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ANTIOXIDANT AND TOXICITY SCREENING OF MALAYSIAN TRADITIONAL HERBS

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ANTIOXIDANT AND TOXICITY SCREENING OF MALAYSIAN TRADITIONAL HERBS

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PENGESAHAN

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ABSTRACT

Malaysia is a biodiverse country rich in many herbs with desirable medicinal value and therapeutic properties. Therefore, the objective of the present study was to screen the antioxidant properties and toxicity effects of selected Malaysian traditional herbs. Eleven plant samples were selected based on their traditional uses and pharmacological properties. These plants were extracted using 80% methanol and screened for their antioxidant activities by both DPPH and FRAP antioxidant assays while the toxicity effects of the plant crude extracts were accessed using Zebrafish (*Danio rerio*) embryo and larvae toxicity assays. Results showed that three out of eleven plant crude extracts which were *Melastoma malabathricum* (leaf), *Polygonum minus* (leaf) and *Ficus deltoidea* (leaf) demonstrated high radical scavenging activities using DPPH assay with IC_{50} values of 120.17 ± 9.25 µg/mL, 118.57 ± 9.86 µg/mL and 267.53 ± 17.24 µg/mL, respectively. In addition, *Melastoma malabathricum* (leaf), *Polygonum minus* (leaf) and *Ficus deltoidea* (leaf) showed the highest total antioxidant activity using FRAP assay with IC_{50} values of 4.75 ± 0.12 µg/ml, 10.91 ± 1.42 µg/ml and 28.92 ± 1.54 µg/ml, respectively. *Melastoma malabathricum* (leaf), *Polygonum minus* (leaf) and *Ficus deltoidea* (leaf) showed moderate to slightly toxicity effects toward zebrafish embryo with LC_{50} values of 210.18 ± 27.36 µg/ml, 185.75 ± 7.84 µg/ml and 39.85 ± 1.73 µg/ml, respectively and LC_{50} values of 106.10 ± 3.54 µg/ml, 357.71 ± 27.96 µg/ml and 180.43 ± 30.22 µg/ml, respectively toward zebrafish larvae. Thus, our present results have shown that these three herbs might have the potential to be developed as herbal products to treat various oxidative-based diseases due to their high antioxidant activities *in vitro* with moderate to slightly toxicity effects *in vivo* using zebrafish embryo model.

ABSTRAK

Malaysia adalah sebuah negara yang kaya dengan kepelbagaian biologi dan mempunyai banyak tumbuhan herba yang mempunyai nilai perubatan dan sifat-sifat terapeutik. Oleh itu, objektif kajian ini adalah untuk menyaring ciri antioksidan dan kesan ketoksikan herba tradisional Malaysia terpilih. Sebelas sampel herba telah dipilih berdasarkan kegunaan tradisional mereka. Sampel herba kemudian diekstrak dengan 80% metanol dan disaring untuk aktiviti antioksidan menggunakan DPPH dan FRAP asai antioksidan manakala kesan ketoksikan diuji dengan menggunakan zebrafish (*Danio rerio*) embrio dan larva asai ketoksikan. Hasil kajian menunjukkan bahawa tiga daripada sebelas ekstraks iaitu *Melastoma malabathricum* (daun), *Polygonum minus* (daun) dan *Ficus deltoidea* (daun) menunjukkan aktiviti antioksidan paling tinggi dengan nilai IC_{50} iaitu 120.17 ± 9.25 µg/mL, 118.57 ± 9.86 µg/mL dan 267.53 ± 17.24 µg/mL masing-masing dalam DPPH asai antioksidan. Di samping itu, *Melastoma malabathricum* (daun), *Polygonum minus* (daun) dan *Ficus deltoidea* (daun) menunjukkan aktiviti antioksidan yang tertinggi dalam FRAP asai dengan nilai-nilai IC_{50} sebanyak 4.75 ± 0.12 µg/mL, 10.91 ± 1.42 µg/mL dan 28.92 ± 1.54 µg/mL, masing-masing. *Melastoma malabathricum* (daun), *Polygonum minus* (daun) dan *Ficus deltoidea* (daun) menunjukkan kesan sederhana ketoksikan kepada embrio zebrafish dengan nilai LC_{50} iaitu 210.18 ± 27.36 µg/mL, 185.75 ± 7.84 µg/mL dan 39.85 ± 1.73 µg/mL masing-masing dan nilai LC_{50} iaitu 106.10 ± 3.54 µg/mL, 357.71 ± 27.96 µg/mL dan 180.43 ± 30.22 µg/mL, masing-masing kepada larva zebrafish. Oleh itu, kajian ini telah menunjukkan bahawa ketiga-tiga herba ini mempunyai potensi untuk dihasilkan sebagai produk herba untuk merawat pelbagai penyakit berasaskan oksidatif kerana mereka mempunyai aktiviti antioksidan yang tinggi dan kesan ketoksikan sederhana kepada model zebrafish.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF ABBREVIATIONS

