ESSENTIAL OILS, PHYTOCHEMISTRY AND ANTIOXIDANT ACTIVITY OF *Syzygium pyrifolium* (Blume) DC. (MYRTACEAE)

NUR HAZWANIE BINTI ABDUL KADIR

UNIVERSITI PENDIDIKAN SULTAN IDRIS

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ABSTRACT

This study aims to identify the essential oils composition, phytochemicals, and antioxidant activity of *Syzygium pyrifolium* (Blume) DC. leaves and bark extracts. The essential oils were obtained using hydrodistillation technique and their chemical compositions were determined by gas chromatography and gas chromatography-mass spectrometry. Phytochemicals were separated using various chromatography techniques and their structures were elucidated using infrared, nuclear magnetic resonance, ultraviolet, and mass spectrometry. Antioxidant activity of the essential oils, extracts, and selected phytochemicals was determined using the total phenolic content and free radical scavenging assay. Results showed that *S. pyrifolium* leaf oil mainly consists of geraniol (73.8%), β-pinene (7.0%), α-pinene (5.7%), and (*E*)-β-ocimene (4.5%). Geraniol was successfully separated from the leaf oil as a pure compound. The bark oil contains cuparene (7.2%), β-elemene (6.8%), longifolene (5.6%) and α-terpineol (4.7%) as major components. Seven compounds were also separated from the leaf and bark extracts of *S. pyrifolium* and successfully identified as 5-hydroxy-3,7,4′-trimethoxyflavone, 5-hydroxy-3,7,3′,4′-tetramethoxyflavone, 5,4′-dihydroxy-3,7,3′-trimethoxyflavone, 5-hydroxy-6,7,4′-trimethoxyflavone, 4′,5,7-trimethoxyflavone, 5,7-dimethoxyflavone, and 3,4,5-trihydroxybenzoic acid. The bark methanolic extract exhibited the highest total phenolic content with gallic acid equivalent to 490 mg/g extract. Meanwhile, leaf and bark methanolic extracts showed strong antioxidant activity in the free radical scavenging assay with percentage inhibition of 88.4% and 89.9%, respectively. In conclusion, leaf and bark oils of *S. pyrifolium* consist of oxygenated monoterpenes and sesquiterpene hydrocarbons as major group components. In addition, leaves and bark methanolic extracts mainly contain flavonoids and phenolic compounds which exhibited strong antioxidant activity. This study implies that extracts of *S. pyrifolium* have potential to prevent free radical harmful effects and may protect against various cardiovascular and inflammatory diseases.

MINYAK PATI, FITOKIMIA DAN AKTIVITI ANTIOKSIDAN DARIPADA *Syzygium pyrifolium* (Blume) DC. (MYRTACEAE)

ABSTRAK

Kajian ini bertujuan untuk mengenal pasti komposisi minyak pati, fitokimia, dan aktiviti antioksidan daripada ekstrak daun dan batang *Syzygium pyrifolium* (Blume) DC. Minyak pati diperoleh menggunakan teknik penyulingan hidro dan komposisi kimianya ditentukan dengan kromatografi gas dan kromatografi gas-spektrometri jisim. Fitokimia telah dipisahkan menggunakan pelbagai teknik kromatografi dan strukturnya telah dikenal pasti menggunakan inframerah, resonans magnet nukleus, ultralembayung, dan spektrometri jisim. Aktiviti antioksidan terhadap minyak pati, ekstrak, dan fitokimia terpilih telah ditentukan menggunakan jumlah kandungan fenolik dan ujian perencatan radikal bebas. Keputusan menunjukkan minyak daun *S. pyrifolium* mengandungi terutamanya geraniol (73.8%), β-pinen (7.0%), α-pinen (5.7%), dan (E)-β-ocimen (4.5%). Geraniol telah berjaya diasingkan daripada minyak daun sebagai sebatian tulen. Minyak batang mengandungi kuparen (7.2%), β-elemen (6.8%), longifolen (5.6%) dan α-terpineol (4.7%) sebagai komponen utama. Tujuh sebatian juga telah diasingkan daripada ekstrak daun dan batang *S. pyrifolium* dan berjaya dikenal pasti sebagai 5-hidroksi-3,7,4′-trimetoksiflavon, 5-hidroksi-3,7,3′,4′-tetrametoksiflavon, 5,4′-dihidroksi-3,7,3'-trimetoksiflavon, 5-hidroksi-6,7,4'-trimetoksiflavon, 4',5,7-trimetoksiflavon, 5,7-dimetoksiflavon, dan asid 3,4,5-trihidroksibenzoik. Ekstrak batang metanol menunjukkan jumlah kandungan fenolik tertinggi setara dengan asid galik 490 mg/g ekstrak. Sementara itu, ekstrak daun dan batang metanol menunjukkan aktiviti antioksidan yang kuat dalam ujian perencatan radikal, masing-masing dengan peratusan perencatan 88.4% dan 89.9%. Kesimpulannya, minyak daun dan batang *S. pyrifolium* terdiri daripada monoterpena beroksigen dan hidrokarbon seskuiterpena sebagai komponen kumpulan utama. Tambahan pula, ekstrak daun dan batang metanol mengandungi flavonoid dan sebatian fenolik yang mempamerkan aktiviti antioksidan yang kuat. Implikasi kajian menunjukkan ekstrak *S. pyrifolium* berpotensi untuk mencegah kesan berbahaya radikal bebas dan boleh melindungi daripada pelbagai penyakit kardiovaskular dan radang.

CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF SCHEME

LIST OF ABBREVIATIONS

α	Alpha
Abs	Absorbance
β	Beta
br	broad
^{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
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
PTLC	Preparative Thin Layer Chromatography
nm	nanometer
R_f	Retention factor
s	singlet
SiO_2	Silica gel

t	triplet
TLC	Thin Layer Chromatography
UV	Ultraviolet

CHAPTER 1

INTRODUCTION

1.1 General Introduction

Natural products, known for their chemical diversity and bioactivity, have continued to serve as models for developing new pharmacological scaffolds. Natural products can be an entire organism, such as a plant, an animal, or a microorganism, that has not been processed or treated beyond a simple preservation method. Besides, natural products also refer to secondary metabolites, which are molecules produced by any living organism (Sarker & Nahar, 2012). Research on natural products has accelerated in recent years due to significant advancements in separation science, spectroscopic techniques, and high-throughput screening technologies. The synergy of these forward-thinking scientific activities has raised the profile and practical importance of natural products in today's society (Molinski, 2014).

Natural products also have been utilized in medicine from prehistoric times to alleviate and treat ailments. It becomes a vital role in ancient traditional medical systems such as Chinese, Ayurvedic, and Egyptian traditional medicine, and they are being used to treat a various of disorders today (Sarker & Nahar, 2012). Traditional herb development is accelerating in Asia, particularly in India, China, Korea, the Philippines, Indonesia, and Thailand. For example, ginseng preparations from Korea and '*jamu*' from Indonesia, are commercially accessible and widely used in traditional medicine (Ahmad, 1993).

Malaysia's rainforests are home to a vast range of plant species, many of which have yet to be discovered, and many are unique and possibly helpful as medicinal sources. Traditional medicine has been practiced in Malaysia since dawn, even before modern pharmaceuticals were utilized to treat illnesses (Abd Kudus, 2010). The usage of plants, particularly herbs, is a prevalent practice by the Malay people of Malaysia. For instance, *akar susun laut* (*Tabernaemontana divaricata*), *akar melur* (*Jasminum sambac*), *bunga raya putih* (*Hibiscus rosa-sinensis*), and *ubi bemban* (*Tabernaemontana divaricata*) have been used to treat cancer in traditional medicine (Nordin & Zakaria, 2016; Zakaria & Mohd, 1994).

Currently, many researchers are attempting to identify more plants with therapeutic properties and the potential to be marketed as herbal treatments. Because of its widespread use in alternative treatments, the Myrtaceae family of plants is regarded to have enhanced therapeutic effects.

1.2 Myrtaceae Family

The Myrtaceae family has nearly about 55,000 species, which were classified into two subfamilies, 17 tribes, and 142 genera. The species are evergreen shrubs or woody trees and are mainly found in North Africa and South America along the Mediterranean. The term "myrtle," which is a common name for various members of the genus *Myrtus*, is frequently applied to a variety of other plants (Rahman, 2018).

Many members of the family have appealing glossy green leaves and brightly colored blooms, making them popular ornamentals. When the plants are young, the leaves are spherical and adhere to the branch tightly, while when the plants are old, the leaves are much longer and thinner (Mitra, 2012). A combination of characteristics distinguishes the family: whole aromatic leaves with oil glands, flower parts in multiples of four or five, ovary half inferior to inferior, numerous brilliantly colored and visible stamens, internal phloem, and vestured pits on the xylem vessels (Ebadollahi, 2013). The leaves are simple, usually opposite, and with a complete border, and oil glands are frequently present, making the leaves scented when crushed. Inflorescences are axillary or terminal, cymose but organized in a various of ways, and have one too many flowers. The flowers are bisexual, actinomorphic, and sometimes polymorphic. The fruit is a capsule, drupaceous berry, or drupe too many seeds (Mitra, 2012).

