CHEMICAL AND PHARMACOLOGICAL STUDIES ON CHEMICAL CONSTITUENTS OF *KAEMPFERIA GALANGA* LINN.

BY

ROZANA OTHMAN

DEPARTMENT OF CHEMISTRY

FACULTY OF SCIENCE

UNIVERSITY OF MALAYA

DISSERTATION PRESENTED FOR THE

DEGREE OF MASTER OF SCIENCE

UNIVERSITY OF MALAYA

KUALA LUMPUR

(NOVEMBER 1998)

ACKNOWLEDGEMENT

I would like to express my greatest appreciation to my two supervisors, Dr. Khalijah Awang, Department of Chemistry, and Associate Prof. Dr. Mohd Rais Mustafa, Department of Pharmacology, University of Malaya, for their beneficial guidance, invaluable advice, inspiring ideas, patience and confidence throughout this study. My gratitude would also be due to Dr. Mustafa Ali Mohd, Department of Pharmacology, for his kind help, ideas, dedication, books and for allowing me the access to his instruments (especially the GC-MS machine).

I would also like to extend my sincere thanks to the following person(s):

1. Professor Dr. A. Hamid A. Hadi for his ideas, guidance and encouragement.
2. Associate Prof. Dr. Halijah Ibrahim for supplying the plant materials.
3. Associate Prof. Dr. Hapipah Mohd Ali and Mr. Jamiludin Hidayat for the supply of the compounds structurally similar to ethyl cinnamate.
4. Prof. Ng. Soon, Cik Norzalida, Mr. Hanafiah and Mr. Jasmi for the access to the NMR instrument.
5. Mr. Shahuddin Hashim for his kind assistance during the pharmacological testings on the rat aorta.
6. Staffs of the phytochemistry laboratory for their help around the laboratory.
7. All of my colleagues in the chemistry, pharmacology and zoology departments for their friendship and help, and also for sharing their experiences with me. Also, thanks for the support, concern and the stories.

Finally, I owe very much to my family, especially my husband, Hermanto for the constant encouragement throughout this study, not to forget his regular 'push' for me to finish this thesis.

And last, though not least, Alhamdulillah.

And though one thought
his journey had ended
Think again
There's a whole world to discover

LIST OF FIGURES

LIST OF SCHEMES

LIST OF TABLES

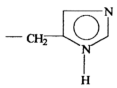
ABBREVIATIONS

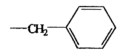
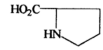
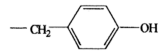
ATP	adenosine triphosphate
$(Ca^{2+})_i$	intracellular Ca^{2+}
$[Ca^{2+}]_i$	intracellular Ca^{2+} concentration
$CaCl_2$	calcium chloride
cAMP	cyclic adenosine monophosphate
CCl_4	carbon tetrachloride
$CDCl_3$	deuterated chloroform
cGMP	cyclic guanosine monophosphate
CH_2Cl_2	dichloromethane
$CHCl_3$	chloroform
cm	centimetre
cm^{-1}	per centimetre
COSY	H-H correlation spectroscopy
δ	chemical shift
DAHP	3-deoxy-*D*-arabino-heptulosonic acid-7-phosphate
DEPT	distortionless enhancement by polarisation transfer
DMSO	dimethylsulphoxide
$\Delta\nu$	difference in frequency
ε	molar absorptivity
ED_{50}	effective dose at 50% activity
EDRF	endothelium-derived relaxing factors
EDTA	ethylenediamine-tetraacetic acid
EGTA	ethyleneglycol-*bis*-(β-aminoethylether)tetraacetic acid
FT	fourier transform
g	gram
GC	gas chromatogram
HETCOR	heteronuclear chemical shift correlation
high $[K^+]$, high K^+	high K^+ concentration
HMBC	heteronuclear multiple bond connectivity
Hz	Hertz

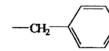
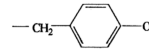
IC_{50}	concentration required to inhibit 50% of muscle contraction
IP_3	inositol-1,4,5-triphosphate
IR	infra red
J	coupling constant (Hz)
KCl	potassium chloride
LC_{50}	concentration required to kill 50% of shrimps
L-type	long lasting type
m	metre
M	molar
m/z	mass/charge
MAP	mean arterial pressure
max	maximum
MeOH	methanol
mg ml^{-1}	milligram per millilitre
$MgCl_2$	magnesium chloride
MHz	megaHertz
ml	millilitre
MLC	myosin light-chain
MLCK	myosin light-chain kinase
MLCP	myosin light-chain phosphate
mm	millimetre
mM	millimolar
MS	mass spectrum
mV	millivolt
µg ml^{-1}	microgram per millilitre
µl	microlitre
NaCl	natrium chloride
$NaHCO_3$	natrium hydrogen carbonate
nm	nanometre
nM	nanomolar
NMR	nuclear magnetic resonance
NO	nitric oxide

