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

MUHAMMAD BIN IBRAHIM

FPSK(p) 2010 5
NUTRIENT COMPOSITION AND ANTIOXIDANT PROPERTIES OF
*MAGIFERA PAJANG KOSTERM* JUICE POWDER AND ITS EFFECTS ON
CARDIOVASCULAR BIOMARKERS IN HYPERCHOLESTEROLEMIC
RABBITS AND HEALTHY HUMAN SUBJECTS

By

MUHAMMAD BIN IBRAHIM

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

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

By

MUHAMMAD BIN IBRAHIM

October 2010

Chairman : Associate Professor Amin Ismail, PhD
Faculty : Medicine and Health Sciences

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 β-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 β-carotene (1.62 ± 0.02 vs 2.30 ± 0.05 µmol/L, p<0.05) and ascorbic acid (78.25 ± 5.69 vs 92.43 ± 4.58 µmol/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 β-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 β-karoten dan 19 mg gallic acid equivalent (GAE) phenolik jumlah, berdasarkan berat basah. Ujian ferric reducing/antioxidant power (FRAP) dan diphenyl picrylhydrazyl (DPPH) menunjukkan serbuk jus bambangan mempunyai kapasiti antioksidan yang tinggi, sebanyak 45% (nilai FRAP adalah 40 mM/100 g) dan 17% (nilai DPPH perencatan 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 yang berbeza secara signifikan menurunkan hepatic 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 hepatic GPx dan SOD, di antara 25% – 62% dan 21% – 43%. Aktiviti GPx dan SOD dalam plasma dan hepatic 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 hepatic 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 β-karoten, sebanyak 71%, 78% dan
83%, secara signifikan. Paras enzim *alkaline phosphatase* (ALP) dan *alanine aminotransferase* (ALT) menurun berikutan dengan penambahan BJP.


Pengambilan 250 ml BJP sehari didapat 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 β-karoten (1.62 ± 0.02 vs 2.30 ± 0.05 µmol/L, p<0.05) and asid askorbik (78.25 ± 5.69 vs 92.43 ± 4.58 µmol/L, p<0.05), masing-masing sebanyak 37% dan 22%. TAS juga didapat meningkat bagi kumpulan BJP dan didapat bercorelasi dengan plasma asid askorbik (r=0.92, p<0.05) dan β-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.
ACKNOWLEDGEMENTS

First and foremost, all thanks to Allah the AL Mighty, for the strength given to me to complete this study. I would like to express my deepest thanks to my main supervisor, Associate Professor Dr. Amin Ismail for his guidance, support and advice throughout my study. His not only my teacher but also as my role model of best researcher. My special thanks to my co-supervisors Associate Professor Dr. Azizah Abdul Hamid and Dr. Azrina Azlan for good comments, motivation and suggestions. I would like to thank the other member of research team, Associate Professor Dr. Hairuszah Ithnin for her assistance during the clinical trial.

I would like to convey my appreciation to Mr. Syed Hasbullah Syed Kamaruddin and Puan Siti Asiyah Yaakob for their help as well as all the lab assistants and staff of Department of Nutrition and Dietetics, Faculty of Medicine and Health Sciences, UPM. Thank you to the International Islamic University Malaysia for granting me the study leave to pursue my postgraduate degree.

Last but not least, I would like to thank my family (especially my mother Salmah Yatim and my late father Hj. Ibrahim Abd. Rahman) and friends (Hasnah Haron, Mohd Hafizan Johar, Abbe Malyki Mohd Jalil and Kong Kin Weng) for their kindness and support. My highest gratitude is dedicated to my wife, Ida Azlina Zakaria and to my lovely daughters, Maisarah Muhammad, Munirah Muhammad, baby Mawaddah Muhammad and my only son Mustaqeem Muhammad for all their patience and love.
I certify that an Examination Committee has met on **October 29th, 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) Regulations 1981. The Committee recommends that the student be awarded the Doctor of Philosophy.

