# PHYTOCHEMICAL COMPOSITION AND ANTIOXIDANT PROPERTIES OF *BACCAUREA ANGULATA* FRUIT JUICE AND ITS EFFECTS ON CARDIOVASCULAR DISEASE BIOMARKERS IN DIET-INDUCED ATHEROSCLEROTIC RABBITS

BY

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# ABSTRACT

Atherosclerosis is the underlying disease process in the blood vessels that usually results in coronary heart disease (CHD) and cerebrovascular disease, which are the most common forms of cardiovascular disease (CVD). A high level of serum cholesterol is mainly identified as an important risk factor in the development and progression of premature atherosclerosis. Previous studies on *Baccaurea angulata* (BA), a Malaysian underutilized fruit, showed that it is very rich in fiber and antioxidants. The present study was thus undertaken to evaluate the potential health benefits of BA fruit juice on cardiovascular disease biomarkers in diet-induced atherosclerotic rabbits. The studies were carried out in three distinct phases. In phase one, the effect of solvents [methanol and phosphate buffered saline (PBS)] using cold extraction was examined on the total phenolic content (TPC), total flavonoid content (TFC), and total carotene content (TCC), free radical scavenging activities and lipid peroxidation inhibition activities of the various fruit parts (skin, pulp and whole fruit) using spectrophotometer. The results indicated that the methanol crude extracts significantly ($p$<0.01) contained higher TPC, TFC and TCC than PBS extracts in all the fruits parts. The edible portion had the highest and most significant ($p$<0.01) TPC (15357.77±150.72 µg gallic acid equivalence (GAE)/g), TFC (37.32±0.55 mg quercetin equivalence (QE)/g), and TCC (6571.43±185.86 µg β-carotene equivalence (BC)/100 g) [dry weight sample] among the methanol crude extracts. In phase two, thirty-five healthy male adult rabbits (*Oryctolagus cuniculus*; New Zealand White strain) with a body weight of 2300–2800 g were used. The rabbits were randomly assigned to one of the seven rabbit groups. Four groups were fed cholesterol diet (1% cholesterol) and 0, 0.5, 1.0, and 1.5 mL of juice per kg of rabbit daily (hypercholesterolemic groups), while the other three groups were fed commercial rabbit pellet and 0, 0.5, and 1.0 mL of juice per kg of rabbit per day (normocholesterolemic groups) for 90 days. Blood samples were taken before and after the experimental period. The serum, aorta and liver homogenates were also analyzed for biochemical biomarkers. The results showed that the physiological dysfunctions of the hemopoietic system, caused by the high-cholesterol diet, were significantly ($p$<0.01) normalized by the administration of BA whole fruit juice, especially at the highest dose (1.5 mL/kg/day). In phase three, histopathological studies were carried out to evaluate the percentage of atherosclerotic lesion accrued using different staining techniques (Hematoxylin & eosin, van Gieson and Sudan IV). The result showed that the supplementation of high-cholesterol diet of the hypercholesterolemic rabbits with only 0.5 mL BA per kg rabbit per day significantly ($p$<0.001) attenuated aortic fatty streak development. Higher BA fruit juice doses used (1.0 and 1.5 mL per kg rabbit per day) also significantly decreased further the development of aortic fatty streaks. Therefore, the phytochemical composition and antioxidant properties of BA whole fruit juice have substantially proved its potential health benefits as an effective hypocholesterolemic, anti-inflammatory and anti-atherosclerotic agent for the management of CVD biomarkers. Further studies may be needed to corroborate these facts.

