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 Raziah

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

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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-*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).** 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-*O*-rhamnoside **(15)** or **(A)**, kaempferol-3-*O*- (4”-*O*-acetyl)rhamnoside **(14)** or **(C)**, kaempferol-3-*O*- (3”, 4”-*O*-diacetyl)rhamnoside **(6)** or **(D)** and kaempferol-3-*O*- (2”, 4”-*O*-diacetyl)rhamnoside **(18)** or **(E)**.

Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia bagi memenuhi keperluan ijazah Master Sains

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-*O*-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)**. Pemprofilan LC-DAD-MS/MS bagi *C. xanthorrhiza* 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)**.

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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:

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

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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.

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SHARIN RUSLAY

Date:

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