CHEMICAL CONSTITUENTS AND BIOACTIVITY OF MALAYSIAN AND INDONESIAN *KAEMPFERIA ROTUNDA*

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A thesis submitted in fulfillment of the
requirements for the award of the degree of
Master of Science (Chemistry)

Faculty of Science
Universiti Teknologi Malaysia

DECEMBER 2009

Dedicated to *Buddha, Dharmma*, *Sangha*,
my beloved father, mother, sisters, brother and friends.

ACKNOWLEDGEMENTS

First of all, I would like to express my deepest appreciation to my supervisor, Prof. Dr. Hasnah Mohd. Sirat, for her guidance, motivation, and more crucially, precious advise to all my research. I am truly grateful for her kindness, all the time and effort she devoted to me.

I would like to extend special thanks to Assoc. Prof. Dr. Farediah Ahmad, Dr. Shajarahtunnur Jamil and Mrs. Norazah Basar for their precious help and guidance.

I would also like to express my gratitude to Universiti Teknologi Malaysia for granting me scholarship and to Department of Chemistry for allowing me to get access to the GC, GC-MS, IR, UV and NMR facility.

I would like to express greatly appreciation to Ms. Mala, Mr. Emrizal and Mr. Oh Boon Thai for their patience, support and discussions. Sincerely thanks to Mr. Farriz, Mr. Syamil, Ms. Azlin, Mrs. Ummu and Mr. Salihin for the friendship and help during the work.

Sincerely thanks to lab assistants especially Mrs. Mekzum, Mr. Azmi and Mr. Hanan who helped me throughout these years.

Special thanks to my parents, my sisters, my brother and Mr. Ting Chee Ming for their love, support and understanding.

PREFACE

This thesis is the result of my work carried out in the Department of Chemistry, Universiti Teknologi Malaysia between July 2007 and June 2009 under the supervision of Prof. Dr. Hasnah Mohd. Sirat. Parts of my work described in this thesis have been reported in the following publications:

1. Yau Sui Feng and Hasnah Mohd. Sirat (2009). Chemical Constituents of *Kaempferia rotunda*. *Mal. J. Sci.* 2009. 28 (special edition): 81-88.

2. Yau Sui Feng and Hasnah Mohd. Sirat (2008). Chemical Constituents of *Kaempferia rotunda.* Paper presented at International Conference on Molecular Chemistry 2008 at University of Malaya, Kuala Lumpur. 25-26 November 2008.

3. Yau Sui Feng and Hasnah Mohd. Sirat (2008). Chemical Constituents of *Kaempferia rotunda*. Poster presented at the 2nd Penang International Conference for Young Chemists 2008 at Universiti Sains Malaysia, Penang. 18-20 June 2008.

ABSTRACT

The essential oils and the phytochemicals of the rhizomes of *Kaempferia rotunda* cultivated in Malaysia and Indonesia have been studied. Hydrodistillation of the fresh rhizomes of *K. rotunda* gave 0.09% and 0.23% oils respectively. These oils were analyzed by GC (Kovats Indices) and GC-MS. The main constituents found in the rhizome oil of Malaysia were bornyl acetate (9.6%), benzyl benzoate (8.4%) and camphor (5.6%), while the rhizome oil from Indonesia was rich in benzyl benzoate (87.7%) and *n*-pentadecane (4.2%). Extractions by soxhlet apparatus were carried out on the dried samples to get the crude extracts. Fractionation and purification on the crude extracts resulted in the isolation of three new cyclohexane oxides and ten known compounds, comprising of cyclohexane oxides, esters, carboxylic acid, labdane diterpene, and flavonoids. Two new compounds identified as 2-(benzoyloxymethyl)phenyl (3-*O*-acetyl)-β-glucopyranoside and 3-debenzoyl-rotepoxide A, together with seven known compounds, crotepoxide, 4-benzoyloxymethyl-3,8-dioxatricyclo[5.1.0.$0^{2,4}$]octane-5,6-diol 5-acetate, 1,6-desoxypipoxide, curcumrinol C, 2'-hydroxy-4,4',6'-trimethoxychalcone and naringenin 4',7-dimethyl ether were isolated from the Malaysian species, while a new compound identified as 3-acetoxy-2-benzoyloxy-1-(benzoyloxymethyl)-cyclohexa-4,6-diene with the seven known compounds namely crotepoxide, 4-benzoyloxymethyl-3,8-dioxatricyclo[5.1.0.$0^{2,4}$]octane-5,6-diol 5-acetate, 1,6-desoxypipoxide, 6-acetylzeylenol, *trans*-docosyl ferulate, benzyl benzoate and benzoic acid were isolated from the Indonesian species. The structures of all compounds were established based on spectral studies using MS, IR, UV and NMR spectroscopies. Naringenin 4',7-dimethyl ether, curcumrinol C, *trans*-docosyl ferulate, and benzoic acid were found for the first time from *K. rotunda* and also the genus of *Kaempferia.* Antibacterial and antioxidant screening assays using disc-diffusion method and 2,2-diphenyl-1-picrylhydrazyl radical (DPPH) method, respectively were carried out on the crude extracts and essential oils. The crude extracts and essential oils of *K. rotunda* from Malaysia and Indonesia did not show activities on antibacterial and antioxidant assay.