The representative species of the Myrtaceae family are aromatic plants with enormous agroindustrial potential, in addition to their ecological importance. Several members of this family are utilized in folk medicine as antidiarrheal, antibacterial,

antioxidant, cleanser, antirheumatic, anti-inflammatory, and antirheumatic agents, as well as lower blood cholesterol (Ebadollahi, 2013). Many valuable goods are produced by myrtle species, including timber (*Eucalyptus* sp.), essential oils and spices (*Melaleuca* sp.), horticultural plants (*Callistemon* and *Leptospermum* sp.), and edible fruits (*Callistemon* sp.) (*Eugenia* and *Myrciaria* species) (Ebadollahi, 2013).

1.3 The Genus *Syzygium*

Syzygium is the largest genus in the Myrtaceae family, with a wide range of species throughout Asia's tropical regions (Rahman, 2018). The genus consists of about 1800 species and can be found mainly in Southeast Asia, Southern China, Australia, New Caledonia, East Africa, Madagascar, the Mascarenhas Islands, the Southwest Pacific Islands, Taiwan, and Southern Japan (Da Costa, 2020). *Syzygium* was placed sixteenth among the 57 largest flowering plant genera, or potentially higher, since many novel species have yet to be discovered (Soh, 2017).

Syzygium is principally found in tropical or subtropical vegetation, ranging from lowland to montane rainforest, swamp, ultramafic forest, savannah to limestone forest. Some species occur in specialized habitats such as along the river, on ultramafic or limestone soil (Soh, 2017). *Syzygium*, 33 rheophytic species with thin leaves, short petioles, flexible twigs, and leaves clustered at the twig ends (Van Steenis, 1981*).* The species have thick, granular bark, glabrous twigs, opposite, entire, penninerved, gland-dotted leaves, and lateral nerves joined, forming a distinct or faint

intramarginal vein. The flowers are bisexual, in terminal or axillary corymbose cymes or panicles. The fruit is a one-celled, berry, and seeds few (Da Costa, 2020).

In the tropical rainforest, *Syzygium* species usually bloom in masses. It is one of the most abundant tree genera in the forest ecosystem, with nectar-rich blooms that bloom in clusters and juicy fruits that are eaten by birds, insects, and small and big mammals (Da Costa, 2020). It is pollinated by birds, bats, and insects, and has a low to high self-compatibility, according to several studies (Parnell et al., 2007).

Syzygium species have a long history of use in traditional medicinal systems (Cock, 2018). For instance, *S. aromaticum* has promising inhibitory activities on fatty acid synthase, which in turn helps in reducing food intake and subsequently induces weight loss. It is also well-known for its anticancer effects and has been the focus of much research (Chua et al., 2019). Besides, *Syzygium* species also renowned for having a high concentration of volatile oils, especially in the parts of the fruit (Ayyanar, 2012). Meanwhile, the species have been reported previously to treat diabetes, diarrhea, stomachaches, colds, and ulcers (Hanif, 2020).

The important ethnomedicinal properties possessed by this genus can be the basis for further research to determine the phytochemical and pharmacological aspects of the above mentioned genus. Table 1.1 lists the various medicinal uses of several *Syzygium* species.

Table 1.1

Medicinal uses of several Syzygium species

Species	Part	Traditional uses
S. cumini	Leaf	Used to treat diabetes**,** opium poisoning, centipede bites, renal problems, dysentery, inflammation, leucorrhoea, stomachache, fever, dermopathy, constipation, and prescribed for nausea, vomiting, bleeding disorders, and metrorrhagia (Hanif, 2020)
	Fruit	Helps in regulating blood sugar levels and possesses a low glycemic index, gastric, anorexia, diuretic, diabetic, chronic diarrhea, hemorrhoids, liver disorders, and abdominal diseases such as loss of appetite, dysentery, and irritable bowel syndrome (Sarma, 2020)
	Bark	To treat dysentery, repeated abortion, and headache (Machodo, 2013)
	Seed	To treat sores and ulcers, cold, cough, fever, skin diseases like rashes, the genitourinary tract ulcers, stoppage of urinary discharge, and intestine infections (Machodo, 2013)
S. aqueum	Leaf	Used for stomach pains and dysentery (Da Costa, 2020)
S. cordatum	Root	Used to treat cough, diarrhea, dysentery, malaria, malaria, wounds, and headache (Maroyi, 2018)

(continue)

