INVESTIGATION OF ESSENTIAL OILS, PHYTOCHEMISTRY AND ANATOMICAL OF *Vitex negundo, Vitex trifolia, Plectranthus amboinicus* and *Plectranthus monostachyus* (LAMIACEAE)

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DISSERTATION PRESENTED TO QUALIFY FOR A
MASTERS IN SCIENCE
(RESEARCH MODE)

FACULTY OF SCIENCE AND MATHEMATICS
UNIVERSITI PENDIDIKAN SULTAN IDRIS

2021

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ACKNOWLEDGEMENT

First and foremost, Alhamdulillah, praises and thanks to Allah, the Almighty, for his countless blessings, throughout my research work to successfully completed the research.

I would like to express my deep and sincere gratitude to Dr. Wan Mohd Nuzul Hakimi Wan Salleh, my supervisor and Assoc. Prof. Dr. Nor Nafizah Mohd Noor, my co-supervisor for giving me the opportunity to do this research under his supervision and providing me with valuable guidance and support throughout this research. I was truly inspired by his hard work and passion. It was a great privilege and honor to work and study under his supervision.

Sincerely thanks to the Department of Chemistry, Faculty of Science and Mathematics, UPSI for the access of laboratory and instruments.

I would also like to give special thanks to my mother, Yaani binti Ab Razak and my family as a whole for their continuous support and understanding when undertaking my research and writing my project. Your prayer for me was what sustained me this far.

Last but not least, special thanks to my lab mates and friends; Natasa, Ammar, Basri, Siddiq, and Mazatul Azrin for their moral support, advice and guidance throughout my research.

ABSTRACT

This study aimed to analyze the essential oils composition, phytochemistry, anatomical and antioxidant activity of the genus *Vitex* (*V. negundo* and *V. trifolia*) and *Plectranthus* (*P. amboinicus* and *P. monostachyus*) from Lamiaceae family. The essential oils were obtained via hydrodistillation technique and their chemical compositions were determined by gas chromatography (GC-FID) and gas chromatography-mass spectrometry (GC-MS). The phytochemicals were obtained using chromatography techniques and their structures were confirmed by spectroscopic data and comparison with literatures. The anatomical study have been investigated on the lamina, midrib and petiole of the leaves part. The antioxidant activity of the essential oils was investigated using DPPH free radical scavenging assay. The study showed a total of 14 and 18 components were identified from the leaf oils of *V. negundo* (92.8%) and *V. trifolia* (91.5%), respectively. The results revealed that the essential oils are made up principally of δ-elemene (43.1%), spathulenol (9.8%), δ-selinene (7.8%) for *V. negundo*, while viridiflorol (42.3%), β-caryophyllene (21.7%), and β-elemene for *V. trifolia.* In the case of *Plectranthus* essential oils, 20 components was found from *P. amboinicus* (91.1%) and 37 components were identified from *P. monostachyus* (98.8%) oils. The major components of *P. amboinicus* oil were carvacrol (54.4%), β-caryophyllene (8.9%), and α-cis-bergamotene (7.7%), whereas *P. monostachyus* oil gave β-caryophyllene (26.2%), germacrene D (12.5%), δ-cadinene (9.2%), and germcarene B (8.8%). In addition, viridiflorol and carvacrol have been successfully isolated from the crude oils, whereas vanillic acid, vanillin, β-sitosterol and β-sitostenone were identified from the crude extracts. The essential oil of *P. amboinicus* displayed strong antioxidant activity with IC_{50} value 32.5 µg/mL. Meanwhile, the oil gland has been found in lamina, midrib and petiole of all essential oils. As conclusion, the composition of the essential oils from four species of Lamiaceae family have shown various chemical components and proved *via* anatomical study. The implication of this study demonstrates the importance of the characterization of Lamiaceae taxa in elucidating phylogenetic relationships as well as the potential of *Plectranthus* essential oils as a source of natural antioxidants.

