



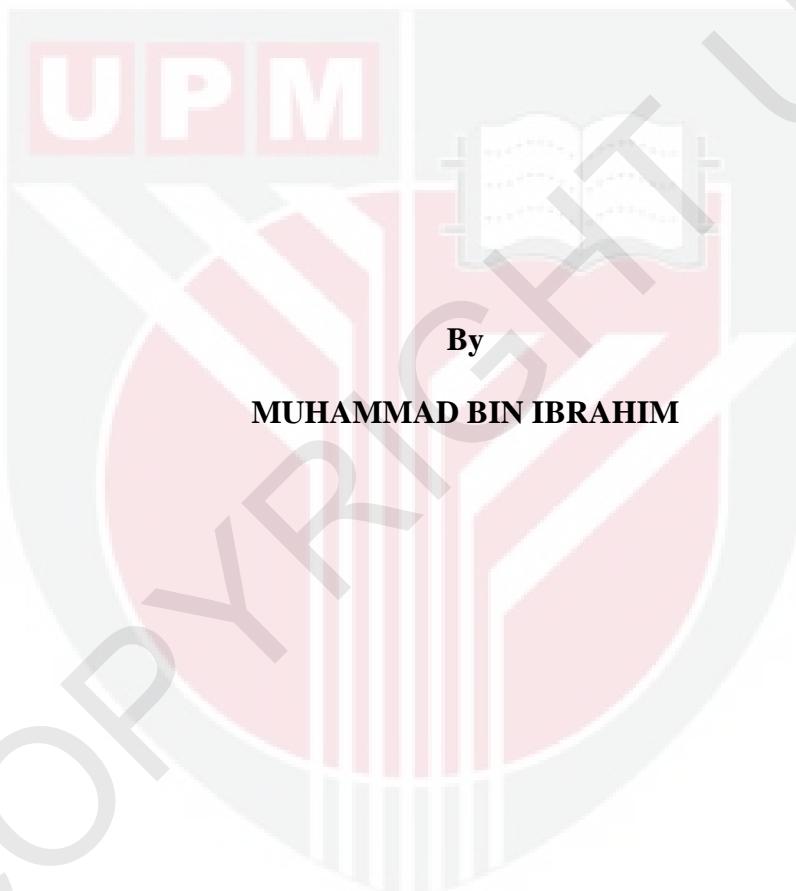
UNIVERSITI PUTRA MALAYSIA

**NUTRIENT COMPOSITION AND ANTIOXIDANT PROPERTIES OF  
*MAGIFERA PAJANG KOSTERM*. JUICE POWDER AND ITS EFFECTS  
ON CARDIOVASCULAR BIOMARKERS IN HYPERCHOLESTOLEMIC  
RABBITS AND HEALTHY HUMAN SUBJECTS**

**MUHAMMAD BIN IBRAHIM**

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

**October 2010**

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

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By

**MUHAMMAD BIN IBRAHIM**

**October 2010**

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The main objective of this study was to determine the nutrient composition, antioxidant properties and effects on cardiovascular biomarkers of *Mangifera pajang* juice powder in hypercholesterolemic rabbits and healthy human subjects. There were three phases in this study. In phase one, the nutritional composition analysis showed that every 100 g of bambangan juice powder (BJP) per wet basis contained 10% moisture, 4% crude protein, 2% crude fat, 76% carbohydrate, 3% ash, 0.8% total dietary fibre (0.1% insoluble and 0.7% soluble dietary fibre), 132 mg ascorbic acid, 36 mg β-carotene and 19 mg gallic acid equivalent (GAE) total phenolics. Ferric reducing/antioxidant power (FRAP) and diphenyl picrylhydrazyl (DPPH) assays showed that BJP had significantly higher ( $p<0.05$ ) antioxidant capacity by 45 (FRAP value of 40 mM/100 g) and 17% (DPPH value of 52% of radical scavenging activity), respectively as compared to bambangan pulp powder (BP) (FRAP value of 27 mM/100 g; DPPH value of 43% of radical scavenging activity).

In phase two, animal experiment was conducted to examine the effects of daily supplementation of BJP at different dosage (5%, 15%, 25% and 35%) on CVD risk biomarkers in hypercholesterolemic rabbits. Supplementation with 25 % of BJP was found to be significantly decreased ( $p<0.05$ ) the total cholesterol (TC), triglycerides (TG) and low density lipoprotein cholesterol (LDL-C) by 37%, 67% and 30%, respectively. Furthermore, supplementation with 25% of BJP had significantly reduced ( $p<0.05$ ) the ratio of TC to HDL-C by 25%. Supplementation with 4 different doses of BJP had significantly decreased ( $p<0.05$ ) plasma malondialdehyde (MDA) by 86%, 89%, 84% and 87%, respectively. Similarly, supplementation with 4 different doses of BJP had significantly ( $p<0.05$ ) decreased the hepatic MDA contents by 80%, 77%, 77% and 83%, respectively. BJP supplementation had significantly reduced ( $p<0.05$ ) the percentage of lesion by 23% to 66% as compared to that of positive control (CD) group and more pronounced in the BJP25 group.

