UNIVERSITI PUTRA MALAYSIA

SEARCH FOR NEW ANTIOXIDANTS AND OTHER RELATED BIOACTIVE COMPOUNDS FROM ZINGIBERACEOUS SPECIES

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By

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UPM

Abstract of the thesis presented to the Senate of Universiti Putra Malaysia in fulfilment of the requirements for the degree of Doctor of Philosophy

SEARCH FOR NEW ANTIOXIDANTS AND OTHER RELATED BIOACTIVE COMPOUNDS FROM ZINGIBERACEOUS SPECIES

By

HABSAH MOHAMAD

November 2002

Chairman: **Prof. Dr. Md. Nordin Hj. Lajis**

Faculty: **Science and Environmental Studies**

Thirty-one species from Zingiberacea family were screened for their antioxidant (FTC method), antimicrobial (disc diffusion method), and antitumour promoting (EBV EA assay method) activities. Three species, *Alpinia zerumbet, Alpinia rafflesiana* and *Etlingera elatior*, were selected for further study, based on their promising preliminary biological activities. Five known compounds were isolated from the rhizomes of *Alpinia zerumbet,* namely 5,6-dehydrokawain, flavokawin B, 1,7-diphenyl-5-hydroxy-6-heptene-3-one, (-)-pinocembrin, and a mixture of stigmasterol and β-sitosterol. From the fruits of *Alpinia rafflesiana*, seven compounds were isolated, namely 5,6-dehydrokawain, flavokawin B, 1,7-diphenyl-5-hydroxy-6-heptene-3-one, (-)-pinocembrin, cardamonin, (-)-pinostrobin, and 2′,3′,4′,6′-tetrahydroxychalcone. This is the first report on the isolation of 2′,3′,4′,6′-tetrahydroxychalcone from *Alpinia*. From the rhizomes of

Etlingera elatior, 11 compounds were isolated, namely stigmast-4-en-3-one, stigmast-4-ene-3,6-dione, stigmast-4-en-6β-ol-3-one, stigmast-4-en-6α-ol-3-one, a mixture of stigmasterol and β-sitosterol, 5α,8α-epidioxyergosta-6,22-dien-3β-ol, 16-hydroxylabda-8(17),11,13-trien-16,15-olide, tetracosanoic acid, 1-(4-hydroxy-3-methoxyphenyl)-7-(4-hydroxyphenyl)-1,6-heptadiene-3,5-dione or demethoxycurcumin 1,7-bis(4-hydroxyphenyl)-2,4,6-heptatrienone, 1,7-bis(4-hydroxyphenyl)-1,4,6-heptatrien-3-one. 16-Hydroxylabda-8(17),11,13-trien-16,15-olide and 1,7-bis(4-hydroxyphenyl)-2,4,6-heptatrienone were identified as new compounds. The structure of these compounds were established based on spectral data and comparison with literature data.

The three diarylheptanoids, demethoxycurcumin, 1,7-bis(4-hydroxyphenyl)-2,4,6-heptatrienone, 1,7-bis(4-hydroxyphenyl)-1,4,6-heptatrien-3-one, were found to have high antioxidant activity. 5,6-Dehydrokawain and (-)-pinocembrin showed weak antioxidant activity. Flavokawin B was found to be cytotoxic to a number of cell lines including MCF-7 and T-47D (Human, mammary carcinoma, positive estrogen receptor). Cardamonin showed cytotoxic activity against CEM-SS cell line. 5,6-Dehydrokawain, 1,7-diphenyl-5-hydroxy-6-heptene-3-one, (-)-pinocembrin, stigmast-4-en-3-one, stigmast-4-ene-3,6-dione, stigmast-4-en-6β-ol-3-one, and tetracosanoic acid showed antitumour promoting activity.

Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia bagi memenuhi keperluan ijazah Doktor Falsafah

