STUDIES ON ESSENTIAL OILS, PHYTOCHEMICALS AND ANTIOXIDANT ACTIVITY OF *Magnolia candollii* H.Keng AND *Magnolia alba* DC. (MAGNOLIACEAE)

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UNIVERSITI PENDIDIKAN SULTAN IDRIS

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***Special dedication of this grateful feeling to**;*

My parents and family, ***Yahaya Mat and Roshidah Musa*** *for the*

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ABSTRACT

The purpose of this study was to investigate the essential oils, phytochemicals, and antioxidant activity of *Magnolia candollii* and *Magnolia alba* (Magnoliaceae). The essential oils were obtained *via* hydrodistillation technique and their chemical compositions were determined by gas chromatography-flame ionization detection (GC-FID) and gas chromatography-mass spectrometry (GC-MS). The phytochemicals were isolated using chromatography techniques and their structures were confirmed by spectroscopic data and comparison with literature. The antioxidant activity was determined through total phenolic content and DPPH free radical scavenging assay. The study showed that the essential oil of *M. candollii* consists of α-pinene (29.7%), elemol (10.2%), β-pinene (8.5%), β-caryophyllene (7.2%), α-terpineol (7.0%), and guaiol (5.4%). In addition, the major components of essential oil from *M. alba* were linalool (65.4%), β-caryophyllene (6.7%), and (*E*)-nerolidol (5.2%). Isolation and purification of *M. candollii* bark and *M. alba* leaf extracts yielded seven phytochemicals identified as licarin A, squalene, parthenolide, vanillin, stigmasterol acetate, friedelin, and β-sitosterol. Besides, linalool was successfully isolated from *M. alba* leaf essential oil. The methanol extract of *M. candollii* showed the highest antioxidant activity with phenolic content value as 380.0 mg GAE/g and 77.2% inhibition in the DPPH free radical scavenging assay. Licarin A demonstrated the highest antioxidant activity among the isolated compounds with percentage inhibition of 72.0% in the DPPH assay. In conclusion, the essential oils of *M. candollii* bark and *M. alba* leaf contain oxygenated monoterpenes and monoterpene hydrocarbons as principal components, respectively. Meanwhile, the phytochemical study of both plant extracts led to identification of different classes of natural compounds including neolignan, sesquiterpene lactone, phenolic, and terpenes. The study has significant implication on natural product research regarding essential oils and phytochemicals with promising antioxidant activity as well as their applications in the pharmaceutical industries.

KAJIAN MINYAK PATI, FITOKIMIA, DAN AKTIVITI ANTIOKSIDAN DARIPADA *Magnolia candollii* H.Keng DAN *Magnolia alba* DC. (MAGNOLIACEAE)

ABSTRAK

Tujuan kajian ini adalah untuk mengkaji minyak pati, fitokimia, dan aktiviti antioksidan daripada *Magnolia candollii* dan *Magnolia alba* (Magnoliaceae). Minyak pati diperoleh melalui teknik penyulingan hidro dan komposisi kimianya ditentukan oleh kromatografi gas-pengesanan pengionan nyalaan (GC-FID) dan kromatografi gas-spektrometri jisim (GC-MS). Fitokimia telah diasingkan menggunakan teknik kromatografi dan strukturnya telah disahkan oleh data spektroskopi dan perbandingan dengan literatur. Aktiviti antioksidan telah dikenalpasti melalui jumlah kandungan fenolik dan ujian perencatan radikal bebas DPPH. Kajian menunjukkan minyak pati *M. candollii* terdiri daripada α-pinen (29.7%), elemol (10.2%), β-pinen (8.5%), β-karyofailen (7.2%), α-terpineol (7.0%), dan guaiol (5.4%). Selain itu, komponen utama minyak pati daripada *M. alba* ialah linalol (65.4%), β-karyofailen (6.7%), dan (*E*)-nerolidol (5.2%). Pengasingan dan penulenan daripada ekstrak batang *M. candollii* dan daun *M. alba* menghasilkan tujuh fitokimia yang dikenalpasti sebagai likarin A, skualena, parthenolid, vanilin, stigmasterol asetat, fridelin, dan β-sitosterol. Selain itu, linalol berjaya diasingkan daripada minyak pati daun *M. alba*. Ekstrak metanol *M. candollii* menunjukkan nilai kandungan fenolik yang paling tinggi iaitu 380.0 mg GAE/g dan 77.2% perencatan dalam asai radikal bebas DPPH. Likarin A menunjukkan aktiviti antioksidan yang paling tinggi dalam kalangan sebatian yang diasingkan dengan peratusan perencatan iaitu 72.0% perencatan dalam asai DPPH. Kesimpulannya, minyak pati batang *M. candollii* dan daun *M. alba* masing-masingnya mengandungi monoterpena beroksigen dan hidrokarbon monoterpena sebagai komponen utamanya. Sementara itu, kajian fitokimia kedua-dua ekstrak tumbuhan membawa kepada pengenalpastian pelbagai kelas sebatian semula jadi termasuk neolignan, seskuiterpena lakton, fenolik, dan terpena. Kajian ini menunjukkan implikasi yang signifikan terhadap penyelidikan sebatian semula jadi mengenai minyak pati dan fitokimia dengan potensi aktiviti antioksidan termasuk aplikasinya dalam industri farmaseutikal.

