UNIVERSITI PUTRA MALAYSIA

BENEFICIAL PROPERTIES OF *Garcinia atroviridis* Griff. FRUIT AND LEAF AQUEOUS EXTRACT IN EXPERIMENTAL ATHEROSCLEROSIS IN RABBITS

NURSAKINAH BINTI ISEMAAIL

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By

NURSAKINAH BINTI ISEMAAIL

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

June 2012

DEDICATION

I would love to dedicate this accomplishment to my lovely parents, Isemaail bin Basri and Fatimah binti Ibrahim; to my precious siblings, Nizamuddin, Nurfadhilah, Najmi and Nurnaimah; to my much beloved husband, Muhammad Nor Fazali bin Fazil; and last but certainly not least to my dear lifelong best friend, Hasnah binti Bahari.

Abstract of thesis presented to the Senate of Universiti Putra Malaysia in fulfilment of the requirement for the degree of Master of Science

BENEFICIAL PROPERTIES OF *Garcinia atroviridis* Griff. FRUIT AND LEAF AQUEOUS EXTRACT IN EXPERIMENTAL ATHEROSCLEROSIS IN RABBITS

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Chairman: Assoc. Prof. Zulkhairi Amom, PhD

Faculty: Medicine and Health Sciences

Numerous plants were proven beneficial in preventing atherosclerosis. The plant *Garcinia atroviridis*, locally known as asam gelugur had been traditionally utilized for various medicinal purpose. The present study aimed to evaluate the potential benefit of *G.atroviridis* fruit and leaf aqueous extracts supplementation by assessing the lipid profiles, antioxidant and liver enzymes, and atherosclerosis development in atherosclerosis-induced rabbits. The fruit and leaf samples were collected fresh from parts of Kedah, Malaysia; cut into pieces, dried in an oven at 30°C for 2 days and powdered. Then, 100 g of fruits and leaves powder were soaked separately in 1000 ml distilled water, incubated in water bath at three temperatures and time settings (40°C for 12 hours; 60°C for 6 hours; 100°C for 15 min) to optimize their antioxidant ability, filtered and spray dried. The antioxidant activity was evaluated using the 1,1-diphenyl-2-picrylhydrazyl (DPPH) scavenging and Ferric Reducing Antioxidant Power assay. Total phenolic content was measured by the Folin Ciocalteu method. The leaf extract (89.2%)

had higher DPPH scavenging activity than vitamin C (88.8%) ($p \geq 0.05$) and almost comparable to synthetic antioxidant butylated hydroxytoluene (94.6%). The fruit and leaf extracts prepared at 60^0C for 6 hours, and 100^0C for 15 min, respectively offered the highest antioxidant activity and phenolic contents, and therefore were supplemented to the rabbits. The extracts were force-fed once daily for 12 weeks at the dose of 50 mg/kg (F50), 100 mg/kg (F100) and 200 mg/kg (F200) for fruits; and 5 mg/kg (L5), 55 mg/kg (L55) and 105 mg/kg (L105) for leaves. Three control groups were excluded from the extract supplementation. All rabbits were fed a 0.5% cholesterol enriched diet, except the normal control group. The simvastatin control group was prescribed 10 mg/kg simvastatin while the positive control group was without any supplementation. Blood samples were collected via marginal ear vein at the beginning and every 4 weeks. As the experiment end, the leaf (L105) and fruit (F50, F100 and F200) supplementations significantly ($p<0.05$) retained the enzyme superoxide dismutase at its initial level. All extracts supplementations significantly reduced ($p<0.05$) lipid profiles (total cholesterol, triglycerides and low density lipoprotein) and liver enzymes (alkaline phosphatase, gamma-glutamyl transpeptidase and aspartate aminotransfarase). Both extracts had effectively prevented liver damage and atherosclerotic plaque formation with at least 25% lower atherogenic index than the positive control group. Hence, this study signifies the potential benefits of *G. atroviridis* fruit and leaf extracts as antioxidants, hypocholesterolemic, hepatoprotective, and anti-atherosclerotic agents.

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

FAEDAH KANDUNGAN EKSTRAK AIR BUAH DAN DAUN *Garcinia atroviridis* Griff DALAM EKSPERIMEN ATEROSKLEROSIS DALAM ARNAB

Oleh

NURSAKINAH BINTI ISEMAAIL

Jun 2012

Pengerusi: Prof. Madya Zulkhairi Amom, PhD

Fakulti: Fakulti Perubatan dan Sains Kesihatan

Pelbagai tumbuhan telah terbukti berkesan mengurangkan risiko penyakit aterosklerosis. Tumbuhan *Garcinia atroviridis,* dikenali sebagai asam gelugur telah digunakan secara tradisi untuk pelbagai tujuan kesihatan. Kajian ini bertujuan menilai potensi faedah pengambilan ekstrak buah dan daun *G. atroviridis* sebagai makanan tambahan, dengan mengkaji profil lemak, enzim antioksida dan hati, serta pertumbuhan aterosklerosis dalam arnab yang dirangsang ke arah atherosklerosis. Sampel buah dan daun segar dikumpul dari beberapa kawasan di Kedah, Malaysia; dipotong, dikeringkan di dalam ketuhar pada suhu 30°C selama 2 hari dan dihancurkan. Kemudian, 100 g serbuk buah dan daun direndam secara berasingan dalam 1000 ml air suling, dieram dalam pengukus pada tiga tetapan suhu dan masa (40°C selama 12jam; 60°C selama 6jam; 100°C selama 15 minit) bagi mengoptimumkan antioksidanya, ditapis dan dikeringkan secara semburan. Aktiviti antioksida diukur menggunakan ujian perambatan 1,1-diphenyl-2-picrylhydrazyl (DPPH) dan asai kemampuan menurunkan ferum (FRAP). Jumlah kandungan fenolik diukur