KAJIAN MINYAK PATI, FITOKIMIA DAN ANATOMI DARIPADA GENUS *VITEX* DAN *PLECTRANTHUS* (LAMIACEAE)

ABSTRAK

Kajian ini bertujuan untuk menganalisis komposisi minyak pati, fitokimia, anatomi dan aktiviti antioksidan genus *Vitex* (*V. negundo* dan *V. trifolia*) dan *Plectranthus* (*P. amboinicus* dan *P. monostachyus*) dari keluarga Lamiaceae. Minyak pati diperoleh melalui teknik penyulingan hidro dan komposisi kimianya ditentukan dengan kromatografi gas (GC-FID) dan kromatografi gas-spektrometri jisim (GC-MS). Sebatian fitokimia diperolehi menggunakan teknik kromatografi dan strukturnya disahkan melalui data spektroskopi dan perbandingan dengan data literatur. Kajian anatomi telah djalankan pada lamina, midrib dan petiole bahagian daun. Aktiviti antioksidan minyak pati dikaji menggunakan ujian radikal bebas DPPH. Kajian menunjukkan sejumlah 14 dan 18 komponen dikenal pasti, masing-masing dari minyak daun *V. negundo* (92.8%) dan *V. trifolia* (91.5%). Hasil kajian menunjukkan minyak pati terdiri terutamanya dari δ-elemen (43.1%), spatulenol (9.8%), δ-selinen (7.8%) untuk *V. negundo*, sementara viridiflorol (42.3%), β-karyofailen (21.7), dan β-elemen untuk *V. trifolia*. Dalam kes minyak pati *Plectranthus*, 20 komponen didapati dari minyak *P. amboinicus* (91.1%) dan 37 komponen dikenal pasti dari minyak *P. monostachyus* (98.8%). Komponen utama minyak *P. amboinicus* adalah carvacrol (54.4%), β-karyofailen (8.9%), dan α-cis-bergamoten (7.7%), manakala minyak *P. monostachyus* memberikan β-karyofailen (26.2%), germakrena D (12.5%), δ-kadinen (9.2%), dan germakrena B (8.8%). Sebagai tambahan, viridiflorol dan carvacrol telah berjaya diasingkan dari minyak mentah, manakala asid vanilik, vanillin, β-sitosterol dan β-sitostenone dikenal pasti dari ekstrak kasar. Minyak pati *P. amboinicus* menunjukkan aktiviti antioksidan yang kuat dengan nilai IC_{50} 32.5 µg/mL. Sementara itu, kelenjar minyak telah dijumpai di lamina, midrib dan petiole dari semua minyak pati. Kesimpulannya, komposisi minyak pati dari empat spesies keluarga Lamiaceae telah menunjukkan pelbagai komponen kimia dan terbukti melalui kajian anatomi. Implikasi kajian ini menunjukkan pentingnya pencirian Lamiaceae taxa dalam menjelaskan hubungan filogenetik serta potensi minyak pati *Plectranthus* sebagai sumber antioksidan semula jadi.

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ABBREVIATIONS

α	Alpha
Abs	Absorbance
β	Beta
^{13}C	Carbon-13
CC	Column Chromatography
$CDCl_3$	Deuterated chloroform
$CHCl_3$	Chloroform
cm	Centimeter
cm^{-1}	Per centimeter
δ	chemical shift
d	doublet
DCM	Dichloromethane
Et_2O	Diethyl ether
EtOAc	Ethyl acetate
GC	Gas Chromatography
GC-MS	Gas Chromatography-Mass Spectrometry
h	Hour(s)
n-Hex	Hexane
^{1}H	Proton
H_2SO_4	Sulfuric acid
HCl	Hydrochloric acid
Hz	Hertz
IR	Infrared
J	Coupling constant
KI	Kovats Index
L	Liter
m	multiplet
M^+	Molecular ion
MeOH	Methanol
MHz	Megahertz

min	Minute(s)
m/z	Mass to charge ion
mg	milligram
m.p	Melting point
$MgSO_4$	Magnesium sulphate
mL	milliliter
mm	millimeter
MS	Mass Spectrometer
NMR	Nuclear Magnetic Resonance
nm	nanometer
PTLC	Preparative Thin Layer Chromatography
s	singlet
SiO_2	Silica gel
t	triplet
TLC	Thin Layer Chromatography

CHAPTER 1

INTRODUCTION

1.1 General Introduction

Plant derived natural products have been practiced in pharmaceutical sectors for synthesizing of valuable products in term of clinical aids for curing and preventing diseases. Decades ago, the plants were used in many ways throughout daily life. The plant benefits humankind in maintaining the health, culinary materials, the roof and huts for protection, cooking and some more. This is scientifically proved the starter of the ethnobotany (Atanasov et al., 2015).

