LC-MS/MS PROFILING AND CHARACTERIZATION OF ACTIVE COMPONENTS FROM MEDICINAL GINGERS (*CURCUMA XANTHORRHIZA* AND *ZINGIBER ZERUMBET*)

By

SHARIN BIN RUSLAY

Thesis Submitted to the School of Graduate Studies, Universiti Putra Malaysia, in Fulfilment of the Requirements for the Degree of Master of Science

January 2006
DEDICATION

This thesis is dedicated to my beloved family

My father, Ruslay bin Jantan

My mother, Saamah bt Haji Hamid

My siblings
Saharuddin
Ruslinah
Surimy
Siti Razia

and

My beloved wife Nur Yuhasliza Abd Rashid

and

Our Prince Muhamad Danial bin Sharin
Abstract of the thesis presented to the Senate of Universiti Putra Malaysia in fulfilment of the requirement for the degree of Master of Science

**LC-MS/MS PROFILING AND CHARACTERIZATION OF ACTIVE COMPONENTS FROM MEDICINAL GINGERS (CURCUMA XANTHORRHIZA AND ZINGIBER ZERUMBET)**

By

SHARIN BIN RUSLAY

January 2006

Chairman:  Professor Md Nordin Hj. Lajis, PhD
Institute:  Bioscience

Ground fresh rhizomes of *Zingiber zerumbet* and *Curcuma xanthorrhiza* were exhaustively extracted using acetone, ethanol and water. Acetone extract of *Z. zerumbet* and ethanol extract of *C. xanthorrhiza* gave highest yield of crude extract. The crude extracts were fractionated between water and hexane, ethyl acetate and butanol. All the crude extracts and fractions were screened for antioxidant activity. Ethyl acetate and butanol fractions exhibited good antioxidant activity, of which ethyl acetate fractions from both plants showed strongest antioxidant activity.

High Performance Liquid Chromatography (HPLC) profiling was done to analyse the peak patterns of extracts and fractions of both plants. Liquid chromatography- UV diode-array and electrospray ionization mass spectroscopy (ESI-MS) have been used to characterize the active fractions of *Z. zerumbet* and *C. xanthorrhiza*. The active fraction (ethyl acetate) from *Z. zerumbet* was analysed to afford kaempferol-3-O-rhamnoside (A) and its isomeric acetyl derivative as kaempferol-3-O-(2’ or 3’-O-acetyl)rhamnoside (B),
kaempferol-3-\(\text{-}O-\text{(}4''\text{-}O\text{-acetyl})\text{rhamnoside (C), kaempferol-3-}\text{-}O(3''\text{,}4'''\text{-}O\text{-diacetyl})\text{rhamnoside (D) and kaempferol-3-}\text{-}O(2''\text{,}4'''\text{-}O\text{-diacetyl})\text{rhamnoside (E). All the structures were confirmed by using various spectroscopic method including HPLC (spiking method), ESI-MS, IR, UV and NMR spectroscopy. Three components, bisdemethoxycurcumin (F), demethoxycurcumin (G) and curcumin (H) were identified from active ethyl acetate fraction of C. xanthorrhiza. The LC-DAD-MS/MS profiling of Z. zerumbet and C. xanthorrhiza have been developed for the first time as per our knowledge.}

Phytochemical studies on the rhizomes of Z. zerumbet have yielded 7 pure compounds. Hexane fraction afforded zerumbone (1), while ethyl acetate fraction gave demethoxycurcumin (9), kaempferol (11), kaempferol-3-\(\text{-}O\text{-rhamnoside (15 or (A), kaempferol-3-}\text{-}O\text{-} \text{(}4''\text{-}O\text{-acetyl})\text{rhamnoside (14 or (C), kaempferol-3-}\text{-}O\text{-} \text{(}3''\text{,} \text{4'''\text{-}O\text{-diacetyl}})\text{rhamnoside (6 or (D) and kaempferol-3-}\text{-}O\text{-} \text{(}2''\text{,} \text{4'''\text{-}O\text{-diacetyl}})\text{rhamnoside (18} or (E).}
PEMPROFILAN DAN PENCIRIAN MELALUI LC-MS/MS TERHADAP KOMPONEN AKTIF DARIPADA ZINGER UBATAN (CURCUMA XANTHORRHIZA DAN ZINGIBER ZERUMBET)

Oleh

SHARIN BIN RUSLAY

Januari 2006

Pengerusi:  Profesor Md Nordin Haji Lajis, PhD
Institut:  Biosains

Kisaran rizom segar daripada Zingiber zerumbet dan Curcuma xanthorrhiza telah diekstrak secara berasingan menggunakan aseton, etanol dan air. Ekstrak aseton dari Z. zerumbet dan ekstrak etanol dari C. xanthorrhiza memberikan hasil ekstrak mentah yang tertinggi berbanding pelarut yang lain. Setiap ekstrak mentah dilakukan fraksinasi diantara air dengan heksana, etil asetat dan butanol. Semua ekstrak mentah dan fraksinya diskrin menggunakan ujian antioksida. Fraksi daripada etil asetat dan butanol menunjukkan aktiviti yang baik dimana fraksi etil asetat dari kedua-dua tumbuhan menunjukkan aktiviti antioksida yang sangat kuat.