# خلاصة البحث


تصلب الشرايين (Atherosclerosis) هو عملية المرض الكامنة في الأوعية الدموية الذي عادة ما يؤدي إلى أمراض القلب التاجية (CHD) والأمراض الدماغية الوعائية، اللتان هما الأكثر شيوعا من أشكال أمراض القلب والأوعية الدموية (CVD) . ويعتبر مستوى عال من الكولسترول في الدم عامل خطر مهم في تطور وتقدم تصلب الشرايين المبكر. وأظهرت الدراسات الأولية على *Baccaurea angulata*، (BA) التي هي فاكهة ماليزية غير المستخدمة بالقدر الكافي، أنه غني جدا من الألياف والمواد المضادة للاكسدة. ولهذا أجريت الدراسة الحالية لتقييم الفوائد الصحية المحتملة من عصير فاكهة BA على المؤشرات الحيوية لمرض القلب والأوعية الدموية في الأرانب المصابة بتصلب الشرايين الناجم عن النظام الغذائي. أجريت الدراسات على ثلاث مراحل متميزة. في المرحلة الأولى، تم فحص تأثير المذيبات [الميثانول والفوسفات مخزنة المالحة [(PBS) باستخدام الاستخلاص البارد على المحتوى الفينولي الكلي (TPC) ، ومجموع محتوى الفلافونويد (TFC)، والمحتوى الكلي للكاروتين (TCC)، أنشطة الكسح الجذور الحرة وأنشطة بيروكسيد الدهون في مختلف أجزاء الفاكهة (الجلد، ولب والفاكهة بكاملها) باستخدام مقياس الطيف الضوئي. وأشارت النتائج إلى أن مقتطفات النفط الخام الميثانول يتضمن بقدر معنوي ($p<0.01$) أعلى TPC، TFC وTCC من المستخلصات PBS في جميع أجزاء الفواكه. وكان للجزء الصالح للأكل أعلى وأهم (150.72 ± 15357.77) TPC (<0.01) ميكروغرام الغال التكافؤ حمض (GAE)/ ز)، TFC (0.55 ± 37.32) ملغ كيرسيتين التكافؤ (QE)/ ز)، و(185.86 ± 6571.43) TCC ميكروغرام β كاروتين التكافؤ (100 / (BC ز) [عينة الوزن الجاف] بين المستخلصات الخام الميثانول. وفي المرحلة الثانية، استخدمت خمسة وثلاثين أرنبا الذكور البالغين (*Oryctolagus cuniculus* ؛ نيوزيلندا سلالة الأبيض) مع وزن الجسم من 2300-2800 غ. وتم تعيين الأرانب عشوائيا إلى واحدة من المجموعات السبعة. تم تغذيتها أربع مجموعات منها الغذائي الكولسترول (1٪ الكوليسترول) و 0، 0.5، 1.0، و 1.5 مل من عصير لكل كيلوغرام من الأرانب يوميا (hypercholesterolemic)، في حين تم تغذية المجموعات الثلاث الأخرى بيليه الأرنب العادي و0، 0.5، و 1.0 مل من عصير لكل كيلوغرام من أرنب يوميا (normocholesterolemic) لمدة 90 يوما. وتم أخذ عينات الدم قبل وبعد فترة التجربة. وقد تم تحليل مصل الدم، وشريان الأورطي، والكبد أيضا عن المؤشرات الكيمياء الحيوية. وأظهرت النتائج أن الخلل الفسيولوجية للدم الناجمة عن النظام الغذائي مرتفع الكولسترول، تم تصحيحها بقدر معنوي ($p<0.01$) بتناول عصير فاكهة BA، ولا سيما في أعلى جرعة (1.5 مل / كغ / يوم). وفي المرحلة الثالثة، أجريت الدراسات النسيجية في تأثير عصير فاكهة BA بكاملها لتقييم نسبة آفة تصلب الشرايين المتراكمة باستخدام تقنيات تلوين مختلفة (الهيماتوكسيلين ويوزين، فان Gieson والسودان IV). وأظهرت النتيجة أن تكملات النظام الغذائي المرتفع الكوليسترول في الدم، للأرانب مفرط كوليستيرول الدم (hypercholesterolemic)، ب-0.5 مل BA يوميا تخفض وتقصر بمستوي معنوي ($p<0.001$) خط الدهنية الأبهري. كما انخفض أعلى جرعات عصير فاكهة BA المستخدمة (1.0 و 1.5 مل لكل كغ أرنب يوميا) بشكل كبير أيضا على تطوير الشرائط الدهنية الأبهري. ولذلك، فإن التكوين الكيميائي النباتي والخصائص المضادة للاكسدة من عصير فاكهة BA بكاملها قد أثبتت إلى حد كبير فوائدها الصحية المحتملة باعتبارها ناقص كولستيرول الدم الفعال، عامل مضاد للالتهابات ومضاد للتصلب الشرايين. قد تكون هناك حاجة إلى مزيد من الدراسات لتأكيد هذه الحقائق.

# APPROVAL PAGE

The thesis of Ahmed Idris Adewale has been approved by the following:

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# DECLARATION

I hereby declare that this thesis 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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*This project is dedicated to Allah and then His beloved Prophet and Messenger Muhammad (S.A.W.), his household, companions and all adherents of the pristine Islam till the day of reckoning.*

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| | |
|---|---|
| % | Percentage |
| ± | Plus-minus |
| × | Multiplication |
| ÷ | Division |
| + | Plus |
| − | Minus |
| < | Less than |
| > | More than |
| g | Gram |
| mg | Milligram |
| kg | Kilogram |
| L | Litre |
| mL | Millilitre |
| µL | Microlitre |
| µmol | Micromole |
| ºC | Degree celcius |

