IMMUNOMODULATORY AND ANTI-METASTATIC ACTIVITIES OF SELECTED ZINGIBERACEAE SPECIES

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ABSTRACT

The use of *Zingiberaceae* rhizomes as herbal medicine has been practised since historical time. There have been many researches on the anti-inflammatory, anti-oxidative, and anti-mutagenic properties on Zingiberaceae. However, scientific knowledge of Zingiberaceae plants in immunomodulatory and anti-metastatic aspects are quite limited. Therefore, the present study was conducted to evaluate the immunomodulatory and anti-metastatic activities of ten selected *Zingiberaceae* species commonly consumed in Malaysia, namely *Alpinia galanga, Boesenbergia rotunda, Curcuma aeruginosa*, *Curcuma domestica*, *Curcuma mangga*, *Curcuma xanthorrhiza*, *Kaempferia galanga*, *Zingiber montanum*, *Zingiber officinale*, and *Zingiber zerumbet*. Each of the rhizomes was extracted with petroleum ether, chloroform and methanol and a total of 30 extracts were subjected for *in vitro* screening tests. The immunomodulatory activities of the extracts were examined using nitric oxide (NO) assay to determine their potentials in inhibiting NO generation in activated macrophage cells, RAW 264.7. In the anti-metastatic aspect, their anti-proliferative and anti-migration potentials against highly metastatic hormone-independent human breast cancer cells, MDA-MB-231 were evaluated using conventional [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide] (MTT) assays and scratch wound assay. Besides that, in order to select the least toxic extract among the active ones, the potential toxicity of the extracts was determined using the Selectivity Index (SX). This was the first report demonstrating that varieties of local *Zingiberaceae* species are potent inhibitor of NO generation in activated macrophages, and also act as potent agents in anti-proliferative and anti-migration of highly metastatic human breast cancer cells, MDA-MB-231. The best activity was demonstrated by the chloroform extract of *Alpinia galanga*, chloroform extract of *Curcuma domestica*, and petroleum ether extract *Zingiber zerumbet*.

The present finding strongly suggests the potential of selected Zingiberaceae in a bioassay guided anti-cancer drug development programme.

ABSTRAK

Rizom *Zingiberaceae* telah digunakan sebagai ubat herba sejak sekian lama. Terdapat banyak kajian tentang ciri aspek anti-inflamasi, anti-oksidatif, dan anti-mutagenik rizom *Zingiberaceae*. Namun, pengetahuan saitifik berkaitan dengan aspek immunomodulator dan anti-metastasis spesis *Zingiberaceae* adalah terhad. Oleh itu, kajian ini dijalankan untuk mengkaji kesan immunomodulator dan anti-metastasis ke atats sepuluh *Zingiberaceae* spesis yang biasanya dimakan oleh penduduk tempatan di Malaysia, iaitu *Alpinia galanga*, *Boesenbergia rotunda*, *Curcuma aeruginosa*, *Curcuma domestica*, *Curcuma xanthorrhiza*, *Kaempferiaa galanga*, *Zingiber montanum*, *Zingiber officinale*, dan *Zingiber zerumbet*. Rizom daripada setiap spesis diekstrak dengan meggunakan petroleum eter, kloroform, dan methanol untuk menghasilkan 30 ektrak yang dikaji secara *in vitro*. Aktiviti immunomodulator ekstrak dikaji dengan menggunakan esei nitrik oksida (NO) dan untuk menentukan potensi setiap ekstrak untuk mengurangkan penghasilan NO dalam sel makrofaj. Bagi aspek anti-metastasis, kesan anti-proliferasi dan anti-migrasi terhadap sel kanser buah dada, MDA-MB-231 dikaji dengan menggunakan esei 'MTT' dan esei 'scratch wound'. Selain itu, 'Selectivity Index' (SX) telah digunakan untuk memilih ekstrak yang paling tidak toksik daripada ektrak yang paling aktif. Ini adalah laporan pertama yang menunjukkan pelbagai jenis *Zingiberaceae* spesis tempatan berpotensi sebagai immunomodulator untuk mengurangkan penghasilan NO generasi dalam sel makrofaj, dan juga bertindak sebagai agen anti-proliferasi dan anti-migrasi terhadap sel MDA-MB-231. Ekstrak kloroform *Alpinia galanga*, *Curcuma domestica*, dan ekstrak petroleum eter *Zingiber zerumbet* menunjukkan aktiviti yang terbaik. Kajian ini mencerminkan potensi *Zingiberaceae* spesis terpilih untuk kanjian lanjut dalam pembangunan ubat anti-kanser.

