



STUDY OF *Baccaurea angulata* (BELIMBING DAYAK)
ON CARDIOVASCULAR DISEASES INDICES AND ITS
TOXICITY EFFECTS

BY

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ABSTRACT

Cardiovascular diseases (CVD) have become a leading cause of death and disability and staggering increase in its incidence has been documented worldwide. Previous conducted research has provided the evidence on effectiveness of antioxidative properties of polyphenols contained in fruits and vegetables at combating various chronic diseases including CVD. *Baccaurea angulata*, locally known as ‘belimbing dayak’ or ‘belimbing hutan’ is one edible fruit species restrictedly distributed in Sabah and Sarawak. This present study was aimed to provide scientific data on nutritional information and health benefits of *B. angulata* juice extract (BAE). Pearson’s correlation was used to obtain correlation whereas analysis of variance (ANOVA) was used to test the mean difference among data. Freeze dried BAE consisted of high carbohydrate followed by moisture, total dietary fiber, ash, and protein. The extract holds high energy density with low water activity (A_w) value for storage stability. Phytochemical screening revealed that BAE contained of 7.90 ± 0.05 mg gallic acid equivalent (GAE)/g of total phenolic acids and 12.74 ± 0.24 mg quercetin equivalent (QE)/g of total flavonoids content and both were strongly correlated to ferric reducing antioxidant power (FRAP) value ($p < 0.01$) but not to superoxide anion/xanthine oxidase dismutase (XOD) scavenging capacity. Male Sprague Dawley rats were induced with high cholesterol diet (normal rat chow enriched with 1 % cholesterol and 0.2 % cholic acid) and supplemented with BAE given orally for 10 weeks. No significant changes in body weight, heart and kidney weight were notified. Liver weight was significantly higher in groups consuming high cholesterol diet ($p < 0.05$) than in normal control with slight decrease in groups supplemented with BAE than hypercholesterolemic control (CP). Plasma and liver total cholesterol (TC), triglycerides (TG), low density lipoprotein cholesterol (LDL-c), and atherogenic index (AI) were markedly increased in CP group with significant values in TC, LDL-c, and AI ($p < 0.05$) and reduced in high density lipoprotein cholesterol (HDL-c). Reduction of plasma and liver TC, TG, LDL-c and AI were observed in dose-dependent manner upon consumption of 100 (TL), 300 (TM) and 500 mg (TH) of BAE/kg body weight/day with significant reduction in plasma TG ($p < 0.05$). Notable increase in plasma HDL-c was found in all groups consuming BAE compared to CP group but liver HDL-c was less affected. The highly reduced plasma and liver *glutathione peroxidase* (GPx) and *superoxide dismutase* (SOD), in CP group were raised in all groups consuming BAE especially by TM group (300 mg BAE/kg bodyweight/day). Remarkably, with reference to normal control, plasma SOD value was successfully restored in TM and TH groups. In determining the effects of post BAE administration towards liver and kidney, no significant changes ($p > 0.05$) in aspartate transaminase (AST), alanine transaminase (ALT), alkaline phosphatase (ALP), total protein, albumin, urea, and creatinine levels were evaluated. Notable increase in AST, ALT, and ALP were observed in CP group due to hypercholesterolemic condition and with reference to normal control, these parameters were least affected in TM group. Furthermore, BAE showed no significant effects towards haematological parameters.