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LIST OF ABBREVIATIONS

α	Alpha
Abs	Absorbance
β	Beta
^{13}C	Carbon-13
CC	Column Chromatography
$CDCl_3$	Deuterated chloroform
$CHCl_3$	Chloroform
cm^{-1}	Per centimeter
COSY	Correlation spectroscopy
1D	1 Dimension
2D	2 Dimension
δ	chemical shift
d	doublet
dd	doublet of doublets
DEPT	Distortionless Enhancement by Polarization Transfer
EIMS	Electron Impact Mass Spectrometry
Et_2O	Diethyl ether
GC	Gas Chromatography
GC-MS	Gas Chromatography-Mass Spectrometry
^{1}H	Proton
HMBC	Heteronuclear Multiple Bond Correlation
HMQC	Heteronuclear Multiple Quantum Coherence

HPLC	High Performance Liquid Chromatography
Hz	Hertz
IR	Infrared
J	Coupling constant
KBr	Potassium bromide
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
s	singlet
SiO_2	Silica gel
t	triplet
TLC	Thin Layer Chromatography

CHAPTER 1

INTRODUCTION

1.1 General Introduction

Nature has played a significant role in human pharmaceutical production, and particularly to bacteria, fungi, and plant natural products. The major source for medical technology discovery is natural products (Corre & Challis, 2009). A natural product is described as anything created by life, including biotic materials, bio-based materials, bodily fluids, and other natural materials once found in living organisms (Abozenadah et al., 2017). Natural products have high genetic variability and special pharmacological or biological activities as a result of thousands of years of natural selection and evolutionary processes that have influenced their utility. Natural materials have a structural diversity that greatly exceeds the capabilities of synthetic organic chemists in the lab. Furthermore, natural products have been used to cure illnesses in both conventional and modern folk medicine (Abozenadah et al., 2017).

Since the beginning, medicinal plants have been used to cure a wide range of illnesses. Approximately 20,000 plant species are known to be used for medicinal purposes (Gullece et al., 2006). Natural ingredients derived from herbs, fungi, bacteria, and other species are also used in medicinal formulations as pure compounds or extracts. Polyphenols, terpenes, fatty acids, and vitamins found in natural sources have shown a variety of bioactivities. For instance, natural skincare formulations that have antioxidant, anti-hyperpigmentation, and antimicrobial effects can help to enhance skin tone, texture, and attractiveness (Emerald et al., 2016).

