPM during weekend to finish up my lab works. After I've got married in December 2014 and transferred to Hospital in Terengganu, I still made an effort to come to UPM to finish up my thesis writing. Along the journey as master student, I had attended a conference (International Anatomical and Biomedical Sciences IABS in August 2015) and get involved in Poster Presentation. I always wondering what happened around and love to know something by doing research. I believe that research could help to give better understanding about Allah's creations and could also been beneficial for human being.

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