Supplementation with BJP had significantly increased ( $p<0.05$ ) the plasma glutathione peroxidase (GPx) and superoxide dismutase (SOD). Similarly, the BJP supplemented groups also showed a significant increment ( $p<0.05$ ) in hepatic GPx and SOD which ranged between 25% to 62% and 21 to 43%, respectively. Plasma and hepatic GPx and SOD activities had significantly increased in BJP25 and BJP35 groups compared to the other BJP supplemented groups. Supplementation with BJP had significantly increased ( $p<0.05$ ) hepatic total antioxidant status (TAS) by 16% to 58%. The levels of plasma ascorbic acid were significantly increased ( $p<0.05$ ) in the BJP25 and BJP35 by 44% and 67%, respectively. Supplementation with 15%, 25%, and 35% of BJP had significantly increased  $\beta$ -carotene by 71%, 78% and 83%.

Lower levels of plasma alkaline phosphatase (ALP) and alanine aminotransaminase (ALT) were registered due to the BJP supplementation.

In phase three, the effects of BJP on CVD risk biomarkers in normocholesterolemic subjects in a cross-over, single-blind, and placebo-controlled trial were carried out. A total of 100 subjects were screened among staff and postgraduate students of the Faculty of Medicine and Health Sciences (FMHS), Universiti Putra Malaysia (UPM), Malaysia. Ethical approval was obtained from Medical Research Ethics Committee, FMHS, UPM and the subjects were provided informed consent letter to participate in the study carried out at the Nutrition Counseling Laboratory at FMHS, UPM. The study consisted of 32 subjects who were observed over a period of 4 weeks. The subjects ranged from 23 to 28 years old with a cholesterol level of 3.92 to 4.83 mmol/L.

Similar to animal findings (Chapter 4), supplementation of 250 ml BJP drink per serving per day led to a significant decrease in TC (18%,  $p<0.05$ ), ratio of TC:HDL cholesterol (48%,  $p<0.05$ ) and MDA (23%,  $p<0.05$ ). However, there were no significant changes for other biomarkers. Compared with placebo, supplementation with BJP increased the concentration of plasma  $\beta$ -carotene ( $1.62 \pm 0.02$  vs  $2.30 \pm 0.05$   $\mu\text{mol/L}$ ,  $p<0.05$ ) and ascorbic acid ( $78.25 \pm 5.69$  vs  $92.43 \pm 4.58$   $\mu\text{mol/L}$ ,  $p<0.05$ ) significantly at the end of the study by 37% and 22% respectively. TAS tend to be greater after consumption of BJP than placebo and positively related ( $r=0.92$ ,  $p<0.001$ ) with plasma ascorbic acid and ( $r=0.73$ ,  $p<0.05$ ) with  $\beta$ -carotene.

In conclusion, these findings indicated that supplementation of BJP in daily diet may have the potential benefits to provide a protection against cardiovascular disease by

lowering the related risk biomarkers and concurrently increasing the antioxidant status in normocholesterolemic subjects. The findings were further supported with the data gathered from animal study where daily supplementation of BJP for 10 weeks had lowered the risk factors for cardiovascular disease evident by the significant correlation ( $p<0.05$ ) with antioxidant properties present in BJP. Therefore, supplementation with BJP is beneficial as an alternative way to provide effective prevention from cardiovascular disease.



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

**KOMPOSISI NUTRIEN DAN CIRI-CIRI ANTIOKSIDAN SERBUK JUS  
*MANGIFERA PAJANG KOSTERM.* DAN KESANNYA KE ATAS  
PARAMETER KARDIOVASKULAR DALAM ARNAB TERARUH  
KOLESTEROL DAN INDIVIDU SIHAT**