PENCARIAN SEBATIAN ANTIOKSIDAN BARU DAN SEBATIAN BIOAKTIF YANG BERKAITAN DARIPADA SPESIES ZINGIBERACEA

Oleh

HABSAH MOHAMAD

November 2002

Pengerusi: **Prof. Dr. Md. Nordin Hj. Lajis**

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Tiga puluh satu spesies daripada keluarga Zingiberaceae telah dikaji untuk menentukan aktiviti antioksidan (kaedah FTC), aktiviti antimikrobial (kaedah pembauran cakera), dan aktiviti anti-penggalakan tumor (kaedah EBV EA). Tiga spesies, iaitu *Alpinia zerumbet, Alpinia rafflesiana* dan *Etlingera elatior*, telah dipilih untuk kajian lebih lanjut, berdasarkan aktiviti biologi yang tinggi daripada ekstrak mentah spesies ini. Lima sebatian yang diketahui telah dipencilkan daripada rizom *Alpinia zerumbet*, , iaitu 5,6-dehidrokawain, flavokawin B, 1,7-difenil-5-hidroksi-6-hepten-3-on, (-)-pinosembrin, dan campuran β-sitosterol dan stigmasterol. Tujuh sebatian telah dipencilkan daripada buah *Alpinia rafflesiana,* iaitu 5,6-dehidrokawain, flavokawin B, 1,7-difenil-5-hidroksi-6-hepten-3-on, (-)-pinosembrin, kardamonin, (-)-pinostrobin dan 2′,3′,4′,6′-tetrahidroksikalkon. Ini merupakan laporan yang pertama mengenai pemencilan 2′,3′,4′,6′-tetrahidroksikalkon daripada *Alpinia.* Sebelas

sebatian telah diasingkan daripada rizom *Etlingera elatior*,, iaitu stigmast-4-en-3-on, stigmast-4-en-3,6-dion, stigmast-4-en-6α-ol-3-on, stigmast-4-en-6β-ol-3-on, campuran β-sitosterol dan stigmasterol, 5α,8α-epidioksiergosta-6,22-dien-3β-ol, 16-hidroksilabda-8(17),11,13-trien-16,15-olida, asid tetrakosanoik, 1-(4-hidroksi-3-metoksifenil)-7-(4-hidroksifenil)-1,6-heptadien-3,5-dion, 1,7-bis(4-hidroksifenil)-2,4,6-heptatrienon dan 1,7-bis(4-hidroksifenil)-1,4,6-heptatrien-3-on. Sebatian 16-hidroksilabda-8(17),11,13-trien-16,15-olida dan 1,7-bis(4-hidroksifenil)-2,4,6-heptatrienon merupakan sebatian baru. Struktur sebatian-sebatian ini dikenalpasti berdasarkan data spektroskopi dan perbandingan dengan data literatur.

Ketiga-tiga sebatian diarilheptanoid, iaitu demetoksikurkumin, 1,7-bis(4-hidroksifenil)-2,4,6-heptatrienon dan 1,7-bis(4-hidroksifenil)-1,4,6-heptatrien-3-on, menunjukkan aktiviti antioksidaan yang tinggi. 5,6-Dehidrokawain dan (-)-pinosembrin menunjukkan aktiviti antioksidaan yang rendah. Flavokawin B didapati sitotoksik terhadap beberapa talian sel termasuk MCF-7 dan T-47D (sel karsinoma mamari manusia, estrogen reseptor positif). Kardamonin didapati sitotoksik terhadap talian sel CEM-SS. Sebatian 5,6-dehidrokawain, 1,7-difenil-5-hidroksi-6-hepten-3-on, (-)-pinosembrin, stigmast-4-en-3-on, stigmast-4-en-3,6-dion, stigmast-4-en-6α-ol-3-on, stigmast-4-en-6β-ol-3-on dan asid tetrakosanoik menunjukkan aktiviti anti-penggalakan tumor.

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I certify that an Examination Committee met on 26th November 2002 to conduct the final examination of Habsah Mohamad on her Doctor of Philosophy thesis entitled "Search for New Antioxidants and Other Related Compounds from Zingiberaceous Species" 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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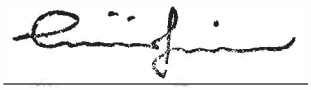

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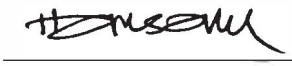
DECLARATION

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



HABSAH MOHAMAD

Date: 31st December 2002

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$[\alpha]_D$	-	specific rotation at sodium D-line
δ	-	chemical shift in ppm
br	-	broad
COSY	-	Correlated Spectroscopy
^{13}C	-	carbon-13
$CHCl_3$	-	chloroform
CH_2Cl_2	-	dichloromethane
d	-	doublet
dd	-	doublet of doublets
DPPH	-	diphenyl-*p*-picrylhydrazyl
EA	-	early antigen
EBV	-	Eipstein-Barr virus
EIMS	-	Electron impact mass spectroscopy
EtOAc	-	ethyl acetate
FGHMBC	-	Field Gradient Heteronuclear Bond Connectivity (by 2D Multiple Quantum NMR)
FGHMQC	-	Field Gradient ^1H-Detected Heteronuclear Multiple Quantum Coherence (via Direct Coupling)
^1H	-	proton
HPLC	-	High performance liquid chromatography
IC	-	inhibition concentration
id	-	internal diameter
IR	-	infrared
J		Coupling constant in Hz
Lit.	-	literature
m	-	multiplet
M	-	molar
MeOH	-	methanol
MID	-	minimum inhibition dose
Mol. wt.	-	molecular weight
mp	-	melting point
MS	-	mass spectrum/ mass spectrometry
NMR	-	nuclear magnetic resonance
NOE	-	Nuclear Overhauser Enhancement
p	-	*para*
s	-	singlet
sh	-	shoulder
t	-	triplet
TMS	-	tetramethylsilane
TPA	-	12-*O*-tetradecanoylphorbol-13-acetate
UV	-	ultraviolet

