ANTIOXIDANT AND ANTI-DIABETIC PROPERTIES, AND PHYTOCHEMICAL PROFILE OF DIFFERENT PARTS OF Mangifera odorata GRIFF. FRUIT

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By

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Chair : Nurul Shazini binti Ramli, PhD
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Mangifera odorata G. or known as “kuini” in Malay represent the hybrid form between *M. indica* (mango) and *M. foetida* (bacang). Mango and its fruit wastes are known for its high nutritive and pharmaceutical value, however there was a lack of scientific data focusing on the nutritional composition and biological activities of *M. odorata* fruit. In this study, peel, pulp and seed kernel of *M. odorata* fruit were investigated for their nutritional composition. Then, the samples were freeze-dried and extracted using several extraction solvents (acetone, ethanol, methanol at 60% v/v and water), and their effect on antioxidants (TPC, TFC, FRAP assay, and DPPH assay) and anti-diabetic activities (α-amylase and α-glucosidase inhibition assay) were determined. Next, the ethanolic extract of peel, pulp, and seed kernel from *M. odorata* was further analysed for their phytochemical profile using ultra-high-performance liquid chromatography electrospray ionization orbitrap tandem mass spectrometry (UHPLC-ESI-Ortbitrap-MS/MS). The results indicated that the seed kernel is rich in fat, protein, carbohydrate, and ash (2.76, 2.62, 43.31 and 1.29 g/100 g fresh fruit, respectively). Meanwhile, the peel contains significantly higher fibre, potassium, sulfur, aluminum, calcium, manganese, iron, boron, β-carotene and ascorbic acid compared to seed kernel and pulp. Results show that the seed kernel has the highest TPC and exhibits the strongest scavenging activity compared to other samples. Among the tested solvents, 60% ethanol extract showed highest reducing power in peel and pulp. In addition, acetone at 60% from peel and pulp extracts showed high scavenging activity and TPC in peel and seed kernel. Anti-diabetic assays revealed that the peel, pulp and seed kernel were not active inhibitors of α-amylase and α-glucosidase. The most abundant compounds identified in *M. odorata* fruit were phenolic acid, ellagic acid, and flavonoid, which mostly found in peel and seed kernel. In conclusion, *M. odorata* by-products (peel and seed kernel) contained a high nutritional value

with good antioxidant properties, hence might be potentially used as a functional food ingredient.

Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia sebagai memenuhi keperluan untuk ijazah Master Sains

SIFAT ANTIOKSIDAN DAN ANTI-DIABETES, SERTA PROFIL FITOKIMIA PADA PELBAGAI BAHAGIAN BUAH *Mangifera odorata* Griff.

Oleh

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Mangifera odorata G. atau lebih dikenali sebagai "kuini" merupakan hibrid di antara *M. indica* (mangga) dan *M. foetida* (bacang). Mangga dan sisa buahnya terkenal dengan nilai pemakanan dan farmaseutikal yang tinggi. Walau bagaimanapun, terdapat kekurangan data saintifik yang fokus kepada komposisi pemakanan dan aktiviti biologi di dalam buah *M. odorata*. Di dalam kajian ini, komposisi pemakanan di dalam kulit, pulpa dan isi biji buah *M. odorata* telah dikaji. Kemudian, sampel buah *M. odorata* telah dibeku-kering dan diekstrak menggunakan beberapa pelarut pengekstrak (aseton, etanol, metanol pada 60% v/v dan air) dan kesannya terhadap antioksidan (TPC, TFC, asai FRAP dan DPPH) dan aktiviti anti-diabetes (asai perencatan α-amilase dan α-glukosidase) telah ditentukan. Seterusnya, profil fitokimia daripada ekstrak etanol daripada kulit, pulpa dan isi biji daripada *M. odorata* juga telah dianalisis menggunakan kromatografi cecair berprestasi ultra-tinggi dengan pengionan semburan-elektro orbitrap serta massa spektrometri (UHPLC-ESI-Ortbitrap-MS/MS). Keputusan menunjukkan bahawa isi biji kaya dengan lemak, protein, karbohidrat, dan abu (2.76, 2.62, 43.31 dan 1.29 g/100 g buah-buahan segar, masing-masing). Sementara itu, kandungan serat, kalium, sulfur, aluminium, kalsium, mangan, besi, boron, β-karotena dan asid askorbik di dalam kulit lebih tinggi berbanding isi biji dan pulpa. Keputusan juga menunjukkan bahawa isi biji mempunyai TPC yang tertinggi dan mempamerkan aktiviti memerangkap yang paling kuat berbanding sampel lain. Di antara pelarut-pelarut yang diuji, etanol 60% ekstrak menunjukkan aktiviti yang tinggi dalam kuasa pengurangan di dalam kulit dan pulpa dan juga aktiviti memerangkap di dalam isi benih. Tambahan pula, aseton pada 60% daripada ekstrak kulit dan pulpa menunjukkan aktiviti memerangkap yang tinggi dan TPC dalam kulit dan isi biji. Asai anti-diabetes mendedahkan bahawa kulit, pulpa dan isi biji tidak aktif dalam merenjatkan enzim α-amilase dan α-glukosidase. Sebatian yang paling banyak dikenal pasti di dalam buah *M. odorata*

