ISOLATION AND CHARACTERIZATION OF SELECTED ANTICANCER COMPOUNDS FROM ACANTHACEAE AND VERBENACEAE MEDICINAL PLANTS

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DECLARATION

I hereby declare that the material in this thesis is of my own effort, except for the quotations, excerpts, equations, summaries and references which have been duly acknowledged.

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ABSTRACT

Abundance in nature, plants are easy to obtain and comprehensively studied for their pharmacological activities in various health issues including complicated diseases such as cancers. Numerous reports on *Ruellia tuberosa* and *Phyla nodiflora* showed to have significance inhibition on human breast cancer, MCF-7 cells however, there is a lack of scientific report on the isolation of anti-cancer compounds from these plants. Therefore, this study was carried out to isolate and characterize targeted anti-cancer compounds from the leaves of *Ruellia tuberosa* and the stems of *Phyla nodiflora* which included the isolation of selected anti-cancer compounds from roots of *Clinacanthus nutans* for the first time. The preliminary MTT assay on MCF-7 cells exhibited the ethyl acetate extract of *Clinacanthus nutans* root with a significant inhibition with IC_{50} value of 20.590±2.318 µg/ml which was comparable to the *Ruellia tuberosa* leaf methanol extract with IC_{50} value of 20.563±2.825 µg/ml. Besides that, the ethyl acetate extract of *Phyla nodiflora* stem showed the weakest anti-cancer effect on MCF-7 cells than the other two extracts with an IC_{50} value of 35.597±3.276 µg/ml. These MTT assay results indicated the capabilities of the extracts for further study in isolation and characterization of selected anti-cancer compounds from the plants. The isolation of lupeol was carried out using maceration of the dried *Clinacanthus nutans* roots in ethyl acetate and successive recrystallization in methanol which yielded 1.138 g of white amorphous powder. The leaves of *Ruellia tuberosa* were extracted using soxhlet extraction in methanol and undergo repeated silica gel column chromatography using hexane and ethyl acetate as the solvents with gradient elution method which yielded 1.628 g of yellow translucent liquid. In addition, the *Phyla nodiflora* stems were extracted using soxhlet extraction in ethyl acetate and repeated silica gel column chromatography was also carried out using hexane and ethyl acetate as the solvents by gradient elution which resulted in 0.064 g of a white solid. From structural elucidation, the white amorphous powder of 1.138 g isolated from *Clinacanthus nutans* root ethyl acetate extract was suggested to be lupeol and furthermore, squalene was determined from the 1.628 g of yellow translucent from *Ruellia tuberosa* leaf methanol extract by comparison to the published literature data. The white solid obtained from *Phyla nodiflora* stem ethyl acetate extract with a yield of 0.064 g was assumed to consist a mixture of three main dietary phytosterols namely stigmasterol, campesterol and β-sitosterol as elucidated by spectroscopic analysis compared to the previous literature data. The MTT assay result revealed that squalene isolated from *Ruellia tuberosa* leaf methanol extract had the highest anti-cancer activity on MCF-7 cells with an IC_{50} value of 4.225±0.590 µg/ml followed by lupeol isolated from *Clinacanthus nutans* root ethyl acetate extract with IC_{50} value of 16.813±1.316 µg/ml. The phytosterols mixture isolated from *Phyla nodiflora* stem ethyl acetate extract had the lowest anti-cancer activity with IC_{50} value of 36.497±2.530 µg/ml in MTT assay. The findings indicate all of the extracts and their isolated compounds showed the potential in causing 50% growth inhibition of the MCF-7 cells in MTT assay. Thus, this study is important for future drug development in creating safer alternative plant-derived medicinal drug than the more adverse effects of chemotherapy.