CHAPTER 1

INTRODUCTION

Antioxidant is a chemical substance extremely useful to humans. It helps us to ward off many kinds of diseases related to lungs, kidneys, heart, cardiovascular system, muscle and brain, and it helps to retard the aging process. Antioxidant has the ability to prevent or delay the formation of free radicals and lipid peroxidation in the human bodies, two main causes of human diseases and aging.

Antioxidants can be obtained from synthesis and natural sources. Antioxidants from natural sources include a wide variety of compounds from a wide range of classes, including plant-based antioxidants; amino acids, peptides and protein hydrolyzates; phytates; phospholipids; and vitamin and enzymes (Shahidi, 1997). Among the plants that have been known to provide antioxidant compounds are those belonging to Zingiberaceae, or the ginger family. Most members of the Zingiberaceae family are recognizable by the characteristic aromatic leaves and fleshy rhizomes when both of them are crushed, and also by the elliptic to elliptic-oblong leaves arranged in two ranks on the leaf-shoot. The plants vary in height and size. The Zingiberaceae family comprises about 1200 species, with 1000 occuring in tropical Asia. The richest area, with 24 genera and about 600 species, is the Malesian region, which includes

Malaysia, Indonesia, Brunei, Singapore, the Philippines and Papua New Guinea. The Peninsular Malaysia itself is estimated to have about 18 genera with more than 160 species (Larsen, 1999).

Through the ages, plants from Zingiberaceae family have been frequently used as raw materials in traditional medicines and spices (Perry, 1980). Species such as *Alpinia oxyphylla*, *Curcuma domestica*, *Curcuma xanthorrhiza*, *Zingiber officinale*, *Zingiber cassumunar* and others have been documented as extremely helpful to the elders (Itokawa *et al.*, 1981a; Burkill, 1966; Tilaar *et al.*, 1991; Ibrahim and Rahman, 1988; Ammon *et al.*, 1992; and Shiobara *et al.*, 1985). To date, considerable studies have shown that Zingiberaceae species did display antioxidant property. For instance, gingerol and diarylheptanoids related compounds isolated from *Zingiber officinale* Rosc. have been observed to have antioxidant activity (Kikuzaki and Nakatani, 1993). In addition, Cassumunins A, B, and C from *Zingiber cassumunar* were claimed to dislay both anti-inflammatory and antioxidant activity (Masuda and Jitoe, 1994). *Zingiber* is not the only genus in which antioxidant and related activities are inherent. The genus of *Curcuma* and *Alpinia* are also potent candidates to supply us with natural antioxidant and related activity compounds. Curcumin isolated from *Curcuma domestica* has been reported to display antioxidant activity (Toda *et al.*, 1985). Curcuminoids from *Curcuma*

xantorrhiza were also declared to have promising antioxidant activity (Masuda *et al.*, 1992). In addition, 1'-acetoxychavicol acetate and 1'-acetoxyeugenol acetate isolated from *Alpinia galanga* were found to have antitumour activity (Itokawa *et al.*, 1987).

Because of the large number of species in the Zingiberaceae family, many of them are still left unstudied, as evidenced by unavailability of such reports. There is a huge opportunity for researchers to discover new antioxidant compounds from these unexplored species. Furthermore, if the plants had been selected for study, only certain parts of the plants were the focus of the research. For example, for *Alpinia zerumbet*, only the leaves (Mpalantinos *et al.*, 1998) and seeds (Hong *et al.*, 1996) have been studied thus far. In addition, only the rhizomes of *Alpinia rafflesiana* that have been studied (Sirat *et al.*, 1996), while for the case of *Etlingera elatior*, only the essential oil of flower shoots that has been studied (Wong *et al.*, 1993.

These previous successful studies, and the easy availability of a collection of the unexplored Zingiberaceae species at the Laboratory of Phytomedicine, Institute of Bioscience, Universiti Putra Malaysia and throughout the country, became the impetus for us to continue searching