UNIVERSITI PUTRA MALAYSIA

ANTIOXIDATIVE PROPERTIES OF SELECTED MALAYSIAN HERBAL PLANTS

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FBSB 2014 25

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MASTER OF SCIENCE
UNIVERSITI PUTRA MALAYSIA

2014

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By

SUMAZIAN BT YUSUF

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

April 2014

DEDICATION

This thesis dedicated to my parents, Yusuf Isa and Haminah Hamat, my sisters, Suriani Yusuf and Suhazilah Yusuf and my brother, Suhaifa Yusuf, last but not least to my beloved husband Mohd Zulkefly Ramli and all of my family members who have been a great support, motivation and prayers since the beginning of my studies.

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

ANTIOXIDATIVE PROPERTIES OF SELECTED MALAYSIAN HERBAL PLANTS

By

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April 2014

Chairperson : Professor Maziah Mahmood, PhD

Faculty : Biotechnology and Biomolecular Sciences

Antioxidant activities of nine herbal plants which are commonly used in Malaysia as folk medicines, namely *Barringtonia racemosa* (putat), *Curcuma domestica* (kunyit), *Kaempferia galanga* (cekur), *Hydrocotyle bonariensis* (pegaga embun), *Centella asiatica* (pegaga), *Piper betel* (sireh), *Polygonum minus* (kesum), *Piper sarmentosum* (kadok) and *Cosmos caudatus* (ulam raja) were examined. All of the herbs selected for all readily available for everyday use with a variety of uses well known since time immemorial. The study was conducted to see the appropriate dose to be used for commercial purposes. The compounds in leaf and rhizome were extracted using hot water (100 ^{0}C), fresh water and ethanol (80%), separately. Total antioxidant content were measured using ferric reducing power (FRAP), 1,1 diphenyl -2- picrylhydrazyl free radical scavenging assay (DPPH) and β-carotene bleaching assay. Subsequently, the relevant antioxidant compounds; total carotenoids, total ascorbic, total flavonoids and total phenolic. Enzymatic antioxidants include superoxide dismutase (SOD), ascorbate peroxidase (APX), glutathione reductase (GR), catalase (CAT) and peroxidase (POX) activities were determined. Anti-aging enzymes activities; anti-tyrosinase, anti-elastase and anti-hyaluronidase were also determined using spectrophotometry method. For FRAP assay, hot water extract of *C. domestica* (623.87 ± 0.01 mmol Fe^{2+}/g DW) showed good activity and fresh water extract of *K. galanga* exhibited a very strong antioxidant properties in DPPH assay with (83.27 ± 0.01%). *H. bonariensis* leaf extract has been recognized to contain high antioxidant activity with (19.04 ± 0.01 %) using β-carotene bleaching assay. The rhizome extract of *C. domestica* showed the highest total carotenoid content of (0.04 ± 0.01 mg/mg FW) and leaf extract of *P. sarmentosum* indicated (1.72 ± 0.01 mg/g FW) for the highest total of ascorbic acid content. Level of total flavonoids in the herbal plants varied between (0.42 ± 0.00 and 6.28 ± 0.01 mg/g DW) and those of total phenolics between (2.78 ± 0.01 and 12.35 ± 0.01 mg/g DW). For enzymatic antioxidant activities, the results showed that *C. domestica* (0.42 ± 0.01 µmol/min/g FW), *P. sarmentosum* (0.14 ± 0.01 µmol/min/g FW), *C. asiatica* (0.76 ± 0.01 µmol/min/g FW), *H. bonariensis* (0.92 ± 0.01 µmol/min/g FW) and *P. minus* (0.38 ± 0.01 µmol/min/g FW) were higher in SOD, APX, GR, CAT and POX activity, respectively. The highest percentage of anti-tyrosinase activity is in fresh water extract of *C. domestica* rhizome (97.86 ± 0.02 %) using L-tyrosine as substrate. *C.*

asiatica leaf exhibited a very good level of elastase and hyaluronidase inhibition with (84.53 ± 0.02%) and (81.01 ± 0.01%), respectively. The findings showed the potential of nine medicinal plants as powerful antioxidant that can be used in producing herbal based product in cosmetically and pharmaceutical.

