



UNIVERSITI PUTRA MALAYSIA

***DIETARY FIBER AND ANTIOXIDANT PROPERTIES OF *Mangifera pajang*
KORT. PEELS AND THEIR SYNERGISTIC HEALTH EFFECTS ON
HYPERCHOLESTEROLEMIC RATS***

FOUAD ABDULRAHMAN SALEH HASSAN

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By

FOUAD ABDULRAHMAN SALEH HASSAN

**Thesis Submitted to the School of Graduate Studies, Universiti Putra Malaysia, in
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April 2012

*ALLAH IS THE ONLY ONE WHO GIVES US THE POWER TO
FIGHT DISHONESTY AND INJUSTICE*

To

*MY BELOVED HOMELAND YEMEN
THE SOULS OF YEMENI AND ISLAMIC WORLD MARTYRS
MY BELOVED PARENTS A SOURCE OF LOVE AND
COMPASSION*

*MY BELOVED FAMILY
MY PRECIOUS FRIEND SADEQ HASAN*

WITH MY LOVE FROM DEEP OF MY HEART

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

DIETARY FIBER AND ANTIOXIDANT PROPERTIES OF *Mangifera pajang* KORT. PEELS AND THEIR SYNERGISTIC HEALTH EFFECTS ON HYPERCHOLESTEROLEMIC RATS

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Chair: Professor Amin Bin Ismail, PhD

Faculty: Medicine and Health Sciences

The fruit of *Mangifera pajang* Kort., known as bambangan is an underutilised fruit that is found in Malaysia (Sabah and Sarawak). Its size is about 3 times as large as commercial mango with a high amount of peel. The study was aimed to characterize the peels and investigate its effect on lowering cholesterol of hypercholesterolemic rat model. Bambangan peels are rich in dietary fiber and have been shown to contain high amount of valuable compounds such as polyphenols that should be strongly considered for exploitation. Dietary fiber (DF) and several properties of bambangan peels related to its nutritional quality were investigated. The physicochemical properties and antioxidant capacity of fiber rich peel powder (FRPP) obtained from bambangan peels were characterized. Chemical composition of soluble dietary fibre (SDF) and insoluble dietary fibre (IDF) for their related polysaccharides using RI-HPLC were also determined. The FRPP had a high amount of DF (72.3 g/100 g dry weight) with a balanced SDF/IDF ratio (1:1.2). The FRPP had a high glucose retardation index, water-holding capacity (WHC), oil-holding capacity (OHC), and swelling property. As FRPP was characterized

for its content of DF, it also analyzed for its content of phenolic compounds in an acidified methanolic extract obtained from fully ripe bambangan (*M. pajang* Kort.) peel. The antioxidant capacity of the FRPP as determined by Ferric Reducing Antioxidant Power (FRAP) and 1, 1-diphenyl-2- picrylhydrazyl (DPPH) assays exhibited strong potency with a high value (44 $\mu\text{g/mL}$) of IC_{50} , due to the presence of associated polyphenols (98.3 mg GAE/g FRPP) as determined by Folin–Ciocalteu method. Sixteen phenolic compounds, were identified and quantified in FRPP using a HPLC-DAD coupled to Quantum-electrospray ionization /mass spectrometry (TSQ Quantum Ultra-ESI-MS) to confirm peaks identification, by comparing their retention times, UV–Vis absorption spectra and mass spectra with authentic standards. Gallic acid, *p*-coumaric acid, ellagic acid, protocatechuic acid and mangiferin were the major compounds among the identified 16 phenolics in *M. pajang* Kort. peels with amount of 20.9, 12.7, 7.3, 5.4 and 4.8 mg/g FRPP, respectively.

Investigation of the potential hypolipidemic effect of FRPP was performed using an animal model of dietary-induced hypercholesterolemia rats. For 6 weeks rats were fed cholesterol-free diets as a negative control, diets supplemented with 2% cholesterol to induce hypercholesterolemia (positive control), fiber control diet containing 5% cellulose as standard DF and test diets supplemented with 70 g of FRPP per kilogram (providing 5% DF) and 97 g/ kg (providing 7% of DF). Beside the nutritional parameters, lipid profile, malondialdehyde (MDA), toxicity parameters, total antioxidant status (TAS), the activity of the antioxidant enzymes glutathione peroxidase (GPX) and superoxide dismutase (SOD) were measured in plasma. In addition, the fecal bile acids excretions were also determined utilizing HPLC-DAD. The consumption of FRPP in

