PHYTOCHEMISTRY AND ANTIOXIDANT ACTIVITY OF SELECTED SPECIES FROM LAURACEAE FAMILY

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ABSTRACT

The purposes of the study were to analyse the essential oils composition, phytochemicals, and antioxidant activity of selected species of the genus *Litsea* (*L. costalis*, *L. machilifolia* and *L. globularia*), *Beilschmiedia* (*B. kunstleri*, *B. insignis* and *B. pahangensis*) and *Cryptocarya* (*C. impressa*, *C. infectoria* and *C. rugulosa*) from Lauraceae 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 analysis and comparison with literature. The antioxidant activity of the essential oils and extracts was determined using DPPH free radical scavenging assay. The study showed that the major components of *L. costalis*, *L. machilifolia* and *L. globularia* essential oils were β-caryophyllene (12.6%), β-sesquiphellandrene (29.5%) and β-caryophyllene (25.2%), respectively. Besides, α-longipinene (11.0%), (*E*)-nerolidol (32.4%) and δ-cadinene were the major components from the essential oils of *B. kunstleri*, *B. insignis* and *B. pahangensis*, respectively. As for *C. impressa*, *C. infectoria* and *C. rugulosa*, the essential oils consisted of α-cadinol (40.7%), β-caryophyllene (25.4%) and bicyclogermacrene (15.6%), respectively. The dichloromethane and methanol extracts of *B. insignis* yielded seven phytochemicals identified as vanillic acid, vanillin, benzyl benzoate, benzoic acid, betulin, β-sitosterol and β-sitostenone. All essential oils displayed weak activity in DPPH radical scavenging assay, while the *L. costalis* methanolic extract showed the strongest activity (IC_{50} 21.9 ppm) comparable to ascorbic acid (IC_{50} 2.9 ppm). In conclusion, the major composition of essential oils was sesquiterpene hydrocarbons, meanwhile carboxylic acids and terpenes were successfully isolated from *B. insignis* extracts. The implication of the study showed the essential oils and extracts of the genus *Litsea*, *Beilschmiedia* and *Cryptocarya* have potential in pharmaceutical applications.

FITOKIMIA DAN AKTIVITI ANTIOKSIDAN DARIPADA SPESIES TERPILIH FAMILI LAURACEAE

ABSTRAK

Tujuan kajian untuk menganalisis komposisi minyak pati, fitokimia dan aktiviti antioksidan bagi spesies terpilih genus *Litsea* (*L. costalis*, *L. machilifolia* dan *L. globularia*), *Beilschmiedia* (*B. kunstleri*, *B. insignis* dan *B. pahangensis*) dan *Cryptocarya* (*C. impressa*, *C. infectoria* dan *C. rugulosa*) daripada famili Lauraceae. Minyak pati diperoleh melalui teknik penyulingan hidro dan kandungan kimianya ditentukan melalui kromatografi gas (GC-FID) dan kromatografi gas-spektrometri jisim (GC-MS). Sebatian fitokimia diperolehi menggunakan teknik kromatografi dan strukturnya disahkan melalui analisis spektroskopi dan perbandingan dengan literatur. Antioksidan aktiviti minyak pati dan ekstrak ditentukan menggunakan asai radikal bebas DPPH. Kajian menunjukkan komponen utama daripada minyak pati *L. costalis*, *L. machilifolia* dan *L. globularia* masing-masingnya adalah β-karyofaillena (12.6%), β-seskuifelandrena (29.5%) dan β-karyofaillena (25.2%). Selain tu, α-longifainena (11.0%), (*E*)-nerolidol (32.4%) dan δ-kadinena (21.9%) merupakan komponen utama minyak pati masing-masingnya daripada *B. kunstleri*, *B. insignis* dan *B. pahangensis*. Bagi *C. impressa*, *C. infectoria* dan *C. rugulosa*, minyak pati masing-masingnya terdiri daripada α-kadinol (40.7%), β-karyopfaillena (25.4%) dan bisiklogermakrena (15.6%). Ekstrak diklorometana dan metanol daripada *B. insignis* menghasilkan tujuh sebatian fitokimia yang dikenalpasti sebagai asid vanilik, vanilin, benzil benzoat, asid benzoik, betulin, β-sitosterol dan β-sitostenon. Kesemua minyak pati mempamerkan aktiviti yang lemah dalam asai radikal bebas DPPH, manakala ekstrak metanol *L. costalis* menunjukkan aktiviti terkuat (IC_{50} 21.9 ppm) berbanding asid askorbik (IC_{50} 2.9 ppm). Kesimpulannya, komposisi utama minyak pati adalah hidrokarbon seskuiterpena, manakala asid karboksilik dan terpena telah berjaya dipencilkan daripada ekstrak *B. insignis*. Implikasi kajian menunjukkan minyak pati dan ekstrak daripada genus *Litsea*, *Beilschmiedia* dan *Cryptocarya* berpotensi dalam aplikasi farmaseutikal.