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

PENILAIAN ANTIOKSIDAN DALAM TUMBUHAN HERBA TERPILIH DI MALAYSIA

Oleh

SUMAZIAN BT YUSUF

April 2014

Pengerusi : Profesor Maziah Mahmood, PhD

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Aktiviti-aktiviti antioksidan di dalam sembilan jenis tumbuhan herba di Malaysia yang popular sebagai ubatan tradisional dikenali sebagai *Barringtonia racemosa* (putat), *Curcuma domestica* (kunyit), *Kaempferia galanga* (cekur), *Hydrocotyle bonariensis* (pegaga embun), *Centella asiatica* (pegaga), *Piper betel* (sireh), *Polygonum minus* (kesum), *Piper sarmentosum* (kadok) dan *Cosmos caudatus* (ulam raja) telah diuji. Kesemua herba yang dipilih kerana semuanya senang didapati untuk kegunaan harian juga dikenali dengan pelbagai kegunaan semenjak dahulu lagi. Kajian dijalankan untuk melihat dos yang sesuai untuk digunakan bagi tujuan komersial. Pengestrakan daun dan rizom ditunjukkan melalui tiga kaedah berlainan iaitu air panas (100 ^{0}C), air dan etanolik (80%). Jumlah antioksidan diukur secara penurunan ferik (FRAP), '1,1 diphenyl -2-picrylhydrazyl (DPPH) pengikatan radikal bebas dan penyekatan pelunturan β-karotin. Untuk kandungan antioksidan yang relevan pula termasuk jumlah karotenoid, asid askorbik, flavonoid dan fenolik. Manakala aktiviti antioksidan enzim pula adalah seperti 'Superoxide dismutase (SOD)', 'Ascorbate peroxidase (PRX)', 'Glutathione reductase (GR)', 'Catalase (CAT)' dan 'Peroxidase (POX)'. Di samping itu, jumlah kandungan gula (hidrolisis dan bukan hidrolisis) serta enzim anti-penuaan seperti 'anti-tyrosinase', 'anti-elastase' dan 'anti-hyaluronidase' juga dianalisa menggunakan spektrofotometer. Untuk kaedah FRAP, estrak air panas *C. domestica* (623.87 ± 0.00 mmol $^{Fe2+}$/g berat kering) menunjukkan aktiviti terbaik dan estrak air *K. galanga* menunjukkan aktiviti antioksidan paling kuat untuk kaedah DPPH iaitu (83.27 ± 0.01%). Manakala *H. bonariensis* didapati mengandungi aktiviti antioksidan paling tinggi iaitu (19.04 ± 0.01%) melalui pelunturan β-karotin. Ekstrak rizom *C. domestica* menunjukkan jumlah karotenoid tertinggi iaitu sebanyak (0.04 ± 0.01 mg/g berat basah) dan estrak daun *P. sarmentosum* menunjukkan (1.72 ± 0.01 mg/g berat basah) untuk kandungan asid askorbik tertinggi. Paras kandungan flavonoid dalam pelbagai tumbuhan herba adalah antara (0.42 ± 0.01 dan 6.28 ± 0.01 mg/g berat kering) manakala jumlah fenolik pula adalah antara (2.78 ± 0.01 dan 12.35 ± 0.01 mg/g berat kering). Aktiviti-aktiviti antioksidan enzim telah didapati didalam *C. domestica* (0.42 ± 0.01 μmol/min/g berat basah), *P. sarmentosum* (0.14 ± 0.01 μmol/min/g berat

basah), *C. asiatica* (0.76 ± 0.01 μmol/min/g berat basah), *H. bonariensis* (0.92 ± 0.01 μmol/min/g berat) dan *P. minus* (0.38 ± 0.01 μmol/min/g berat basah) masing-masing tertinggi didalam SOD, APX, GR, CAT dan POX. Peratusan aktiviti anti-tyrosinase yang tertinggi adalah (97.86 ± 0.02%) didalam estrak air rizom *C. domestica* dimana L-tyrosine digunakan sebagai substrat. Ekstrak-ekstrak daun *C. asiatica* yang berbeza menunjukkan perencatan enzim elastase dan hyaluronidase yang sangat baik iaitu masing-masing dengan (84.53 ± 0.02%) dan (81.01 ± 0.01%). Penemuan ini telah menunjukkan potensi sembilan ekstrak herba sebagai antioksidan yang berkuasa dan berguna dalam penghasilan produk-produk yang berasaskan herba di dalam industri kosmetik dan farmaseutikal.

