UNIVERSITI PUTRA MALAYSIA

TOTAL PHENOLIC CONTENT, ANTIOXIDANT AND ANTIDIABETIC PROPERTIES OF SEED COATS OF SELECTED BEANS AND TESTA OF COCONUT (Cocus nucifera L.)

ADEKOLA KHADIJAT ADETOLA

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By

ADEKOLA KHADIJAT ADETOLA

Thesis Submitted to the School of Graduate Studies, Universiti Putra Malaysia, in Fulfillment of the Requirements for the Degree of Master of Science

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DEDICATION

To my beloved parents, Prof. and Dr. (Mrs) F.A. Adekola

For their endless support, prayers and encouragement.

Abstract of thesis presented to the Senate of Universiti Putra Malaysia in fulfillment of the requirement for the Degree of Master of Science

TOTAL PHENOLIC CONTENT, ANTIOXIDANT AND ANTIDIABETIC PROPERTIES OF SEED COATS OF SELECTED BEANS AND TESTA OF COCONUT (*Cocus nucifera* L.)

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Chairman : Professor Abu Bakar Saleh, PhD
Faculty : Biotechnology and Biomolecular Sciences

Diabetes mellitus is one of the most common metabolic disorders affecting the global population; management of this disorder still remains inadequate owing to the side effects of synthetic hypoglycaemic drugs available. This study aims at comparing the seed coats of four varieties of beans (red kidney bean, red bean, black-eyed pea, black bean) and testa of coconuts (mature and tender coconut) in terms of their antioxidant and antidiabetic properties. Hundred (100) gram portions of the milled bean seed coats and coconut testas were soaked, centrifuged and filtered to obtain crude extracts. Quantification of the phenolic acids and flavonoids in the extracts was carried out using high performance liquid chromatography coupled with diode array detection. The total phenolic content, total flavonoid content, antioxidant potentials (DPPH, ABTS and FRAP) and the α-amylase and α-glucosidase inhibitory activities of the crude extracts were studied *in vitro*. The results showed that the red kidney bean seed coat (RKB) {DPPH IC_{50}=63.60±3.50 µg/mL, ABTS IC_{50}=111.30±0.60 µg/mL, FRAP=204.71±2.87 mmol} and tender coconut testa (TCO) {DPPH IC_{50}=47.40±7.00 µg/mL, ABTS IC_{50}=125.70±6.70 µg/mL, FRAP=546.10±36.90 mmol} exhibited the highest antioxidant activities. Both extracts had also shown strong inhibition towards α-glucosidase activity (RKB, IC_{50}=19.90±5.67 and TCO, IC_{50}=4.84±1.43 µg/mL) and followed by mild inhibition towards α-amylase activity (RKB, IC_{50}=120.5±15.4 and TCO, IC_{50}=532.8±68.0 µg/mL). The total phenolic content of RKB seed coat and TCO testa extracts were 21.80±0.50 and 44.607±0.56 mg gallic acid equivalents per gram of seed coat while the flavonoid contents were 24.38±1.22 and 67.597±7.00 mg quercetin equivalents per gram of seed coat respectively. The chromatography results showed that the extracts contained appreciable level of some phenolic acids (0.01±0.01 - 5.747±0.54 mg/g) and flavonoids (0.05±0.00 - 7.25±0.06 mg/g) including gallic acid, ellagic acid, caffeic acid, chlorogenic acid, quercetin, catechin, epigallocatechin gallate and rutin. In the *in vivo* study, antidiabetic effects of TCO and RKB on streptozotocin induced diabetic rats were evaluated using various biochemical parameters. Forty-nine (49) rats were randomly divided into seven groups of seven rats each for normal control, diabetic untreated and five diabetic treated groups. Administration of extracts at a dose of 200 and 400 mg/kg body weight (b.wt.) were carried out daily for 14 days. The results showed that both TCO and RKB extracts at the different doses were able to significantly ($p<0.05$) reduce hyperglycaemia. Infact, TCO demonstrated the most remarkable reduction of 1.5 fold decrease at the dose of 200 mg/kg b.wt when compared to the normal control group. Treatments using these two extracts also reduced the levels of cholesterol, urea, bilirubin, creatinine, alanine transaminase (ALT), aspartate transaminase (AST) and total protein by trace amounts. These results suggest that red kidney bean seed coat and tender coconut testa would have higher potential as nutraceuticals and could serve as natural alternative sources for antidiabetic remedy.

Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia sebagai memenuhi keperluan untuk Ijazah Sarjana Sains

JUMLAH KANDUNGAN FENOLIK, ANTIOKSIDAN DAN CIRI-CIRI ANTIDIABETIK JENIS KULIT BENIH KACANG DAN TESTA KELAPA (*Cocus nucifera* L.)

Oleh

ADEKOLA KHADIJAT ADETOLA

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Pengerusi : Profesor Abu Bakar Saleh, PhD
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Diabetes mellitus adalah salah satu gangguan metabolik yang paling biasa yang memberi kesan kepada penduduk global; pengurusan penyakit ini masih tidak mencukupi disebabkan oleh kesan-kesan sampingan ubat-ubatan sintetik hipoglisemia yang ada. Kajian ini bertujuan untuk membandingkan kulit benih empat jenis kacang (kacang panggang, kacang merah, kacang hitam bermata, kacang hitam) dan testa kelapa (matang dan muda) dari segi antioksidan dan ciri-ciri antidiabetes. Seratus bahagian (100) gram kulit benih kacang (RKB) dan testas kelapa yang (TCO) dikisar telah direndam, sentrifuj dan ditapis untuk mendapatkan ekstrak mentah. Kuantifikasi asid fenolik dan flavonoid dalam ekstrak telah dijalankan dengan menggunakan kromatografi cecair berprestasi tinggi dipasangkan dengan pengesanan diod. Jumlah kandungan fenolik, jumlah kandungan flavonoid, potensi antioksidan (DPPH, ABTS dan FRAP) dan aktiviti perencatan α-amilase dan α-glucosidase bagi ekstrak mentah telah dikaji secara *in vitro*. Hasil kajian menunjukkan bahawa ekstak kulit kacang panggang {DPPH IC_{50}=63.60±3.50 µg/mL, ABTS IC_{50}=111.30±0.60 µg/mL, FRAP=204.71±2.87 mmol} dan testa kelapa muda {DPPH IC_{50}=47.40±7.00 µg/mL, ABTS IC_{50}=125.70±6.70 µg/mL, FRAP=546.10±36.90 mmol} telah mempamerkan aktiviti antioksidan yang tertinggi. Kedua-dua ekstrak juga telah menunjukkan perencatan kuat ke arah aktiviti α-glucosidase (RKB, IC_{50}=19.90±5.67 and TCO, IC_{50}=4.84±1.43 µg/mL) dan diikuti oleh perencatan sederhana terhadap aktiviti α-amilase (RKB, IC_{50}=19.90±5.67 and TCO, IC_{50}=4.84±1.43 µg/mL). Jumlah kandungan fenolik RKB kulit benih dan TCO testas ekstrak masing-masing adalah 21.80 ± 0.50 dan 44.607 ± 0.56 mg bersamaan asid galik/g kulit benih manakala jumlah kandungan flavonoid RKB kulit benih dan TCO testas ekstrak masing-masing adalah 24.38±1.22 and 67.597±7.00 mg bersamaan quercetin/g kulit benih. Keputusan kromatografi menunjukkan bahawa ekstrak mengandungi beberapa asid fenolik (0.01±0.01 - 5.747±0.54 mg/g) dan flavonoid (0.05±0.00 - 7.25±0.06 mg/g)

