PHYTOCHEMISTRY AND BIOLOGICAL ACTIVITIES OF *Lindera subumbelliflora* Kosterm. AND *Lindera caesia* Reinw. ex Fern.-Vill

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UNIVERSITI PENDIDIKAN SULTAN IDRIS

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NUR NABILAH BINTI MOHD ZAINI

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Whom I love and those who love me

This humble work is a sign of my love to all of you

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ABSTRACT

The purpose of this study was to investigate the essential oils, phytochemicals, and biological activities of *Lindera subumbellifora* and *Lindera caesia* (Lauraceae). The essential oils were obtained from the leaves part using hydrodistillation technique and their chemical compositions were determined using gas chromatography (GC-FID) and gas chromatography-mass spectrometry (GC-MS) methods. Cold extraction of the leaves and roots of *L. subumbellifora* was employed followed by isolation of phytochemicals using gravity column chromatography techniques. The chemical structures of phytochemicals were confirmed by comparison with literature and spectroscopic data. The antioxidant and antibiofilm activities were investigated using DPPH free radical scavenging and semi-quantitative static biofilm assay, respectively. A total of 28 components (99.6%) were successfully identified from the leaf oil of *L. subumbelliflora* which mainly consisted of β-eudesmol (14.6%), *cis*-α-bergamotene (11.0%), α-copaene (8.5%), dodecen-1-ol (8.5%), and (*E*)-nerolidol (8.3%). Meanwhile, the leaf oil of *L. caesia* constituted a total of 39 components (96.7%), dominated by terpinen-4-ol (26.3%), *neo*-intermedeol (23.2%), and eudesma-4,11-dien-3-one (10.4%). Isolation studies on the leaves and roots extracts of *L. subumbelliflora* had yielded 5,6-dehydrokawain, pinostrobin, β-sitosterol, pinocembrin, 4-hydroxy-3-methoxyacetophenone, ferulic acid, quercetin, and syringic acid. In antioxidant activity, the methanolic root extract of *L. subumbelliflora* and quercetin displayed significant activity with IC_{50} values of 77.4 and 55.2 µg/mL, respectively. In antibiofilm activity, the methanolic root extract of *L. subumbelliflora* displayed substantial inhibitory activity against *Streptococcus mutans* (99.2%) and *Candida albicans* (49.2%), while quercetin showed highly effective inhibitory activity against *Streptococcus mutans* (98.4%). In conclusion, the essential oils of *L. subumbellifora* and *L. caesia* contain sesquiterpene hydrocarbons and oxygenated sesquiterpenes as principal components, respectively. Meanwhile, the isolation study of *L. subumbellifora* extracts led to discovery of dihydrochalcones, steroid, phenolics, and flavonol. These findings suggest the potential applications of *Lindera* species in preventing oral biofilm formation and their therapeutic potential in drug development.

FITOKIMIA DAN AKTIVITI BIOLOGI DARIPADA *Lindera subumbelliflora* Kosterm. DAN *Lindera caesia* Reinw. ex Fern.-Vill