ACKNOWLEDGEMENTS

Alhamdulillah, praise be to Allah for His blessing for me to finish up this thesis. This task would not be complete and possible without help, co-operation and encouragement of a large number of peoples in my life. Special thanks to my supervisor, Prof Dr. Maziah Mahmood and my co supervisor, Dr Syahida Ahmad for their guidance, encouragement, help and patience throughout the whole project.

My graceful thank to all my lab mates for their help, comments, critics and recommendation. Special thanks also to Mohd Hakiman for the help on editing my thesis. I also would like to thank to Universiti Putra Malaysia for Grant Research Fellowship (GRF) for two years of financial support. Lastly, I would like to express my appreciation to my family for their encouragement, understanding, advice and support.

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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Abs	Absorbance
$AlCl_3$	Aluminium chloride
APX	Ascorbate peroxidase
BHA	Butylated hyroxyanisole
BHT	Butylated hydrotoluene
BSA	Bovine Serum Albumin
CAT	Catalase
Cu	Copper
Cd	cadmium
℃	Degree Celcius
DCPIP	2,6-dichloroindophenol
DPPH	2, 2-diphenyl-1-picrylhydrazyl
DNA	Deoxyribonucleic acid
DNS	3,5-dinitrosalicylic acid
DNTB	5, 5-dithio-bis-(2-nitrobenzoic acid)
DW	Dry weight
EDTA	Ethylenediaminetetraacetic acid
et al.,	*at alli* and other people
Fe	Ferum
Fe^{2+}	Ferrous
$FeCl_3$	Ferum chloride
FRAP	Ferric reducing antioxidant power

FW	Fresh weight
G	Gram
GAE	Gallic acid
GlcNAc	N-acetyl-D-glucosamine
GlcUAc	d-glucuronic acid
GR	Glutathione reductase
GSH	Glutathione disulphide
GSSG	Glutathione sulfhydryl
HCl	Hydrochloric acid
Hr	Hour
H_2O	Water
H_2O_2	Hydrogen peroxide
KCN	Potassium cyanide
KOH	Potassium hydroxide
Mg	Milligram
Mg/L	Milligram per liter
Min	Minute
Ml	Milliliter
mM	Millimolar
Mn	Manganese
Mg	Microgram
Ml	Microliter
Mmol	Micromol
Na_2CO_3	Sodium carbonate
NADPH	Glutamate synthase

$NaNO_2$	Sodium nitrate
NaOH	Sodium hydroxide
NBT	Nitroblue tetrazolium salt
Ni	Nickel
nm	Nanometer
O_2^-	Superoxide
Pb	Plumbum
POX	Peroxidase
PVPP	Polyvinylpolypyrrolidone
%	Percentage
rpm	Rotation per minute
ROS	Reactive oxygen species
SD	Standard deviation
SOD	Superoxide dismutase
TPTZ	Tripyridyltriazine
UV	Ultraviolet
v/v	volume per volume
w/v	weight per volume
Zn	Zinc

CHAPTER 1

INTRODUCTION

Medicinal herbs have long been used as natural source of medicines. Since a long time ago in the past era, herbs were used in traditional therapy, cosmetics and health supplements. According to WHO report, between the years of 1991 to 2001, Malaysia is among the nine countries in this world that gave big amount of sales in herbal medicine where the market of herbal and natural products in Malaysia was estimated to worth approximately RM 10 billion and the rate were increasing about 8% per year in 2008 (Effendy *et al*., 2012).

Plants are sources of antioxidants that counteract reactive oxygen species (ROS) which include activated and/or free radical oxygen compounds, such as singlet oxygen, superoxide anion radical, hydroxyl radical and hydrogen peroxide (Huda-Faujan *et al*., 2007). These species can attack tissues in the dermis or epidermis which cause skin aging and DNA damage (Shon *et al*., 2003). Therefore, antioxidants from plant origins are increasing in demand in the market as source of natural antioxidants since artificial antioxidants such as butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT), and tertiary butylhydroquinone (TBHQ) which are widely used in the industry are toxic and carcinogenic.

