



DETERMINATION OF NUTRIENT COMPOSITION
AND TOXICITY OF *Baccaurea angulata*

BY

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A dissertation submitted in fulfilment of the requirement for
the degree of Master of Health Sciences

Kulliyyah of Allied Health Sciences
International Islamic University Malaysia

DECEMBER 2013

ABSTRACT

The purpose of this study is to explore the potential of physicochemical and antioxidant characteristics of *Baccaurea angulata* fruit juice and to determine an acceptable dose to be consumed. Size of *B. angulata* fruits was measured and separated into whole fruit, berry and skin portions. Samples were blended, filtered and freeze-dried. Lyophilized juice was analyzed for nutrient and chemical compositions, with antioxidant capacity quantifications. Results showed freeze-dried skin (FDS) had the highest moisture (21.04 %) and ash (10.04%) content, protein (2.12%), total fat (2.09%), and water activity (0.467 a_w), compared to freeze-dried whole fruit (FDWF) and freeze-dried berry (FDB). FDWF had the highest carbohydrate (74.12%) and total dietary fibre (6.3 %) content while FDB had highest content of crude fibre (0.36%) and gross energy (304.09kcal/100 g) as compared to other samples. Meanwhile, FDS showed highest value in diphenyl-picryl-hydrazyl (DPPH) assay (102.66mg AA/100 g) and trolox equivalent antioxidant capacity (TEAC) assay (847.46mg TE/100 g) values. Toxicity study on normal rats was carried out using FDWF sample. Animals in both acute and sub-chronic studies demonstrated no significant changes in general behaviour, growth, and relative organ weights. A significant increment of red blood cells ($8.93 \times 10^6/\mu\text{L}$) was observed in low dose female group with decrease of white blood cells ($5.67 \times 10^3/\mu\text{L}$) and lymphocyte ($4.33 \times 10^3/\mu\text{L}$). In liver function test, total protein and alkaline phosphatase were markedly increased in male treated group (300 mg/kg), 36.50g/L and 47.90U/L, respectively. The low dose female group also showed decrement in same parameters, 63.70g/L and 73.50U/L. However, gross and microscopic appearance of the organs showed no significant pathological changes. There was no evidence of any tissue injury. In acute study, lethal dose (LD_{50}) for FDWF was determined to be $>5,000$ mg/kg. Therefore, the no-observable adverse effect level for *B. angulata* was 1,200 mg/kg administered orally for 13 weeks. In conclusion, *B. angulata* whole fruit juice has the potential to be utilized for preparation of a health drink as it is safe and offers opportunity to be developed for nutraceutical and functional fruit product.