The Leipzig Catalogue of Vascular Plants (LCVP) stated 351,180 vascular plant species and 6160 natural hybrids across 13,460 genera, 564 families and 84 orders (Freiberg et al., 2020). There are at least 250,000 species of flowering plants in the world and about 150,000 of them are found in the tropics. An accurate estimation

in South-East Asia recorded about 35,000 vary species. Whereas alone in Malaysia found 8,000 species. In approximately, 7411 plant species have been documented in Peninsular Malaysia and Sabah, respectively and 1300 medicinal plant species have been reported in the tropics endemic in Malaysia which possess medicinal values whether in conservative methods or by traditional practices (Abu Bakar et al., 2018).

Traditional medicine is an integral part of Malaysian's culture and has been accomplished by numerous ethnic groups long before the introduction of the recent medicine. Much of the knowledge is still leading in the culture of the numerous ethnic groups and unrecorded. This statement was proved by the classical works of Burkill, represented the ancient of the old folks and traditional practices on medicine uses of plants (Escalona et al., 2015). Malaysia has been categorized as biodiversity-rich nations with the diversity of medicinal plants. The most popular medicinal plants from Malaysia are *Momordica charantia*, *Andrographis paniculata*, *Ficus deltoidea*, *Eurycoma longifolia*, *Labisia pumila*, *Melastoma malabathricum*, *Orthosiphon stamineus* and *Piper sarmentosum* (Asiah et al., 2015).

Presently, numerous experts are endeavoring to classify new plants which have medicinal values and have the prospective to be commercialized as herbal medicines. Lamiaceae is one of the plant families which is thought to have importance medicinal values due to its wide used in many traditional medicines.

1.2 Lamiaceae Family

The Lamiaceae is known as the mint family which also the one among of flowering plants family. They have traditionally been deliberated narrowly associated to Verbenaceae, however phylogenetic studies recommended that numerous genera categorised in Verbenaceae belong instead in Labiatae then current Lamiaceae. The Labiatae was given because the flowers typically have fused tubular petals into an upper lip and a lower lip. Although this is still deliberated an acceptable alternative name, most botanists now refer to Lamiaceae (Ebadollahi et al., 2020). The classification of the family is recently fully revised by Bramley et al. (2019) in the Flora Malesiana.

The Lamiaceae family is a cosmopolitan distribution, growing over the entire planet and particularly high dispersion in the Mediterranean region. The Lamiaceae is containing about 236 genera and has been stated to 7,534 species species (Yuan et al., 2010). For their habitat, they prefer the open fields. Lamiaceae cultivated as ornamental and herbs. Some are shrubs, trees or, rarely in form of vines. The largest genera are *Salvia*, *Scutellaria*, *Stachys*, *Plectranthus*, *Hyptis*, *Teucrium*, *Vitex*, *Thymus*, and *Nepeta* (Tamokou et al., 2017).

Due to their aromatic odour and nice flavour, several species of this family are used in the culinary to the come out the satisfaction of the gourmets. The acquainted by people with the merits of basil (*Ocimum basilicum*), oregano (*Origanum vulgare*), thyme (*Thymus vulgaris*), and rosemary (*Salvia rosmarinus*) as smell and taste garnishes in the numerous dishes. Others spices in Lamiaceae family are mint

(*Mentha*), sage (*Salvia officinalis*), savoury (*Satureja*), marjoram (*Origanum majorana*), and perilla (*Perilla frutescens*) enhance the best taste of foods (Burkill, 1966).

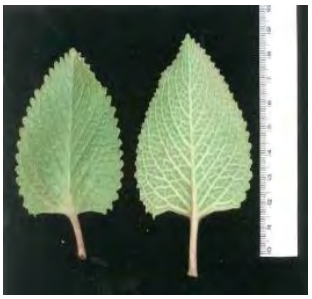
Medicinal properties of the Lamiaceae species are frequently ascribed to their abundance of volatile components. The best qualities of the Lamiaceae are praised to have an incarnating effect on the psyche because of these qualities the volatile oils are used internally as well as externally. For example, the rosemary oil is used as extra therapy in diabetes. Besides, thyme is well known as one of the spices also give a medicinal purpose in relieving a common cold. Other than that, mint and lavender are cultivated also for their oil. Furthermore, their aromatic volatile oils widely used in perfumery and food productions as active elements or as taste and cologne (Khoury et al., 2016).

In facts of the great satisfaction in the most of applications and routines, the Lamiaceae comprise aromatic carbohydrates such as phenols, which have an antiseptic in addition to an aromatic action. With the aid of the terpenes, which the plants have, they defend themselves against insects, fungi, and bacteria. Other elements are phosphorus, magnesium, calcium, potassium, and molybdenum (Lukhoba et al., 2006).