Oleh

**MUHAMMAD BIN IBRAHIM**

**Oktober 2010**

**Pengerusi : Profesor Madya Amin Ismail, PhD**

**Fakulti : Perubatan dan Sains Kesihatan**

Objektif utama kajian ini adalah untuk menentukan komposisi nutrien, antioksidan serbuk jus bambangan and kesannya ke atas parameter kardiovaskular dalam arnab diaruh kolesterol dan individu sihat. Terdapat tiga fasa dalam kajian ini. Dalam fasa pertama, analisa komposisi nutrien ke atas serbuk jus bambangan (BJP) menunjukkan setiap 100 g serbuk jus bambangan mengandungi 10% kelembapan, 4% protein kasar, 2% lemak kasar, 76% karbohidrat, 3% abu, 0.8% gentian diet (0.1% gentian diet tak larut dan 0.7% gentian diet larut), 132 mg asid askorbik, 36 mg  $\beta$ -karoten dan 19 mg *gallic acid equivalent* (GAE) phenolik jumlah, berdasarkan berat basah. Ujian *ferric reducing/antioxidant power* (FRAP) dan *diphenylpicrylhydrazyl* (DPPH) menunjukkan serbuk jus bambangan mempunyai kapasiti antioksida yang tinggi, sebanyak 45% (nilai FRAP adalah 40 mM/100 g) dan 17% (nilai DPPH perencutan radikal bebas adalah 52%) berbanding dengan serbuk pulpa

bambangan (nilai FRAP adalah 27 mM/100 g; nilai DPPH perencatan radikal bebas adalah 43%).

Dalam fasa kedua, kajian menggunakan haiwan dijalankan untuk menentukan kesan pengambilan BJP pada dos yang berbeza (5%, 15%, 25% dan 35%) ke atas parameter risiko kardiovaskular dalam arnab diaruh kolesterol. Penambahan 25% BJP didapati menurunkan paras kolesterol jumlah (TC), trigliserida (TG) dan lipoprotein berketumpatan rendah-kolesterol (LDL-C), 37%, 67% dan 30% secara signifikan. Tambahan pula, penambahan 25% BJP secara signifikan ( $p<0.05$ ) menurunkan nisbah TC kepada HDL-C sebanyak 25%. Penambahan dengan 4 dos BJP yang berbeza secara signifikan menurunkan plasma malondialdehid (MDA), sebanyak 86%, 89%, 84% dan 87%. Penambahan dengan 4 dos BJP berbeza menurunkan hepatik MDA sebanyak 80%, 77%, 77% and 83%. Penambahan BJP juga secara signifikan menurunkan peratusan pembentukan plak atherosklerotik.

Penambahan BJP dalam dos yang berbeza secara signifikan meningkatkan aktiviti enzim *glutathione peroxidase* (GPx) dan *superoxide dismutase* (SOD). Kumpulan arnab yang ditambah dengan BJP menunjukkan peningkatan hepatik GPx dan SOD, di antara 25% – 62% dan 21% – 43%. Aktiviti GPx dan SOD dalam plasma dan hepatik menunjukkan peningkatan yang ketara dalam kumpulan BJP25 dan BJP35 berbanding dengan kumpulan lain. Penambahan BJP dengan dos yang berbeza secara signifikan ( $p<0.05$ ) meningkatkan status hepatik antioksida jumlah (TAS) sebanyak 16% – 58%. Paras asid askorbik juga meningkat secara signifikan dalam kumpulan BJP25 dan BJP35, masing-masing sebanyak 44% dan 67%. Penambahan dengan 15%, 25% dan 35% BJP meningkatkan paras  $\beta$ -karoten, sebanyak 71%, 78% dan

83%, secara signifikan. Paras enzim *alkaline phosphatase* (ALP) dan *alanine aminotransferase* (ALT) menurun berikutan dengan penambahan BJP.

Dalam fasa ketiga, penilaian dilakukan terhadap kesan pengambilan BJP ke atas parameter kardiovaskular di kalangan subjek yang mempunyai tahap kolesterol yang normal. Kajian klinikal dilakukan melalui kaedah silang menggunakan plasebo sebagai kumpulan kawalan. Kesemua 100 orang subjek disaring dari kalangan staf dan pelajar pasca siswazah di Fakulti Perubatan dan Sains Kesihatan (FPSK), UPM. Kelulusan etika telah diperolehi dari Jawatankuasa Etika Penyelidikan Perubatan, FPSK, UPM dan subjek yang terpilih diminta untuk menyatakan persetujuan untuk terlibat dalam kajian ini dengan menandatangani surat kebenaran. Kajian dilakukan di Makmal Kaunseling Pemakanan, FPSK, UPM. Seramai 32 orang yang terpilih untuk kajian ini yang berumur di antara 23 – 28 tahun dan paras kolesterol di antara 3.92 – 4.83 mmol/l.