ABSTRAK

Tujuan kajian ini adalah untuk mengkaji minyak pati, fitokimia, dan aktiviti biologi *Lindera subumbellifora* dan *Lindera caesia* (Lauraceae). Minyak pati diperolehi daripada bahagian daun menggunakan teknik penyulingan hidro dan komposisi kimianya ditentukan dengan menggunakan kaedah kromatografi gas (GC-FID) dan kromatografi gas-spektrometri jisim (GC-MS). Pengekstrakan sejuk daun dan akar *L. subumbellifora* digunakan diikuti dengan pengasingan fitokimia menggunakan teknik kromatografi lajur graviti. Struktur kimia fitokimia telah disahkan dengan perbandingan antara literatur dan data spektroskopi. Aktiviti antioksidan dan antibiofilem telah disiasat masing-masing menggunakan penghapusan radikal bebas (DPPH) dan ujian biofilem statik separa-kuantitatif. Sebanyak 28 komponen kimia (99.6%) berjaya dikenalpasti daripada minyak daun *L. subumbelliflora* yang kandungan utamanya terdiri daripada β-eudesmol (14.6%), *cis*-α-bergamoten (11.0%), α-kopaen (8.5%), dodecen-1-ol (8.5%), dan (*E*)-nerolidol (8.3%). Manakala minyak daun *L. caesia* terdiri daripada 39 komponen (96.7%), didominasi oleh terpinen-4-ol (26.3%), *neo*-intermedeol (23.2%), dan eudesma-4,11-dien-3-on (10.4%). Kajian pengasingan terhadap ekstrak daun dan akar *L. subumbelliflora* telah menghasilkan 5,6-dihidrokawain, pinostrobin, β-sitosterol, pinocembrin, 4-hidroksi-3-metoksiasetofenon, asid ferulik, kuersetin, dan asid siringik. Dalam aktiviti antioksidan, ekstrak akar metanol *L. subumbelliflora* dan kuersetin menunjukkan aktiviti ketara dengan nilai IC_{50} masing-masing 77.4 dan 55.2 µg/mL. Dalam aktiviti antibiofilm, ekstrak akar metanol *L. subumbelliflora* menunjukkan aktiviti perencatan yang besar terhadap *Streptococcus mutans* (99.2%) dan *Candida albicans* (49.2%), manakala kuersetin menunjukkan aktiviti perencatan yang berkesan terhadap *Streptococcus mutans* (98.4%). Kesimpulannya, minyak pati *L. subumbellifora* dan *L. caesia* masing-masing mengandungi hidrokarbon seskuiterpena dan seskuiterpena beroksigen sebagai komponen utama. Sementara itu, kajian pengasingan ekstrak *L. subumbellifora* membawa kepada penemuan dihidrokalkon, steroid, fenolik, dan flavonol. Penemuan ini mencadangkan potensi spesies *Lindera* dalam mencegah pembentukan biofilem oral dan terapeutik dalam pembangunan ubat.

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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 centimetre
COSY	Correlation spectroscopy
1D	1 Dimension
2D	2 Dimension
δ	Chemical shift
d	doublet
DCM	Dichloromethane
dd	doublet of doublets
DEPT	Distortionless Enhancement by Polarization Transfer
EIMS	Electron Impact Mass Spectrometry
Et_2O	Diethyl ether
EtOAc	Ethyl acetate
GC	Gas Chromatography
GC-MS	Gas Chromatography-Mass Spectrometry

GC-FID	Gas Chromatography-Flame Ionisation Detector
^{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
$MgSO_4$	Magnesium sulphate
mL	milliliter
mm	millimeter
MS	Mass Spectrometer
NaCl	Sodium chloride
NMR	Nuclear Magnetic Resonance

nm	nanometer
s	singlet
SiO_2	Silica gel
t	triplet
TLC	Thin Layer Chromatography
PTLC	Preparative Thin Layer Chromatography

CHAPTER 1

INTRODUCTION

1.1 General Introduction

Since the beginning, ancient medical knowledge has been transferred and maintained in every corner of this civilised globe. Almost all early discoveries in medicine came from the field of herbs and natural remedies. Because at the time there was not sufficient information either concerning the reasons for the illnesses or concerning which plant and how it could be utilized as a cure, everything was based on experience. In time, the reasons for the usage of specific medicinal plants for the treatment of certain diseases were being discovered; thus, the medicinal plants' usage gradually abandoned the empiric framework and became founded on explicatory facts (Petrovska, 2012).

Natural products are produced by the living organism through biosynthesis mechanisms (Zhou et al., 2021). Certain illnesses are typically treated by medicinal

plants, which also serve as a possible source for pharmaceuticals. In the past, natural compounds and their structural analogues have significantly influenced pharmacology, particularly for the treatment of cancer and infectious disorders. Nowadays, natural products have undergone a million years of evolution and chemical diversification, which has led to a variety of biological activities and drug-like qualities (Maria & Christoph, 2020).

Malaysia boasts a vast array of plant species and famed in the ancient medical tradition. There is a wide variety of medicinal plants in Malaysia that are used to create potent nutritional, folk medicine, and aesthetic preparations. The most common traditional Malaysian medicinal plants are *Aloe vera* (lidah buaya), *Andrographis paniculata* (hempedu bumi), *Eurycoma longifolia* (tongkat ali), *Labisia pumila* (kacip fatimah), *Ficus deltoidea* (mas cotek), *Cymbopogon nardus* (serai wangi), *Centella asiatica* (pegaga), *Melastoma malabathricum* (senduduk), *Morinda citrifolia* (mengkudu), *Phyllanthus niruri* (dukung anak), *Momordica charantia* (peria), *Orthosiphon stamineus* (misai kucing), and *Piper sarmentosum* (kaduk) (Abu Bakar et al., 2018).