خلاصة البحث

تعد الأمراض القلبية الوعائية السبب الرئيسي في الموت والعجز وقد تم توثيق زيادة مذهلة في حدوث هذه الأمراض في أنحاء العالم. لقد وفرت الدراسات السابقة أدلة على الخواص المضادة للأكسدة للبوليفينولات المتواجدة في الخضار والفواكه في مكافحة مختلف الأمراض المزمنة بما في ذلك الأمراض القلبية الوعائية. نبات (*Baccaurea angulata*) والمعروف محليا باسم (belimbing dayak) أو (belimbing hutan) هو أحد الفواكه الصالحة للأكل ويتواجد حصريا في ولايتي صباح وسراوك. تهدف هذه الدراسة لتوفير بيانات علمية للفوائد الغذائية والطبية لعصير خلاصة ال (*B. angulata*) والتي نسميها اصطلاحا (BAE) في هذه الدراسة. استخدم فحص الارتباط الاحصائي بيرسون لدراسة العلاقة بين البيانات كما استخدم فحص تحليل التباين انوفا (ANOVA) لدراسة الاختلاف بين المتوسطات. بينت النتائج أن الخلاصة المجفدة لل (BAE) تحتوي كميات كبيرة من السكريات يليها المحتوى المائي ثم الألياف ثم الرماد فالبروتين. فالخلاصة تحتوي طاقة عالية مع فعالية مائة قليلة مما يزيد الثباتية خلال التخزين. تم الكشف من خلال الفحص الكيميائي النباتي للخلاصة أن محتوى الفينولات الكلية كان 7.90 ± 0.05 ميليغرام مكافئ حمض الغاليك ومحتوى الفلافونويدات الكلية كان 12.74 ± 0.24 ميليغرام مكافئ الكورسيتين. كلا الفينولات والفلافونويدات كانا مرتبطين بشكل كبير مع طاقة مضادة الأكسدة للحديد المختزل (FRAP) مع قيمة المتغير الاحصائي ($p < 0.01$) ولكنهما لم يبديا ترابطا مع القدرة الكاسحة ل أنيون / كراتين أكسيداز الفائق (XOD). تم دراسة تأثير الخلاصة النباتية على جردان التجارب البيضاء حيث تم تعريضها لحمية عالية الكوليسترول من خلال تغذيتها على طعام الجرذان الطبيعي والمزود بالكوليسترول وحمض الكوليك بنسبة 1% و 0.2% على التوالي وقد أعطيت الخلاصة لهذه الجرذان فمويا لمدة 10 أسابيع. لم يلاحظ أي تغير هام في وزن الجسم أو الكلية أو القلب بينما ازداد وزن الكبد بشكل ملحوظ احصائيا في المجموعات التي أخضعت لحمية عالية الكوليسترول بالمقارنة مع المجموعات الشاهد ($p < 0.05$) مع انخفاض طفيف في المجموعات المزودة بالخلاصة النباتية مقارنة بالمجموعات الشاهد عالية الكوليسترول (CP). كان هناك زيادة ملحوظة في كوليسترول الكبد والبلازما الكلي (TC) و ثلاثي الغليسيرول (TG) والبروتين الشحمي منخفض الكثافة (LDL-C) ومؤشر تصلب الشرايين (AI) في مجموعة ال (CP) وكانت الزيادة في مستويات ثلاثي الغليسيرول (TG) والبروتين الشحمي منخفض الكثافة (LDL-C) ومؤشر تصلب الشرايين (AI) ملحوظة احصائيا مع ($p < 0.05$) كما لوحظ انخفاض في نسبة البروتين الشحمي عالي الكثافة (HDL-C). أدى الاعطاء الفموي للخلاصة بجرعات 100 (TL) و 300 (TM) و 500 (TH) ملغ / كيلوغرام من وزن جسم الجرذ في اليوم الى انخفاض في تراكيز كوليسترول الكبد والبلازما الكلي (TC) و ثلاثي الغليسيرول (TG) والبروتين الشحمي منخفض الكثافة (LDL-C) (C) ومؤشر تصلب الشرايين (AI) وكان انخفاض كوليسترول البلازما الكلي TG ملحوظا احصائيا مع ($p < 0.05$). كما تم تسجيل ارتفاع ملحوظ في نسب البروتين الشحمي عالي الكثافة البلازمي HDL-C عند كل المجموعات المزودة بالخلاصة النباتية BAE مقارنة مع مجموعة ال (CP) وعلى العكس كان تأثير البروتين الشحمي عالي الكثافة الكبدي HDL-C قليلا وغير ملحوظ. تم عكس تأثير الحمية عالية الكوليسترول على تراكيز بيروكسيد الجلوتاتيون (GPx) وفوق أكسيد الديسموتاز (SOD) من خلال اعطاء الخلاصة النباتية BAE حيث ارتفعت تراكيز كلا الأنزيمات خاصة عند مجموعة (TM) التي عولجت بجرعة 300 ملغ / كغ من وزن الجرذ في اليوم من الخلاصة النباتية BAE. وقد تم بشكل ملحوظ والمقارنة مع المجموعات الطبيعية الشاهد إعادة نسبة ال (SOD) البلازمي الى القيم الطبيعية عند مجموعات ال (TM) وال (TH). بالنسبة لتأثير ما بعد اعطاء الخلاصة النباتية BAE على الكبد والكلية لم تتم ملاحظة أي تغيرات مهمة في مستويات السبارتات ترانسأميناز (AST) والألانين ترانسأميناز (ALT) وفوسفاتاز الكلوي (ALP) والبروتين الكلي والألبومين والبولية والكرياتينين. تم تسجيل زيادة ملحوظة في نسب (AST) و (ALT) و (ALP) في مجموعة (CP) بسبب الظروف عالية الكوليسترول كما كانت هذه المعلمات اقل تأثرا في مجموعة ال (TM) بالمقارنة مع الشاهد الطبيعي. وعلاوة على ذلك لم تبد الخلاصة النباتية BAE أي تأثير على المعلمات الدموية.

