UNIVERSITI PUTRA MALAYSIA

GENETIC DIVERSITY OF SELECTED AROMATIC PLANTS IN AYER HITAM FOREST RESERVE, SELANGOR

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GENETIC DIVERSITY OF SELECTED AROMATIC PLANTS IN AYER HITAM FOREST RESERVE, SELANGOR

By

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DEDICATION

This is dedicated to my family and friends.

My deepest gratitude to my beloved parents; Mr. Mat Desa @ Mohd Wari Bin Haron and Mrs. Laili Binti Mohd Hashim, and to my family members; Nor Lailinda Hanum Binti Mat Desa, Mohd Azizi Bin Mat Desa, Nor Lailian MaimonHayu Binti Mat Desa, Nor Lily Maria Hadijah Binti Mat Desa and Mohamad Azrai Fitri Bin Mat Desa for their supports and loves. Not to be forget, my late sister Nor Lailenney Marlina Hanim Sara Binti Mat Desa, our love and pray always be with you.

I also dedicate this dissertation to my supervisor, Associate Professor Dr. Rozi Mohamed and my Forest Biotechnology's Colleagues.
Last but not least, I dedicate this dissertation to Dr. Razak for his wise and knowledge in evaluating this documentation of the project.

May God Bless All of Us

ABSTRACT

Malaysia is rich in aromatic plant species but they have not been fully explored. During an exploration in October 2015 at Ayer Hitam Forest Reserve (AHFR), Selangor, several plant species were identified from their ability to produce strong aroma. They included *Trema angustifolia* (Cenderai/Mengkirai), *Cinnamomum iners* (Medang Teja), *Actinodaphne sphaerocarpa* (Medang Payung), *Syzygium polyanthum* (Serai Kayu), *Prismatomeris glabra* (Tongkat Haji Samad), *Monocarpia marginalis* (Mempisang) and *Chromolaena odorata* (Kapal Terbang). Morphology of each species was described and DNA samples were isolated to provide for genetic information. DNA samples were extracted using FavorPrep Plant Genomic DNA Extraction Mini Kit. The DNA *psb*A-*trn*H region was amplified in PCR and sequenced. Sequence analysis and searches data at GeneBank's database revealed that many of Malaysian aromatic plant species have not been sequenced. This is the first report on genetic diversity of aromatic plants from AHFR. More efforts should be taken to document these plant species for potential use in producing aromatic-essential products.

ABSTRAK

Malaysia kaya dengan kepelbagaian spesis tumbuhan aromatik, namun masih belum diteroka sepenuhnya. Dalam satu ekspedisi pada Oktober 2015 di Hutan Simpan Ayer Hitam (HSAH), Selangor, beberapa spesis tumbuhan telah dikenalpasti berupaya menghasilkan aroma yang kuat. Spesies-spesies tersebut adalah *Trema angustifolia* (Cenderai/Mengkirai), *Cinnamomum iners* (Medang Teja), *Actinodaphne sphaerocarpa* (Medang Payung), *Syzygium polyanthum* (Serai Kayu), *Prismatomeris glabra* (Tongkat Haji Samad), *Monocarpia marginalis* (Mempisang) dan *Chromolaena odorata* (Kapal Terbang). Ciri-ciri morfologi kesemua species direkod dan sampel DNA digunakan bagi merekod maklumat genetik. Sampe DNA diekstrak dengan menggunakan *FavorPrep Plant Genomic DNA Extraction Mini Kit*. Struktur DNA *psb*A-*trn*H dikuatkan melalui PCR dan disusun. Analisis turutan DNA dan hasil carian data di pangkalan data GeneBank menunjukkan bahawa masih banyak spesis tumbuhan aromatik yang belum disusun dan direkod. Ini merupakan laporan pertama tentang kepelbagaian genetik spesis tumbuhan aromatik di HSAH. Tindakan lanjut dalam pendokumentasian spesis-spesis ini perlu diambil bagi kegunaan masa hadapan terutama dalam pembangunan produk berasaskan tumbuhan aromatik.

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APPROVAL SHEET

I certify that this research project report entitled Genetic Diversity of Selected Aromatic Plants in Ayer Hitam Forest Reserve, Selangor by Mohamad Azren Putra Bin Mat Desa has been examined and approved as a partial fulfillment of the requirements for the degree of Bachelor of Forestry Science in the Faculty of Forestry, Universiti Putra Malaysia.