termasuk asid galik, asid ellagik, asid kafeik, asid klorogenik, kuersetin, katekin, epigallokatekin gallate dan rutin. Dalam kajian *in vivo*, kesan antidiabetik TCO dan RKB pada streptozotocin teraruh tikus diabetik telah dinilai menggunakan pelbagai parameter biokimia. Empat puluh sembilan tikus secara rawak dibahagikan kepada tujuh kumpulan iaitu tujuh tikus setiap satu untuk kawalan normal, kencing manis dan kumpulan diabetes terawat. Perawatan ekstrak pada dos 200 dan 400 mg/kg b.wt. telah dijalankan setiap hari selama 14 hari. Hasil kajian menunjukkan bahawa kedua-dua ekstak TCO dan RKB pada dos yang berbeza dapat ($p<0.05$) mengurangkan hiperglisemia dengan ketara sebanyak 1.5% kali ganda penurunan. Malah, TCO menunjukkan pengurangan yang paling luar biasa pada dos 200 mg/kg b.wt. Rawatan menggunakan kedua-dua ekstrak juga telah mengurangkan tahap kolesterol, urea, bilirubin, kreatinin, alanine transaminase (ALT), aspartate transaminase (AST) dan jumlah protein oleh jumlah ekstrak yang rendah. Keputusan ini menunjukkan bahawa kulit kacang panggang merah kacang dan testa kelapa muda mempunyai potensi yang lebih tinggi sebagai nutraseutikal dan boleh menjadi sumber semula jadi alternatif bagi perawatan antidiabetik.

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I certify that a Thesis Examination Committee has met on 17 May 2017 to conduct the final examination of Adekola Khadijat Adetola on her thesis entitled "Total Phenolic Content, Antioxidant and Antidiabetic Properties of Seed Coats of Selected Beans and Testa of Coconut (*Cocus nucifera* L.)" in accordance with the Universities and University Colleges Act 1971 and the Constitution of the Universiti Putra Malaysia [P.U.(A) 106] 15 March 1998. The Committee recommends that the student be awarded the Master of Science.

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This thesis was submitted to the Senate of the Universiti Putra Malaysia and has been accepted as fulfillment of the requirement for the degree of Master of Science The members of the Supervisory Committee were as follows:

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LIST OF ABBREVIATIONS

ABTS	2,2'-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid)
ALT	Alanine transaminase
AST	Aspartate transaminase
b.wt.	Body weight
BB	Black beans
BEP	Black-eyed peas
DM	Diabetes mellitus
DNA	Deoxyribose nucleic acid
DNS	3,5-dinitrosalicyclic acid
DPPH	2,2-diphenyl-1-picrylhydrazyl
ED	Erectile dysfunction
FCR	Folin-Ciocalteu reagent
FRAP	Ferric reducing antioxidant power
g/L	Grams per litre
GAE	Gallic acid equivalent
HPLC-DAD	High performance liquid chromatography with Diode array detection
IDF	International diabetes federation
IDDM	Insulin dependent diabetes mellitus
IC_{50}	Inhibitory concentration
IU/L	International units per litre
kg	Kilograms
MafA	MAF BZIP Transcription Factor A
MCO	Mature coconut

mL	Millilitres
µL	Microlitre
mm	Millimetres
mg	Milligrams
µM	Micromolar
mM	Millimolar
µg	Micrograms
mg/dL	Milligrams per decilitre
nm	Nanometres
NHMS	National health and morbidity survey
NIDDM	Non-insulin dependent diabetes mellitus
OGTT	Oral glucose tolerance test
PC	Phenolic compounds
Pdx-1	Pancreatic and duodenal homeobox gene 1
QE	Quercetin equivalent
RB	Red beans
RKB	Red kidney beans
RNS	Reactive nitrogen species
ROS	Reactive oxygen species
STZ	Streptozotocin
TCO	Tender coconut
TPC	Total phenolic content
TPTZ	Tri (2-pyridyl)-1,3,5-triazine
U/L	Units per litre
WHO	World health organisation

