

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

BY

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

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ABBREVIATIONS

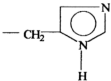
ATP	adenosine triphosphate
$(\text{Ca}^{2+})_i$	intracellular Ca^{2+}
$[\text{Ca}^{2+}]_i$	intracellular Ca^{2+} concentration
CaCl_2	calcium chloride
cAMP	cyclic adenosine monophosphate
CCl_4	carbon tetrachloride
CDCl_3	deuterated chloroform
cGMP	cyclic guanosine monophosphate
CH_2Cl_2	dichloromethane
CHCl_3	chloroform
cm	centimetre
cm^{-1}	per centimetre
COSY	H-H correlation spectroscopy
δ	chemical shift
DAHP	3-deoxy- <i>D</i> -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- <i>bis</i> -(β -aminoethylether)tetraacetic acid
FT	fourier transform
g	gram
GC	gas chromatogram
HETCOR	heteronuclear chemical shift correlation
high $[\text{K}^+]$, 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
<i>J</i>	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 phosphatase
mm	millimetre
mM	millimolar
MS	mass spectrum
mV	millivolt
µg ml ⁻¹	microgram per millilitre
µl	microlitre
NaCl	sodium chloride
NaHCO ₃	sodium hydrogen carbonate
nm	nanometre
nM	nanomolar
NMR	nuclear magnetic resonance
NO	nitric oxide

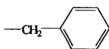
PE	phenylephrine
pet. ether	petroleum ether
ppm	parts per million
R _f	retention factor
ROC	receptor-operated non-selective Ca ²⁺ channel
R _t	retention time
SR	sarcoplasmic reticulum
tlc	thin layer chromatography
TMS	tetramethylsilane
2D	two dimensions
UV	ultra violet
v/v	volume per volume
var	variants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

Amino acids

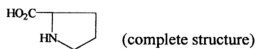


<u>abbreviation</u>	<u>name</u>	<u>structure of R</u>
Ala	alanine	—CH ₃
Arg	arginine	—CH ₂ CH ₂ CH ₂ NHC(=NH)NH ₂
Asp	aspartic acid	—CH ₂ CO ₂ H
Gly	glycine	—H
His	histidine	
Ile	isoleucine	—CH(CH ₃)CH ₂ CH ₃
Leu	leucine	—CH ₂ CH(CH ₃) ₂

Phe phenylalanine



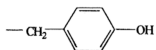
Pro proline



Ser serine



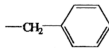
Tyr tyrosine



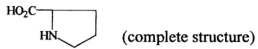
Val valine



Phe phenylalanine



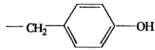
Pro proline



Ser serine



Tyr tyrosine



Val valine



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-lysine, 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 berpanduan 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: sinamaldehyd, *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 sinamaldehyd 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.