hypercholesterolemic condition improved the animals' blood lipid profile, liver and kidney functions and reduced lipid peroxidation marker with increasing the excretion of bile acid. Thus, suggested that the potential contribution of FRPP in a cardiovascular risk reduction. Hypercholesterolemia and hypertriglyceridemia were recognized as a result of the cholesterol-rich diets. FRPP exhibited an important hypolipidemic action, returning triglyceride (TG) levels in hypercholesterolemic animals to normal values. The hypocholesterolemic effect of FRPP with dose of 7% was observed, reducing total and low-density lipoprotein cholesterol (−63%, −76%, respectively). Decreased lipid peroxidation in plasma as a consequence of FRPP intake was found in hypercholesterolemic treated groups. Increased the fecal bile acids excretion as a result of FRPP intake was observed in all treated groups. This could be attributed to the high DF content. All these favorable findings might be related to its DF content and the natural presence of antioxidant polyphenols that prevent lipid peroxidation. Findings of the present study had indicated that the incorporation of FRPP into hypercholesterolemic diet improved the lipid profile and reduced lipid peroxidation. It might defend against cellular damage and contribute to a reduction of cardiovascular risk. The findings in the current study might be related to the synergistic effect of SDF (33% d.w) and polyphenols in FRPP.

FRPP properties showed that peels of *Mangifera pajang* Kort. are rich source of DF and other bioactive compounds that could be used as functional food ingredients. It also exhibited an important health effect as lowering cholesterol thus would put forward the potential application of bambangan peels to be incorporated into food formulation.

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

SERAT DIETARI DAN CIRI ANTIOKSIDAN KULIT BUAH *Mangifera pajang* KORT. DAN KESAN SINERGISTIK KE ATAS KESIHATAN TIKUS YANG HIPERKOLESTEROL

Oleh

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Buah *Mangifera pajang* Kort. dikenali sebagai bambangan, merupakan buah terpinggir yang boleh ditemui di Malaysia (Sabah and Sarawak). Saiznya 3 kali lebih besar daripada mangga komersial dan mempunyai kulit yang tebal. Kajian ini bertujuan untuk mencirikan kulitnya dan mengkaji kesannya merendahkan kolesterol model tikus yang hiperkolesterolemik. Kulit bambangan kaya dengan serat dietari dan telah menunjukkan kandungan komponen bernilai tinggi seperti polifenol. Komponen ini perlu dipertimbangkan untuk dieksploitasi. Serat dietari (DF) dan pelbagai ciri berkaitan dengan kualiti pemakanan kulit bambangan dikaji. Ciri fizikokimia dan kapasiti antioksidan serbuk kulit berfiber tinggi (FRPP) serta komposisi kimia serat dietari larut air (SDF) dan serat dietari tidak larut air (IDF) FRPP ditentukan dengan kromatografi cecair bertekanan tinggi-pengesan indeks refraktif (HPLC-RI).

Kandungan DF dalam FRPP adalah tinggi (72.3 g/100 g berat kering) dengan nisbah SDF/IDF yang seimbang (1:1.2). FRPP mempunyai indeks perencatan dialisis glukosa, keupayaan pegangan air (WHC), keupayaan pegangan minyak (OHC) dan sifat mengembang yang tinggi. Analisis kandungan komponen fenolik dalam ekstrak metanol berasid yang diperoleh daripada kulit buah bambangan masak sepenuhnya (*Mangifera pajang* Kort.) juga dijalankan. Kapasiti antioksidan FRPP seperti yang ditentukan dalam ujian kuasa antioksidan penurunan ferik (FRAP) dan 1, 1-difenil-2- pikrilhidrazil (DPPH) menunjuk nilai potensi IC₅₀ yang tinggi (44 µg/mL), ini dijelaskan oleh kehadiran polifenol sebanyak 98.3 mg kesamaan asid galik (GAE)/g FRPP yang ditentukan dengan ujian *Folin-Ciocalteu*. Enam belas komponen fenolik dalam kulit buah bambangan telah dikenalpasti dan ditentukan dengan kromatografi cecair bertekanan tinggi-pengesan sinaran fotodiod (HPLC-DAD) yang dilengkapi dengan pengionan kuantum-elektrospray/spektrometri jisim (TSQ Quantum Ultra-ESI-MS) untuk pengesahan puncak, berdasarkan perbandingan masa retensi, spektra penyerapan sinaran ultraungu-nyata dan spektra jisim dengan rujukan piawai. Asid galik, asid *p*-kumarik, asid elagik, asid protokatekuik dan mangiferin merupakan komponen fenolik utama yang dikenalpasti dalam kulit buah bambangan dengan kandungan masing-masing adalah 20.9, 12.7, 7.3, 5.4 dan 4.8 mg/g FRPP.