w/v	Weight per volume
%	Percentage

CHAPTER 1

INTRODUCTION

1.1 General Introduction

Diabetes mellitus (DM) is an endocrine disorder of carbohydrate, protein and lipid metabolism characterised by elevated blood glucose levels which occurs as a result of defect in insulin secretion, insulin action or both (Yusoff *et al*., 2015; De Souza *et al*., 2010). It has become rampant due to increasing rates of obesity among the youths resulting from sedentary lifestyles as well as a steady increase of those with a parental history of type II diabetes (Amutha & Mohan, 2015). Statistics show that over 180 million people are suffering from diabetes worldwide with about 5% deaths each year. If not properly checked, the number of people suffering from this disorder may double by 2030 (Jemain *et al.,* 2011). According to the International Diabetes Federation, about 366 million people were affected with diabetes worldwide in 2011 (Lacroix & Li-Chan, 2014), and is expected to escalate to an alarming figure of 642 million by 2040 (Jaacks *et al.,* 2016). Malaysia which is a multi-ethnic nation comprising 3 major Asian races (Malays, Chinese and Indians) with a population of about 25 million has a high risk of being prone to diabetes epidemic owing to a major shift in lifestyles and ageing of the population (Yun *et al.,* 2007). The recent National Health and Morbidity Survey (NHMS) of Malaysia showed that the prevalence of diabetes in adults aged ≥18 years has increased steadily from 15.2% in 2011 to 17.5% in 2015 ("Institute for Public Health," 2015; "Institute for Public Health," 2011).

Diabetes mellitus is a major health problem; hence control of hyperglycaemia is important as if left untreated can increase the vulnerability of several macro vascular and micro vascular complications such as, coronary vascular disease, hypertension, stroke, cardiomyopathy and nephropathy, retinopathy, neuropathy (Jayaraj *et al.,* 2013; Chakrabarti & Rajagopalan, 2002). Recent reports have showed that high postprandial plasma glucose level is more deleterious than fasting blood glucose as it can cause serious complications and also increase mortality rate. Hence, there is a need to control the level of postprandial blood glucose (Ye, Song, Yuan, & Mao, 2010).

One of the effective therapeutic approaches for the treatment of diabetes is to lower the postprandial hyperglycaemia level by suppressing absorption of glucose through the inhibition of the carbohydrate-hydrolyzing enzymes namely, α-amylase and α-glucosidase (De Souza *et al*., 2010; Nickavar & Abolhasani, 2013). Inhibitors of α-amylase and α -glucosidase such as acarbose, can help in reducing the postprandial blood glucose rise by prolonging the enzymatic hydrolysis of complex carbohydrate, thereby delaying glucose absorption (Shobana *et al.,* 2009). A number of inhibitors of α-amylase and α-glucosidase have been isolated from medicinal plants to serve as an orthodox drug with increased potency and mild side effects when compared with the existing modern drugs (Kazeem *et al*., 2013).

Another key approach to inhibit the debilitating progression of diabetic health complications is through the complementary use of antioxidants (Mahomoodally & Muthoora, 2014). It has been postulated that oxidative stress induced by reactive oxygen species (ROS) can induce cell membrane disintegration and mutilation of protein, lipid and deoxyribose nucleic acid (DNA) which can initiate or promote the development of several chronic diseases such as; diabetes mellitus (Hashim *et al.*, 2013). Evidence has shown that hyperglycaemic condition is the cause of oxidative stress due to an increment in the production of free radicals in the mitochondria. This has been associated with the pathogenesis of beta cell dysfunction, insulin resistance and occurrence of diabetic complications. Antioxidants such as vitamins E and C have been proven to exhibit antioxidant protective effects against reactive oxygen species and prevention of diabetic microvascular complications in animal models (Yusoff *et al*., 2015).

