

**IDENTIFICATION OF CHANGES IN THE SERUM
PROTEOME OF SPONTANEOUSLY HYPERTENSIVE RATS
(SHR) FOLLOWING ADMINISTRATION OF AQUEOUS
EXTRACTS FROM *Ficus deltoidea***

NUR ATIQAHA HAIZUM BINTI ABDULLAH

**FACULTY OF SCIENCE
UNIVERSITY OF MALAYA
KUALA LUMPUR**

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FROM *Ficus deltoidea***

NUR ATIQAHA HAIZUM BINTI ABDULLAH

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following administration of aqueous extracts from *Ficus deltoidea*

Field of Study: Natural Product Biochemistry

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ABSTRACT

Ficus deltoidea or better known as Mas Cotek is an herbaceous plant found around the coast of Peninsular Malaysia especially in Terengganu and Kelantan. This plant is believed to have the capability in helping treat or controlling diseases including hypertension. Hypertension is a major disease that affects by many people all over the world. It is one of the main diseases that contribute to high rate of mortality. Even though modern medicine have been used to treat hypertension, some people still believe that traditional medicinal practices such as taking natural products along with healthy lifestyle will significantly improve hypertension. In this study, the capability of *F. deltoidea* aqueous extracts in controlling hypertension were evaluated *in vitro*; using Angiotensin Converting Enzyme (ACE) inhibitory activity assay and through an *in vivo* study; by looking at the alteration of serum protein profiles in spontaneously hypertensive rats (SHR). Different parts of the plants obtained from two *F. deltoidea* varieties were used: fruits from the small-leaf variety (SF) and leaves from the big-leaf variety (BL). Changes in serum protein profiles were studied using proteomics techniques involving Sodium Dodecyl Sulphate- Polyacrylamide Gel Electrophoresis (SDS-PAGE), two-dimensional gel electrophoresis (2-DE), Matrix-assisted Laser Desorption / Ionization-Time of Flight / Time of Flight mass spectrometry (MALDI-TOF/TOF) and Surface-Enhanced Laser Desorption / Ionization-Time of Flight mass spectrometry (SELDI-TOF). Based on the results obtained, *F. deltoidea* showed positive effects towards the inhibition of ACE with the IC₅₀ values of 0.13 and 0.18 mg/ml for SF and BL respectively. Thus, both samples were considered to be equally efficient as ACE inhibitory agent. Results had also

demonstrated significant alterations in the serum protein profiles expressions of treated SHR rats as compared to normal and diseased rats (non-treated SHR). Seven serum proteins were confidently identified by MALDI-TOF/TOF which includes plasma retinol binding protein, serum albumin fragment, complement c3 precursor, haptoglobin, Apolipoprotein H, alpha 1 macroglobulin (A1M) and alpha 1 antiproteinase. All of these proteins showed possible association with hypertension in which A1M being the most related. SELDI-TOF univariate analysis revealed the alterations towards low molecular weight (LMW) serum proteins (2,500-40,000 kDa). Three protein peaks were altered following treatment with BL and seven with SF. SELDI-TOF multivariate analysis suggested several LMW serum proteins as potential biomarker candidates for hypertension; proteins with the m/z 33086 and 25706 (following SF treatment) and m/z 1617.5 and 5059.7 (following BL treatment). This study provides useful information that will greatly enhance the investigation of the exact mechanism of *F. deltoidea* in combating hypertension. These scientific evidences support the use of this plant as potential antihypertensive agent as claimed traditionally.