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LIST OF SYMBOLS AND ABBREVIATIONS

˚C	degrees Celsius
%	percentage
[ ]	concentration
<	less than
>	more than
≤	less than or equal to
≥	more than or equal to
µg/mL	microgram per millimetre
µm	micrometre
µL	micro litre
µM	micromole
cm	centimetre
g	gram
g/L	gram per litre
h	hour
mg	milligram
mg/mL	milligram per millilitre
min	minute
mm	millimetre
mm/h	millimetre per hour
mL	millimetre
nm	nanometre
M	mole
rpm	revolutions per minute
et al.	et alia (and other)
i. e.	id est (that is)
L	litre
P	probability
(PG)E_2	prostaglandin
[NF]-κB	nuclear factor
$_L$-NAME	N^G-nitro-$_L$-arginine methyl ester
$_L$-NIO	*N*-iminoethyl-$_L$-ornithine
$_L$-NNA	N^G-nitro-$_L$-arginine
cNOS	constitutive nitric oxide synthase
dH_2O	distilled water
eNOS	endothelial nitric oxide synthase
iNOS	inducible nitric oxide synthase
nNOS	neuronal nitric oxide synthase
ABS	absorbance
ANOVA	analysis of variance

ATCC	American Type Culture Collection
CD	costimulatory molecule
CO_2	carbon dioxide
COX	cycloxygenase
CI	Cytotoxicity Index
DMEM	Dulbeccos's Modified Eagle's Medium
DMSO	dimethyl sulphoxide
ECM	extracellular matrices
EMT	epithelial mesenchymal transition
Eq.	equation
FBS	foetal bovine serum
FDA	Food and Drug Administration
H_2PO_4	phosphoric acid
H_2SO_4	sulphuric acid
HCl	hydrochloric acid
HOAc	acetic acid
IKK	IκB-β kinase
IC_{50}	cytostatic concentrations
IFN-γ	interferon-gama
IL	interleukin
IR	inhibitory rate
KH_2PO_4	potassium dihydrogen phosphate
KI	potassium iodide
LN_2	liquid nitrogen
LPS	lipopolysaccharide
MAPK	mitogen-activated protein kinase
MC_A	absolute migration capability
MCP	monocyte chemoattractant protein
MDA-MB-231	human breast cancer cell
MEM	minimum Essential Medium Eagle
MMP	matrix metalloproteinase
MRC-5	human lung fibroblast cell
MTT	3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide
Na_2HPO_4	sodium hydrogen phosphate
NaCl	sodium chloride
$NaHCO_3$	sodium bicarbonate
$NaNO_2$	sodium nitrite
NaOH	sodium hydroxide
NADPH	nicotinamide adenine dinucleotide phosphate-oxidase
NO	nitric oxide

NO_2^-	nitrite
NOS	nitric oxide synthase
NK	natural killer
O_2^-	superoxide anion
OD_{540}	optical density at 540 nm
$ONOO^-$	peroxynitrite
PBS	phosphate buffer saline
R_f	retardation factor
RAW 264.7	murine macrophages cell
RNS	reactive nitrogen species
ROS	reactive oxygen species
S.E.M	standard error of mean
SPSS 16.0	Statistical Package for the Social Sciences Version 16.0
TJ	tight junction
TLC	thin-layer chromatography
TLR	toll-like receptor
TNF-α	tumour necrosis factor-alpha
UV	ultraviolet