ABSTRAK

PEMENCILAN DAN PENCIRIAN SEBATIAN-SEBATIAN ANTI-KANSER TERPILIH DARI TUMBUHAN UBATAN ACANTHACEAE DAN VERBENACEAE

Kelimpahan dalam alam semula jadi, tumbuhan adalah mudah diperolehi dan dikaji secara menyeluruh mengenai aktiviti farmakologi dalam pelbagai masalah kesihatan termasuk penyakit rumit seperti kanser. Banyak laporan pada Ruellia tuberosa dan Phyla nodiflora menunjukkan kepentingan dalam perencatan pada kanser payudara manusia, sel MCF-7 bagaimanapun, terdapat kekurangan laporan saintifik mengenai pemencilan sebatian anti-kanser dari tumbuhan tersebut. Oleh itu, kajian ini dijalankan untuk memencilkan dan mencirikan sebatian-sebatian anti-kanser daripada daun Ruellia tuberosa dan batang Phyla nodiflora yang termasuk pemencilan sebatian anti-kanser terpilih dari akar Clinacanthus nutans buat kali pertama. Dalam kajian ini, ekstrak etil asetat akar Clinacanthus nutans menunjukkan nilai IC_{50} 20.590±2.318 µg/ml iaitu setanding dengan ekstrak metanol duan Ruellia tuberosa dengan nilai IC_{50} 20.563±2.825 µg/ml. Selain itu, ekstrak etil asetat batang Phyla nodiflora menunjukkan aktiviti anti-proliferatif yang paling lemah pada MCF-7 sel berbanding dengan dua ekstrak yang lain dengan nilai IC_{50} 35.597±3.276 µg/ml. Pemencilan lupeol dengan menggunakan kaedah pemaseratan daripada ekstrak etil asetat akar kering Clinacanthus nutans dan penghabluran semula secara berturut-turut dalam metanol telah menghasilkan 1.138 g sebatian amorfus putih. Daun Ruellia tuberosa yang telah diekstrak dengan menggunakan pengekstrakan soxhlet dalam metanol dan menjalani berulang dengan heksana dan etil asetat mengikut kaedah kecerunan yang menghasilkan 1.628 g cecair lut kuning. Di samping itu, batang Phyla nodiflora diekstrak dengan menggunakan pengekstrakan soxhlet dalam etil asetat dan berulang kromatografi turus gel silika juga dijalankan dengan menggunakan heksana dan etil asetat juga dengan kaedah kecerunan yang menghasilkan 0.064 g pepejal putih. Dari struktur elusidasi, amorfus putih 1.138 g dipencilkan daripada ekstrak etil asetat akar Clinacanthus nutans disyorkan sebagai lupeol dan tambahan pula, squalene telah ditentukan daripada 1.628 g cecair lut kuning yang terpencil terutamanya daripada ekstrak metanol daun Ruellia tuberosa dibandingkan dengan data sastera sebelumnya. Pepejal putih yang diperolehi daripada ekstrak etil acetate batang Phyla nodiflora dengan hasil 0.064 g diandaikan terdiri daripada campuran tiga fitosterol utama iaitu stigmasterol, campesterol dan β-sitosterol oleh analisis spektroskopi juga berbanding dengan data sastera sebelumnya. Keputusan asai MTT mendedahkan bahawa squalene yang dipencilkan daripada ekstrak methanol daun Ruellia tuberosa mempunyai aktiviti anti-kanser yang tertinggi pada sel MCF-7 dengan IC_{50} 4.225±0.590 µg/ml diikuti oleh lupeol yang dipencilkan daripada ekstrak etil asetat akar Clinacanthus nutans dengan IC_{50} 16.813±1.316 µg/ml. Fitosterol campuran dipencilkan daripada ekstrak etil asetat batang Phyla nodiflora mempunyai aktiviti anti-kanser yang paling lemah dengan IC_{50} 36.497±2.530 µg/ml berbanding dengan squalene dan lupeol dalam MTT assai. Kajian ini menunjukkan semua daripada ekstrak dan sebatian terpencil mereka memaparkan potensi dalam menyebabkan 50% perencatan pertumbuhan pada sel MCF-7 dalam asai MTT. Oleh itu, kajian ini adalah penting dalam menghasilkan ubat perubatan alternatif dari tumbuhan yang lebih selamat berbanding dengan kesan kemoterapi.