adalah asid fenolik, asid elagik, dan flavonoid yang kebanyakkannya berada di dalam kulit dan isi biji. Kesimpulannya, sisa daripada buah *M. odorata* (kulit dan isi biji) mengandungi nilai pemakanan yang tinggi disamping sifat-sifat antioksidan yang baik, oleh itu ia mungkin berpotensi digunakan sebagai bahan fungsian makanan.

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

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TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF ABBREVIATIONS

HPLC	High Performances Liquid Chromatography
UHPLC	Ultra High Pressure Liquid Chromatography
FLD	Postcolumn Fluorescence Derivation
H_20	Water
UV-Vis	Ultraviolet Visible
LC-MS	Liquid Chromatography Mass Spectrometry
DF	Dietary Fibre
v/v	Volume per volume
w/v	Weight per volume
DNA	Deoxyribonucleic acid
%	Percentage
Nm	Nanometer
Rpm	Revolutions per minute
Α	Alpha
Β	Beta
Δ	Delta
ɣ	Gamma
M	Meter

Cm	Centimetre
G	Gram
Mg	Milligram
µg	Microgram
Kcal	Kilocalorie
RE	Retinol Equivalent
Q-tof	Quadrupole Time of Flight
ESI	Electrospray Ionization
m/z	Mass to charge
˚C	Degree Celsius
V	Volt
IC_{50}	50% Inhibitory Concentration
Min	Minute
L	Litre
mL	Millilitre
µL	Microliter
M	Molar
Mm	MilliMolar
IU	International unit

Psi	Pound-force per square inch
PDA	Photodiode Array
HSD	Honestly Significant Difference

CHAPTER 1

INTRODUCTION

1.1 Background

Free radical is a highly reactive and unstable entity. It can generate redox reactions in the cell components by passing their unpaired electron (Asmat et al., 2016). It is composed of oxygen (reactive oxygen species, ROS) and nitrogen (reactive nitrogen species, RNS) that being produced in the cellular system (Rahimi-Madiseh et al., 2016; Devasagayam et al., 2004). The increase of production or insufficient removal of ROS/RNS by the endogenous antioxidant would lead to oxidative stress (Matsuda & Shimomura, 2013), which may interfere with the function and structure of the cellular protein, lipids and nucleic acid (Rani et al., 2016). This situation can cause the cellular disturbance and trigger the development of several chronic diseases such as cancer, cardiovascular diseases (CVD) and diabetes (Rani et al., 2016). Numerous studies revealed the involvement of oxidative stress in the pathogenesis of diabetes by altering their biomarkers such as enzymatic systems (Patel et al., 2013), lipid peroxidation (Pérez-Matute et al., 2009), impaired glutathione metabolism (Maritim et al., 2003) and decreased Vitamin E levels (Maritim et al., 2003).

Diabetes mellitus (DM) is a metabolic disorder which associates with the insulin resistant or insufficient production of insulin secreted by β-cells in the pancreas (Rochette et al., 2014). The complications of DM can lead to several diseases such as heart diseases, stroke, blindness, neuropathy, retinopathy and nephropathy and kidney diseases (Rahimi- Madiseh et al., 2016). DM is becoming a major public health concern due to its increasing prevalence among adults aged 18 years from 4.7% in 1980 to 8.5% in 2014 globally (WHO, 2016; Kim et al., 2003). Similarly, in Malaysia, the rising trend was evident in the past two decades, where the prevalence was nearly tripled since 1996 (Institute for Public Health, 2015). In 2015, approximately 2 million adults had type 2 DM, affected 17.5% Malaysian populations (Institute for Public Health, 2015).