Table 1.1 *(continue)*

Species	Part	Traditional uses
S. cordatum	Bark	To treat amenorrhea, anemia, chest complaints, emetics, gonorrhea, respiratory ailments, sexually transmitted infections, sores, and tuberculosis (Maroyi, 2018)
	Leaf	Used to treat colds, fever, gastro-intestinal complications, herpes simplex, herpes zoster, pre-hepatic jaundice, skin rash, and stomach problems (Maroyi, 2018)
	Fruit	To treat the wound in the mouth (Maroyi, 2018)
S. polyanthum	Leaf	Used for antiulcer, anti-diabetes, anti-inflammatory, and antidiarrhea treatment (Hamad, 2017)
S. caryophyllatum	Fruit	Treating diabetes (Watsara, 2020)
S. aromaticum	Clove	Used to relieve the stomach pain, muscle cramps, nerve conditions, nausea, diarrhea, hernia, toothache, skin counterirritant, and inflammation (Bhowmik, 2012)
S. samarangense	NM	For the treatment of fever and diarrhea (Ghayur, 2006)
S. malaccense	Bark	Used for stringent, treat cracked tongue, itching, and diuretic, blood pressure, respiration, alleviate edema dysentery, antibiotic, mouth ulcer and used by women with irregular menstruation (Nigam, 2012)
S. polycephalum	Fruit	Used for curing high blood sugar (Nigam, 2012)
S. guineense	NM	Antihypertensive properties (Cock, 2019)

(continue)

Table 1.1 *(continue)*

Species	Part	Traditional uses
S. curanii	Leaf	Cure for high blood sugar (Nigam, 2012)
S. jambos	Flower	Tonic for the brain and liver, diuretic, fever, diarrhea, dysentery, diabetics, anesthetic, and catarrh (Nigam, 2012)
	Leaf	The decoction is applied to eyes sore, emetic and cathartic, relieve asthma, bronchitis, and hoarseness (Nigam, 2012)
S. lineare	Leaf	Astringent, refrigerant, diuretic, to cool the body, and increase stamina (Nigam, 2012)
S. suboriculare	NM	To treat coughs, colds, diarrhea, and dysentery (Cock, 2019)
S. moorei	NM	Antiseptic properties (Cock, 2019)
S. francisii	NM	Antiseptic properties (Cock, 2019)
S. grande	NM	Antiseptic properties (Cock, 2019)
S. forte	NM	Antiseptic properties (Cock, 2019)

*NM – not mentioned

Few numbers of studies on the phytochemicals of *Syzygium* species have been reported and showed that the species are rich in tannins, alkaloids, steroids, flavonoids, terpenoids, fatty acids, and phenols (Srivastava & Chandra, 2013). In this study, *S. pyrifolium* has been selected to investigate of essential composition, phytochemicals, and biological activity.

Syzygium pyrifolium, locally known as ‘kelat’ or ‘jambu hutan’ in Malaysia, is mainly distributed in Malaysia, Thailand, Singapore, and Indonesia. *Syzygium pyrifolium* is one of the dominant plants in the primary forest at To Daeng Peat Swamp Forest, Narathiwat Province, Thailand (Yanbuaban et al., 2007).

Figure 1.1. Syzygium pyrifolium (Blume) DC.

1.4 Problem Statement

Medicinal plants from the *Syzygium* species appear to be of major importance due to their wide range of phytochemicals and biological properties that have been reported. In Malaysia, several *Syzygium* species have not been thoroughly explored chemically or pharmacologically. However, several *Syzygium* species have been reported for the phytochemical studies such as *S. aromaticum*, *S. cumini*, and *S. samarangense*. This study focus on *S. pyrifolium*. Until now, no report on this species. Given the relevance of this genus's therapeutic benefits in treating various ailments, it's evident that more

research is needed to be explored. Thus, investigations on the essential oils composition, isolation of phytochemicals, and the biological activity of *S. pyrifolium* were conducted. The outcomes of the study contribute to the improvement of human wellness and the field of pharmaceutical products 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 *S. pyrifolium*.
2. To isolate the major component of essential oil and phytochemicals of the extracts from *S. pyrifolium* and characterized spectroscopically.
3. To determine the antioxidant activity of the essential oils, crude extracts, and isolated phytochemicals from *S. pyrifolium*.

1.6 Scopes of Study

The study was separated into three parts. The first part was the extraction of essential oils by the 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 phytochemicals using various chromatographic methods. The structures of the isolated phytochemicals were analyzed spectroscopically using IR, NMR (1D and 2D), and MS. Finally, the antioxidant activity of the essential oils, crude extracts, and phytochemicals were carried out using total phenolic content and DPPH free radical scavenging assay.