خلاصة البحث

هدفت هذه الدراسة إلى دراسة الخصائص الفيزيائية والكيميائية المضادة للأكسدة في عصير ثمرة الـ (*Baccaurea angulata*) بالإضافة لتحديد الجرعة التي يمكن استهلاكها للإستفادة منها. تم تحديد حجم الثمار وفصل الثمرة (الفاكهة) وحببات التوت التي في داخلها بالإضافة للغلاف الخارجي، ثم بعد ذلك تم خلط العينات وعمل عصير منها وتم تنقيتها ومن ثم وضعها في مجفف التجفيد. بعد ذلك تم تحليل المحتوى الغذائي والكيميائي للعصير المجفف بالتجميد و فحص امكانيته كمضاد أكسده. النتائج أظهرت أن مستخلص القشرة الخارجية المجفف بالتجميد (FDS) يحتوي على أعلى نسبة رطوبة (21.04%) و رماد (10.04%) ونسبة بروتين (2.12%) ، ومجموع كلي من الدهون بنسبة (2.09%) ، وماء (aw 0.467) و بالمقارنة بالمستخلص المجفف بالتجميد للثمرة ككل (FDWF) و حبة التوت المجففة بالتجميد (FDB) وُجد أن FDWF تحتوي على أقصى مُحتوى كربوهيدرات بنسبة (74.12%) وألياف غذائية بنسبة (6.3%) ، بينما الـ FDB فكانت نسبة الألياف فيها تعادل (0.36%) وسعرات حرارية (304.09kcal/100 g) مقارنة بالعينات الأخرى. من جهة أخرى ظهر أن الـ FDS يحتوي على أعلى قيمة عند قياسه باختبار Diphenyl-Picryl-Hydrazyl (DPPH) تعادل (102.66mg AA/100 g) و (847.46mg TE/100 g) باستخدام اختبار (TEAC) trolox equivalent antioxidant capacity. دراسة السمية تم اجراؤها على فئران المختبر الطبيعية (الغير مريضة) وتم تعريضها لعينة من الـ FDWF ، الحيوانات في الدراسة أظهرت أنه لا يوجد أي تغييرات أساسية على السلوك العام للحيوان سواء على صعيد نموا أو على تغير وزن الأعضاء. كما أظهرت الدراسة زيادة ملحوظة في خلايا الدم الحمراء (8.93 $\times 10^6/\mu\text{L}$) في مجموعة اناث الفئران التي تعرضت لجرعة منخفضة ، بالإضافة لذلك لوحظ نقص في خلايا الدم البيضاء ($5.67 \times 10^3/\mu\text{L}$) والخلايا الليمفاوية ($4.33 \times 10^3/\mu\text{L}$). أما اختبارات وظائف الكبد فأظهرت زيادة ملحوظة في المجموع الكلي للبروتين الفوسفاتيز القاعدي في مجموعة الفئران الذكور المعالجة (300 mg/kg) و (47.90U/L و 36.50g/L على التوالي. مجموعة الفئران التي تعرضت للجرعات المنخفضة أظهرت زيادة في نفس العوامل أيضاً (63.70g/L و 73.50U/L. على الرغم من ذلك فإن الدراسة الميكروسكوبية والمقطعية للأعضاء لم تُظهر أي تغييرات مرضية ، ولم يظهر أي دليل على أن هناك مجروح في الأنسجة. وفي دراسة الحدة وجرعة القتل (LD_{50}) للـ FDWF تم تحديدها لتكون $>5,000$ مليجرام لكل كيلوجرام. وبالنتيجة لم يكن هناك أي تأثير مُعاكس للـ *B. angulata* حيث أنه تم تقديم جرعة 1,200 مليجرام لكل كيلوجرام ولمدة 13 أسبوع. تُخلص هذه الدراسة إلى أن عصير الثمار ككل لنبات الـ *B. angulata* يُمكن أن يُستخدم ويُستفاد منه لتحضير المشروبات الصحية المغذية ، كما أنه آمن ويُقدم فرصة لتطوير علاج غذائي ومنتج غذائي فعال.