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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
DCM	Dichloromethane
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
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
R_f	Retention factor
s	singlet
SiO_2	Silica gel
t	triplet
TLC	Thin Layer Chromatography

CHAPTER 1

INTRODUCTION

1.1 General Introduction

Plants are believed to have existed before the era of humankind. Humans have been using plants for their benefit since the early beginning of human history. Plants have so many usages as they are the source of food, shelter, and utensil as well as treatment for discomfort and diseases (Newman et al., 2000). Today, plants still serve as the main supplier for human development and ensure our survival as they are the main source of oxygen on the planet. Over the last few decades, many types of research have been carried on using plants as the main ingredient to produce alternative medicine (Ali et al., 2015).

Medicinal plants are the local heritage in many developing countries. The traditional medicinal system and drugs are being widely performed commonly in

India and China, as well as East Asian and African countries. They are also important as raw materials of several chemical drugs and also used for antimicrobials, anti-inflammatory, antituberculosis, antiviral treatments (Kumar et al., 1997). Modern drug-based medicine that is widely used nowadays proposed a variety of side effects, hence people have to find an alternative way to treat them by using traditional medicine from plants. Presently, most of the clinical drugs are from plant extracts and their derivatives (Igdir et al., 2013).

In Malaysia, many medicinal plants have been used as traditional forms of Malay, Chinese and Indian medicine. These plants can be found in herbal products and as part of the traditional Malaysian health care system because of their therapeutic efficacy (Alsarhan et al., 2014). The most popular Malaysian medicinal plants are *Aloe vera* (lidah buaya), *Labisia pumila* (kacip fatimah), *Ficus deltoidea* (mas cotek), *Centella asiatica* (pegaga) and *Piper sarmentosum* (kaduk). Several Malaysian medicinal plants are used for health preparations. The roots of *Eurycoma longifolia* (tongkat ali) is one of the main ingredients in preparing these mixtures. *Andrographis paniculata* (hempedu bumi), a mixture of buds from several types of *lime* and *kancing baju* are used to treat diabetes, whereas *Orthosiphon grandifloras* (misai kucing) are used for hypertension (Burkill, 1966; Ahmad, 2015).

Currently, numerous researchers are currently striving to discover more plants which have medicinal qualities and have the capability to be marketed as herbal remedies. Lauraceae is one of the families of plants that are considered to have elevated therapeutic benefits due to its extensive used in various alternative medicines.

1.2 Problem Statement

Due to their limited range of phytochemicals and biological properties that have been documented, medicinal plants from the Lauraceae family seem to be of considerable significance. However, several Malaysian species of Lauraceae have not been extensively studied, both chemically and biologically. Nine species from the Lauraceae family have been selected for this study. Among them, six species was not reported yet on essential oils composition, whereas seven species was not described yet on phytochemistry. Taking into account the significance of the traditional uses of this genus in the management of several diseases, it is clear that there is a need to explore a wider range on the studies of essential oils, phytochemistry and their biological activity. Thus, studies on investigation concerning the extraction of essential oils, the isolation of phytochemicals, and the biological activity of the species selected. The research findings will contribute to the development in nutraceutical and potential in the pharmaceutical industry.

1.3 Objectives of Study

The objectives of the study are:

1. To investigate the chemical compositions of the essential oils from nine species of the Lauraceae family.
2. To isolate the phytochemicals from *B. insignis* extracts and characterized using spectroscopic techniques.
3. To determine the antioxidant activity of the essential oils and extracts.

1.4 Scopes of Study

The study was separated into three sections. The first section was the extraction of essential oils using the hydrodistillation method from the leaves of *L. costalis*, *L. machilifolia*, *L. ferruginea*, *B. kunstleri*, *B. insignis*, *B. pahangensis*, *C. impresssa*, *C. infectoria*, and *C. rugulosa*. The essential oil composition were examined using GC, GC-MS, and Kovats Indices in order to classify the chemical components. The second section was the isolation of the phytochemicals from *B. insignis* extracts using various chromatography methods such as column chromatography and preparative thin layer chromatography. The structures of the isolated phytochemicals were elucidated spectroscopically using IR, NMR, and MS. Lastly, the antioxidant evaluation was carried out using DPPH free radical scavenging method on the essential oils and crude extracts.