



UNIVERSITI PUTRA MALAYSIA

***NUTRITIONAL AND ANTIOXIDATIVE PROPERTIES OF
UNDERUTILIZED *Ziziphus mauritiana* L. (BIDARA) FOR
NUTRACEUTICAL POTENTIAL***

FATIN NOR AMIRAH BINTI MOHD JAILANI

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By

FATIN NOR AMIRAH BINTI MOHD JAILANI

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

February 2021

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

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Faculty: Biotechnology and Biomolecular Sciences

Nowadays, researchers around the globe are extensively searching for potential safe alternative drugs and antioxidant. The need for this alternative natural resource is due to the harmful side effects of current commercial synthetic drugs and antioxidant. The underutilized plant might be the key to discovering the presence of potential bioactive compounds for medicinal purposes or nutraceutical products. However, due to the scarcity of information and lack of study on many underutilized plants, the potential antioxidants compound remains undiscovered. *Ziziphus mauritiana* is one of Malaysian underutilized plant which had been used locally as traditional treatments and also for Islamic practises. This plant is believed to have good nutritional content and carries antioxidative properties. Thus, this study is carried out to investigate the nutritional composition and physicochemical value of *Z. mauritiana* extracts associated with the antioxidant capacity of the different extraction solvents. The nutritional composition of *Z. mauritiana* leaves, fruit and seed were determined using proximate analysis. The physicochemical properties of *Z. mauritiana* leaves and fruit were measured based on their pH value, total acidity (TA) and total soluble solid content (TSS) while antioxidant capacity was analyzed using TPC, TFC, FRAP and DPPH assays. Based on the results, leaves showed the highest percentage of ash (9.06%) and crude protein (14.59%) while the moisture content (88.32%) was found the highest in fruit. The crude fiber (48.12%), fat (1.89%), carbohydrate (63.24%) and energy (411.61 kJ) were the highest in the seed. The results also showed that *Z. mauritiana* fruit had a higher value of TSS (11.70°Brix) and TA (0.32%) than the leaves while the pH of the leaves (5.47) was higher than fruit (4.77). The phytochemical compound of squalene (46.69%) in leaves, 5-hydroxymethylfurfural (35.04%) in fruit and conipheryl alcohol (21.45%) in seed were the major compounds found using GC-MS analysis. The antioxidant capacity of *Z. mauritiana* in different

solvent extracts showed that the highest TPC, TFC and FRAP value was found in leaves water extract (287.71 mgGAE/g), in fruit water extracts (119.75 mgQAE/g) and leaves acetone extract (10.06 mgFe/g) respectively. While the strongest free radical scavenging activity against DPPH was shown in the leaves water extract (4.02 $\mu\text{g/ml}$). According to principal component analysis (PCA), flavonoid compound has shown a significant positive correlation with ferric reducing power by 0.760 while the extracts can be classified into three distinctive groups of potential phenolic, flavonoid and weak scavenging activity against DPPH. Thus, the underutilized plant of Malaysian *Z. mauritiana* has the potential to be used as an alternative source of nutraceutical product.



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sebagai memenuhi keperluan untuk Ijazah Master Sains

**NILAI PEMAKANAN DAN SIFAT ANTIPENGOXIDA TUMBUHAN NADIR
Ziziphus mauritiana L. (BIDARA) UNTUK KEUPAYAAN NUTRASUTIKAL**

Oleh

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Pada masa kini, para penyelidik di seluruh dunia giat memperluaskan pencarian alternatif ubatan dan antioksidan yang selamat. Keperluan untuk mencari sumber alternatif semula jadi ini disebabkan oleh kesan sampingan yang berbahaya daripada ubatan dan antioksidan tiruan komersial. Tumbuhan nadir mungkin menjadi kunci untuk menemukan sebatian yang mempunyai potensi bagi tujuan perubatan atau produk nutrasetikal. Namun, disebabkan kekurangan maklumat dan kajian mengenai tumbuhan nadir ini, potensi sebatian antioksidan kekal tersembunyi. *Ziziphus mauritiana* adalah salah satu tumbuhan nadir di Malaysia yang sering digunakan secara tempatan untuk perubatan tradisi dan juga untuk amalan dalam agama Islam. Tumbuhan ini dipercayai mempunyai kandungan nutrisi yang baik dan sifat antipengoksida. Oleh itu, kajian ini telah dijalankan untuk mengkaji komposisi nutrisi dan nilai fizikokimia ekstrak *Z. mauritiana* berhubung kait dengan keupayaan antioksidan di dalam pelarut ekstrak yang berbeza. Komposisi pemakanan daun, buah dan biji *Z. mauritiana* telah ditentukan menggunakan analisa terhampir. Sifat fizikokimia yang terdapat pada daun dan buah *Z. mauritiana* diukur berdasarkan nilai pH, jumlah keasidan (TA) dan jumlah pepejal terlarut (TSS) manakala keupayaan antioksidan telah dianalisa menggunakan kaedah TPC, TFC, FRAP dan DPPH. Berdasarkan keputusan kajian, daun telah menunjukkan peratusan tertinggi abu (9.06%) dan protein (14.59%) manakala kandungan lembapan (88.32%) didapati paling tinggi di dalam buah. Serat (48.12%), lemak (1.89%), karbohidrat (63.24%) dan tenaga (411.61 kJ) adalah yang tertinggi di dalam benih. Hasil kajian menunjukkan bahawa buah *Z. mauritiana* mempunyai nilai TSS (11.70 °Brix) dan TA (0.32%) yang lebih tinggi berbanding daun manakala pH daun (5.47) adalah lebih tinggi daripada buah (4.77). Kompaun fitokimia squalene (46.69%) di dalam daun, 5-hydroxymethylfurfural (35.04%) di dalam buah dan alkohol koniferil (21.45%) di dalam benih adalah kompaun utama yang telah

