UNIVERSITI PUTRA MALAYSIA

GENETIC DIVERSITY, VOLATILE CONSTITUENTS, CYTOTOXICITY AND GENOTOXICITY OF SELECTED AROMATIC PLANT SPECIES FROM AYER HITAM FOREST RESERVE

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By

SITI RAHIMAH BINTI JUMAAT

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

September 2017

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DEDICATION

In the name of Allah S.W.T., the most Benevolent and ever Merciful

All praise be to Allah S.W.T.

Special dedicated to:

My Parents

JUMAAT WAGIMIN & MISNAH ROSLAM

and

My Sisters

SITI NOOR FADZLINA JUMAAT

NOOR MAISURAH JUMAAT

NOOR JALILAH JUMAAT

NOOR MASTURAH JUMAAT

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

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September 2017

Chairman : Associate Professor Rozi Mohamed, PhD
Faculty : Forestry

Medicinal and aromatic plants (MAPs) are widely valued for their aromas, tastes, and remedies of various human illnesses. The Ayer Hitam Forest Reserve (AHFR) contains a high diversity of MAPs, which potential has not been explored. This study was conducted to assess the genetic diversity of MAPs at AHFR and to identify the chemical composition of several of them. Ten species of MAPs from eight families (Annonaceae, Asteraceae, Burseraceae, Lauraceae, Myrtaceae, Piperaceae, Rutaceae, and Thymelaeaceae) were collected based on morphological identification and subjected to DNA sequencing using the intergenic spacer region *psb*A-*trn*H and the nuclear ribosomal internal transcribed spacer 2 (ITS2). They were selected based on their medicinal and aromatic properties, which have been utilized by the aborigines at AHFR. DNA sequences of both regions had high similarities to their respective species in the Genbank. Phylogenetic trees were constructed and analyzed using Maximum Likelihood (ML) criteria together with 61 additional sequences of MAPs incorporated from the Genbank. Through molecular approach, species identity of these MAPs can be ascertained before employing their properties.

The volatile constituents and toxicity content of three aromatic plant species, *Syzygium polyanthum* Wight (Walp.), *Monocarpia marginalis* (Scheff.) J. Sinclair, and *Chromolaena odorata* (L.) R.M. King & H. Rob, were determined, via Gas Chromatography (GC) with Flame Ionization Detector (FID). These species were chosen because of their distinct sensory characteristics with pleasant and strong odor of the leaves and stems. Altogether, 116 compounds were identified. Sesquiterpenes like *α*-cadinol, *α*-cedrene, cubenol, farnesol, ledol, trans-*β*-nerolidol, and *γ*-muurolene were abundantly found in both

essential oils and crude extracts of three species. These genuine aromatic compounds display their potential on the significant properties primarily for fragrance and pharmaceutical purposes. To validate the safety consumption and application of these plant extracts, toxicity evaluations were carried out on human Peripheral Blood Mononuclear Cells (PBMCs). Three plant extracts were toxic to human PBMCs; the essential oils of *M*. *marginalis* and *C*. *odorata*, and hexane extract of *C*. *odorata* yielded IC_{50} and LD_{50} values of 76 mg/mL and 6,913 mg/kg, 14 mg/mL and 3,684 mg/kg, and 2.45 mg/mL and 1,927 mg/kg, respectively. The LD_{50} values indicated that the *M*. *marginalis* and *C*. *odorata* extracts were classified as slightly and moderately hazardous, respectively, for the oral and dermal hazardous levels according to the category of toxic chemicals set forth by the World Health Organization. A detailed toxicity evaluation via comet assay proved that all of the extracts of *M*. *marginalis* and *C*. *odorata* induced significant DNA damage ($p < 0.05$), while none of the *S*. *polyanthum* extracts exerted genotoxicity. Screening the phytochemical and toxicity contents of plants is crucial before their appropriateness for human consumption and application can be considered.

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

KEPELBAGAIAN GENETIK, KANDUNGAN MERUAP, SITOTOKSISITI DAN GENOTOKSISITI DARIPADA SPESIES TUMBUHAN BERAROMA TERPILIH DARI HUTAN SIMPAN AYER HITAM