Members of the examination Committee were as follows:

**Zaitun Yassin, PhD**  
Associate Professor  
Faculty of Medicine and Health Sciences  
Universiti Putra Malaysia  
(Chairman)

**Asmah Rahmat, PhD**  
Professor  
Faculty of Medicine and Health Sciences  
Universiti Putra Malaysia  
(Internal Examiner)

**Rokiah Yusof, PhD**  
Associate Professor  
Faculty of Medicine and Health Sciences  
Universiti Putra Malaysia  
(Internal Examiner)

**Manohar Garg**  
Professor  
Nutraceuticals Research Group  
University of Newcastle  
Australia  
(External Examiner)

________________________________________

**BUJANG KIM HUAT, PhD**  
Professor and Deputy Dean  
School of Graduate Studies  
Universiti Putra Malaysia

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 Doctor of Philosophy. The members of the Supervisory Committee are as follows:

**Amin Ismail, PhD**  
Associate Professor  
Faculty of Medicine and Health Sciences  
Universiti Putra Malaysia  
(Chairman)

**Azrina Azlan, PhD**  
Lecturer  
Faculty of Medicine and Health Sciences  
Universiti Putra Malaysia  
(Member)

**Azizah Abdul Hamid, PhD**  
Associate Professor  
Faculty of Food Science and Technology  
Universiti Putra Malaysia  
(Member)

_______________________________  
HASANAH MOHD GHAZALI, PhD  
Professor and Dean  
School of Graduate Studies  
Universiti Putra Malaysia

Date:
DECLARATION

I hereby declare that the thesis is based on my original work except for quotations and citations which have been duly acknowledge. 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
# TABLE OF CONTENTS

<table>
<thead>
<tr>
<th>Section</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>ABSTRACT</td>
<td>ii</td>
</tr>
<tr>
<td>ABSTRAK</td>
<td>vi</td>
</tr>
<tr>
<td>ACKNOWLEDGEMENT</td>
<td>x</td>
</tr>
<tr>
<td>APPROVAL</td>
<td>xi</td>
</tr>
<tr>
<td>DECLARATION</td>
<td>xiii</td>
</tr>
<tr>
<td>LIST OF TABLES</td>
<td>xviii</td>
</tr>
<tr>
<td>LIST OF FIGURES</td>
<td>xx</td>
</tr>
<tr>
<td>LIST OF ABBREVIATION</td>
<td>xxi</td>
</tr>
</tbody>
</table>

## CHAPTER

<table>
<thead>
<tr>
<th>1. INTRODUCTION</th>
<th>1</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.1 The background of the study</td>
<td>1</td>
</tr>
<tr>
<td>1.2 Statement of the problem</td>
<td>6</td>
</tr>
<tr>
<td>1.3 Significance of the study</td>
<td>8</td>
</tr>
<tr>
<td>1.4 The scope of the study</td>
<td>10</td>
</tr>
<tr>
<td>1.5 Objectives of the study</td>
<td>11</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>2. LITERATURE REVIEWS</th>
<th>13</th>
</tr>
</thead>
<tbody>
<tr>
<td>2.1 Overview and trends of cardiovascular disease</td>
<td>13</td>
</tr>
<tr>
<td>2.1.1 Cardiovascular disease in selected developed countries</td>
<td>14</td>
</tr>
<tr>
<td>2.1.2 Cardiovascular disease in selected developing countries</td>
<td>15</td>
</tr>
<tr>
<td>2.1.3 Cardiovascular disease in Asia</td>
<td>16</td>
</tr>
<tr>
<td>2.1.4 Cardiovascular disease in Malaysia</td>
<td>20</td>
</tr>
<tr>
<td>2.1.5 Cardiovascular disease and gender</td>
<td>23</td>
</tr>
<tr>
<td>2.2 Cardiovascular risk factors</td>
<td>24</td>
</tr>
<tr>
<td>2.2.1 Total cholesterol and hypercholesterolemia</td>
<td>24</td>
</tr>
<tr>
<td>2.2.2 Low density lipoprotein cholesterol</td>
<td>30</td>
</tr>
<tr>
<td>2.2.3 Triglyceride and hypertriglyceridemia</td>
<td>33</td>
</tr>
<tr>
<td>2.2.4 High density lipoprotein cholesterol</td>
<td>34</td>
</tr>
<tr>
<td>2.2.5 Ratio of total cholesterol to high density lipoprotein cholesterol</td>
<td>36</td>
</tr>
<tr>
<td>2.3 Atherosclerosis and coronary artery disease</td>
<td>37</td>
</tr>
<tr>
<td>2.4 Free radicals and cardiovascular disease</td>
<td>40</td>
</tr>
<tr>
<td>2.5 Lipid peroxidation and cardiovascular disease</td>
<td>41</td>
</tr>
<tr>
<td>2.6 Liver and kidney biomarkers related to cardiovascular disease</td>
<td>42</td>
</tr>
<tr>
<td>2.6.1 Liver enzymes</td>
<td>42</td>
</tr>
<tr>
<td>2.6.2 Kidney function parameters</td>
<td>44</td>
</tr>
<tr>
<td>2.7 Fruits and vegetables and cardiovascular health</td>
<td>45</td>
</tr>
<tr>
<td>2.7.1 In vitro studies</td>
<td>46</td>
</tr>
<tr>
<td>2.7.2 Animal studies</td>
<td>48</td>
</tr>
<tr>
<td>2.7.3 Human studies</td>
<td>51</td>
</tr>
</tbody>
</table>
2.8 Fruits and vegetables intake among adults in Malaysia compared to other countries
2.9 Mango
  2.9.1 Mango and its role in the global market
  2.9.2 Types of Mango
  2.9.3 Nutritional composition and antioxidant properties of Mango
  2.9.4 Mango and cardiovascular health
2.10 Antioxidant and cardiovascular health
  2.10.1 Dietary antioxidants
  2.10.2 Vascular antioxidant enzymes

