UNIVERSITI PUTRA MALAYSIA

ANTIOXIDANT AND VASCULAR RELAXATION ACTIVITIES OF TROPICAL PLANT EXTRACTS

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By

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April 2003

Chairman : Professor Suhaila Mohamed, Ph.D.

Faculty : Food Science and Biotechnology

In this study, the methanolic extracts of twelve plants which are the leaves of noni (*Morinda citrifolia* L.), maman (*Gynandropsis gynandra* L.), cashew (*Anacardium occidentale* L.), sweet potato (*Ipomoea batatas* L.), papaya (*Carica papaya* L.), mint (*Mentha arvensis* L.), semambu (*Azadirachta indica*), betel (*Piper betle* L.); lemongrass stalk (*Cymbopogon citrates* Stapf.), bird chili fruit (*Capsicum frutescens* L.), roselle calyx (*Hibiscus sabdariffa*), and oil palm frond (*Elaeis guineensis* Jacq.) were analyzed for *in vitro* antioxidant and vascular activities. Their total phenolic content was also estimated. Plant extract showing high antioxidant and vascular relaxing activities were further tested for their *in vivo* antioxidant and cholesterol lowering effects in hypercholesterolemic New Zealand White rabbits.

In vivo antioxidant test (ferric reducing/antioxidant potential [FRAP] assay) showed the highest activity in cashew leaf extract (4.3 mmol FRAP/g d.w.) and the lowest in lemongrass extract (0.2 mmol FRAP/g d.w). The antioxidant activity of the cashew leaf extract was equivalent to those of green tea extract while lemongrass extract's antioxidant activity was equivalent to those of purified apigenin. The total phenols ranged from 55-256 mg gallic acid equivalent / g dry weight (d.w.) extracted samples with the highest concentration in oil palm frond extract. In this study, the antioxidant activity of the extracts did not correlate with the total phenolic contents, mainly due to the heterogeneity of the samples tested.

For vascular activity, sweet potato, betel, cashew, maman, mint, oil palm frond, semambu, bird chili, and papaya extracts showed more than 50 % relaxing effects in Wistar Kyoto rat's isolated aortic ring preparation. Most of the relaxing effects were endothelium-dependent with nitric oxide as the main mediator. Prostacyclin and endothelium-derived hyperpolarizing factors (EDHF) effects were also observed in small amounts. For the smaller vessels, mesenteric arteries, the extent of relaxation is reduced, where only oil palm frond, betel and lemongrass extracts showed more than 50 % relaxing effect. It is observed that in the smaller vessel, the EDHFs effects are more prominent, especially in the betel and lemongrass extracts.

Oil palm frond was selected for the *in vivo* study for its high phenolic content and high vascular relaxing activities in both blood vessel preparations. This study showed that oil palm frond supplementation in the diets of hypercholesterolemic rabbits led to a delayed

increment in serum total cholesterol levels. There was no significant antioxidant and toxicity effect on liver, kidney and muscle observed.

This study shows that most of the selected plants possess a high antioxidant and vascular relaxing activities. Antioxidants in food are important in minimizing food deterioration i.e. rancidity which affect the taste and textures of food containing lipids. In human, dietary antioxidants help to reduce oxidative stress, which is associated with the etiology of several chronic diseases such as hypertension, cancer, aging diseases, atherosclerosis and coronary artery diseases. Similarly, consumption of plants possessing vascular relaxing activities may restore and/or improve vascular functions and hence protect against vascular diseases such as hypertension.

Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia sebagai memenuhi keperluan untuk ijazah Master Sains

AKTIVITI ANTIOSIDAN DAN VASODILATOR EKSTRAK TUMBUHAN TROPIKAL

Oleh

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Dalam kajian ini, ekstrak metanol dua-belas tumbuhan iaitu daun noni (*Morinda citrifolia* L.), maman (*Gynandropsis gynandra* L.), gajus (*Anacardium occidentale* L.), keledek (*Ipomoea batatas* L.), betik (*Carica papaya* L.), pudina (*Mentha arvensis* L.), semambu (*Azadirachta indica*) dan sirih (*Piper betle* L.); batang serai (*Cymbopogon citrates* Stapf.), buah cili burung (*Capsicum frutescens* L.) kelopak roselle (*Hibiscus sabdariffa*) dan daun kelapa sawit (*Elaeis guineensis* Jacq.) telah diuji untuk aktiviti-aktiviti antioksida dan vaskular *in vitro*. Jumlah kandungan fenol juga dianggarkan. Ekstrak tumbuhan yang menunjukkan aktiviti antioksida dan vaskular yang tinggi selanjutnya diuji untuk kesan antioksida dan penurunan kolesterol *in vivo* dalam arnab New Zealand White.

Ujian antioksida *in vivo* (kajian ferric reducing/antioxidant potential [FRAP]) menunjukkan aktiviti yang tertinggi dalam ekstrak daun gajus (4.3 mmol FRAP/g berat kering [b.k]). Aktiviti antioksida ekstrak daun gajus adalah setara dengan aktiviti yang ditunjukkan oleh ekstrak teh hijau manakala aktiviti antioksida ekstrak serai adalah setara dengan yang terdapat pada apigenin. Jumlah kandungan fenol pula mempunyai julat di antara 55-256 mg GAE (gallic acid equivalent) / g b.k. dengan kandungan yang tertinggi dalam ekstrak daun kelapa sawit. Dalam kajian ini, aktiviti antioksida ekstrak-ekstrak dengan kandungan fenol tidak menunjukkan sebarang korelasi, yang berkemungkinan besar disebabkan oleh kepelbagaian sampel tumbuhan.

Untuk aktiviti vaskular, ekstrak daun keledek, sirih, gajus, pudina, kelapa sawit, semambu, buah cili dan daun betik menunjukkan kesan relaksasi yang melebihi 50 % dalam penyediaan gelung aorta tikus Wistar Kyoto. Kebanyakan kesan relaksasi adalah bergantung kepada kehadiran endotelium yang mana nitrik oksida merupakan pengantara utama. Sebilangan kecil daripada kesan relaksasi adalah disebabkan oleh prostasiklin dan faktor hiperpolarisi endotelium (EDHF). Untuk arteri mesenteri, iaitu salur darah yang lebih kecil, kesan relaksasi berkurangan jika dibandingkan dengan gelung aorta, dengan hanya ekstrak daun kelapa sawit, sirih dan serai yang menunjukkan kesan relaksasi melebihi 50 %. Pemerhatian menunjukkan bahawa dalam salur darah yang lebih kecil, kesan EDHF adalah lebih ketara, terutamanya bagi ekstrak sirih dan serai.

Daun kelapa sawit telah dipilih untuk kajian *in vivo* kerana ia mempunyai kandungan fenol dan kesan relaksasi vaskular dalam kedua-dua penyediaan salur darah. Dalam kajian ini, penambahan daun kelapa sawit ke dalam makanan arnab yang

hiperkolesterolemik menjurus kepada penangguhan dalam penambahan paras kolesterol di dalam serum. Tiada kesan antioksida dan ketoksikan yang signifikan ke atas hati, buah pinggang, dan otot ditunjukkan.

Kajian ini menunjukkan yang kebanyakan tumbuhan yang dipilih mempunyai aktiviti-aktiviti antioksida dan relaksasi vaskular yang tinggi. Antioksida di dalam makanan adalah penting untuk meminimakan kerosakan makanan iaitu ketengitan yang mempengaruhi rasa dan tekstur makanan yang mengandungi lemak. Pada manusia, antioksida dari makanan membantu mengurangkan tekanan oksida, yang dikaitkan dengan pembentukan beberapa jenis penyakit kronik seperti hipertensi, barah, penyakit yang berkaitan dengan penuaan, aterosklerosis dan penyakit koronari arteri. Sehubungan dengan itu, pemakanan yang mengandungi tumbuhan yang mempunyai aktiviti relaksasi vaskular yang tinggi mungkin dapat mengekalkan dan/atau memperbaiki fungsi vaskular dan seterusnya memberi perlindungan daripada penyakit vaskular seperti hipertensi.