Oleh

SITI RAHIMAH BINTI JUMAAT

September 2017

Pengerusi : Profesor Madya Rozi Mohamed, PhD
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Tumbuhan ubatan dan beraroma (MAPs) berkembang secara meluas dan bernilai kerana aroma, rasa, dan kegunaan mereka dalam merawat pelbagai penyakit manusia. Hutan Simpan Ayer Hitam (HSAH) mengandungi kepelbagaian MAPs yang tinggi, di mana potensinya masih belum diterokai. Kajian ini dijalankan untuk menilai kepelbagaian genetik MAPs di HSAH dan untuk mengenalpasti komposisi kimia daripada beberapa spesies. Sepuluh spesies MAPs daripada lapan keluarga (Annonaceae, Asteraceae, Burseraceae, Lauraceae, Myrtaceae, Piperaceae, Rutaceae, dan Thymelaeaceae) telah dikumpulkan dari HSAH berdasarkan pengenalan morfologi dan tertakluk kepada penjujukan DNA dengan menggunakan *psb*A-*trn*H dan nuklear ribosom penjarak jujukan dalam 2 (ITS2). Spesies yang dipilih adalah berdasarkan ciri-ciri perubatan dan aromatik mereka yang telah digunakan oleh orang asli di AHFR pada zaman dahulu. Jujukan DNA daripada kedua-dua rantau mempunyai persamaan tinggi dengan spesies masing-masing yang terdapat di *GenBank*. Pokok filogenetik kedua-dua rantau telah dibina dan dianalisis menggunakan kriteria *Maximum Likelihood* (ML) bersama-sama dengan 61 jujukan tambahan diperbadankan dari *GenBank*. Melalui pendekatan molekul ini, identiti spesies MAPs ini boleh ditentukan sebelum memanipulasi nilai mereka.

Kandungan meruap dan tahap ketoksikan daripada tiga MAPs, *Syzygium polyanthum* Wight (Walp.), *Monocarpia marginalis* (Scheff.) J. Sinclair, dan *Chromolaena odorata* (L.) R.M. King & H. Rob, telah dikenal pasti melalui gas kromatografi (GC) dengan pengesanan pengionan nyalaan (FID). Spesies-spesies ini dipilih kerana ciri-ciri mereka iaitu bau yang menyenangkan terutama pada daun dan batang. Sebanyak 116 kandungan telah dikenalpasti.

Sesquiterpena seperti *α*-cadinol, *α*-cedrene, cubenol, farnesol, ledol, trans-*β*-nerolidol, dan *γ*-muurolene telah banyak dijumpai dalam kedua-dua minyak pati dan ekstrak mentah dalam ketiga-tiga spesies. Sebatian aroma tulen memaparkan potensi mereka kepada sifat-sifat yang ketara terutamanya untuk tujuan wangian dan farmaseutikal. Bagi mengesahkan keselamatan dalam penggunaan ekstrak tumbuhan, Penilaian ketoksikan dalam spesies tumbuhan telah dijalankan ke atas sel darah putih manusia (PBMCs). Tiga ekstrak tumbuhan didapati toksik pada PBMCs; minyak pati *M*. *marginalis* dan *C*. *odorata*, dan ekstrak heksana *C*. *odorata* menghasilkan nilai IC_{50} dan LD_{50} masing-masing sebanyak 76 mg/mL dan 6,913 mg/kg, 14 mg/mL dan 3,684 mg/kg, dan 2.45 mg/mL dan 1,927 mg/kg. Nilai LD_{50} menunjukkan bahawa *ekstrak M. marginalis* dan *C. odorata* diklasifikasikan sebagai sedikit berbahaya dan sederhana berbahaya untuk tahap berbahaya lisan dan kulit mengikut kategori bahan kimia toksik yang ditetapkan oleh Pertubuhan Kesihatan Sedunia (WHO). Satu penilaian ketoksikan terperinci melalui *comet assay* membuktikan bahawa kesemua ekstrak *M*. *marginalis* dan *C*. *odorata* mendorong sejumlah besar kerosakan DNA yang ketara ($p < 0.05$), manakala tiada ekstrak daripada *S*. *polyanthum* yang mengalami kesan genotoksik. Saringan kandungan fitokimia dan ketoksikan tumbuhan adalah penting sebelum kesesuaiannya untuk kegunaan manusia boleh dipertimbangkan.

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I certify that a Thesis Examination Committee has met on 14 September 2017 to conduct the final examination of Siti Rahimah binti Jumaat on her thesis entitled "Genetic Diversity, Volatile Constituents, Cytotoxicity and Genotoxicity of Selected Aromatic Plant Species from Ayer Hitam Forest Reserve" 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 Science.

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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:

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Name of Member of Supervisor Committee: Ummi Hani Abdullah

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α-	Alpha
β-	Beta
γ-	Gamma
δ-	Delta
τ-	Tau
°C	Degree celsius
µg	Microgram
µL	Microliter
µm	Micrometre
AHFR	Ayer Hitam Forest Reserve
CCD	Charged coupled device
cm	Centimetre
dbh	Diameter at breast height
DMSO	Dimethyl sulfoxide
DNA	Deoxyribonucleic acid
E	East
eV	Electric vehicle
FID	Flame ionization detector
g	Gram
GC	Gas chromatography
h	Hour
IC_{50}	Inhibitory concentration
ITS2	Internal transcribed spacer 2
LD_{50}	Lethal dose
m	Metre
mg	Milligram
min	Minute
ML	Maximum likelihood
mL	Millilitre
MAPs	Medicinal and aromatic plants
MS	Mass Spectrometry
MTT	3-(4,5-Dimethylthiazol-2-Yl)-2,5-Diphenyltetrazolium Bromide
N	North
NaCl	Sodium chloride
NaOH	Sodium hydroxide
NCBI	National Center for Biotechnology Information
ng	Nanogram
nm	Nanometre
OTM	Olive tail moment
RI	Retention index
rpm	Revolutions per minute
RPMI	Roswell Park Memorial Institute
s	Second
SISFEC	Sultan Idris Shah Forestry Education Centre
TAE	Tris base, acetic acid and EDTA