# LIST OF ABBREVIATIONS

| | |
|---|---|
| ABC | ATP-binding cassette |
| ALP | Alkaline phosphatase |
| ALT | Alanine transaminase |
| AST | Aspartate transaminase |
| ATP | Adenosine triphosphate |
| BC | β-carotene equivalence |
| BHA | Butylated hydroxyanisole |
| BHT | Butylated hydroxytoluene |
| CHD | Coronary heart disease |
| CVD | Cardiovascular disease |
| DALY | Disability-adjusted life years |
| ECM | Extracellular matrix |
| ECs | Endothelial cells |
| FBC | Full blood count |
| GAE | Gallic acid equivalence |
| Hb | Hemoglobin |
| Hct | Hematocrit |
| HDL-c | High-density lipoprotein cholesterol |
| HMG-CoA | 3-hydroxy-3-methylglutaryl coenzyme A |
| IMT | Intima-media thickness |
| LDL | Low-density lipoprotein |
| LUC | Large unstained cells |

| | |
|---|---|
| MPV | Mean platelet volume |
| NCD | Non-communicable disease |
| NCEP | National Cholesterol Education Program |
| NHMS | National Health and Morbidity Surveys |
| NO | Nitric oxide |
| Ox-LDL | Oxidized low-density lipoprotein |
| PLT | Platelet count |
| QE | Quercetin equivalence |
| RBC | Red blood cells |
| SMCs | Smooth muscle cells |
| TCC | Total carotene content |
| TFC | Total flavonoid content |
| TPC | Total phenolic content |
| UHPLC | Ultra high-performance liquid chromatography |
| WBC | White blood cells |
| WHO | World Health Organization |

# CHAPTER ONE

# INTRODUCTION

## 1.1 BACKGROUND OF THE STUDY

The dominant and burgeoning contributor to the global burden of disease, in recent times, is non-communicable disease (NCD) or chronic disease (Magee & Narayan, 2013; Snowdon, Malakellis, Millar & Swinburn, 2014). NCD alone, in contrast to other causes combined, was responsible for 63% (36 million) from the 57 million deaths that occurred globally in 2008 (Chintamunnee & Mahomoodally, 2012; Hughes, Hancock & Cooper, 2012).

On the other hand, among the NCD sub-groups, the leading number-one cause of death worldwide, whose global epidemicity is still evolving and which largely contributes to the chronic disease cluster, is cardiovascular disease (CVD), the disease of the heart and blood vessels. Though, the major impact of CVD is felt by both the developed and developing nations, the death rates from it are declining in most developed and high-income countries while the trends are increasing in most developing, low- and middle-income countries. Thus, CVD is no longer the disease of only the affluent and industrialized nations as it has been long conceived.

Inter alia, these changes in the global disease pattern have been attributed to aging populations (due to improved childhood nutrition and decline in infectious and communicable diseases), globalization and/or westernization as well as rapid urbanization and industrialization. Paradoxically, the developed and high-income countries have the potential to increase their resource allocations to health. They equally have access to equitable and effective health care services. The low- and

middle-income countries, however, are increasingly plagued with very limited resource availability, technological know-how, low-cost health delivery and less access to equitable and effective health care services to treat effectively and manage CVD. According to the reports of Institute of Medicine [IOM] (Fuster & Kelly, 2010), 82% of 17.1 million deaths from CVD reported in 2004 were from low- and middle-income countries, out of which 7.2 million were due to coronary heart disease (CHD), and another 5.7 million were due to stroke (World Health Organization, 2011; Thon, Yein & Lian, 2012). The World Health Organization (WHO) has also projected a combined death toll of 24 million from heart diseases and stroke by 2030 (Reinhardt, 2005). It has also been projected that Southeast Asia would have the largest percentage increase in CVD-related deaths by 2030 (World Health Organization, 2011).

The major and leading causes of preventable morbidity and mortality in Malaysia, as in many other developing and developed countries, have also shifted from communicable to non-communicable diseases (Akter et al., 2010; Cheah, Lee, Khatijah & Rasidah, 2011). In Malaysia, which is still considered a middle-income country, the prevalence of CVD risk factors has also risen. For instance, the rates of diabetes, hypertension and hypercholesterolemia reportedly rose from 8.3%, 33.0% and 5.0%, respectively, in 1996 to 14.9%, 42.6% and 24.0%, respectively, in 2006, while in-hospital CVD deaths shot up from 15.7% in 2006 to 25.4% in 2009 (Rasiah et al., 2013). According to a report from the Ministry of Health in Malaysia in 2005, cerebrovascular disease and coronary heart disease have been identified as the two leading causes of death (Annual report, 2005), both of which were also classified among the top 10 causes of hospitalization in government hospitals. Ideally, these

non-communicable diseases are preventable through modification of risk factors and lifestyle changes (Cheah et al., 2011).