ABSTRAK

Ficus deltoidea atau lebih dikenali sebagai Mas Cotek merupakan sejenis tumbuhan herba yang banyak dijumpai di sekitar persisiran pantai Semenanjung Malaysia terutamanya di Terengganu dan Kelantan. Tumbuhan ini dipercayai berkebolehan untuk membantu merawat atau mengawal pelbagai jenis penyakit termasuk hipertensi. Hipertensi adalah penyakit utama yang dideritai oleh manusia di seluruh dunia. Ia adalah salah satu penyakit yang menyumbang kepada kadar kematian yang tinggi. Walaupun ubat-ubatan moden telah digunakan untuk merawat hipertensi, masih ramai yang mempercayai penggunaan ubatan tradisional seperti pengambilan produk tradisional beserta amalan gaya hidup sihat mampu memperbaiki tahap hipertensi dengan berkesan. Dalam kajian ini, kebolehan ekstrak akues *F. deltoidea* untuk mengawal hipertensi telah dinilai secara *in vitro*; menggunakan esei perencatan aktiviti Enzim Penukaran Angiotensin (Angiotensin Converting Enzyme - ACE) dan secara *in vivo*; dengan melihat kepada perubahan profail protein di dalam serum tikus-tikus hipertensif spontan (Spontaneously hypertensive rats – SHR). Bahagian berlainan pada pokok yang diperolehi daripada dua variasi *F. deltoidea* telah digunakan; buah-buah daripada variasi berdaun kecil (SL) dan daun-daun daripada variasi berdaun besar (BL). Perubahan pada profil protein serum telah dikaji menggunakan teknik-teknik proteomik melibatkan kaedah satu dimensi (Sodium Dodecyl Sulphate-Polyacrylamide Gel Electrophoresis - SDS-PAGE), dua dimensi gel elektroforesis (2-DE), ‘Matrix-assisted Laser Desorption / Ionization-Time of Flight / Time of Flight’ (MALDI-TOF/TOF) dan ‘Surface-enhanced Laser Desorption/ionization-Time of Flight’ (SELDI-TOF). Berdasarkan keputusan yang diperolehi, *F. deltoidea* menunjukkan kesan positif

terhadap perencatan aktiviti ACE dengan nilai IC_{50} 0.13 dan 0.18 mg/ml bagi SF dan BL masing-masing. Maka, kedua-dua sampel dianggap mempunyai kesan setara sebagai agen perencat ACE. Keputusan juga menunjukkan perubahan signifikan terhadap profil protein serum bagi tikus SHR yang dirawat berbanding tikus normal dan berpenyakit (SHR tidak dirawat). Tujuh protein serum telah dikenalpasti dengan menggunakan MALDI-TOF/TOF iaitu protein pengikat retinol plasma, pecahan albumin serum, prekursor komplemen c3, haptoglobin, Apolipoprotein H, alpha 1 macroglobulin (A1M) dan alpha 1 antiproteinase. Kesemua protein ini menunjukkan kebarangkalian adanya perkaitan dengan hipertensi dimana A1M dilihat mempunyai perkaitan yang sangat kuat. Analisis univariat SELDI-TOF memperlihatkan perubahan terhadap protein serum dengan berat molekul yang kecil (low molecular weight - LMW) (2,500-40,000 kDa). Pengekspresan tiga puncak protein telah berubah selepas dirawat dengan BL manakala tujuh puncak protein telah berubah selepas dirawat dengan SF. Analisis multivariat SELDI-TOF mencadangkan beberapa serum protein LMW yang berpotensi sebagai calon penandabiologi ; protein-protein dengan m/z 33086 dan 25706 (selepas rawatan dengan SF) serta m/z 1617.5 dan 5059.7 (selepas rawatan dengan BL). Kajian ini memberikan maklumat berguna yang akan memacu penyelidikan bagi menjelaskan mekanisme sebenar bagaimana *F. deltoidea* menentang hipertensi. Kajian saintifik ini adalah sebahagian bukti yang dapat menyokong penggunaan tumbuhan ini sebagai agen hipertensi sepertimana yang dinyatakan secara tradisional.

