UNIVERSITI PUTRA MALAYSIA

CHEMICAL COMPOSITION, ANTIOXIDATIVE AND CHOLESTEROL LOWERING PROPERTIES OF SELECTED MALAYSIA SEAWEEDS

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FSTM 2008 15

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By

PATRICIA MATANJUN

Thesis Submitted to the School of Graduate Studies, Universiti Putra Malaysia, in Fulfilment of the Requirements for the Degree of Doctor of Philosophy

December 2008

To my husband Andre,

daughter Amanda, sons Eric and Isaac

Abstract of thesis presented to the Senate of Universiti Putra Malaysia in fulfillment of the requirement for the degree of Doctor of Philosophy

CHEMICAL COMPOSITION, ANTIOXIDATIVE AND CHOLESTEROL LOWERING PROPERTIES OF SELECTED MALAYSIA SEAWEEDS

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DECEMBER 2008

Chairman : Professor Dr. Suhaila Mohamed, PhD

Faculty : Food Science and Technology

The objectives of this study were to evaluate *in vitro* antioxidant activity and total phenolic screenings of eight species of Malaysia seaweeds (*Kappaphycus alvarezii*, *Eucheuma denticulatum*, *Halymenia durvillaei*, *Caulerpa lentillifera*, *Caulerpa racemosa*, *Dicyota dichotoma*, *Sargassum polycystum* and *Padina* spp.), determine chemical composition of three selected edible seaweeds and investigate effects of these seaweeds on antioxidative, cholesterol-lowering, and their effects on biochemical, morphological and histological characteristics of selected tissues of rats fed on high-cholesterol/high-fat (HCF) diets. *In vitro* antioxidant activities of the eight species of seaweeds were evaluated using TEAC (trolox equivalent antioxidant capacity) and FRAP (ferric reducing antioxidant power) assays. Total phenolic contents of these seaweeds were determined using Folin-Ciocalteu assay. Red seaweed *K. alvarezii*, green seaweed *C. lentillifera* and brown seaweed *S. polycystum* were selected based on their high *in vitro* antioxidant activity, and further evaluated for their chemical composition,

in vivo antioxidant activity and cholesterol-lowering effects in Sprague Dawley rats fed with HCF diet for 16 weeks. Chemical analysis of seaweeds comprised of proximate composition, dietary fiber, vitamin C, vitamin E (α-tocopherol), minerals, carotenoids, chlorophylls, fatty acids and amino acids. Animal experimental diets comprised of eight groups: normal diet (N, control group), HCF diet (HCF group), normal diet supplemented with 5% seaweeds (N+KA, N+CL and N+SP groups), and HCF diet supplemented with 5% seaweed (HCF+KA, HCF+CL and HCF+SP groups). Effects of seaweeds in preventing hypercholesterolaemia and peroxidation in rats were studied via assessing the plasma lipids and, plasma and organs malondialdehyde (MDA) concentrations. Likewise, activities of antioxidant enzymes such as superoxide dismutase (SOD), glutathione peroxidase (GSH-Px) and catalase (CAT) were accessed as indices of oxidative stress. Biochemical markers for liver, heart and kidney damage such as alanine aminotransferase (ALT), aspartate aminotransferase (AST), γ-glutamyltransferase (GGT), creatinine kinase (CK), CK-MB isoenzyme, urea, creatinine and uric acid were measured. Somatic index and descriptive histological changes in the liver, heart, kidney, brain, spleen and eye of the experimental rats were also performed, while quantitative histology was restricted only to necrosis in the liver, kidney and brain. The results showed that administration of *K. alvarezii* and *C. lentillifera* reduced (P<0.05) plasma low-density lipoprotein cholesterol and triglyceride, and increased (P<0.05) plasma high-density lipoprotein cholesterol thus improving the atherogenic index of rats fed a HCF diet. These seaweeds were shown to reduce body weight gain in rats fed a HCF diet in the following order *S. polycystum*>*C. lentillifera*>*K. alvarezii*. However, *K. alvarezii* and *C. lentillifera* were more effective than *S. polycystum* in

improving the antioxidant status by reducing (P<0.05) lipid peroxidation and increasing (P<0.05) antioxidant enzymes in liver, heart and kidney of rats fed the HCF diet. Histological examinations demonstrated consumption of all three seaweeds did not exert any damage to the liver, heart, kidney, brain, spleen and eyes in normal rats. In conclusion, *K. alvarezii* and *C. lentillifera* showed hypolipidaemic effects, improve antioxidant status and exert a protective effect in mitigating the cardiac, hepatic, renal and brain abnormalities in rats fed HCF diet. The presence of high dietary fiber especially soluble fiber, omega-3 fatty acids such as eicosapentaenoic acid (C20:5ω3), and antioxidant compounds such as polyphenols, vitamin C, α-tocopherol, carotenoids and selenium may probably contributed to the cholesterol-lowering and antioxidant efficacy of these seaweeds.

Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia sebagai memenuhi keperluan untuk ijazah Doktor Falsafah

KOMPOSISI KIMIA, CIRI-CIRI ANTIOKSIDAN DAN PENURUNAN KOLESTEROL RUMPAI LAUT MALAYSIA TERPILIH

Oleh

PATRICIA MATANJUN

DISEMBER 2008

Pengerusi : Profesor Dr. Suhaila Mohamed, PhD

Fakulti : Sains dan Teknologi Makanan

Objektif-objektif kajian ini adalah untuk menilai aktiviti antioksidan *in vitro* dan menyaring kandungan fenolik lapan spesis rumpai laut Malaysia (*Kappaphycus alvarezii*, *Eucheuma denticulatum*, *Halymenia durvillaei*, *Caulerpa lentillifera*, *Caulerpa racemosa*, *Dicyota dichotoma*, *Sargassum polycystum* dan *Padina* spp.), menentukan komposisi kimia tiga rumpai laut terpilih yang boleh dimakan dan menyelidik kesan rumpai laut ini ke atas antioksidan, penurunan kolesterol, dan kesan biokimia, morfologi serta ciri-ciri histologi tisu-tisu terpilih tikus-tikus yang diberi makan diet tinggi-kolesterol/tinggi-lemak (HCF). Aktiviti antioksidan *in vitro* lapan spesis rumpai laut dinilai melalui ujian TEAC (kapasiti antioksidan equivalen troloks) dan FRAP (kuasa antioksidan penurunan ferik). Kandungan jumlah fenolik rumpai laut dinilai melalui ujian Folin-Ciocalteu. Rumpai laut merah *K.. alvarezii*, rumpai laut hijau *C. lentillifera* dan rumpai laut perang *S. polycystum* telah dipilih berdasarkan aktiviti antioksidan *in vitro* yang tinggi dan dinilai selanjutnya dari segi komposisi kimia,

aktiviti antioksidan *in vivo* serta penurunan kolesterol ke atas tikus Sprague Dawley yang diberi makan diet HCF selama 16 minggu. Komposisi kimia rumpai laut terdiri daripada komposisi proksimat, serabut diet, vitamin C, vitamin E (α-tokoferol), mineral, karotenoid, klorofil, asid lemak dan asid amino. Eksperimen diet haiwan merangkumi lapan kumpulan: diet biasa (kumpulan kawalan, N), diet HCF (kumpulan HCF), diet biasa ditambah 5% rumpai laut (kumpulan N+KA, N+CL dan N+SP) dan diet HCF ditambah 5% rumpai laut (kumpulan HCF+KA, HCF+CL dan HCF+SP). Kesan rumpai laut untuk mencegah hiperkolesterolemia dan peroksidaan dalam tikus dikaji melalui ukuran lipid plasma, dan kepekatan malondialdehid (MDA) dalam plasma serta organ. Begitu juga untuk aktiviti enzim antioksidan seperti superoksid dismutase (SOD), glutation peroksidase (GSH-Px) dan katalase (CAT) telah dinilai sebagai penunjuk untuk tekanan oksidatif. Penanda biokimia untuk kerosakan hati, jantung dan buah pinggang seperti alanin aminotrasferase (ALT), aspartat aminotransferase (AST), γ-glutamiltransferase (GGT), kreatinin kinase (CK), isoenzim CK-MB, urea, kreatinin dan asid urik telah disukat. Indeks somatik dan perubahan histologi diskriptif dalam hati, jantung, buah pinggang, otak, limpa dan mata tikus kajian juga telah dilakukan, sementara histologi kuantitatif dihadkan hanya kepada nekrosis dalam hati, buah pinggang dan otak. Keputusan menunjukkan *K.. alvarezii* dan *C. lentillifera* menurunkan (P<0.05) plasma kolesterol lipoprotein ketumpatan rendah dan trigliserid, serta meningkatkan (P<0.05) plasma kolesterol lipoprotein ketumpatan tinggi, oleh itu memperbaiki indeks atherogenik tikus yang diberi makan diet HCF. Rumpai laut ini didapati menurunkan penambahan berat badan mengikut susunan *S. polycystum*> *C. lentillifera*>*K. alvarezii*. Walaubagaimanapun, *K. alvarezii* dan *C. lentillifera* didapati