ABSTRAK

Kajian terhadap komposisi minyak pati dan fitokimia ke atas *Kaempferia rotunda* yang ditanam di Malaysia dan Indonesia telah dilakukan. Sebanyak 0.09% dan 0.23% hasil minyak pati masing-masing diperolehi daripada penyulingan hidro terhadap rizom segar *K. rotunda*. Minyak pati ini seterusnya dianalisis dengan menggunakan KG kapilari (Indeks Kovat) dan KG-SJ. Sebatian utama dalam minyak pati rizom dari Malaysia terdiri daripada bornil asetat (9.6%), benzil benzoat (8.4%), dan kamfor (5.6%), manakala minyak pati rizom dari Indonesia didapati kaya dengan benzil benzoat (87.7%) dan *n*-pentadekana (4.2%). Pengekstrakan soxhlet telah dijalankan terhadap rizom kering untuk mendapatkan ekstrak mentah. Pengasingan daripada ekstrak telah menemukan tiga sebatian baru dan sepuluh sebatian yang telah diketahui, merangkumi sebatian sikloheksana oksida, ester, asid karboksilik, diterpena labdana dan flavonoid. Dua sebatian baru yang diasingkan daripada spesies Malaysia dikenali sebagai 2-(benzoiloksimetil)fenil (3-*O*-asetil)-β-glukopiranosida dan 3-debenzoilrotepoksida A, bersama dengan tujuh sebatian yang diketahui, iaitu krotepoksida, 4-benzoiloksimetil-3,8-dioksatrisiklo[5.1.0.$0^{2,4}$]oktana-5,6-diol 5-asetat, 1,6-desoksipipoksida, kurkumrinol C, 2'-hidrosi-4,4',6'-trimetosikalkon and naringenin 4',7-dimetil eter. Satu sebatian baru dikenali 3-asetoksi-2-benzoiloksi-1-(benzoiloksimetil)sikloheksa-4,6-diena, manakala sebatian-sebatian yang telah diketahui, iaitu krotepoksida, 4-benzoiloksimetil-3,8-dioksatrisiklo[5.1.0.$0^{2,4}$]oktana-5,6-diol 5-asetat, 1,6-desoksipipoksida, 6-asetil-zeilenol, *trans*-dokosil ferulat, benzil benzoat dan asid benzoik telah diasingkan daripada spesies Indonesia. Struktur bagi semua sebatian telah dikenalpasti secara spektroskopi melalui MS, IR, UV dan NMR. Naringenin 4',7-dimetil eter, kurkumrinol C, *trans*-dokosil ferulat, dan benzoik asid telah ditemui pertama kali daripada *K. rotunda* khususnya daripada genus *Kaempferia*. Ujian antibakteria dan antioksidan melalui teknik pembauran cakera dan radikal bebas DPPH telah dijalankan ke atas ekstrak mentah dan minyak pati. Ekstrak mentah dan minyak pati *K. rotunda* dari Malaysia dan Indonesia didapati tidak menunjukkan perencatan aktiviti terhadap bakteria dan radikal bebas DPPH.

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LIST OF ABBREVIATIONS

br	broad
CC	Column Chromatography
COSY	Correlation Spectroscopy
^{13}C	Carbon-13
$CDCl_3$	Deuterated chloroform
CD_3COCD_3	Deuterated acetone
$CHCl_3$	Chloroform
CIMS	Chemical Ionization Mass Spectrometry
DPPH	2,2-Diphenyl-1-picrylhydrazyl
d	doublet
dd	doublet of doublet
ddd	doublet of doublet of doublet
DCM	Dichloromethane
DEPT	Distortionless Enhancement by Polarization Transfer
D_2O	Deuterium oxide
EtOAc	Ethyl acetate
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
IC_{50}	Inhibition Concentration at 50%
J	coupling constant