melalui kaedah Folin Ciocalteu. Aktiviti antioksida ekstrak daun (89.2%) adalah lebih tinggi daripada vitamin C (88.8%) ($p \geq 0.05$) dan hampir sama dengan antioksida tiruan *butylated hydroxytoluene* (94.6%). Ekstrak buah yang disediakan pada suhu 60^0C dalam tempoh pengeraman 6 jam, serta ekstrak daun yang disediakan pada 100^0C dalam 15 minit mempunyai aktiviti antioksida dan kandungan fenolik paling tinggi, maka ia diberikan sebagai makanan tambahan kepada arnab. Ekstrak tersebut disuapkan secara paksa sekali sehari selama 12 minggu pada dos 50 mg/kg (F50), 100 mg/kg (F100) dan 200 mg/kg (F200) bagi buah; 5 mg/kg (L5), 55 mg/kg (L55) dan 105 mg/kg (L105) bagi daun. Tiga kumpulan kawalan telah dikecualikan dari pemberian ekstrak. Semua arnab diberi makanan yang diperkaya 0.5% kolesterol, kecuali kumpulan kawalan normal. Kumpulan kawalan simvastatin (SC) telah diberi 10 mg/kg simvastatin manakala kumpulan kawalan positif tidak diberi apa-apa rawatan. Sampel darah dikumpul melalui salur vena telinga pada permulaan dan setiap 4 minggu. Setelah eksperimen berakhir, didapati pemberian daun (L105) dan buah (F50, F100 and F200) telah mengekalkan enzim *superoxide dismutase* secara signifikan ($p<0.05$) di tahap asal. Semua pemberian ekstrak telah menurunkan profil lemak (jumlah kolesterol, trigliserida dan lipoprotein ketumpatan rendah) serta enzim hati (*alkaline phosphatase*, *gamma-glutamyl transpeptidase* dan *aspartate aminotransfarase*) secara signifikan ($p<0.05$). Kedua-dua ekstrak itu juga telah berkesan menghalang kerosakan hati dan pembentukan plak aterosklerosis, dengan index aterosklerosis sekurang-kurangnya 25% lebih rendah dari kumpulan kawalan positif. Maka, kajian ini telah menunjukkan potensi faedah buah dan daun *G.atroviridis* sebagai antioksida, agen pengawal kolesterol, pelindung organ hati dan anti-aterosklerosis.

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In the name of ALLAH, the most gracious and the most merciful, as through HIS blessings I have been able to complete this thesis as required. This thesis is the end of my long journey in obtaining the degree of Masters of Science in Physiology. My deepest and sincere gratitude to my main supervisor, Assoc. Prof. Dr. Zulkhairi bin Haji Amom, from the Department of Human Anatomy, Faculty of Medicine and Health Sciences, Universiti Putra Malaysia for opening the door of further education for me and giving me the opportunity to pursue this study. His wide knowledge and guidance have been of great value for me. I would also like to express my gratitude to my co-supervisor, Dr. Norhafizah binti Mohtaruddin for her detailed and constructive comments, understanding and personal guidance, which provided a good basis for the present thesis. I would also like to thank the lecturers and staffs of the Faculty of Medicine and Health Sciences, UPM and the Forest Research Institute of Malaysia (FRIM), Kuala Lumpur who were involved in giving me assistance either directly or indirectly in the completion of this thesis and the experiments. Also many thanks to all the members of my research group for extensive help and lovely memories of company throughout the duration of my study. My appreciation also goes to the Ministry of Higher Education for funding my research through the Fundamental Research Grant Scheme (FRGS). Finally, my warmest and special gratitude goes to my parents and all my family members, my husband and my best friend for their continuous support and love. I am highly in debt to them and wish nothing but the best for them in the future journey of their lives.

APPROVAL

I certify that an Examination Committee has met on 18th of June 2012 to conduct the final examination of **Nursakinah binti Isemaail** on her Master of Science thesis entitled "**Beneficial Properties of *Garcinia atroviridis* Griff Fruit and Leaf Aqueous Extract in Experimental Atherosclerosis in Rabbits**" in accordance with Universiti Pertanian Malaysia (Higher Degree) Act 1980 and Universiti Pertanian Malaysia (Higher Degree) Regulations 1981. The Committee recommends that the student be awarded the Master of Science.

Members of the Examination Committee were as follows:

Sabrina binti Sukardi, PhD
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Date:

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

Zulkhairi Hj Amom, PhD
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Universiti Putra Malaysia
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School of Graduate Studies
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Date:

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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NURSAKINAH BINTI ISEMAAIL

Date: 18 June 2012

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