ABSTRAK

Penyakit kardiovaskular mencatatkan peningkatan insiden yang serius di seluruh dunia dan menjadi punca utama kematian dan hilang upaya. Berdasarkan kajian yang telah dijalankan, sifat antioksidan polifenol yang terkandung dalam buah-buahan dan sayur-sayuran menunjukkan keberkesanan dalam memerangi pelbagai penyakit kronik termasuk penyakit kardiovaskular. *Baccaurea angulata*, lebih dikenali sebagai buah belimbing dayak atau belimbing hutan, adalah salah satu spesies buah-buahan hutan yang boleh dimakan dan boleh didapati di Sabah dan Sarawak. Penyelidikan ini bertujuan untuk menyediakan data saintifik tentang maklumat pemakanan dan manfaat kesihatan dari penggunaan jus ekstrak *B. angulata* (BAE). Ujian kolerasi Pearson dan analisis varians (ANOVA) digunakan bagi menentukan hubungan antara data kajian. Hasil analisis menunjukkan serbuk sejuk kering BAE mengandungi nilai karbohidrat yang tinggi diikuti kelembapan, jumlah serat, abu, dan protein. Serbuk ekstrak mengandungi kepadatan tenaga yang tinggi dengan aktiviti air yang rendah (A_w) menunjukkan kestabilan ekstrak bagi tujuan penyimpanan. Saringan fitokimia menunjukkan bahawa BAE mempunyai jumlah asid fenolik sebanyak 7.90 ± 0.05 mg persamaan setara asid galik dan jumlah flavonoid sebanyak 12.74 ± 0.24 mg persamaan setara quercetin bagi setiap gram ekstrak dan nilai kedua-duanya berkolerasi dengan aktiviti antioksidan yang disaring dengan ujian kebolehan penurunan ferum pada plasma (FRAP) ($p < 0.01$) tetapi tidak berkolerasi dengan ujian anion kapasiti perencat xantin oksidase dismutase (XOD). Tikus jantan Sprague Dawley telah diberikan diet berkolesterol tinggi (diperkaya dengan kolesterol 1 % dan 0.2 % asid kolik) untuk menjadikannya hiperkolesterolemik dan ditambah dengan BAE, diberikan secara oral selama 10 minggu. Tiada perubahan signifikan direkodkan bagi berat badan, jantung dan ginjal. Berbanding kawalan normal, terdapat perbezaan signifikan bagi berat hati dalam kumpulan diet kolesterol tinggi ($p < 0.05$) dengan sedikit penurunan pada kumpulan yang menerima diet tambahan BAE berbanding kawalan hiperkolesterolemik (CP). Kadar kolesterol (TC), trigliserida (TG), kolesterol lipoprotein berketumpatan rendah (LDL-c) dari plasma dan hati, dan indeks atherogenik (AI) meningkat secara signifikan ($p < 0.05$) dalam kumpulan CP disamping penurunan nilai kolesterol lipoprotein ketumpatan tinggi (HDL-c). Pengurangan kadar TC, TG, LDL-c di dalam plasma dan hati beserta nilai AI dapat diperhatikan selari dengan peningkatan nilai dos BAE disamping penurunan TG plasma yang signifikan ($p < 0.05$). Kenaikan ketara plasma HDL-c diperhatikan dalam semua kumpulan yang menerima diet tambahan BAE berbanding dengan kumpulan kawalan CP, namun tiada perubahan direkodkan di dalam hati. *Glutathion peroxidase* (GPx) dan *superoxide dismutase* (SOD), menunjukkan peningkatan aktiviti dalam semua kumpulan yang menerima diet tambahan BAE terutamanya oleh kumpulan dos sederhana (300 mg/kg berat badan sehari). Merujuk kepada kawalan normal, nilai plasma SOD berjaya dipulihkan dalam kumpulan dos sederhana dan tinggi. Tiada perubahan yang signifikan ($p > 0.05$) pada kadar enzim aspartate transaminase (AST), alanine transaminase (ALT), alkaline fosfatase (ALP), jumlah protein, albumin, urea, dan tahap kreatinin yang dinilai menunjukkan penggunaan BAE tidak mendatangkan kesan sampingan kepada hati dan buah pinggang. Peningkatan nilai AST, ALT, dan ALP dalam kumpulan kawalan positif disebabkan oleh keadaan hiperkolesterolemik dan dengan merujuk kepada kawalan normal, parameter berkenaan paling kurang menunjukkan kesan dalam kumpulan dos sederhana. Diet tambahan BAE juga tidak memberikan kesan sampingan yang signifikan pada parameter hematologi.