ABSTRAK

Kajian ini dilakukan untuk menentukan ciri-ciri fizikokimia dan sifat antipengoksida jus buah *Baccaurea angulata* disamping menentukan dos yang sesuai untuk diminum. Buah *B. angulata* diukur secara fizikal dan kemudian diasingkan kepada bahagian keseluruhan buah, isi buah dan kulit buah. Sampel dikisar, ditapis, dan kemudian produk dikering sejukbeku. Jus yang diekstrak ditentukan komponen kimia pemakanan, serta ditentukan kapasiti antipengoksidaan. Hasil menunjukkan ekstrak kering kulit buah (FDS) mengandungi jumlah kandungan kelembapan (21.04 ± 0.20 %) dan abu (10.04 ± 0.97 %), kandungan protein (2.12 ± 0.21 %), lemak (2.09 ± 1.89 %), serta aktiviti air ($0.467 a_w$) tertinggi berbanding ekstrak kering keseluruhan buah (FDWF) serta isi buah (FDB). FDWF menunjukkan kandungan karbohidrat (74.12 ± 0.32 %) serta gential diet (6.3 %) paling tinggi manakala FDB pula mengandungi paling banyak gential kasar (0.36 ± 0.21 %) dan jumlah tenaga (304.09 ± 10.36 kcal/100 g) berbanding dengan sampel-sampel yang lain. Selain itu, FDS juga mempunyai nilai diphenyl-picryl-hydrazyl (DPPH) (102.66 ± 2.12 mg AA/100 g) dan trolox equivalent antioxidant capacity (TEAC) (847.46 ± 10.57 mg TE/100 g) paling tinggi. Seterusnya, ujian ketosikan keatas tikus normal dijalankan menggunakan sampel FDWF. Haiwan kajian akut dan sub-kronik tidak menunjukkan perubahan signifikan ($p > 0.05$) pada perilaku umum, ketumbesaran serta berat relatif organ. Peningkatan signifikan ($p < 0.05$) pada sel darah merah ($8.93 \pm 0.33 \times 10^6/\mu\text{L}$) diperhatikan pada tikus betina kumpulan dos rendah (300 mg/kg) serta penurunan bilangan sel darah putih ($5.67 \pm 0.45 \times 10^3/\mu\text{L}$) dan limfosit ($4.33 \pm 0.42 \times 10^3/\mu\text{L}$). Dalam ujian fungsi hati, jumlah protein dan alkaline fosfatase masing-masing meningkat dengan ketara ($p < 0.05$) pada tikus jantan kumpulan rawatan dos 300 mg/kg iaitu 36.50 ± 2.65 g/L dan 47.90 ± 4.48 U/L. Manakala, tikus betina kumpulan dos rendah menunjukkan penurunan bagi parameter-parameter tersebut iaitu masing-masing, 63.70 ± 5.28 g/L dan 73.50 ± 12.91 U/L. Pemerhatian secara kasar dan mikroskopik organ utama haiwan berkenaan tidak menunjukkan sebarang perubahan patologi. Maka, ini membuktikan bahawa tidak berlaku sebarang kecederaan pada tisu haiwan kajian. Oleh itu, melalui kajian akut, nilai dos maut (LD_{50}) untuk FDWF ditentukan sebagai $> 5,000$ mg/kg. Manakala “no observable adverse effect level” (NOAEL) untuk *B. angulata* adalah 1,200 mg/kg yang diberi secara oral selama 13 minggu. Kesimpulannya, jus *B. angulata* berpotensi untuk dijadikan sebagai persediaan minuman kesihatan kerana buah ini didapati selamat dan menawarkan pelbagai peluang untuk dimajukan sebagai produk nutrasetikal.

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.

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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.

Darina Binti Ibrahim

Signature

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**STUDY OF NUTRIENT COMPOSITION AND TOXICITY OF *Baccaurea
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Date

*I dedicate this thesis especially to my parents, Ibrahim Bin Jawi and Hapsah Abd
Hamid for their endless love and prayers for their youngest child in the family.*

ACKNOWLEDGEMENTS

In the name of Allah the Most Beneficent and the Most Merciful

Alhamdulillah. I sincerely would like to state my gratitude to Most Beneficent and Most Merciful creator, Allah S.W.T for permitting me to complete this project within duration of study, as well as giving me this sweetness of joy after two years of sweat and tears. This thesis has finally completed therefore I would like to thank all who gave meendless support throughout this project.

First and foremost, a special thank to my supervisor, Asst. Prof. Dr. Muhammad Ibrahim, and co-supervisor, Asst. Prof. Dr. Norazlanshah Hazali. I really appreciate the advice and guidance that they have given me from the beginning until the end of this study. Their continuous encouragement pushed me to continue my research until the last page of this project.

Not to forget all science officers, assistant science officers, and medical laboratory technologies, from Kulliyyah of Allied Health Sciences, Kulliyyah of Science, and Kulliyyah of Medicine. I would like to express my gratitude to them for sharing their valuable knowledge and experiences. Their expertise and assistance were very helpful during the process of this project.

I would like to uttermy appreciation to allpostgraduate friends, especially Nurhazni who had assisted from beginning of this project until this thesis is submitted. Lastly, I would like to articulate gratefulness to my familymembers, Dayana, Doreen, Daniel, and Dottie for their endless support and understanding. Their love and patience did not falter during my long term of absence due to my full commitment towards this study. Thank you for all of your prayers. At last, I have accomplished my mission