KBr	Potassium bromide
KI	Kovats Index
lit.	Literature
LWT	Lebensm.-Wiss. u.-Technol / Food Science and Technology
MIC	Minimum Inhibition Concentration
MS	Mass Spectrometry
mM	millimolar
m/z	mass to charge ion
MeOH	Methanol
m.p.	melting point
$MgSO_4$	Magnesium sulphate
MHz	Megahertz
m	multiplet
NMR	Nuclear Magnetic Resonance
nm	nanometer
NaOH	Sodium hydroxide
NaCl	Sodium chloride
Ph	Phenyl
PE	Petroleum ether
ppm	parts per million
q	quartet
R_f	retention factor
RP-VLC	Reversed Phase Vacuum Liquid Chromatography
SD	Standard Deviation
s	singlet
t	triplet
tr	trace
TLC	Thin Layer Chromatography
UV	Ultraviolet
VLC	Vacuum Liquid Chromatography
μM	micromolar
δ	chemical shift
c	concentration

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CHAPTER 1

INTRODUCTION

1.1 General Introduction

Malaysia with its tropical forest is blessed with high biological diversity, which enclosed over 10% of the world's total number of species, with some of them are unique only to Malaysia. Among more than 7,000 species of angiosperms and 600 species of ferns in Malaysia, about 12 to 18% of trees, shrubs and herbs are reported to have medicinal properties [1]. The usage of medicinal plant products has attracted interest since the past decade. The beneficial medicinal effects of plant materials typically result from the secondary products in the plant [2]. Many biologically active plant-derived compounds were discovered as a result of chemical studies through isolation of active compounds from traditional medicine [3]. Our Malaysian flora represents a huge, barely untapped reserve of natural resources which is believed to contain substances with therapeutic potentials that yet to be explored.

Research projects related to the natural products carried out in Malaysia included: the chemistry and technology of palm oil, natural pesticides, natural flavours and pharmalogical testing of medicinal plants [4]. Development of organic chemistry has been closely associated with the chemistry of natural products. Many techniques of extraction, separation, structure determination and synthesis have been developed to understand the structural variation among the natural products. Research in this area has certainly led to better understanding of the structural

requirements for a variety of physiological activities, leading to the synthesis and modification of several lead compounds and analogues.

The study of bioactive natural products constituents is the first step in drug discovery programs, while the eventual outcomes of blockbuster drugs may not be that easily realised in view to the high cost and research effort [5]. Despite all these, natural products drug discovery programs are developed all over the world, mainly because of the high chemical diversity from natural products as compared to synthetics. The potential of these natural products is largely unknown and endangered plants have added the urgency for more vigorous screening programs. With the currently available tools of extraction, chromatographic separation and structure identification, a fresh look at the well-studied plants may also be rewarding. In Malaysia, local universities and research institutes are embarking on their own programs which involve a concerted multidisciplinary approach in the discovery of bioactive agents from plant-derived natural products. Certain novel strategies have been carried out based on the expertise and funds available. The screening programs implemented and administered are aimed to discover new compounds from the Malaysian flora for use in the pharmaceutical and related industries. This in turn, will help in the development and transfer of technology, which can be achieved through collaborative programs.

1.2 Objectives

Phytochemical studies reported in the literature review are mostly carried out on the *Kaempferia* species of Thailand. Literature search revealed only a few reports on the studies of *K. rotunda* compared to other species. Therefore, this research will focus on the chemical compositions of essential oil and chemical constituents the rhizomes of *K. rotunda* cultivated in Kempas, Johor and Indonesia. In addition, antioxidant and antimicrobe activities of the rhizomes of *K. rotunda* will also be investigated.

The first objective is to extract the essential oils from the fresh rhizomes of *Kaempferia rotunda* and to analyze the chemical compositions of the essential oils. The second objective is to extract phytochemicals from the dried rhizomes, to purify and identify their structures. The third goal is to evaluate the antioxidant and antimicrobe activities of the essential oils, crude extracts and the pure isolated compounds.

1.3 Scope of Study

This research focuses on the study of the essential oils compositions of the fresh rhizomes and phytochemicals from the dried rhizomes of *Kaempferia rotunda* from Malaysia and Indonesia. The fresh rhizomes will be extracted by hydrodistillation technique to obtain the essential oil. The dried rhizomes will be extracted using soxhlet with different polarity of solvents. The crude extracts will be fractionated by using vacuum liquid chromatography (VLC), followed by purification of the fractions using gravity column chromatography (CC) or chromatotron or versa-flash column chromatography and or recystallization to obtain the pure compounds. The compositions of the essential oils will be analyzed using GC and GC-MS, while the chemical constituents from dried samples will be characterized using spectroscopic methods including MS, IR, ^{1}H NMR, ^{13}C NMR, 2D NMR (COSY, HMQC, HMBC) and UV. The biological activities such as antioxidant and antibacterial will be carried out on the crude extracts, essential oils and the pure isolated compounds.