APPROVAL PAGE

I certify that I have supervised and read this study and that in my opinion, it conforms to acceptable standards of scholarly presentation and is fully adequate, in scope and quality, as a thesis for the degree of Master of Health Sciences (Nutrition Science).

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DECLARATION

I hereby declare that this dissertation is the result of my own investigations, except where otherwise stated. I also declare that it has not been previously or concurrently submitted as a whole for any other degrees at IIUM or other institutions.

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CARDIOVASCULAR DISEASES INDICES AND ITS TOXICITY EFFECTS**

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Affirmed by Nurhazni binti Khir Jauhari

.....
Signature

.....
Date

To my beloved family;

Khair Jauhari Alias (ayah)

Saadah Ismail (ibu)

Hazwani & Roy Hendra Putra

Muhammad Hafizi

Nurhaziqah

Adheem & Airis.

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LIST OF ABBREVIATIONS AND SYMBOLS

| | |
|-------|--|
| AI | Atherogenic index |
| AIHW | Australian Institute of Health and Welfare |
| ALP | <i>Alkaline phosphatase</i> |
| ALT | <i>Alanine transaminase</i> |
| AST | <i>Aspartate transaminase</i> |
| ATP | Adenine triphosphate |
| AU | Absorbance unit |
| CAT | <i>Catalase</i> |
| COX-2 | <i>Cyclooxygenase</i> |
| CGPx | <i>Cellular glutathione peroxidase</i> |
| CHD | Coronary heart diseases |
| CN | Normocholesterolemic/normal control |
| CP | Hypercholesterolemic control |
| Cm | Centimeter |
| CVD | Cardiovascular diseases |
| DMF | Dimethyl sulfoxide |
| DNA | Deoxyribonucleic acid |
| ECG | -(-)epicatechin-3-gallate |
| EGCG | Epigallocatechin gallate |
| EGPx | <i>Extracellular glutathione peroxidase</i> |
| FAO | Food and Agriculture Organization |
| FRAP | Ferric reducing antioxidant capacity of plasma |

| | |
|---------|---|
| GIGPx | <i>Gastrointestinal glutathione peroxidase</i> |
| GPx | <i>Glutathione peroxidase</i> |
| GSH | Glutathione |
| GST | <i>Glutathione S-transferase</i> |
| G | Gram |
| Hb | Hemoglobin |
| HCT | Haematocrit |
| HDL-c | High density lipoprotein cholesterol |
| HMG-CoA | 3-hydroxy-3-methylglutaryl-CoA |
| HOCl | Hydrochlorous acid |
| HSDA | Sodium N-(2-hydroxyl-3-sulphopyrpyl)-3,5-dimethoxyaniline |
| IDF | Insoluble dietary fiber |
| IL | Interleukin |
| Inos | Inducible nitric oxide synthase |
| IU | International unit |
| KCl | Potassium chloride |
| LDH | Lactate dehydrogenase |
| Kg | Kilogram |
| LDL-c | Low density lipoprotein cholesterol |
| LPS | Lipopolysaccharides |
| L | Liter |
| MDA | <i>Melondialdehyde</i> |
| Mg | Miligram |
| ml | Mililiter |