3 NUTRITIONAL COMPOSITION AND ANTIOXIDANT PROPERTIES OF BAMBANGAN FRUITS
  3.1 Introduction
  3.2 Materials and methods
    3.2.1 Chemicals and reagents
    3.2.2 Fruit sampling and preparation
    3.2.3 Determination of physical characteristics of bambangan fruits
    3.2.4 Preparation of samples
    3.2.5 Sample extraction
    3.2.6 Proximate analysis
    3.2.7 Estimation of ascorbic acid and β-carotene using high performance liquid chromatography (HPLC) method
    3.2.8 Determination of total phenolics content
    3.2.9 Antioxidant capacity determination
    3.2.10 Statistical analysis
  3.3 Results and discussion
    3.3.1 Physical characteristics of bambangan fruits
    3.3.2 Proximate composition of bambangan pulp and bambangan juice powders
    3.3.3 Antioxidant properties of bambangan pulp and bambangan juice powders
  3.4 Conclusions

4 EFFECTS OD BAMBANGAN JUICE POWDER ON CARDIOVASCULAR BIOMARKERS IN HYPERCHOLESTEROLEMIC RABBITS
  4.1 Introduction
  4.2 Materials and methods
    4.2.1 Chemicals
    4.2.2 Preparation of bambangan fruits and bambangan juice powder
    4.2.3 Preparation of bambangan juice powder mixture
    4.2.4 Preparation of cholesterol diet
    4.2.5 Animal study
    4.2.6 Blood sample collection
<table>
<thead>
<tr>
<th>Section</th>
<th>Title</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>4.2.7</td>
<td>Biochemical analysis and atherosclerotic plaque assessment</td>
<td>103</td>
</tr>
<tr>
<td>4.2.8</td>
<td>Statistical analysis</td>
<td>113</td>
</tr>
<tr>
<td>4.3</td>
<td>Results</td>
<td>113</td>
</tr>
<tr>
<td>4.3.1</td>
<td>Effects of bambangan juice powder on body and organ weights</td>
<td>113</td>
</tr>
<tr>
<td>4.3.2</td>
<td>Effect of bambangan juice powder supplementation on plasma lipids and atherogenic index levels</td>
<td>115</td>
</tr>
<tr>
<td>4.3.3</td>
<td>Effect of bambangan juice powder supplementation on malondialdehyde levels</td>
<td>120</td>
</tr>
<tr>
<td>4.3.4</td>
<td>Effect of bambangan juice powder supplementation on atherosclerotic lesions</td>
<td>120</td>
</tr>
<tr>
<td>4.3.5</td>
<td>Effects of bambangan juice powder on plasma and hepatic glutathione peroxidase</td>
<td>125</td>
</tr>
<tr>
<td>4.3.6</td>
<td>Effect of bambangan juice powder on plasma and hepatic superoxide dismutase</td>
<td>128</td>
</tr>
<tr>
<td>4.3.7</td>
<td>Effect of bambangan juice powder on plasma and hepatic of total antioxidant status</td>
<td>131</td>
</tr>
<tr>
<td>4.3.8</td>
<td>Effect of bambangan juice powder on plasma liver enzymes</td>
<td>134</td>
</tr>
<tr>
<td>4.3.9</td>
<td>Effect of bambangan juice powder on plasma antioxidants</td>
<td>136</td>
</tr>
<tr>
<td>4.3.10</td>