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VZV	-	Varicella-Zoster Virus
HSV	-	Herpes Simplex Virus
HER2	-	Human Epidermal Growth Factor Receptor 2
ILC	-	Invasive Lobule Carcinoma
IDC	-	Invasive Ductal Carcinoma
SERM	-	Selective Estrogen Receptor Modulator
LCIS	-	Lobular Carcinoma *In-Situ*
TGF-β	-	Transforming Growth Factor-β
TDLUs	-	Terminal Duct Lobular Units
EAC	-	Erich's Ascites Carcinoma
NK	-	Natural Killer Cell
DCIS	-	Ductal Carcinoma *In-Situ*
K-562	-	Human Erythroleukemia Cell Line
GSH	-	Glutathione
CAT	-	Catalase
SOD	-	Superoxide Dismutase
RAJI	-	Human Burkitt's Lymphoma cell line
DPPH	-	2,2-diphenyl-1-picrylhydrazyl
CSC	-	Cancer Stem Cell
H460	-	Human Lung Carcinoma
MDA-MB231	-	Human Breast Adenocarcinoma
STZ	-	Streptozotocin

LVEF	-	Left Ventricular Ejection Fraction
MTT	-	3-(4,5-dimethyl2-thiazolyl)-2,5-diphenyltetrazolium bromide
MCF-7	-	Human Breast Cancer Cell Line
ATTC	-	American Type Culture Collection
TLC	-	Thin Layer Chromatography
VLC	-	Vacuum Liquid Chromatography
GC	-	Gas Chromatography
FTIR	-	Fourier-Transform Infrared
MP	-	Melting Point
NMR	-	Nuclear Magnetic Resonance
MS	-	Mass Spectrometry
CO_2	-	Carbon Dioxide
µg	-	microgram
ml	-	millilitre
kg	-	kilogram
δ	-	chemical shift
MHz	-	Megahertz
ppm	-	part per million
J	-	coupling constant
Hz	-	Hertz
FBS	-	Ferum Bovine Serum
rpm	-	Revolutions per minute
PBS	-	Phosphate-Buffered Saline
nm	-	nanometre

OD	-	Optical Density
MCG-803	-	Human Stomach Cancer
SK-N-AS	-	Human Neuroblastoma Cell Line
HT-29	-	Human Colorectal Adenocarcinoma Cell Line
HMG-CoA	-	3-hydroxy-3 methylglutaryl-coenzyme A
CINV	-	Chemotherapy-Induced Nausea and Vomiting
FEC	-	Fluorouracil, Cyclophosphamide, Epirubicin
FAC	-	Fluorouracil, Doxorubin, Cyclophosphamide
CMF	-	Cyclophosphamide, Methotrexate, Fluorouracil
Io	-	Intensity of the light entering the sample
It	-	Intensity of the light exiting the sample
T	-	Transmittance
NF-κB	-	Nuclear Factor kappa B
AIs	-	Aromatase Inhibitors
ER+	-	Positive Oestrogen Receptor
OS	-	Overall Survival
PFS	-	Progression-Free Survival
EGFR	-	Epidermal Growth Factor Receptor
HER1	-	Human Epidermal Growth Factor Receptor 2
EI	-	Electron Impact
COSY	-	Correlation Spectra
HSQC	-	Heteronuclear Single Quantum Coherence
HMBC	-	Heteronuclear Multiple Bond Correlation
hv	-	Photon's Energy

ε	-	Absorptivity
i.d.	-	internal diameter
±	-	plus minus
g	-	gram
µM	-	micromolar
EtOAc	-	Ethyl Acetate
MeOH	-	Methanol