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LIST OF ABBREVITION

AHFR	Ayer Hitam Forest Reserve
BLAST	Basic Loci Alignment Tool
bp	Base pair
cpDNA	Chloroplast DNA
DNA	Deoxyribonucleic acid
MAPs	Medicinal and Aromatic Plants
MEGA	Molecular Evolutionary Genetics Analysis
NCBI	National Centre for Biotechnology Information
NJ	Neighbor-Joining
PCR	Polymerase Chain Reaction
RNase	Ribonuclease
SISFEC	Sultan Idris Shah Forestry Education Centre
sp.	Species
TAE	Tris-acetate-EDTA
UCMP	University of California Museum of Paleontology
UPM	Universiti Putra Malaysia
USA	United State of America
UV	Ultraviolet

CHAPTER ONE

INTRODUCTION

1.1 General Background

Plants have various types of uses. Due to the variety uses of plants, they are playing big role as economic contributor in most of the countries that relaying on agriculture-based products. Other than as source of food, plants are also used as sources for non-food industrial products. Plants are the provider of textiles and fabric materials, which we make clothes from them. The raw materials especially woods are used world widely such as for constructions, buildings, crafts and furniture. Plants are also used in the production of biomass for energy resource. In other hand, many plant species are cultivated for their secondary metabolites, which useful for production of fine chemicals and other specialty products (Lubbe & Verpoorte, 2011).

Plants that used primarily for their medicinal or aromatic properties in pharmacy and perfumery are defined as medicinal and aromatic plants (MAPs) (Planta Europa, 2010). These including trees, herbs, shrubs, creepers, climbers and epiphytes. Medicinal and aromatic plants (MAPs) can be used for the production of essential oils, pharmaceutical, herbal health products, dyes and colorants, cosmetic and personal care products, plant protection products and intermediate products produced from the primary products (Lubbe & Verpoorte, 2011).

In Malaysia, the development of aromatic plants is still unclear. But it is known that most of the aromatic plants are used by the locals as traditional medicines, ornamental plants and source of foods. There is no record that the aromatic plants being planted in large scale for trading, except for *Aquilaria sp.* The *Aquilaria sp.* are being planted for their agarwood, which economically give great income for the people.

This study focused on seven selected species of aromatic trees in Ayer Hitam Forest Reserve, Puchong, Selangor. The selected species are *Trema angustifolia* (Cenderai/Mengkirai), *Cinnamomum iners* (Medang Teja), *Actinodaphne sphaerocarpa* (Medang Payung), *Syzygium polyanthum* (Serai Kayu), *Prismatomeris glabra* (Tongkat Haji Samad), *Monocarpia marginalis* (Mempisang) and *Chromolaena odorata* (Kapal Terbang). Most of the species come from different families, with few sharing the same family classification. This forest reserve is house for 430 tree species from 230 genus and 72 families (Faridah Hanum, 1999). The most diverse families are from Euphobiacea (39 species), Myrtaceae (26 species) and Lauraceae (23 species).

1.2 Problem Statement

AHFR encompasses varies plants species from different genus and families. Out of them, there are a few species that can be categorized as aromatic plants. Yet, there is no specific documentation about aromatic plants in AHFR. Plant lists and documentation are usually done via categorizing and classifying the plants by their species, genus and families. This is giving some difficulties to identify aromatic plants species in AHFR. In term of laborious, the lack of expertise in this molecular study by our locals, especially on aromatic plants can cause its less in genetic information.

Species identification by DNA sequencing method is useful and advantageous. This method serves as a supporting aid to the conventional plant identification through morphology characteristics. This method does not require the whole plants for species identification, but just needs a piece of dried or fresh tissue. Through this method, we can distinguish a group of aromatic plants in a short time by performing both botanical observation and molecular study. The results are useful information for conservation and economic purpose in the future. Aromatic plants contain extractives which give big contributions to economic development. This molecular study is important to establish the relatedness between the selected aromatic plants species.

1.3 Objectives

The objectives of this study are:

I. To identify and select aromatic plants species in the AHFR via present publications and site-visits.

II. To collect selected aromatic plants species samples and document their morphological characteristics.

III. To study the phylogenetic relationship of the selected aromatic plants species by using PCR analysis.

1.4 Justification of Study

The results of this study might help to improve and develop method in species identification and classification based on morphological and molecular information. Through this study, the molecular information can help in species identification and classification. Commonly, species identification and classification are based on morphological information such as leaves, flowers, fruits and bark's scale. Yet, the identification of species based on morphological information may be wrong as its need broad knowledge in botany's field. Thus, the DNA sequences database can be reference as supporting information in plant identification.

Phylogenetic information is important and can be used for varies of purposes. It can be used for analysis of morphological and several kinds of molecular data. Furthermore, this information can be used in making estimation of evolutionary relationships and the relatedness among the selected aromatic plants species. However, the accurate estimates of phylogenetic relationships between the selected species are still lacks. This is a limitation that must be faced by ecology community from apply the evolutionary information within their field and studies. Therefore, this study is quite useful and has its own priority due to its importunateness in supplying molecular information.

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