Potensi kesan hipolipidemik FRPP dilaksanakan dengan menggunakan model haiwan tikus yang diaruh hiperkolesterol dengan diet. Dalam masa enam minggu, tikus yang diberi diet tanpa kolesterol dijadikan sebagai kawalan negatif; tikus yang diberi diet dengan 2% kolesterol untuk menjadikan hiper-kolesterolemia dijadikan sebagai kawalan positif; diet kawalan mengandungi 5% selulosa sebagai DF rujukan; diet kajian

mengandung 70 g FRPP/kg (membekalkan 5% DF) dan 97 g FRPP/kg (membekalkan 7% DF). Selain parameter pemakanan, profil lipid, paras malondialdehid (MDA), parameter ketoksikan, status jumlah antioksidan (TAS) serta aktiviti enzim antioksidan glutathione peroksidase (GPX) dan superoksida dismutase (SOD) dalam plasma ditentukan. Tambahan pula, perkumuhan asid hempedu dalam najis juga ditentukan dengan HPLC-DAD. Pengambilan FRPP dalam tikus hiper-kolesterolemik memberi kesan positif ke atas profil lipid darah, fungsi hati dan buah pinggang, serta penurunan penanda pengoksidaan lipid dengan peningkatan perkumuhan asid hempedu. Maka ini mencadangkan potensi FRPP dalam penurunan risiko penyakit kardiovaskular. Hiper-kolesterolemia dan hiper-trigliseridemia dalam tikus dikenal pasti sebagai kesan diet kaya dengan kolesterol. Serbuk kulit berfiber tinggi (FRPP) telah mempamerkan kesan hipo-lipidemik, dengan penurunan paras trigliserida (TG) dalam tikus hiper-kolesterolemik ke paras normal. Kesan hipo-kolesterolemik FRPP juga dilihat dalam tikus yang diberi suplementasi 7% DF, dengan penurunan paras jumlah kolesterol dan lipoprotein-kolesterol berketumpatan rendah (LDL) (-63% dan -76% masing-masing). Penurunan pengoksidaan lipid dalam plasma merupakan kesan pengambilan FRPP. Peningkatan perkumuhan asid hempedu dalam najis sebagai kesan pengambilan FRPP didapati dalam semua kumpulan diberi suplementasi FRPP. Ini boleh dijelaskan dengan kandungan DF yang tinggi dalam FRPP. Kesemua kesan positif suplementasi FRPP boleh dikaitkan dengan kandungan DF dalam FRPP dan kehadiran semulajadi antioksidan polifenol dalam FRPP yang mencegah pengoksidaan lipid. Kajian menunjukkan bahawa suplementasi FRPP dalam diet hiper-kolesterolemik memberi kesan positif ke atas profil lipid plasma dan menurunkan pengoksidaan lipid. Suplementasi FRPP mungkin memberi pertahanan terhadap kerosakan sel dan

mengurangkan risiko penyakit kardiovaskular. Kesan positif suplementasi FRPP dalam kajian ini boleh dikaitkan dengan kesan sinergi kandungan SDF (33% FRPP) dan polifenol yang tinggi.

Ciri FRPP telah menunjukkan bahawa kulit *Mangifera pajang* Kort. adalah sumber kaya DF dan komponen bioaktif lain yang boleh digunakan sebagai bahan makanan berfungsi. Ia juga telah menunjukkan kesan penting bagi kesihatan dengan menurunkan paras kolesterol, maka dengan itu kulit buah tersebut boleh diaplikasikan bersama ke dalam formulasi makanan.

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I certify that a Thesis Examination Committee has met on 18-04-2012 to conduct the final examination of Fouad Abdulrahman Saleh Hassan on his thesis entitled “Dietary Fiber and Antioxidant Properties of *Mangifera pajang* Kort. Peels and Their Synergistic Health Effects on Hypercholesterolemic Rats” in accordance with the Universities and University Colleges Act 1971 and the Constitution of the Universiti Putra Malaysia [P.U. (A) 106] 15 March 1998. The Committee recommends that the student be awarded the Doctor of Philosophy degree.

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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.

FOUAD ABDULRAHMAN SALEH HASSAN

Date: 18 April 2012

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