UNIDO	United Nations Industrial Development Organization
UPM	Universiti Putra Malaysia
UV	Ultraviolet
WHO	World Health Organization

CHAPTER 1

INTRODUCTION

1.1 General

Plants have an extensive history of medicine, perfumery, and cosmetics utilization, but only recently, the economic value of such plants has been appreciated (Schippmann *et al.,* 2002). Approximately 75% of rural people in the world depend on medicinal plants for their principal health care (Debnath *et al.,* 2006). From recent estimation conducted by World Health Organization (WHO), more than 3.5 billion people in developing countries have been utilizing medicinal plants for various health treatments (Xego *et al.,* 2016).

In Southeast Asia, there are approximately 6,500 species regarded as herbs and spices (Narayanaswamy and Ismail, 2015). Malaysia is one of the tropical rainforest countries that has plentiful sources of medicinal and aromatic plants (MAPs) (Roslen *et al.,* 2014). There are over 6,000 MAPs species from tropical forest across the country, and 1,300 species from 550 genera are in Peninsular Malaysia. Most of these MAPs are useful as human daily resources and dietary supplements. Local community has been applying these natural products extensively until now. Hence, these resources should not be wasted without maximum utilization on commercial basis (Zakaria and Mohd, 2010).

During human antiquity, various ailments have been treated using nature materials. Nature is extensively important as source of medicine and huge amounts of natural sources have been commercialized as modern drugs. The acceptance of medicinal plants has increased because of the effectiveness, reasonable, and belief that natural cures are more reliable than conventional drugs (Alsarhan *et al.,* 2014). An increased reliance towards medicinal plants can be attributed to the development of plant-based drugs and chemotherapeutics from plants, and herbal remedies for the conservation of good health. Although the preparation of the medicines are quite complicated, medicinal plant remedies have become mainstream for the treatment of almost all minor illness and several major ailments (Augustino *et al.,* 2014).

The usages of aromatic plants are widely spread throughout the world as food flavorings, cosmetics and fragrance. In Malaysia, aromatic plants are primarily utilized in food and beverages, spices, perfumes, and toiletries. The cosmetics products derived from aromatic plants are regulated under Sale of Drugs Act 1952 (Revised 1989) and the Control of Drugs and Cosmetics Regulations 1984

(amended 2009) (Adnan and Othman, 2012). Several plants with their known aromatic and medicinal properties are exploited and composed as commercial products like skin and hair care as well as medicated cosmetics (Narayanaswamy and Ismail, 2015). However, the development of MAPs products is time-consuming because the commercial supply of MAPs requires consistency in quality and volume (Sher *et al.,* 2015). World Health Organization's commercial assurance has taken measures by producing proper guidelines on pharmaceutical manufacturing of MAPs (WHO, 2003).

1.2 Problem Statements

Documentation of MAPs throughout the world are continuing constantly. However, in these few years, forest urbanization and deforestation have been operated illegally resulting in an enormous loss of forest products particularly MAPs (Ong *et al.,* 2011). In Malaysia, only few documentations of MAPs have been found, which limit the research on the conservation and utilization of their properties. Limited information of MAPs documentation in Ayer Hitam Forest Reserve (AHFR) has been discovered. In the past decades, there are only 98 species of medicinal plants are found in AHFR. Documenting the indigenous knowledge through ethnobotanical studies is important for sustainable utilization of MAPs products. Several active compounds have been discovered from plants based on ethnobotanical information. It is of common knowledge that chemical composition of a plant is highly affected by various factors such as the method of extraction, solvent used, and experimental conditions such as temperature and length of incubation, and many more. Despite the beneficial effects attributed to MAPs, growing awareness has been existing on the hazards associated with the use of plants and their extracts as remedies. With the diverse growth factors and environments, the chemicals should be clarified and toxicity tested, including both cellular and genomic levels.

1.3 Justification

MAPs can also be lethal to humans and animals due to the presence of toxic compounds in the plant itself. Species screening is significant to extract the potential toxins that could be dangerous for consumption hence revising their appropriateness to enter the market. Therefore, species documentation is a mandatory for further use in establishing aromatic and medicinal plant properties. The candidate components that are appealing for final use as novel aromas in fragrance industries can be discovered. This aspect of the extract has never been explored before from any of the studied species.

1.4 Objectives

The general objective of this study is to determine several aromatic plant species properties using chemical approaches including essential oil and crude extractions, and Gas Chromatography Flame Ionization Detector (GC-FID) technique.

The specific objectives of this study include:

1. To discover the genetic diversity of ten MAPs from AHFR using DNA sequencing of the *psb*A-*trn*H and ITS2 regions.
2. To determine the volatile constituents of the selected aromatic plant species via GC-FID.
3. To evaluate the cytotoxicity and genotoxicity levels of the selected aromatic plant species on human Peripheral Blood Mononuclear Cells (PBMCs).

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