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I certify that an Examination Committee has met on 15th April 2003 to conduct the final examination of Irine Runnie ak Henry Ginjom on her Master of Science thesis entitled "Antioxidant and Vascular Relaxation Activities of Tropical Plant Extracts" in accordance with Universiti Pertanian Malaysia (Higher Degree) Act 1980 and Universiti Pertanian Malaysia (Higher Degree) Regulations 1981. The committee recommends that the candidate be awarded the relevant degree. Members of the Examination Committee are as follows:

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DECLARATION

I hereby declare that the thesis is based on my original work except for quotations and citations which have been duly acknowledged. I also declare that it has not been previously or concurrently submitted for any other degree at UPM or other institutions.

.....

IRINE RUNNIE AK HENRY GINJOM

Date: 14/8/2003

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LIST OF ABBREVIATIONS

ACE	Angiotensin converting enzyme
Ach	Acetylcholine
AIDS	Acquired Immune Deficiency Syndrome
ALT	Alanine aminotransferase
ATP	Adenosine triphosphate
BHA	Butylated hydroxyanisole
BHT	Butylated hydroxytoluene
BMI	Body Mass Index
C	Cholesterol control group
CAD	Coronary artery disease
CaM	Ca^{2+}/calmodulin
cAMP	Cyclic adenosine monophosphate
cGMP	Cyclic guanosine monophosphate
CI	Confidence interval
CoQ10	Coenzyme Q10
CSIRO	Commonwealth Scientific and Industrial Research Organisation
Cu-Zn SOD	Copper-zinc superoxide dismutase
CVD	Cardiovascular diseases
DAD	Diode array detection
DNA	Deoxyribonucleic acid

DTNB	5,5`-Dithio-bis (2-Nitribenzoic) Acid
EC-SOD	Extracellular superoxide dismutase
EDCF	Endothelium derived contraction factor
EDHF	Endothelium derived hyperpolarizing factor
EDRF	Endothelium-derived relaxing factor
EDTA	Ethylene diamine tetraacetic acid
eNOS	Endothelial nitric oxide synthase
FFA	Free fatty acid
FRAP	Ferric Reducing/Antioxidant Potential
GAE	Gallic acid equivalent
GGT	Gamma glutamyl transpeptides
GSH	Glutathione
GSH-Px	Glutathione peroxidase
HCl	Hydrochloric acid
HDL	High-density lipoprotein
H_2O	Water
H_2O_2	Hydrogen peroxide
His	Histamine
HMG-CoA	3-hydroxy-3-methylglutaryl-CoA
HOCl	Hypochlorous acid
$HOCl^-$	Hypochlorite
HPLC	High performance liquid chromatography

HPLC-MS	High performance liquid chromatography with mass spectrophotometer
INDO	Indomethacin
KCl	Potassium chloride
LC-MC	Liquid chromatography with mass spectrophotometer
LDL	Low-density lipoprotein
LDL-C	Low-density lipoprotein cholesterol
$LO^{\bullet}$	Lipid alkoxyl
$LOO^{\bullet}$	Lipid peroxyl
M	Molar
MAB	Mesenteric arterial bed
MDA	Malonaldehyde
Mn-SOD	Manganase superoxide dismutase
N	Normal control group
NA	Noradrenaline
Na_2HPO_4	Disodium phosphate
NaCl	Sodium chloride/saline
NADPH	Nicotinamide adenine dinucleotide phosphate, reduced form
NO	Nitric oxide
$NO^{\bullet}$	Nitric oxide radical
NOLA	Nω-Nitro-L-Arginine
NOS	Nitric oxide synthase