| | |
|------------------|--|
| Mm | Milimolar |
| MOH | Ministry of Health Malaysia |
| NAD ⁺ | Nicotinamide adenine dinucleotide (reduced form) |
| NADH | Nicotinamide adenine dinucleotide |
| NBT | Nitroblue tetrazolium chloride |
| NCD | Noncommunicable diseases |
| NCEP | National Cholesterol Education Program |
| Ox-LDL | Oxidized low density lipoprotein |
| PC | Phosphatidylcholine |
| PHGPx | <i>Phospholipid hydroperoxide glutathione peroxidase</i> |
| RBC | Red blood cells |
| RNS | Reactive nitrogen species |
| ROS | Reactive oxygen species |
| SD | Standard deviation |
| SDF | Soluble dietary fiber |
| SEM | Standard error of mean |
| SOD | <i>Superoxide dismutase</i> |
| SM | Sphingomyelin |
| TC | Total cholesterol |
| TFC | Total flavonoid content |
| TG | Triglycerides |
| TH | Treatment high dose |
| TL | Treatment low dose |
| TM | Treatment medium dose |

| | |
|------------------|-----------------------------------|
| TNF | Tumor necrosis factor |
| TPC | Total phenolic content |
| TPTZ | 2,4,6-tripyridyl-s-triazine |
| UV | Ultra violet |
| VCMC | Vascular smooth muscle cells |
| WBC | White blood cells |
| WHO | World Health Organization |
| XOD | <i>Xanthine oxidase dismutase</i> |
| $O_2^{\bullet-}$ | Superoxide anion |
| OH^{\bullet} | Hydroxyl radical |
| ROO^{\bullet} | Peroxyl radical |
| H_2O_2 | Hydrogen peroxide |
| NO^{\bullet} | Nitric dioxide |
| NO_2 | Nitrogen dioxide |
| ONO^- | Peroxynitrite |
| % | Percentage |
| < | Less than |
| > | More than |
| °C | Degree celcius |
| α | Alpha |
| β | Beta |
| Γ | Gamma |

CHAPTER ONE

INTRODUCTION

Cardiovascular disease (CVD), a group of heart and blood vessel disorder has been a leading cause of death both at global and in Malaysia for years. According to World Health Organization (WHO) (2011), an estimation of 17.3 million morbidity cases reported due to cardiovascular diseases, representing 30 % of global death in 2008. By 2030, mortality number due to coronary heart diseases and ischemic heart diseases (stroke) is projected to reach more than 26.3 million making CVD as the single leading cause of death. Interaction between multiple risk factors such as smoking, physical inactivity, and the most common practice- unhealthy diet, generate early mechanism of developing CVD (Clinical Practice Guidelines, 2008; Mackay, Mensah, Mendis, and Greenlund, 2004).

Previous research proposed that an increase in oxidative stress is believed to be the underlying cause for many chronic and degenerative diseases including cardiovascular disease (Kritchevsky, 1999; Greig and Maxwell, 2001; Mayne, 2003). Indeed, Tribble (1999) and Southon (2000) reported an inverse association of disease development with high intake of dietary antioxidant consumption from fruits and vegetables. Phenolic compounds had been rigorously studied for their bioactivity and health effects involving *in vitro* and *in vivo* together with pre-clinical studies. Mayne (2003) further reported the essence of antioxidant to overcome lipid oxidation by free radical species which prevent development of CVD. In addition, Vita (2005) explained that actions of such antioxidants such as polyphenols and carotenoids can be benefitted at inhibiting cholesterol synthesis and improves endothelial functions.

SIGNIFICANT OF STUDY

Many therapeutically useful bioactive compounds and micronutrients are available within our food source such as fruits and vegetables as proven in numerous studies (Yan, Teng, and Jhi, 2006; Mahattanatawee, 2006; Lin T., Lin H., Chen, Lin M., Chou, and Wang, 2007; Abdul Ghaffar, Prasad, Weng, and Ismail, 2009). Fruits and vegetables that are usually being consumed contain high caretenoids, vitamin C, polyphenols and other beneficial micronutrients. These have become an integral part of daily human diets and provide readily accessible antioxidants (Mahattanatawee, 2006) which provide beneficial protective effects against CVD development (Liu, Mensen, Lee, Cole, Hennekens, et al., 2000; Nakamura, Nagata, Oba, Takatsuka, and Shimizu, 2008).