In this study, two genus from Lamiaceae has been selected to be investigated which are *Vitex* sp. and *Plectranthus* sp. Each genus would be representative by two species and the descriptions for each species are shown in Table 1.1. In addition, the scientific classification of both genus are revealed in Table 1.2.

Table 1.1

The selected species of the Lamiaceae family

Species	Description
Vitex negundo 	**Local name:** *Chinese chaste tree* **Distribution:** Tropical Eastern, Southern Africa and Asia **Medicinal uses:** In Malaysia, it is used in traditional herbal medicine for women's health, as well as dealings the menstrual cycle, fibrocystic breast disease and post-partum therapies (Geetha, 1994).
Vitex trifolia 	**Local name:** *Lemuni* **Distribution:** Widespread from North Australia east to Tahiti and north via Indonesia and the Philippines to China, India and Sri Lanka **Medicinal uses:** The leaves are used to give female illnesses in the Cook Islands, and used to dismiss fever in Samoa. Besides, the dried leaves are scorched to prevent mosquitos (Aeri et al., 2020)
Plectranthus amboinicus 	**Local name:** *Bangun-bangun* **Distribution:** Throughout tropical Africa, Asia, Australia, and the Americas, including Brazil **Medicinal uses:** In Malaysia, the leaves extract is given after childbirth, and the juice to manage cough (Arumugam et al., 2016)

(continue)

Table 1.1 *(continue)*

Species	Description
Plectranthus monostachyus 	**Local name:** *Monkey's Potato* **Distribution:** Tropical and subtropical Asia to northern Australia **Medicinal uses:** According to folk medicine, the leaf sap is taken internally for fever, cough, headache, colic and convulsions. It is thought to have a calming, sedative effect, as well as improving appetite and strengthening the stomach (Irsyam & Mountara, 2018)

Table 1.2

Scientific classification of the genus Vitex and Plectranthus

Kingdom	Plantae	Plantae
Clade	Tracheophytes	Tracheophytes
Clade	Angiosperms	Angiosperms
Clade	Eudicots	Eudicots
Clade	Asterids	Asterids
Order	Lamiales	Lamiales
Family	Lamiaceae	Lamiaceae
Subfamily	Premnoideae	Ocimeae
Genus	*Vitex*	*Plectranthus*

1.3 Problem Statement

Despite a conservative uses of various species in Lamiaceae family, lots of the plants come in with the same species whether in the same family such as *Vitex* and *Plectranthus* have not been researched properly especially in Malaysia. Besides, abroad of Lamiaceae species not been explored thoroughly on their chemical and biological studies to support their significance in medicinal uses. In addition, many studies pointed out on the importance of morphological characters in delimitation and identification in some Lamiaceae species.

The anatomical characters are important for characterization of Lamiaceae taxa. Besides, these features play an important role in elucidating phylogenetic relationships in many taxa. Most current review of the family in the Flora Malesiana (Bramely et al. 2019) were not much discuss on the anatomical characters and phytochemical constituents including essential oil.

Therefore, the study that is involving the extraction and analysis of the essential oils as well as the relationship of anatomical characteristics of the leaves and their essential oils will be studied. Furthermore, the antioxidant activity of the essential oils will be examined and contributed to the improvement for pharmacological applications hereafter.

1.4 Objectives of Study

The purposes of this study are:

1. To investigate the chemical compositions of the essential oils from *V. negundo*, *V. trifolia*, *P. amboinicus*, and *P. monostachyus.*
2. To isolate the components from the essential oils and crude extracts of selected species followed by characterizations using IR, NMR and MS.
3. To study the anatomical of the leaves and their relationship with the essential oils.
4. To evaluate the antioxidant activity of the essential oils.

1.5 Scopes of Study

The study was separated into four parts. The first part was the extraction of the essential oils from the leaves of *V. negundo*, *V. trifolia*, *P. amboinicus*, and *P. monostachyus* by using hydrodistillation method. The chemical compositions of the essential oils were analyzed using GC, GC-MS and Kovats Indices. The second part was to isolate the components from the essentials and crude extracts of selected species followed by characterization the structures using spectroscopic methods such as IR, 1D/2D NMR, and MS. The third part was to determine the anatomical study of the leaves and their essential oils relationship using microscope. Finally, the antioxidant activity of the essential oils were assessed using DPPH radical scavenging assay.