Malaysia has a diverse range of plant species and a traditional medicinal system. In Malaysia, a total of 1,230 species have been reported as medicinal plants used in alternative medicine (Zakaria & Mohd, 1994). According to previous research, the presence of unique phytochemicals in particular Malaysian plants has shown a good treatment for living beings. For instance, the stem of *Amaranthus spinosus* (bayam berduri) and the leaves of *Carica papaya* (betik) are two of the most popular plants used by locals to treat gastric problems (Alsarhan et al., 2014). In addition, the leaves of *Aloe vera* (lidah buaya) are used to heal sunburns, restore dead skin, and cells (Manvitha & Bidya, 2014). Besides, the leaves and roots of *Syzygium polyanthum* (serai kayu), *Michelia champaka* (cempaka), and *Citrus hystrix* (limau purut) have also been used in diabetes and malarial fever (Abdulrahman et al., 2018). Therefore, one of the most persuasive reasons for this wide array of chemical variety, which exists as a subset of biological variety is the chemical adaptation that these tropical plants go through time by becoming herbal remedies or for medicinal purposes (Mitra et al., 2007).

Presently, experts are finding more plants with therapeutic properties and the potential to be sold as herbal remedies. Magnoliaceae is one of the plant families that is thought to enhance traditional medicine which existed for decades due to their valuable natural sources of fragrance and bioactive phytochemicals.

1.2 Magnoliaceae Family

Magnoliaceae, a family of *Magnolia* and order of Magnoliales consists of about 17 genera and 300 species. It is mainly dispersed in mild and tropical Asian and American continents. Magnolioideae and Liriodendroideae are the two subfamilies that constitute the Magnoliaceae family. Several genera have been recognized such as *Alcimandra, Lirianthe, Manglietia, Michelia, Pachylarnax, Parakmeria, Talauma,* and *Yulania*, whereas the genus *Magnolia* and *Liriodendron* are restricted to China (Xia et al, 2018).

Magnoliaceous plants are of extraordinary value in botanical research. Hypothetically, they as the classic agent of primitive taxa are the key materials for the inquire of the root and advancement of angiosperms, and for the recreation of the characteristic framework of angiosperms (Sima et al. 2001). Magnoliaceae trees and shrubs have two ranked stipulate leaves that enclose young buds, while the flowers are hermaphrodite and actinomorphic (Yashasvi, 2021). Magnoliaceae is closely related to the Annonaceae in terms of floral structure and organization (Yashasvi, 2021). Essentially, they are an evergreen broad-leaved woodlands and deciduous broad-leaved timberlands from tropical to mild zones as well as celebrated trees for decorative, timber, therapeutic, and fragrance (Sima et al. 2001).

Most of the genus of the Magnoliaceae family have a woody plant with primitive flowering. Several plants in this family are valuable for traditional uses. For instance, the bark of *Magnolia officinalis* is very popular in China as a remedy for flatulent dyspepsia, cough, and asthma (Pu et al., 1990). *Michelia champaka* has been used in traditional medicine to treat postpartum depression, fever, diabetes, and hypertension (Abdulrahman et al., 2018), whereas the root bark of *Liriodendron tulipifera* is used as a febrifuge in paroxysmal fevers (Herrick, 1995). This family also economically used for timber such as *Michelia excelsa* wood, is a valuable commercial timber known as whitewood (Yashasvi, 2021).

1.3 Genus *Magnolia*

According to Frodin and Govaerts (1996), the genus *Magnolia* is divided into three subgenera which are *Magnolia*, *Yulania* and *Gynopodium*. The genus *Magnolia* is comprising of about 219 species and is widely distributed in Asian and American regions (Mabberley, 2017). These species are geologically located in the Southeastern US, Mexico, Central America, the Caribbean, and Southeast Asia. The most common *Magnolia* species such as *M. salicifolia, M. kobus*, *M. macrophylla*, *M. ashei*, *M. acuminate*, *M. grandiflora, M. virginiana*, and *M. liliiflora* which are native in Japan, Korea, Southeastern US, Mexico, and China (Myers, 2020).