lebih berkesan daripada *S. polycystum* dalam memperbaiki status antioksidan dengan menurunkan (P<0.05) peroksidaan lipid dan meningkatkan (P<0.05) enzim antioksidan dalam hati, jantung dan buah pinggang tikus yang diberi makan diet HCF. Pemeriksaan histologi mendapati pengambilan *K. alvarezii*, *C. lentillifera* dan *S. polycystum* tidak mengakibatkan kerosakan kepada hati, jantung, buah pinggang, otak, limpa dan mata tikus-tikus normal. Kesimpulannya, *K. alvarezii* dan *C. lentillifera* didapati memberi kesan hipolipidemik, memperbaiki status antioksidan dan kesan perlindungan dengan meringankan ketidaknormalan kardiak, hepatik, renal dan otak tikus yang diberi makan diet HCF. Kehadiran serabut diet yang tinggi khususnya serabut larut, asid lemak omega-3 seperti asid eikosapentaenoik (C20:5ω3), dan sebatian antioksidan seperti polifenol, vitamin C, α-tokoferol, karotenoid dan selenium mungkin menyumbangkan kesan penurunan kolesterol dan keberkesanan antioksidan rumpai laut ini.

ACKNOWLEDGEMENTS

First and foremost I would like to thank the Almighty for his blessings and wisdom to enable me to complete this thesis.

I would like to take this opportunity to express my deepest gratitude to my supervisor, Prof. Dr. Suhaila Mohamed for her invaluable suggestions, guidance, motivation and patience throughout the research. I would also like to express my special gratitude to Assoc. Prof. Dr. Noordin Mohamed Mustapha and Assoc. Prof. Dr. Kharidah Muhammad for their constant guidance, support, tremendous encouragement and constructive comments.

Furthermore, I would like to thank the Dean of School of Food Science and Nutrition at Universiti Malaysia Sabah (UMS), Assoc. Prof. Dr. Mohd Ismail Abdullah, for providing additional research funding that assisted in the completion of my research works, as well as for his constant encouragement. I would also like to thank the Ministry of Science, Technology and Innovation of Malaysia (MOSTI) for funding part of this research project, and also to Public Service Department/UMS for the SLAB scholarship.

Special thanks to all the staff of Faculty of Food Science and Technology, and Faculty of Veterinary Medicine at Universiti Putra Malaysia (UPM) and National Institute of Pharmaceutical and Nutraceutical, especially staffs of Animal House, Post Mortem Laboratory, Histopathology Laboratory, Haematology and Clinical Biochemistry Laboratory, and Food Biochemistry Laboratory who had contributed in one way or

another towards the success of this research. Also, I would like to thank Borneo Marine Research Institute at UMS, and Sabah Fisheries Department for supplying the seaweeds and Dr. Charles Vairappan for collection of seaweeds and technical assistance. Also, thank you to Prof. Dr. Cheng Hwee Ming to allow me to use the facilities at the Antioxidant Laboratory at University Malaya.

Special thanks to my friends, Radhiah, Siti Anarita, Imilia, Juliana, Intan, Syida, Yang Hayati, Ooi Kock, Boon Kuan, Zailawati, Sanaz, Dr. Lee and Dr. Yasmin whose help, suggestions, comments and moral supports have helped in the improvement and completion of this thesis. Also thank you to everyone who had contributed in one way or another to the success of this research.

Lastly but not least, my heartfelt gratitude goes to my husband, children and family for their continuous encouragement, patience, understanding, support and unwavering love throughout the years of my study.

I certify that an Examination Committee has met on **16 December 2008** to conduct the final examination of **Patricia Matanjun** on her **Doctor of Philosophy** thesis entitled **"Chemical Composition, Antioxidative and Cholesterol Lowering Properties of Selected Malaysia Seaweeds"** 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 degree of Doctor of Philosophy.

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This thesis was submitted to the Senate of Universiti Putra Malaysia and has been accepted as fulfilment of the requirement for the degree of **Doctor of Philosophy**. The members of the Supervisory Committee were as follows:

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DECLARATION

I declare that the thesis is my original work except for quotations and citations which have been duly acknowledged. I also declare that it has not been previously, and is not concurrently, submitted for any other degree at Universiti Putra Malaysia or at any other institution.

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PATRICIA MATANJUN

Date:

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