%	Percentage
abs	Absorbance
AD	Alzheimer’s disease
ALS	Amyotrophic lateral sclerosis
C. longa	*Curcuma longa*
C. nutans	*Clinacanthus nutans*
Cat	Catalase
cm	Centimeter
DPPH	2,2-diphenyl-1-picrylhydrazyl
DPPH-H	2,2-diphenyl-1-picrylhydrazine
E3M	Embryo media
et al	And friends
F. deltoidea	*Ficus deltoidea*
FRAP	Ferric reducing antioxidant power assay
g	Gram
g/mL	Gram per milliliter
GPx	Glutathione peroxidase
GR	Glutathione reductase
H_2O_2	Hydrogen peroxide
$HO_2^{\cdot}$	Hydroperoxyl radical
HOCL	Hypochlorus
hpf	Hours post fertilization
IC_{50}	Half inhibitory concentration
LC_{50}	Half lethal concentration
m	Meter
M. koenigii	*Murraya koenigii*

M. malabathricum	*Melastoma malabathricum*
mg/mL	Milligram per milliliter
mM	Millimolar
µg/mL	Microgram per milliliter
µL	Micromolar
nm	Nanometer
NO^-	Nitroxyl anion
$NO^·$	Nitric oxide
NO^+	Nitrosanium cation
NOS	Nitric oxide synthase
$O_2^{·-}$	Superoxide radical
$OH^·$	Hydroxyl radical
$ONOO^-$	Peroxynitrite
P. foetida	*Paederia foetida*
P. macrocarpa	*Phaleria macrocarpa*
P. minus	*Polygonum minus*
PD	Parkinson's disease
ppm	Parts per million
RO_2^-	Peroxyl radical
RNS	Reactive nitrogen species
ROS	Reactive oxygen species
SOD	Superoxide dismutase
TPTZ	2,4,6-tri(2-pyridyl)-5-triazine
TR	Thioredoxin reductase
v/v	Volume over volume
w/w	Weight over volume

CHAPTER 1

INTRODUCTION

For the past decades or so, increasing deaths caused by oxidative-based diseases such as cancer, diabetes, neurodegenerative disorder, cardiovascular disease, inflammation as well as aged-related disease have drawn attentions from many authorities. Many studies have been done to identify and discover the origin cause of oxidative-based diseases (Harman, 1992). Free radical, an unstable and highly reactive molecular species has an unpaired electron in its atomic orbital. They act as oxidants and they are able to accept electrons from other molecular species. It is ironic to find out that free radical source from oxygen, which is an important element in respiration metabolism can cause adverse effect in the body at certain situation. The production of free radicals come from external sources as well as internal sources. The generation and accumulation of free radicals increase with age and also other condition such as chronic inflammation, injuries, trauma and toxins. The antioxidant defense system are so crucial that they are responsible in removing excess free radicals in human body. However, oxidative stress occurs when production of free radicals exceed the elimination of free radicals which will result in a wide range of biological molecules damage like lipid peroxidation, DNA damage and protein oxidation (Lobo *et al*., 2010; Niki, 2008).

Antioxidant is a stable compound that is responsible in scavenging excess free radicals. They are radical scavengers, reductants and metal chelating agents. There are two types of antioxidants exist in human body, the enzymatic antioxidants (superoxide dismutase, catalase and glutathione peroxidase) and, non-enzymatic antioxidants such as vitamins (ascorbic acid, alpha-tocopherol and beta-carotene), melatonin, selenium, glutathione, and so much more (Lu and

Holmgren, 2013). Synthetic antioxidants such as BHA (Butylhydroxyanisole) and BHT (Butylhydroxytoulene) were available in the market and they were widely used in food production, therapeutical and cosmetic industry. However, studies showed that the over consumption of these synthetic antioxidants bring carcinogenic effects to body. They can cause cancer. Drugs utilized as treatment for oxidative-based diseases like neurodegenerative disease, cardiovascular disorder and cancer showed low effectiveness and some of them can cause side effects. Therefore, attention was focus on alternative sources such as natural antioxidants (Papas, 1999). High antioxidant properties of vegetables, fruits and herbs were contributed by their bioavailable secondary metabolites. These secondary metabolites are polyphenol compounds, flavonoids, vitamins and so on. Also, natural products show pharmacological effects like antioxidant, anti-inflammatory, anti-microbial, anti-viral, anti-fungal and anti-carcinogenic properties due to their unique phytochemicals (Krishnaiah *et al*., 2007).

Malaysia was blessed because of its rich rainforest biodiversity. Up to 2000 species of herbs from Malaysia have been identified to have medicinal value and therapeutical properties. Examples of herbs that have been developed into herbal products are Tongkat Ali (*Eurycoma longifolia*) and Kacip Fatimah (*Labisia pumila*) (Kadir *et al.*, 2013).

Zebrafish (*Danio rerio*) has been recognized worldwide as an ideal model organism for drug discovery and development due to its unique and favourable features. Their high fecundity, observable ex-utero embryonic development, transparent body and most of all, high level of physiological and anatomical homology with human allow researchers to evaluate the impact and toxicity of a potential toxic chemical compound. At the same time, high similarities in gene homologs allow them to be used for human disease studies (Allen and Neely, 2010).

It is believed that there are many herbs in Malaysia have not yet been explored. Investigations have to be taken to discover herbs with high antioxidant activities which have potential to be developed into herbal product in the future. At the same time, their toxic potentials should be evaluated using zebrafish model organism.

The objectives of the study are:

1) To evaluate the antioxidant properties of plant crude extracts using DPPH and FRAP antioxidant assays.
2) To screen the toxic potential of plant crude extracts via *in vivo* toxicity assays using zebrafish (*Danio rerio*) embryo and larvae.

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