The genus embraces both deciduous and evergreen trees and shrubs with a height of 9 to 31 meters tall, with most species are thin, smooth bark and large leaves and flowers. The woods are soft and light in color and are used in making crates, boxes,

and furniture (Britannica, 2019). *Magnolia* species have unique ornamental values, strong anti-pollution ability, and wide adaptability especially in China, Japan, Thailand, and India (Shen et al., 2008). It is also important for the economy as natural aroma and bioactive compounds. The species is easily recognizable as they are valued for their large and fragrant white, yellow, pink, or purple flowers, frequently smooth and shinning leaves, and cone-like fruits. The flowers, usually cuplike and fragrant, are located at the branch tips and have three sepals, 6 to 12 petals arranged in 2 to 4 series, and many spirally arranged stamens. The seeds, usually reddish, often hang pendulously by slender threads (Britannica, 2019; Ghosh et al., 2021).

Herbal medicines continue to rapidly use by many people across the world for the treatment of various health and to cure illness. In this regard, the use of *Magnolia* species as herbal medicines has also been widely embraced in many developed countries with complementary and alternative medicines (Anquez-Traxler, 2011). For example, in ancient Chinese and Japanese medication, *Magnolia* bark is an ingredient in *Hange-koboku-to*, which consists of five plant extracts, and in *Saiboku-to*, which consists of ten plant extracts. These extracts are used to decrease anxiety and nervous tension and boost sleep. Besides, some researchers reported that the bark and flower buds of *Magnolia* are employed for weight loss, digestion, constipation, inflammation, anxiety, stress, depression, fever, headache, stroke, and asthma (Kuribara et al., 2000).

In earlier reports, the traditional medicines of *Magnolia* species was used in various parts of the world. *Magnolia* species can be marketed as fresh or dried products depending on their use and consumer's preference (Diaz-Maroto et al., 2002). This shows that *Magnolia* species have economic importance in forest products for used as

timber and herbal medicine. Table 1.1 shows the medicinal uses of several *Magnolia* species.

Table 1.1

Medicinal uses of several Magnolia species

Species	Part	Traditional Uses
M. insignis	Leaves	Treating chest, abdominal pain, indigestion, asthma, and dysentery (Vo, 2012)
M. ovata	Flower	Treating fever, cough, scabies, toothache, stomachache, rheumatism, and diabetes (Ghosh et al., 2021)
M. coco	Flower	Prevent age-related diseases and antiskin aging cosmeceutical (Kato et al., 2017)
M. sieboldii	Leaves	Treating inflammatory diseases such as rhinitis, pneumonia, and endometritis (Oyungerel et al., 2014)
M. grandiflora	Flower	Used for a bitter tonic, antimalarial, cold, headache, and stomachache (Baez et al., 2012)
	Flower	Used as a stimulant, diaphoretic, anti-inflammatory, antiseptic agent as well as pain control, anxiety, and nervous disturbances (Khare, 2008)
	Bark	Use as a remedy against itching (Bushnell, 1909)
	Flower	Treatment of cardiovascular diseases and cancer (Lu et al., 2012)
M. flos	Leaves	Treating sinusitis, nasal congestion, and hypersensitive skin (Chen et al., 2020)

(continue)

Table 1.1 *(continue)*

Species	Part	Traditional Uses
M. obovata	Bark	Treatment of gastrointestinal disorders, anxiety, allergic diseases, and bronchial asthma (Youn et al., 2008)
	Bark	Relaxes muscle tension to improve vitality stasis (Sumino et al., 2012)
M. kobus	Flower	Treatments of headaches and colds (Matsutani & Shiba, 1975)
M. linn	Bark	Treatment of thrombotic stroke, depression, anxiety, inflammatory, and neuronal diseases (Watanabe et al., 1983)
M. virginiana	Bark	Treat various ailments, bitter tonics for autumnal fever, and rheumatism (Bolyard, 1981)
	Seed	Applied as a laxative and sudorific in a warm decoction or as an agent against paroxysms of intermittent fever in cold decoctions, powder, or tinctures (Bolyard, 1981)
	Leaves	To prevent chills, warm the blood, and colds (Speck, 1941)
	Root	Used as a diaphoretic in the treatment of rheumatism, pleurisy, cough, consumption, utilized against remittent, intermittent, and typhoidal fever (Price, 1802)
M. officinalis	Stem	Treating thrombotic stroke, typhoid fever, headache, alleviate gastric, and abdominal distension (Xu et al., 2009)