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ABBREVIATIONS

%	percent
°C	degree celsius
µg	microgram
µl	Microliter
µmol	micromole
2-DE	two-dimensional gel electrophoresis
A1AT	alpha 1 antiproteinase
A1M	alpha 1 macroglobulin
ACE	angiotensin converting enzyme
ACN	acetonitrile
ACTB	actin
ACTH	adrenocorticotropic hormone
ALBU	serum albumin precursor
ALLHAT	Antihypertensive and Lipid-Lowering Treatment to Prevent Heart Attack Trial
Ang I	Angiotensin I
Ang II	Angiotensin II
APOH	Apolipoprotein H
APS	ammonium persulfate
AT ₁	Angiotensin II receptor type 1
BL	large/big type leaves of <i>Ficus deltoidea</i>
BP	blood pressure
BPS	Biomarker Pattern Software™
CART	Class and Regression Tree
CBB	coomassie brilliant blue
CHAPS	3-[(3-cholamidopropyl)dimethylammonio]-1-propanesulfonate
CID	collision-induced dissociation
CLUS	clusterin precursor
CM10	a type of weak-positive ion exchanger protein microarrays

CO3	complement c3 precursor
Cp	captopril
CVD	cardiovascular disease
DBP	diastolic blood pressure
ddH ₂ O	double distilled water
DHB	2,5-dihydroxybenzoic acid
DIGE	Differential Gel Electrophoresis
DNA	deoxyribonucleic acid
DOCA	deoxycorticosterone acetate
DTT	dithiothreitol
EDM	Expression Dynamic Mapping application
FDR	False Discovery Rate
FETUA	alpha 2H glycoprotein
g	Gram
H ₃ BO ₃	boric acid
H50	a type of hydrophobic protein microarrays
HCL	hydrochloric acid
HHL	N-Hippuryl-His-Leu hydrate
HPLC	High Performance Liquid Chromatography
HPT	haptoglobin
IC ₅₀	half maximal inhibitory concentrations
ID	identification
IEF	isoelectric focusing
IMAC	immobilized metal affinity capture
INTERMAP	The International Collaborative Study of Macronutrients and Blood Pressure
IP ₃	inositol trisphosphate
IPG	immobilized ph gradients
JNC 7	The 7 th Report of the Joint National Committee
kDa	kilo Dalton
keV	kiloelectron Volt
kg	kilogram
KH ₂ PO ₄	Potassium dihydrogen phosphate

L	Liter
LMWP	low molecular weight proteins
M	Molar
m/z	mass to charge ratio
MALDI-TOF/TOF	Matrix Assisted Laser Desorption / Ionisation –Time of Flight/ Time of Flight
mg	milligram
ml	mililiter
mM	miliMolar
mm Hg	milimeter mercury
mRNA	Messenger ribonucleic acid
MS	mass spectrometry
MS/MS	tandem mass spectrometry
MW	molecular weight
NaCl	sodium chloride
NaOH	sodium hydroxide
nm	nanometer
NR	Normotensive rats
NTA	nitrilotriacetic acid
PAGE	polyacrylamide gel electrophoresis
pI	isoelectric point
ppm	parts per million
Q10	A type of strong anion exchanger protein microarrays
RETBP	plasma retinol binding protein
R _m	relative mobility
S/N	Signal to noise
SBP	systolic blood pressure
SD	Sprague Dawly rats
SDS	sodium dodecyl sulfate
SDS-PAGE	Sodium Dodecyl Sulfate – Polyacrylamide Gel Electrophoresis
SELDI - TOF	Surface Enhanced Laser Desorption / Ionisation- Time of Flight
SF	small type fruits of <i>Ficus deltoidea</i>

SHR	Spontaneously Hypertensive Rats
SHR-BL	Spontaneously Hypertensive Rats treated with big type leaves extract
SHR-Cp	Spontaneously Hypertensive Rats treated with captopril
SHR-SF	Spontaneously Hypertensive Rats treated with small type fruits extract
SPA	sinapinic acid
TEMED	tetramethylethylenediamine
TFA	trifluoroacetic acid
VSMCs	vascular smooth muscle cells
β_2 GI	β_2 -Glycoprotein I