Pemprofilan melalui Kromatografi Cecair Prestasi Tinggi (KCPT) telah dilakukan untuk menganalisis corak puncak bagi ekstrak dan fraksi kedua-dua sampel. Kromatografi Cecair –UL diod-array dan spektroskopi jisim pengionan elektrospray (ESI-MS) digunakan untuk mencirikan fraksi aktif bagi Z. zerumbet dan C. xanthorrhiza. Fraksi yang aktif (etil asetat) dari Z. zerumbet telah di kenalpasti sebagai kaempferol-3-0-rhamnoside (A) dan terbitan asetil isomernya ialah kaempferol-3-O-(2” or 3”-O-
acetyl)rhamnoside (B), kaempferol-3-O-(4”-O-acetyl)rhamnoside (C), kaempferol-3-O-
(3”,4”-O-diacetyl)rhamnoside (D) and kaempferol-3-O-(2”,4”-O-diacetyl)rhamnoside
(E). Semua strukturnya telah disahkan menggunakan pelbagai kaedah spektroskopi
termasuk (KCPT (spiking method), ESI-MS, IR, UV dan NMR. Tiga komponen telah
dikenalpasti daripada fraksi aktif (etil asetat) C. xanthorrhiza iaitu bisdimetoksikurkumin
(F), dimetoksikurkumin (G) dan kurkumin (H). Pemprofillan LC-DAD-MS/MS bagi C.
exanthorrhiza dan Z. zerumbet yang telah dijalankan adalah yang pertama dilaporkan.

Kajian fitokimia ke atas rizom Z. zerumbet menghasilkan 7 sebatian tulen. Fraksi heksana
menghasilkan zerumbon (1), sementara fraksi etil asetat memberikan dimetoksikurkumin
(9), kaempferol (11), kaempferol-3-O-rhamnoside (15) atau (A), kaempferol-3-O-(4”-O-
acetyl)rhamnoside (14) atau (C), kaempferol-3-O-(3”,4”-O-diacetyl)rhamnoside (6) atau
(D) dan kaempferol-3-O-(2”,4”-O-diacetyl)rhamnoside (18) atau (E).
ACKNOWLEDGEMENTS

In the name of Allah, most Gracious, most Merciful only by His grace and mercy this thesis can be completed.

I wish to express my most sincere acknowledgement and deepest appreciation to my supervisor, Prof. Dr. Mohd Nordin Hj. Lajis, for his professional guidance, encouragement and constructive criticisms from the beginning of this research till the final review of the manuscript. He was always there to provide everything I needed in the laboratory. I would also like to thank him for providing financial support during the period of study through the IRPA research.

I am also grateful to the members of my supervisory committee, Assoc. Prof. Dr. Khozirah Shaari and Assoc. Prof. Dr. Daud Israf Ali, in their capacities as members of the Supervisory Committee. Thank you for the comments and suggestion which contributed a lot towards the improvement of the final manuscript.

I am also indebted to the all staffs of the Natural Products Laboratory, IBS Universiti Putra Malaysia for their help and cooperation. Much appreciation also goes to Mrs. Nur Yuhasliza, Mr. Shamsul, Mr. Salahuddin, Mrs. Zurina, Mrs. Julia, Mrs. Normayati, Mrs. Mazina, Mr. Sagi, Mr. Guru, Mat Lip, Nazrul, Uwi, and all my lab mates for making my time an enjoyable one.
I certify that an Examination Committee has met on 24th January 2006 to conduct the final examination of Sharin Ruslay on his Master of Science thesis entitled “LC-MS/MS Profiling and Characterization of Active Components from Medicinal Gingers (Curcuma xanthorrhiza and Zingiber zerumbet) ” in accordance with Universiti Pertanian Malaysia (Higher Degree) Act 1980 and Universiti Pertanian Malaysia (Higher Degree) Regulations 1981. The Committee recommends that the candidate be awarded the relevant degree. Members of the Examination Committee are as follows:

GWENDOLINE EE CHENG LIAN, PhD
Associate Professor
Faculty of Science
Universiti Putra Malaysia
(Chairman)

ASPOLLAH HAJI SUKARI, PhD
Associate Professor
Faculty of Science
Universiti Putra Malaysia
(Internal Examiner)

IRMAWATI RAMLY, PhD
Associate Professor
Faculty of Science
Universiti Putra Malaysia
(Internal Examiner)

HASNAH MOHD SIRAT, PhD
Professor
Faculty of Science
Universti Teknologi Malaysia
(External Examiner)

HASANAH MOHD. GHAZALI, PhD
Professor/ Deputy Dean
School of Graduate Studies
Universiti Putra Malaysia