<td>Effect of bambangan juice powder on hepatic malondialdehyde</td>
<td>138</td>
</tr>
<tr>
<td>4.4</td>
<td>Discussion</td>
<td>140</td>
</tr>
<tr>
<td>4.4.1</td>
<td>Effect of bambangan juice powder on body and organ weights</td>
<td>140</td>
</tr>
<tr>
<td>4.4.2</td>
<td>Hypolipidemic effect of bambangan juice powder</td>
<td>141</td>
</tr>
<tr>
<td>4.4.3</td>
<td>Antiatherogenic effect of bambangan juice powder</td>
<td>144</td>
</tr>
<tr>
<td>4.4.4</td>
<td>Effect of bambangan juice powder on malondialdehyde levels</td>
<td>145</td>
</tr>
<tr>
<td>4.4.5</td>
<td>Antiatherosclerotic effect of bambangan juice powder</td>
<td>146</td>
</tr>
<tr>
<td>4.4.6</td>
<td>Effect of bambangan on plasma and hepatic antioxidant enzymes and total antioxidant status</td>
<td>148</td>
</tr>
<tr>
<td>4.4.7</td>
<td>Effect of bambangan juice powder on plasma liver enzymes</td>
<td>150</td>
</tr>
<tr>
<td>4.4.8</td>
<td>Effect of bambangan juice powder on plasma antioxidants</td>
<td>151</td>
</tr>
<tr>
<td>4.4.9</td>
<td>Effect of bambangan juice powder on lipid peroxidation</td>
<td>153</td>
</tr>
<tr>
<td>4.5</td>
<td>Conclusions</td>
<td>154</td>
</tr>
</tbody>
</table>
**5 EFFECTS OF BAMBANGAN JUICE POWDER DRINK ON CARDIOVASCULAR BIOMARKERS IN NORMOCHESTEROLEMIC SUBJECTS**

156

5.1 Introduction 156
5.2 Materials and methods 156
  5.2.1 Chemicals and reagents 159
  5.2.2 Preparation of bambangan juice powder drink 159
  5.2.3 Sample size calculation 160
  5.2.4 Recruitment of subjects 160
  5.2.5 Study design and diet 162
  5.2.6 Collection and preparation of blood samples 163
  5.2.7 Blood pressure and anthropometric measurement 166
  5.2.8 Measurement of biochemical parameters 166
  5.2.9 Statistical analysis 167
5.3 Results 167
  5.3.1 Baseline characteristics of the subjects 167
  5.3.2 Nutrient intake and physical activity 169
  5.3.3 Body weight and blood pressure 170
  5.3.4 Biochemical analysis 171
5.4 Discussion 176
  5.4.1 Body weight and blood pressure 177
  5.4.2 Plasma glucose level 178
  5.4.3 Plasma lipid level 179
  5.4.4 Plasma malondialdehyde level 182
  5.4.5 Effect of bambangan juice powder supplementation on ascorbic acid and β-carotene 183
  5.4.6 Effect of bambangan juice supplementation on total antioxidant status, glutathione peroxidase and superoxide dismutase enzymes 186
  5.4.7 Effect of bambangan juice powder supplementation on liver enzymes and kidney function tests 188
5.5 Conclusions 188

**6 SUMMARY, GENERAL CONCLUSION AND RECOMMENDATION FOR FUTURE RESEARCH**

190

6.1 Summary 190
6.2 General conclusion 194
6.3 Limitation of study 196
6.4 Recommendation for future research 197

REFERENCES 199
APPENDICES 237
BIODATA OF STUDENT 257
LIST OF PUBLICATIONS 258