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LIST OF ABBREVIATIONS

ABTS	2,2'-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid)
ALT	Alanine transferase
ALP	Alkaline phosphatase
AOAC	Association of Official Analytical Chemists
AST	<i>Aspartate transferase</i>
DPPH	1, 1-diphenyl-2-picrylhydrazyl
DPX	Distyrene plasticizer xylene
EDTA	Ethylenediaminetetraacetic acid
FDB	Freeze-dried berry
FDWF	Freeze-dried whole fruit
FDS	Freeze-dried skin
GGT	Gamma glutamate transferase
H&E	Haematoxylin and Eosin
HDL	High density lipoprotein
IDF	Insoluble dietary fibre
LD ₅₀	Median lethal dose
LDL	Low density lipoprotein
MCH	Mean corpuscular haemoglobin
MCHC	Mean corpuscular haemoglobin concentration
MCV	Mean corpuscular volume
NADPH	Nicotinamide Adenine Dinucleotide Phosphate
NOAEL	No Observable Adverse Effect Level
OECD	Organization of Economic Co-operation and Development

RBC	Red blood cell
RDW	Red blood cell distribution width
ROS	Reactive Oxygen Species
SDF	Soluble dietary fibre
SST	Serum separating tube
TEAC	Trolox equivalent antioxidant capacity
WBC	White blood cell
WHO	World Health Organization

LIST OF SYMBOLS

”	inches
cm	centimetre
°C	degree Celsius
fL	femtolitre
g	gram
kg	kilogram
L	litre
μL	microlitre
μmol	micromol
μM	micromolar
mbar	millibar
mg	milligram
mL	millilitre
mm	millimetre
mmol	millimol
mM	millimolar
nm	nanometre
N	Normality
pg	picogram
U/L	unit per litre
w/v	weight per volume
a _w	water activity

CHAPTER ONE

INTRODUCTION

1.1 STUDY BACKGROUND

Consumption of assorted fruits is highly recommended in order for the body to benefit a variety of nutrients and therapeutic components such as antioxidants. This is because each fruit contains diverse types of phytochemical compounds as well as different compound concentrations due to geographical distribution. People are beginning to change their daily diet as the recognition of the benefits of fruit-based products increases among the worldwide population. This is because previous research had discovered the protective effect of phenolic compounds in fruits against free radicals (Sass-Kiss et al., 2005; Cieslik, et al., 2006; Lin et al., 2007). Therefore, recent research interests have been directed towards exploring and utilizing natural components of fruits, including underutilized species, in a form of natural food products or supplements as a preventive approach to reduce the prevalence of non-communicable diseases such as cardiovascular disease (CVD) (Muhammad, Prasad, Amin, Azrina, and Azizah, 2010).

Exploration on underutilized fruits gives researchers a new perspective about these fruits. Compared to commercial fruits, underutilized fruits in Malaysia are expected to possess high antioxidant capacity. Examples of such fruits include belimbing buluh (*Averrhoa bilimbi*), cerapu (*Garcinia prainiana*), jambu mawar (*Syzygium jambos*), buah Melaka (*Phyllanthus emblica*), sentol (*Sandoricum macropodum*), bacang gelok (*Mangifera foetida*), and jentik-jentik (*Baccaurea polyneura*) (Emmy et al., 2009). The phenolic content of these fruits was more than

500 mg/GAE per 100 g of edible portion while their antioxidant capacity consists of more than 70 %.

Baccaurea angulata is also another type of underutilized fruit. This fruit is from Euphorbiaceae family that possesses essential nutrients (Voon, Patricia, and Chin, 1990) and probably has substantial antioxidant activity similar to *B. polyneura* (Emmy et al., 2009) that may provide protection against degenerative diseases such as CVD. To combat with these diseases, it could be suggested to utilize this fruit in preparation of health drink in a concentrated form in order to deliver a better effect against free radicals. However, when consume concentrated extract, it could be a concern because this extract could be overdose and thus inflict a toxicity effect to the consumer. Hence, toxicological evaluation is crucial to ensure this fruit is safe to be consumed. Safety assessment of fruits is also important because these fruits had exposed to all sorts of hazards whether it is in a form of biological, chemical, or physical hazard. Besides that, bioactive compounds that are contained within these fruits also need to be identified and the concentration to be quantified. This is because phenolic compounds have the potential to react with natural biochemical substances which would then produce byproducts that maybe toxic to the human body.