Management of diabetes include; insulin action enhancement at the target tissues, use of sensitizers (biguanides, thiozolidinediones), use of sulfonylureas (glibenclamide, glimepiride) to stimulate endogenous insulin secretion and using specific enzyme inhibitors such as; acarbose, miglitol to reduce the demand for insulin (Uddin *et al*., 2014). However, a number of antidiabetic agents currently available for the management of diabetes including alpha glucosidase inhibitors, meglitinides are usually associated with serious side effects such as, hypoglycaemia, weight gain, gastro intestinal cramps and liver disorders (Lacroix & Li-Chan, 2014; Shobana *et al*., 2009). Hence, there is a need for antidiabetic therapies of natural origin that are safe and more effective with minimal side effects (Lacroix & Li-Chan, 2014).

Natural antidiabetic remedies from plants, fruits and other dietary sources are gaining awareness because of minimal side effects when compared to some of the modern drugs. This has drawn scientists towards exploring natural antidiabetic remedies due to their ability to stimulate insulin secretion, improve glucose uptake in peripheral tissues and impede the activities of digestive enzymes (Mohamad Jemain et al., 2011). It has been reported that over 1100 plant species so far have been investigated on to treat diabetes (Nickavar & Abolhasani, 2013).

The coconut palm (*Cocos nucifera*) is an essential member of Arecaceae (palm family) family. It is the most widely grown palm around the globe (Agyemang-Yeboah, 2011). Coconut is usually referred to as 'tree of heaven' because it provides useful and diverse products for the benefit of people. More than 93 countries in the world grow coconut in an area of 12 million hectares with production of 59.98 million tonnes of nuts annually. According to Food and Agricultural organization, one of the largest coconuts producing country is Indonesia with production of about 18 million tonnes followed by Philippines and India. Some of the edible products that can be obtained from coconut include; coconut milk, copra, coconut oil. Consumption of coconut is associated with several health benefits such as; increasing the absorption of nutrients, cures kidney and liver diseases, reduction of blood sucrose level (Sangamithra *et al*., 2013). The health benefits of coconut such as the ketogenic and glycaemic properties of coconut oil which has been found to enhance insulin secretion and utilize blood glucose have been studied extensively. Testa is the brown part covering coconut kernel which is usually obtained as a by-product during preparation of coconut products (Agyemang-Yeboah, 2011). Testas are however, getting wasted as studies on their health benefits have not yet received wide publicity.

Dry beans (*Phaseolus spp. L*) are very essential grain legumes both from economic and nutritional viewpoints because they are abundant sources of protein, complex carbohydrates, dietary fibres and minerals (López *et al*., 2013). Intake of legumes regularly has many advantageous physiological effects in controlling and preventing a number of metabolic diseases such as diabetes mellitus and colon cancer (Siddiq *et al.,* 2010). In recent years, special concern has been manifested towards the anti-hyperglycaemic potential of dietary waste sources such as; cereals, coconut and legumes. Since most of the phenolic compounds are concentrated in the seed coat, the objective of this study was to compare brown testa of different coconut types with four varieties of bean seed coats with respect to total phenolic content, antioxidant and antidiabetic properties.

1.2 Problem statement

Diabetes mellitus is a growing public health concern posing serious threat worldwide. Modern drugs available for the management of this disorder are associated with a number of side effects; hence there is a need to focus more on natural antidiabetic remedies which are safer and more effective. This study will focus on the antidiabetic potentials of coconut testa and bean seed coat.

1.3 Hypothesis

- ❖ The coconut testa and bean seed coat extracts will show antioxidant properties.
- ❖ The coconut testa and bean seed coat extracts will exhibit significant inhibition of α-amylases and α-glucosidases.
- ❖ The coconut testa and bean seed coat extracts will show anti-hyperglycaemic effect on diabetic Sprague-dawley rats.

1.4 Objectives of the study

- To measure the antioxidant properties of the coconut testa and beans seed coat extracts.
- To quantify the phenolic compounds in coconut testa and bean seed coat extracts using reverse-phase high performance liquid chromatography (HPLC).
- To evaluate α-amylase and α-glucosidase inhibitory activities in the coconut testa and beans seed coat extracts.

- To determine the antidiabetic effect of the coconut testa and bean seed coat extracts on the Sprague-dawley rats.

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