Similarly, in Malaysia, an alarming rise in traditional CVD risk factors prevalence has been reported by the National Health and Morbidity Surveys (NHMS), which shows that more Malaysians are at risk of acquiring cardiovascular disease (Nuur, Jamaiyah & Selvarajah, 2012). Clustering of risk factors, that is, the presence of multiple risk factors in one patient is known to be associated with increased risk of heart-related diseases (Wilson, Kannel, Silbershatz & D'Agostino, 1999; Wilson, D'Agostino, Parise, Sullivan & Meigs, 2005). Nuur et al. (2012) also reported that cardiovascular risk factor clusters were consistently seen in all Malaysian states and Federal Territories, with the Peninsular showing a higher overall prevalence of clustering. The fact that many reports have shown that different risk profiles exist for sub-populations with demographic variations demonstrates that cardiovascular disease burden is not distributed equally.

Atherosclerosis is the underlying disease process in the blood vessels that usually results in CHD and cerebrovascular disease, which are the most common forms of CVD. It is a progressive blood vessel's disease known and defined as the hardening, thickening and loss or reduction in the elasticity of the arteries, which later results in reduced blood supply, thrombosis (clot formation) and tissue or organ damage. Though, its clinical manifestations usually appear and become palpable in middle and old ages; the disease process always commences from childhood. The accumulation of cellular waste products, fibrous elements, calcium, phosphate, fatty substance, cholesterol and other substances in the inner lining of an artery are the main features of atherosclerosis (Chumark et al., 2008; Kang, 2014). The cholesterol and fatty material that are deposited as plaques inside the lumen of medium- and

large-sized blood vessels, during the early process of atherosclerosis, later cause the inner surface of the blood vessels to become irregular and the lumen to become narrow, and thus making it harder for blood to flow through (stenosis). The less pliability of blood vessels, eventually, causes the plaque rupture, which triggers the blood clot formation. The development of such blood clot in a coronary artery causes a heart attack while its development in a carotid artery causes a stroke. Thus, both heart attack and stroke are mainly caused by a blockage that prevents blood from flowing to the heart and brain, respectively, which are usually acute events. Though, the most common reason for the blockage is a build-up of fatty deposits on the inner walls of the blood vessels that supply the heart and brain. Stroke, however, can also be caused by bleeding from a blood vessel in the brain or from blood clots.

Some of the common processes known to be involved in atherosclerosis include hypercholesterolemia, oxidation and inflammation (Lowenstein & Matsushita, 2004). The pivotal role being played by both the oxidized low-density lipoprotein (ox-LDL) and endothelial dysfunction in the pathogenesis of atherosclerosis has also been well reported (Lusis, 2000; Kabiri, Asgary & Setorki, 2011; Gómez et al., 2014).

Atherosclerosis is a major cause of morbidity and mortality from CVD in much of the world‘s population. It is recently described as a chronic inflammatory disease of the arterial walls at predisposed sites, as a result of an interaction between plasma lipoproteins, several cells (such as monocyte-macrophage, T-lymphocyte, endothelial cells and smooth muscle cells), and the extracellular matrix. A substantial body of evidence has, however, established that the oxidation of low-density lipoprotein (LDL) is one of the major mechanisms for the pathogenesis and progression of atherogenesis as well as the eventual rupture of the atherosclerotic plaque (atheroma). And over the past decades, an endless list of data from experimental animals,

laboratory investigations, genetic forms of hypercholesterolemia and epidemiologic data has indicated that elevated blood LDL cholesterol, known as hypercholesterolemia (Fan & Watanabe, 2003; Meisinger, Baumert, Khuseyinova, Loewel & Koenig, 2005; Hasan et al., 2014) alone or in conjunction with other factors is a major cause of CHD. Additionally, the third report of The National Cholesterol Education Program (NCEP) Expert panel on detection, evaluation, and treatment of high blood cholesterol in adults (Adult Treatment Panel III), in line with earlier similar prospective cohort studies, like the seven countries study, and the Framingham heart study, identified elevated LDL cholesterol as the primary target of cholesterol-lowering therapy, having reviewed and reported many recent clinical trials, which perspicuously show that LDL-lowering therapy reduces the risk for CHD (Adult Treatment Panel III, 2001; Mascarenhas-Melo et al., 2013).

Human health requires a normal supply of cholesterol being an abundant metabolite and a *sine qua non* structural component of mammalian cell membranes. Cholesterol also plays an indispensable and highly important role in many of the body metabolic activities such as cell differentiation, nerve conduction, membrane fluidity, normal embryonic development and steroid synthesis, in addition to being the precursor of a variety of biologically active molecules, like bile acids, vitamin D and steroid hormones. Cholesterol poses remarkable detrimental effect on cell function, tissue development and the whole-body physiology if it is supplied insufficiently. An uncontrolled build-up of cholesterol in cells, however, disrupts membranes, facilitates apoptosis and results in other pathological consequences. Hyperlipidemia, for instance, is caused by the accumulation of excessive cholesterol in the blood, as established by epidemiological, clinical and animal studies. The body maintains cholesterol homeostasis through *de novo* synthesis, intestinal absorption, and biliary