Date:
This thesis submitted to the Senate of Universiti Putra Malaysia and has been accepted as fulfillment of the requirement for the degree of Master of Science. The members of the Supervisory Committee are as follows:

**MD. NORDIN LAJIS, PhD**  
Professor  
Institute of Bioscience  
Universiti Putra Malaysia  
(Chairman)

**KHOZIRAH SHAARI, PhD**  
Associate Professor  
Institute of Bioscience  
Universiti Putra Malaysia  
(Member)

**DAUD ISRAF ALI, PhD**  
Associate Professor  
Institute of Bioscience  
Universiti Putra Malaysia  
(Member)

---

**AINI IDERIS, PhD**  
Professor/Dean  
School of Graduate Studies  
Universiti Putra Malaysia

Date:
DECLARATION

I hereby declare that the thesis is based on my original work except for quotations and citations which have been dully acknowledgement. I also declare that it has not been previously or concurrently submitted for any other degree at UPM or other institutions.

SHARIN RUSLAY

Date:
# TABLE OF CONTENTS

<table>
<thead>
<tr>
<th>Section</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>DEDICATION</td>
<td>ii</td>
</tr>
<tr>
<td>ABSTRACT</td>
<td>iii</td>
</tr>
<tr>
<td>ABSTRAK</td>
<td>v</td>
</tr>
<tr>
<td>ACKNOWLEDGEMENTS</td>
<td>vi</td>
</tr>
<tr>
<td>APPROVAL</td>
<td>vii</td>
</tr>
<tr>
<td>DECLARATION</td>
<td>viii</td>
</tr>
<tr>
<td>TABLE OF CONTENTS</td>
<td>x</td>
</tr>
<tr>
<td>LISTS OF TABLES</td>
<td>xi</td>
</tr>
<tr>
<td>LIST OF FIGURES</td>
<td>xiii</td>
</tr>
<tr>
<td>LIST OF ABBREVIATIONS</td>
<td>xv</td>
</tr>
</tbody>
</table>

## CHAPTER

1 INTRODUCTION  
Chemical Characterization and Profiling of Natural Products  
Antioxidant from Natural Sources  
*Zingiber zerumbet* and *Curcuma xanthorrhiza*  
Objectives of Study  
General Ideas  

2 LITERATURE REVIEW  
*Zingiber zerumbet*  
*Curcuma xanthorrhiza*  
Profiling and Characterization of Natural Products  

3 EXPERIMENTAL  
3.1 Instruments  
3.2 Materials  
3.3 Extraction Methods  
3.4 Screening Methods  
3.5 Profiling Methods  
   HPLC Analysis of *Zingiber zerumbet*  
   HPLC Analysis of *Curcuma xanthorrhiza*  
   LCMS/MS Profiling and Characterization  
3.6 Phytochemical studies  
   Extraction and Isolation of the Constituents from *Z. zerumbet*  
   Isolation Compound from Hexane Fraction of *Z. zerumbet*  
   Isolation of Zerumbone (1)  
   Isolation of Constituents from EtOAc Fraction of *Z. zerumbet*  
   Isolation of Demethoxycurcumin (9)  
   Isolation of Kaempferol and their Derivative  
   Isolation of Kaempferol (11)  


Isolation of Kaempferol-3-O- (3”, 4”-O-diacetyl) rhamnoside (6), Kaempferol-3-O- (2”, 4”-O-diacetyl) rhamnoside (18) 26
Isolation of Kaempferol-3-O- (4”-O-acetyl) rhamnoside (14) 28
Isolation of Kaempferol-3-O-rhamnoside (15) 29

4 RESULTS AND DISCUSSIONS 31
4.1 Extraction and Partitioning of Processes of Z. zerumbet and C. xanthorrhiza 31
   Extraction Methodology 31
4.2 Bioassay of Antioxidant Activity 34
   Antioxidant Activity of Z. zerumbet and C. xanthorrhiza fractions 34
   Screening for Antioxidant Activity 34
4.3 Profiling 37
   HPLC Profiling from Z. zerumbet and C. xanthorrhiza 37
   LC-MS Analysis and Characterization of Active Fractions (EtOAc) from Z. zerumbet 52
   LC-MS Analysis and Characterization of Active Fractions (EtOAc) from C. xanthorrhiza 57
4.4 Phytochemical studies 61
   Isolation of Compounds from Z. zerumbet 61
   Characterization of 2,6,9-Humulatrien-8-one (Zerumbone) (1) 61
   Characterization of Demethoxycurcumin (9) 72
   Characterization of Kaempferol (11) 79
   Characterization of Kaempferol-3-O-rhamnoside (15) 83
   Characterization of Kaempferol-3-O- (4”-O-acetyl) rhamnoside (14) 94
   Characterization of Kaempferol-3-O- (3”, 4”-O-diacetyl) rhamnoside (6) 108
   Characterization of Kaempferol-3-O- (2”, 4”-O-diacetyl) rhamnoside (18) 123

5 CONCLUSION 138

BIBLIOGRAPHY 140
BIODATA OF THE AUTHOR 143