(continue)

Table 1.1 *(continue)*

Species	Part	Traditional Uses
M. officinalis	Branch	Treatment of digestive disturbances, reduce the symptom of cough and asthma (Xu et al., 2009)
	Root	Cure syndromes caused by emotional distress and emotional turmoil (Xu et al., 2009)
	Bark	Used to treat abdominal distention, vomiting, diarrhea, food accumulation, constipation, phlegm, fluid retention, and cough resulting from asthma (Luo et al., 2012)
	Bark	Used for deobstruent, tonic, stomachic, quieting, and anthelmintic (Stuart, 1969)
M. acuminata	Bark	A remedy against toothache, curing stomachache and cramps (Moerman, 1998)
	Fruit	Preparation of a strong and bitter tasting fever (Michaux, 1805)

Two species have been selected for this study which are *Magnolia candollii* and *M. alba*. The descriptions for each species are shown in Table 1.2.

Table 1.2

The selected species of the genus Magnolia

Species	Image	Description
Magnolia candollii	 (3.7119° N, 101.7366° E)	Local name: *Si tekwok* (Malaysia), *Cempaka gonda* (Jawa), *Cempaka telur* (Borneo) Distribution: China (Hainan), Southeast Asia, Peninsular Malaysia, Borneo, Jawa, Philippines, Sulawesi Medicinal uses: Used to treat Alzheimer's disease, anxiety, cancer, digestion problems, liver problems, menstrual cramps, respiratory disorders and weight loss (Nooteboom & Chalermglin, 2000).
Magnolia alba	 (3.7119° N, 101.7366° E)	Local name: *Cempaka Putih* (Malaysia) Distribution: Jawa Indonesia, Peninsular Malaysia, Singapore, Borneo Medicinal uses: Used to treat headache, sinusitis, cough, inflammation, flatulence, nausea, and vaginal discharge (Khairan et al. 2021)

1.4 Problem Statement

Magnoliaceae plants appear to be of major importance due to their wide range of phytochemicals and biological characteristics that have been reported. Several Malaysian Magnoliaceae species have not been thoroughly explored chemically nor biologically. Two species from the genus *Magnolia* were selected and investigated in this study which are *M. candollii* and *M. alba*. Given the relevance of this genus' therapeutic benefits in the treatment of a variety of ailments (asthma, constipation, edema, abdominal distension, and malaria), it's evident that more research is needed. As a matter of fact, investigations on the extraction of essential oils, the isolation of phytochemicals, and the biological activity of the selected species have been conducted. To the best of our knowledge, no report has been published on *M. candollii*. Thus, the outcomes of the study may contribute to the pharmaceutical industry in the future.

1.5 Objectives of Study

The objectives of the study are:

1. To investigate the chemical compositions of the essential oils of *Magnolia candollii* (bark) and *M. alba* (leaf) using GC-FID and GC-MS.

2. To isolate the major component of *M. alba* (leaf) essential oil and phytochemicals of *M. candollii* (bark) and *M. alba* (leaf) extracts and identified spectroscopically (IR, NMR, and MS).

3. To determine the antioxidant activity (TPC and DPPH free radical scavenging) of the essential oils, crude extracts, and selected phytochemicals.

1.6 Scopes of Study

The study was separated into three parts. The first part was on the extraction of the essential oils (leaf and bark) by hydrodistillation method from two *Magnolia* species. The chemical compositions of the essential oils were analysed using GC, GC-MS, Kovats Indices, and multivariate statistical analysis. The second part was to isolate the phytochemicals from *Magnolia* extracts (leaf and bark) as well as the major components from essential oils (leaf) by using various chromatographic methods. The structures of the isolated phytochemicals were analysed spectroscopically using IR, NMR (1D and 2D), and MS. Finally, the antioxidant activity of the essential oils, crude extracts, and selected phytochemicals were performed using DPPH free radical scavenging assay and total phenolic content.