Pengambilan 250 ml BJP sehari didapati menurunkan secara signifikan paras TC sebanyak 18%, nisbah TC kepada HDL-C sebanyak 48% dan MDA sebanyak 23%. Walaubagaimanapun, tiada perubahan kepada parameter kajian yang lain. Berbanding dengan kawalan, pengambilan BJP meningkatkan kepekatan plasma  $\beta$ -karoten ( $1.62 \pm 0.02$  vs  $2.30 \pm 0.05$   $\mu\text{mol/L}$ ,  $p<0.05$ ) and asid askorbik ( $78.25 \pm 5.69$  vs  $92.43 \pm 4.58$   $\mu\text{mol/L}$ ,  $p<0.05$ ), masing-masing sebanyak 37% dan 22%. TAS juga didapati meningkat bagi kumpulan BJP dan didapati berkorelasi dengan plasma asid askorbik ( $r=0.92$ ,  $p<0.05$ ) dan  $\beta$ -karoten ( $r=0.73$ ,  $p<0.05$ ).

Kesimpulannya, penemuan ini menunjukkan pengambilan BJP dalam diet harian mempunyai potensi untuk mencegah penyakit kardiovaskular dengan menurunkan parameter risiko kepada penyakit kardiovaskular dan meningkatkan status antioksida dalam subjek kolesterol normal. Penemuan ini juga disokong oleh data yang diperolehi daripada kajian haiwan yang menunjukkan pengambilan BJP selama 10 minggu menurunkan faktor risiko kepada penyakit kardiovaskular yang berkemungkinan disumbangkan oleh antioksida yang terdapat dalam BJP. Oleh itu, pengambilan BJP adalah berpotensi sebagai satu kaedah alternatif untuk mencegah penyakit kardiovaskular.

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I certify that an Examination Committee has met on **October 29<sup>th</sup>, 2010** to conduct the final examination of **Muhammad Bin Ibrahim** on his **Doctor of Philosophy** thesis entitled "**Nutrient Composition, Antioxidant Properties and Effects on Cardiovascular Biomarkers of Mangifera pajang Juice Powder in Hypercholesterolemic Rabbits and Healthy Human Subjects**" in accordance with Universiti Pertanian Malaysia (Higher Degree) Act 1980 and Universiti Pertanian Malaysia (Higher Degree) Regulatins 1981. The Committee recommends that the student be awarded the Doctor of Philosophy.

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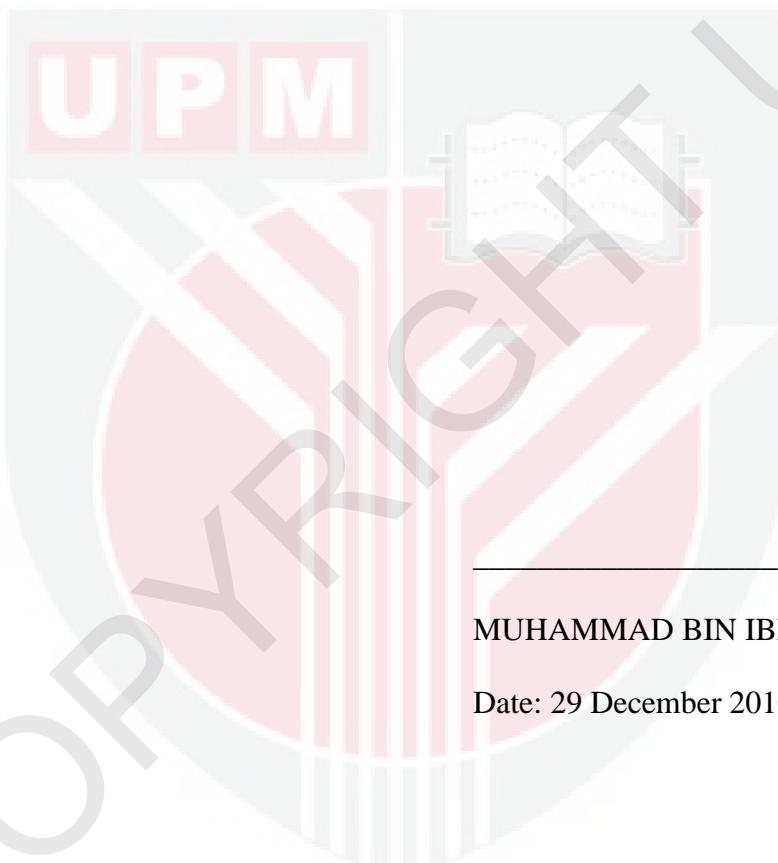
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Date:

## **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, and not concurrently, submitted for any other degree at Universiti Putra Malaysia or at any other institutions.



MUHAMMAD BIN IBRAHIM

Date: 29 December 2010

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