According to earlier research, the presence of specific phytochemicals in a variety of Malaysian plants has proven to be an effective treatment for a better quality of life. For instance, eupatorin and sinensetin isolated from *Orthosiphon stamineus* have anti-inflammatory properties, which may be utilized in the development of novel anti-inflammatory treatments (Laavola et al., 2012). Besides, curcumin found in *Curcuma longa* roots can help in the management of oxidative and inflammatory conditions, metabolic syndrome, arthritis, anxiety, and hyperlipidemia (Giordano & Tommonaro,

2019). Meanwhile, the roots of *Panax ginseng* contain ginsenosides which show effects on diabetes, fatigue, pulmonary problems, dyspepsia, vomiting, and ulcers (Kang & Min, 2012). Furthermore, *Centella asiatica* leaf contains active ingredients, kaempferol and quercetin that are able to treat skin damage and improve blood circulation (Gray et al., 2018). In addition, *Marinda citrifolia* is having the active compounds, scopoletin and rutin, which can act as antioxidant, anticancer, and anti-inflammatory agents (Jamaludin et al., 2021).

Many species of ethnobotanical plants still need to be thoroughly studied in order to serve as a source for prospective pharmaceuticals. For now, many scientists are attempting to identify more plants which have medicinal values and have the potential to be commercialized as herbal medicines. Hence, additional research should be conducted on different plant species, particularly those that have medicinal value for usage as herbal treatments. Lauraceae is one of the plant families which are believed to have high medicinal value due to its wide use in many traditional medicines.

1.2 Problem Statement

Plants from the family Lauraceae showed potential medicinal applications due to their wide range of phytochemicals and biological characteristics that have been reported previously. *Lindera* is a genus of flowering plants in the family Lauraceae that have shown an extensive range of phytochemicals and biological features of Lauraceae plants, and they tend to be of great importance. Earlier, phytochemical studies of *Lindera* species had successfully yielded sesquiterpenoids, alkaloids, butanolides,

lucidones, flavonoids, and phenylpropanoids. These phytochemicals have shown a variety of pharmacological and biological characteristics including anticancer, antihypertensive, anti-inflammatory, and analgesic properties. Nevertheless, several Malaysian *Lindera* species have not been properly investigated, both chemically and physiologically.

To the best of our knowledge, this is the first study reporting on the essential oils composition of *L. subumbelliflora* and *L. caesia*, as well as the phytochemistry and their biological activities. In view of the difficulty of treating infections caused by *Staphylococcus aureus* and *Candida albicans* which produces biofilms, the need for novel and effective therapies is urgent. For this reason, the development of novel therapeutic strategies, such as the use of medicinal plants with antioxidant or antimicrobial activity with a focus on inhibition or eradication of biofilm, is of great interest. This study aims to fill this gap by investigating the antioxidant and antibiofilm properties of *Lindera* species, thereby introducing a novel approach to combating dental biofilms and improving oral health. Hence, these efforts will enable a comprehensive exploration of its pharmacological importance and potential applications in drug discovery.

1.3 Objectives of Study

1. To investigate the chemical composition of the essential oil from the leaves of *L. subumbelliflora* and *L. caesia* using gas chromatography (GC-FID) and gas chromatography-mass spectrometry (GC-MS).

2. To isolate the phytochemicals from the leaves and roots extracts of *L. subumbelliflora* using various chromatography techniques and characterized using spectroscopy methods (IR, MS, and NMR).
3. To determine the antioxidant and antibiofilm potential of the essential oils, crude extracts, and isolated phytochemicals.

1.4 Scopes of Study

The study was divided into three parts. The first part was the extraction of the essential oils by hydrodistillation method from the leaf part of *L. subumbelliflora* and *L. caesia.* The chemical compositions of the essential oils were analysed using GC-FID, GC-MS, and Kovats Indices. The second part was the isolation of the phytochemicals from the leaves and root extracts of *L. subumbelliflora* using various chromatographic methods such as column chromatography. The structures of the isolated phytochemicals were analysed spectroscopically using IR, NMR (1D and 2D), and MS. Finally, the antioxidant and antibiofilm activities of the essential oils, crude extracts, and selected phytochemicals were investigated using DPPH radical scavenging and semi-quantitative static biofilm, respectively.