There is no obstructive or healing method for diabetic patient (Rahimi Madiseh et al., 2016). Hence, the main focus of diabetes management is to slow the progression of diabetes complications. These include the medical-nutrition therapy (MNT) and pharmacological approach. The pharmacological approach involved several anti-hyperglycemia medications that have been widely used for treating the diabetic person (Hussein et al., 2015). Nowadays, the focus of diabetes management is on the prevention of the overproduction of reactive species (Ceriello et al., 2016). However, synthetic drugs were reported to produce undesirable side effects such as hypoglycemia and atherogenesis (Rahimi-

Madiseh et al., 2016). Therefore, as the development of type 2 diabetes and its complication are associated with oxidative stress, the antioxidant approach will be the alternative way of prevention and management of DM. Antioxidant comprises both enzymatic and non-enzymatic which include vitamins, polyphenols and several enzymes (Rolo & Palmeira, 2006). Plants, especially tropical fruits are important sources of antioxidants (Alothman et al., 2009). In fact, several available drugs are derived from plant (Fabricant & Farnsworth, 2001). Apart from that, plant has the advantage of having no or a few side effects compared to synthetic drugs (Kafash-Farkhad et al., 2013).

Mangifera species is a tropical plant that is proven to have pharmacological properties in anticancer (Noratto et al., 2010), anti-diabetic (Bhowmik et al., 2009), decrease kidney damage (Amien et al., 2015) and anti-inflammatory (Dhananjaya & Shivalingaiah, 2016). The mango fruit (*Mangifera indica* L.) and its wastes, is one of the extensively studied *Mangifera* species, owing to abundant nutrients and phytochemicals that benefit human health (Torres-León et al., 2016). In Malaysia, Porcher (1995) reported that eight underutilized *Mangifera* species have been discovered and one of them is *M. odorata* from the family *Anacardiaceae*. This fruit is primarily cultivated in Southeast Asia and is commonly called as "kuini" in Malaysia and consumed by local communities (Lim, 2012). The fruit's color is green to yellowish-green, with dark brown spots (Lim, 2012). The pulp is orange-yellow, firm, fibrous, sour-sweet, juicy with a pungent smell and taste of turpentine (Lim, 2012). The fruit is the hybrid between *M. foetida* (bacang) and *M. indica* (mango) (Teo et al., 2002). The pulp of *M. odorata* is highly nutritious where it contains higher protein and calcium compared to other *Mangifera* species and also contains an acceptable amount of carotenoids (Mirfat et al., 2015; Khoo et al., 2008). Besides, the pulp of *M. odorata* contains higher isoflavones and total phenolic content (TPC) than *M. pajang* (bambangan) and *M. foetida* (Ikram et al., 2009; Khoo & Ahmad, 2008). Mirfat et al. (2015) also reported that this fruit (pulp) exhibited higher antioxidant activity than *M. pajang*. The nutritional and antioxidant potential of pulp from *M. odorata* compared to other *Mangifera* species can be excellent candidates for other functional bioactivities such as anti-diabetic activity and eventually as food ingredients in the food industry.

1.2 Problem Statement

Numerous studies reported the potent antioxidant activities of *M. odorata* fruit (Ikram et al., 2009; Khoo et al., 2008); however little information is available regarding the nutritional and functional properties of the fruit wastes (peel and seed kernel). The fruit wastes could be the excellent candidate for functional bioactivity due to the accumulation of valuable bioactive compounds as previously shown by mango seed kernel and peel (Jahurul et al., 2015), which believed to possess protective effects against oxidative stress-related diseases. Moreover, to the best author's knowledge, studies on the effect of extraction solvents on the antioxidant and anti-diabetic activities and recovery of functional components from different parts of *M. odorata* fruit were scarce. Different part of plant materials contains varying quantities of bioactive compounds which have different chemical characteristics and polarity that may or may not soluble in particular solvent (Michiels et al., 2015). Besides, different polarity of solvent play a significant role in determining the efficiency of the extraction and the activity of the obtained extract (Barchan et al., 2014). Hence, for the accurate quantification of antioxidant potential, the extraction of polyphenols of *M. odorata* fruit must be personalized. The results from this study could provide scientific evidence of health-promoting properties of *M. odorata* fruits and give insights to researchers to further explore the potential of fruits waste in the food industry to eventually benefit the farmer, consumer and food scientist.

1.3 Objectives

This present study is designed with objectives as stated:

1. To determine the proximate composition, sugars, minerals content and antioxidant vitamins (vitamin A, C, and E) of peel, pulp and seed kernel of *M. odorata* fruit.

2. To determine the effect of different extraction solvents (acetone, ethanol, methanol at 60% v/v and water) on total phenolic content (TPC), total flavonoid content (TFC), antioxidant activity (Ferric reducing antioxidant power (FRAP) assay and 2,2-diphenyl-1-picrylhydrazyl (DPPH) free radical scavenging assay) and anti-diabetic properties (α-amylase and α-glucosidase inhibition assay) of peel, pulp and seed kernel of *M. odorata* fruit.

3. To identify the polyphenols profiles of peel, pulp and seed kernel of *M. odorata* fruit using ultra-high-performance liquid chromatography electrospray ionization orbitrap tandem mass spectrometry (UHPLC-ESI-Orbitrap-MS/MS).

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