PE	phenylephrine
pet. ether	petroleum ether
ppm	parts per million
R_f	retention factor
ROC	receptor-operated non-selective Ca^{2+} channel
Rt	retention time
SR	sarcoplasmic reticulum
tlc	thin layer chromatography
TMS	tetramethylsilane
2D	two dimensions
UV	ultra violet
v/v	volume per volume
var	variants
VDC	voltage-dependent Ca^{2+} channel

Amino acids

abbreviation	name	structure of **R**
Ala	alanine	$-CH_3$
Arg	arginine	$-CH_2CH_2CH_2NHC(=NH)NH_2$
Asp	aspartic acid	$-CH_2CO_2H$
Gly	glycine	$-H$
His	histidine	
Ile	isoleucine	$-CH(CH_3)CH_2CH_3$
Leu	leucine	$-CH_2CH(CH_3)_2$

Phe	phenylalanine	
Pro	proline	 (complete structure)
Ser	serine	$-CH_2OH$
Tyr	tyrosine	
Val	valine	$-CH(CH_3)_2$

Phe	phenylalanine	
Pro	proline	 (complete structure)
Ser	serine	$-CH_2OH$
Tyr	tyrosine	
Val	valine	$-CH(CH_3)_2$

ABSTRACT

A phytochemical study on the constituents of *Kaempferia galanga* Linn., belonging to the family of Zingiberaceae, was performed. Using various isolation techniques such as solvent extraction, chromatography and recrystallization, three compounds were isolated: ethyl cinnamate, *p*-methoxycinnamic acid and ethyl *p*-methoxycinnamate. Structural elucidation of these compounds were carried out by spectral methods. Sixty-two volatile components of this plant were also studied using GC-MS.

Bioassay-guided fractionation of the crude CH_2Cl_2 extract on repeated chromatographies using the vascular muscles of rat thoracic aorta afforded the active constituent, ethyl cinnamate, which showed inhibitory effects on phenylephrine and high K^+-induced contractions. Mechanisms of vasorelaxant action of ethyl cinnamate were also compared to several compounds which possessed structurally similar moiety to it, and they were: cinnamaldehyde, *N*-cinnamalidene-*p*-fluoroaniline, *N*-cinnamalidene-*p*-methoxyaniline, *N*-cinnamalidene-lysin, methyl cinnamate and cinnamyl cinnamate. The results of this study suggested that these compounds inhibited both Ca^{2+} influx into the cells via voltage-dependent Ca^{2+} channel and receptor-operated Ca^{2+} channel, and Ca^{2+} release from intracellular stores. Moreover, the vasorelaxant actions of ethyl cinnamate and cinnamaldehyde might also partially involve the release of endothelium-derived relaxing factor (EDRF) and prostacyclin from endothelial cells. Ethyl cinnamate and ethyl *p*-methoxycinnamate were also tested for cytotoxicity against KB cells. The former showed low activity, while the latter revealed strong activity.

ABSTRAK

Satu kajian fitokimia terhadap kandungan *Kaempferia galanga* Linn., yang tergolong kepada famili Zingiberaceae, telah dijalankan. Dari kaedah-kaedah pengasingan seperti pengekstrakan pelarut, kromatografi dan penghabluran semula, tiga sebatian telah diasingkan: etil sinamat, asid *p*-metoksisinamik dan etil *p*-metoksisinamat. Penentuan struktur sebatian-sebatian ini telah dijalankan dengan kaedah spektra. Enam puluh dua komponen mudahruap tumbuhan ini juga telah dikaji dengan menggunakan GC-MS.

Pemisahan berpandukan ujikaji bio yang dijalankan ke atas ekstrak CH_2Cl_2 mentah, dengan kaedah kromatografi yang berulang-ulang dan menggunakan otot aorta tikus, telah menghasilkan konstituen aktif, etil sinamat, yang mempamerkan kesan halangan terhadap pengecutan-yang-dijanakan-oleh-fenilefrina dan K^+ tinggi. Mekanisma aktiviti pengenduran-vaso oleh etil sinamat telah dibandingkan dengan beberapa sebatian yang mempunyai ciri struktur yang serupa dengannya, iaitu: sinamaldehid, *N*-sinamalidin-*p*-floroanalina, *N*-sinamalidin-*p*-metoksianalina, *N*-sinamalidin-lisina, metil sinamat dan sinamil sinamat. Keputusan menyarankan bahawa sebatian-sebatian tersebut menghalang kemasukan Ca^{2+} ke dalam sel-sel melalui terusan-Ca^{2+} yang bergantung kepada voltan dan kepada reseptor, dan juga pelepasan Ca^{2+} dari stor intraselular. Dan lagi, aktiviti pengenduran-vaso oleh etil sinamat dan sinamaldehid juga mungkin sedikit sebanyak melibatkan pelepasan faktor-pengenduran dari endotelium dan prostasiklina dari sel-sel endotelial. Etil sinamat dan etil *p*-metoksisinamat juga telah diuji untuk keracunan-sel terhadap sel-sel KB. Sebatian yang pertama menunjukkan aktiviti yang lemah, manakala sebatian yang kedua menunjukkan aktiviti yang tinggi.