In Malaysia, 20% (3,000 species) of the *angiosperma* and *gymnosperma* plant species have been used for traditional treatments for ages and was reported to have medicinal benefits for a long time ago (Zainol *et al*., 2003). Since herbs have high commercial value, improvement of its quality is important due to its medicinal properties. It was reported that 10-15% of the known higher plant species had been investigated for their important bioactive compounds (Zarate *et al*., 2001). Due to the potential of plants for medicinal and cosmetically purposes, the biochemical compounds need to be investigated using various techniques because there is lack of information on its biochemical constituents that has been reported. Plant antioxidants are believed to play a role in delaying aging process, because of the several compounds such as flavonoids that contribute to antioxidative properties (Harbone and Williams, 2000). This compound was commonly being used as skin improvement products and their applications in cosmetic industries have also been established. Vitamin C and β-carotene also contribute to antioxidant properties (Chanwitheesuk *et al*., 2005; Lisiewska *et al*., 2006). In addition, the compounds can be produced at reasonabe low costs, especially when the right natural sources have been identified, for example, anti-wrinkle products which contain elastase and hyaluronidase inhibitors have been sold in the market.

During aging process, the elasticity of the skin decreases because of elastase enzyme that leads to sagging decreases. At the same time, hyaluronic acid also decreases, causing the skin to become dry and wrinkle. The best way to maintain youthful skin is to degrade the enzymes that can cause aging. Tyrosinase is an enzyme inhibitor that can inhibit melanisation process of human skin (Lim et al., 2009). There are a few numbers of synthetic and natural tyrosinases that have been determined but some activities are not significant or suitable which do not comply with the safety regulations for food and cosmetic additives. Therefore, there is a need to find and determine new tyrosinase inhibitors with high activities and decrease the side effects at the same time (Yan *et al*., 2009). Herbal products are known to have interesting benefits, and they are largely unexplored sources of new and potential drugs. It is for this reason that the emphasis has been given on the potential of herbal medicines for new skincare cosmetics development (Kiken and Cohen, 2002). There are certain plant metabolites which are believed to have the capability to inhibit tyrosinase, hyaluronidase and elastase enzymes such as whitening cream and lotion from *Areca cathecu* or locally known as 'pinang' (Binic *et al*., 2013).

There are many potential herbs that can be commercialized in Malaysia and most of them are subjected to extensive research. In this study, nine species of medicinal herbs were determined the total antioxidants and anti-aging inhibitors activities. They were *Barringtonia racemosa* (Lecythidaceae) or (putat), *Curcuma domestica* (Zingiberaceae) or (kunyit), *Kaempferia galanga* (Zingiberaceae) or (cekur), *Hydrocotyle bonariensis* (Apiaceae) or (pegaga embun), *Centella asiatica* (Apiaceae) or (pegaga), *Piper betel* (Piperaceae) or (sireh), *Piper sarmentosum* (Piperaceae) or (kadok), *Polygonum minus* (Polygonaceae) or (kesum) and *Cosmos caudatus* (Compositae) or (ulam raja). The selection of plant samples for phytochemical screening is generally based on their own special characteristics for traditional anti-aging treatments. All these nine plants also can be found easily in market and popular among Malaysian peoples. This study may provide information regarding the ranges of antioxidative activity and general antioxidant compounds of the selected herbal plants toward the standardization. The informations in this study will be useful in preparing the herbal formulations for health supplements.

The biochemical properties of selected herbs were carried out by analysing the important biochemical compounds which include total antioxidant activities using different method such as Ferric Reducing Antioxidant Power (FRAP), Free Radical Scavenging Assay (DPPH), total flavonoids and phenolic. Besides that, β-carotene, carotenoids and ascorbic acid also determined. In addition, there are enzyme activities such as superoxide dismutase, ascorbate peroxidase, glutathione reductase, catalase, peroxidase and enzyme inhibitors which include tyrosinase, hyaluronidase and elastase activity.

The objectives are:

1. To evaluate the total antioxidant contents of selected Malaysian herbs such as FRAP, DPPH and β-carotene.

2. To examine the content of antioxidant compound: total carotenoid, total ascorbic acid, total flavonoids and total phenolic.

3. To determine activity of the enzymatic antioxidants such as superoxide dismutase, ascorbate peroxidase, glutathione reductase, catalase and peroxidase.

4. To evaluate the tyrosinase, hyaluronidase and elastase enzyme inhibitors in the selected Malaysian herbs.

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