Current mortality and morbidity status indirectly reflects inefficiency of the conventional treatments such as drug therapy and current pharmacological approach together with surgical treatments. Thus, recent research interest has directed towards exploring and utilizing natural ingredients of underutilized fruits in form of natural food product or supplement as a prevention approach to reduce CVD's risk factors. Furthermore, recognition on the benefits of natural products such as fruit juice is increasing among worldwide population and becoming a part of regular diet and more preferable than conventional drugs therapy (Sin, Yusof, Sheikh Abdul Hamid, and Abdul Rahman, 2006; Apak, Guclu, Demirata, Ozyurek, Celik et al., 2007). Many studies conducted had successfully provide evidences on dietary antioxidants from fruits at lowering cholesterol levels (Anila et al., 2002; Hirunpanich, Utaipa, Morales, Bunyaphatsara, Sato et al., 2006; Mohd Adzim Khalili, Norhayati, Rokiah, Asmah, Siti Muskinah, and Abdul Manaf, 2009).

Liver response as such indicated by cholesterol levels is being influenced by diet intake and subjected to body homeostatic mechanism. In animal studies, it is considered as critical and reliable indicator for dietary lipidemic evaluation (Kahlon and Chow, 2000). In addition, sufficient evidences have documented that feeding high cholesterol diet resulted in elevated levels of total cholesterol, low density lipoprotein cholesterol and triglycerides in various animal models such as in hypercholesterolemic rats (Gorinstein, Caspi, Libman, Katrich, Lerner, and Trakhtenberg, 2004; Edijala, Asagba, Eviramremu, and Atomatofa, 2005; Valcheva-Kuzmanova, Kuzmanov, Mihova, Krasnaliev, Borisova, and Belcheva, 2007). Several other studies successfully showed hypolipidemic effect and enhanced plasma antioxidants in animal models treated with antioxidants derived from fruits sample of different species (Anila and Vijayalakshmi, 2002; 2003).

Persistent increase in morbidity and mortality status of cardiovascular diseases and chronic diseases despite modern pharmacological approach has directed a large scale of research at finding as many natural antioxidants source as possible (WHO, 2011). Many are emphasizing on the essence of preserving the antioxidant and other nutrient contents to be consumed as an integral part of daily human diets. Prevention of CVD occurrence is best be addressed by locally planned strategies to suits the lifestyle and settings of public people to avoid unwanted stress that might lead to other health diseases. In Malaysia, Disease Control Division of Ministry of Health Malaysia (MOH) has designated primary prevention strategies which include public health awareness and healthy living- food and lifestyle campaign to promote early prevention against development of CVD. This includes generation of evidence and intervention of functional foods with health benefits derived from locally conducted

research which utilising local natural resources to improve cost effective application and technologies.

Malaysia is well known for its versatility of natural resources with many is yet to be explored especially those that grow in wild. Despite the endemic species distribution, systematic research approach has proven high nutritional values including antioxidants properties contained in underutilized fruits (Abu Bakar, Mohamed, Rahmat, and Fry, 2009; Emmy Hainida, Khoo, Abbe Maleyki, Amin, Salma et al., 2009). This study involved *Baccaurea angulata* (*B. angulata*) or locally known as 'belimbing dayak' or 'belimbing utan'- one among hundreds of Malaysia exotic fruits. To date, there is very limited scientific information on *B. angulata*, thus, this study aimed to evaluate the nutritional and chemical compositions of *B. angulata* together with its possible antioxidant capacity and health effects. Further, with respect to its endemic distribution and seasonal availability, the study on this fruit with particular preservation technique is essential in order to making it available and accessible for public and throughout the year. This study puts one step forward in promoting practical dietary strategy in managing the CVD incidence. Moreover, it is also an indirect effort to commercialize Malaysia local fruit and to conserve one of national heritage.

OBJECTIVES

General Objective

The general objective of this research was to study the effects of *B. angulata* prepared from aqueous whole fruit extract in induced hypercholesterolemic rats.

Specific Objectives

1. To determine phytochemical and antioxidant properties of underutilized *B. angulata* juice extract powder.
2. To evaluate the effects of *B. angulata* juice extract suspension on plasma and liver lipid profiles in hypercholesterolemia rats.
3. To determine the effects of *B. angulata* juice extract suspension on plasma and liver antioxidant enzymes in hypercholesterolemia rats.
4. To examine the toxicity effects in hypercholesterolemia rats due to consumption of *B. angulata* juice extract suspension.