1.2 STATEMENT OF PROBLEM

Malaysia is blessed with not less than 370 species of edible tropical fruits due to its geographical location near to the equatorial line (Rukayah, 2002). Some of these fruits are exported around the world in order to fulfil demands from consumers. For example, star fruit type B10, produced by MARDI, is a popular fruit in Europe and has dominated about 60 % market in Europe with export value of more than RM 30 billion per year (Mohd Feroz, 2012). Other commercial fruits that are exported

include mango, watermelon, mangosteen, rambutan and many others. Underutilized fruits do not receive much attention due to the lack of demand from consumers which brings about its market value. Consequently, farmers and providers are forced to remove these underutilized fruit trees and replace them with commercialize fruit trees. This could push underutilized fruit trees into the brink of extinction.

It is important to study the safety evaluation of fruits in order to obtain a comprehensive profile of fruits all over the world so that it is safe to be consumed. For example, there were cases which found that star fruit (*Averrhoa carambola*) had caused acute renal failure to patients who were previously diagnosed with good health (Neto et al., 2009). This is because star fruit contain oxalate that could form crystals as it reacts with calcium in the human body. Star fruit is a common fruit among Malaysians and it is still consume in its raw form or as fruit juice.

Cardiovascular diseases are the leading cause of mortality, and are major contributors to the burden of diseases worldwide. For over 17.3 million of death reported per year was due to CVDs, which covers about 31 % from main causes of death recorded globally (WHO, 2011). In Malaysia, the Ministry of Health (2011) made a report in Health facts 2010, that CVDs were located at the top (25.35 %) of a list consisting of principal causes of death documented in Ministry of Health`s hospitals. In addition, 6.88 % cases from ten principle causes of hospitalisation in Ministry of Health`s hospitals in Malaysia were due to cardiovascular diseases (2011). Therefore, in order to fulfil recommended intake of fruits per day by Malaysian Dietary Guideline (2010), toxicity profiling of underutilized fruits is essential so that Malaysians are safe to consume these fruits while protecting consumers from risk factors of non-communicable diseases.

1.3 SIGNIFICANCE OF STUDY

This study was conducted to explore the potential of one of underutilized fruits in Malaysia, *B. angulata* whole fruit juice. This investigation is necessary in order to find the nutraceutical value of this fruit. Besides that, this exploration is needed before the fruit is extinct from over disposal by farmers due its lack of market value. Moreover, the nutrient values that were discovered can act as a guide in producing health supplement products from *B. angulata*. Hence, it can be recommended to be incorporated in daily meal preparations.

In addition, many studies conducted had successfully showed evidences on dietary antioxidants from fruits could lower lipid profile in tested animal (Hirunpanich et al., 2006; Mohd Adzim Khalili et al., 2009). Thus, this has increase acknowledgement in worldwide population on the benefit of consuming fruits. Currently, research interest has been directed towards exploring and utilizing natural components from fruits especially those that are underutilized, to produce natural food products or supplements as a prevention approach to reduce CVD's risk factors (Muhammad et al., 2010).

The present study will reveal an additional antioxidant property that could be added to the portfolio of health benefits offered by *B. angulata* extract. Thus, it can strengthen or enrich the information regarding nutritional compositions of underutilized fruits in Malaysia food composition database. This will contribute comprehensive antioxidant content database of a wide range of Malaysian fruits and would be useful for epidemiological research and dietary guidelines formulation. Plus, it helps to establish data on toxicity effect of local underutilized fruits in normal rats. In addition, able to produce economically attractive ingredients that can be benefited to small and medium scale industries.

1.4 OBJECTIVES

1.4.1 General Objective

To determine the nutrient composition and toxicity of “belimbing dayak” (*B. angulata*)

1.4.2 Specific Objectives

1. To determine the nutrient compositions and antioxidant property of different parts of *B. angulata* fruit.
2. To determine the degree of toxicity of *B. angulata* powdered aqueous suspension on experimented rats based on haematological, biochemical parameters, and histological examinations.
3. To determine the acceptable safety dose of *B. angulata* powder in aqueous suspension.