ditemui menggunakan analisis GC-MS. Keupayaan antioksidan ekstrak *Z. mauritiana* di dalam pelarut yang berbeza telah menunjukkan nilai tertinggi iaitu TPC, TFC dan FRAP dalam ekstrak air daripada daun (287.71 mgGAE/g), ekstrak air daripada buah (119.75 mgQAE/g) dan dalam ekstrak aseton daripada daun (10.06 mgFe/g). Selain itu, aktiviti perencatan radikal bebas yang terkuat terhadap DPPH telah ditunjukkan dalam ekstrak air daripada daun (4.02 µg/ml). Berdasarkan analisa komponen utama (PCA), sebatian flavonoid mempengaruhi kuasa penurunan ferric disebabkan oleh hubung kait positif yang ketara (0.760) dan juga kesemua ekstrak dapat diklasifikasikan kepada tiga kumpulan khas yang mempunyai potensi fenolik, flavonoid dan aktiviti perencatan terhadap DPPH yang lemah. Oleh itu, tumbuhan nadir di Malaysia iaitu *Z. mauritiana* mempunyai potensi untuk digunakan sebagai sumber alternatif produk nutraseutikal.

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LIST OF ABBREVIATIONS

ANOVA	Analysis of variance
AOAC	Association of Analytical Chemist
°C	degree celcius
cm	centimeter
DNA	deoxyribonucleic acid
E	East
Fe	ferrous
g	gram
GAE	gallic acid equivalent
GC-MS	gas chromatography-mass spectrometry
IC50	50% inhibition concentration
kJ	kilojoules
μl	microliter
μm	micrometer
m	meter
M	molar
mg	milligram
ml	milliliter
mM	millimolar
mm	millimeter
min	minute
N	North

NaOH	Sodium hydroxide
nm	nanometer
NIST	National Institute of Standards and Technology
QAE	quercetin equivalent
rpm	revolution per minute
RT	retention time
TA	Total acidity
TFC	Total flavonoid content
TPC	Total phenolic content
TSS	Total soluble solid
TPTZ	2,4,6-Tripyridyl-s-triazne
UV-vis	ultra violet-visible
w/v	Weight per volume

CHAPTER 1

INTRODUCTION

1.1 Research Background

Globally, researchers are avidly studying the phytochemicals from various plant species due to their great interest on discovering new plant phytochemical compound that have the potential to be developed as a source of active ingredient in nutraceutical products. Many of this promising plant species are known to fall under category of underutilized plant which defines as neglected plant species of indigenous ancient crop that had been used by local with many undiscovered potentials. In addition, underutilized plant plays many important roles such as enhancing the agrobiodiversity in agricultural development, maintaining the ecosystem stability due to the underutilized plant ability to cope with environmental changes, securing food resource, replenishing nutrition deficit and opening up new market opportunities to local community (International Plant Genetic Resources Institute (IPGRI), 1999). However, the main reason for the underutilized plant to remain undiscovered is due to the scarcity of knowledge on this plant health benefit properties that restraining its potential to be utilized as a promising material for nutraceutical product. The underutilized plant such as Ceri Terengganu (*Lepisanthes fruitocosa*), Dabai (*Canarium odontophyllum*), Kebayau (*Dacryodes rostrata*), Sentul (*Sandoricum macropodum*), Kuini (*Mangifera odonata*) and Bidara (*Ziziphus mauritiana*) are indigenous to Malaysia (Ikram *et. al.*, 2009; Mohd Shukri *et. al.*, 2013) and these underutilized plant were found to have a remarkable nutritive value (phytochemicals and antioxidant) which carry many health benefits. *Ziziphus mauritiana* is one of the promising underutilized plants that come from the family of *Rhamnaceae* and has a common name of Indian jujube or better known as Bidara by local people in Malaysia. This fruit-bearing tree of *Z. mauritiana* is a prominent native plant of Malaysia and also other South East Asia countries such as Thailand, Indonesia and Vietnam (Orwa *et. al.*, 2009) which was found distributed in warm climate region throughout Africa and Asia. Moreover, *Z. mauritiana* plant is commonly found grew in the wild forest of Malaysia apart from being planted as an ornamental plant for residential area and mosque compound. This underutilized plant is known to this date for only being used in traditional home remedies and being consumed as food source due to its nutritive value for many generations.

Bidara leaves are popular among Muslim community in Malaysia for the usage as material for Islamic spiritual treatment and tools during the process of cleansing dead bodies. It is a common material used in Islamic therapeutic centres in Malaysia as a treatment for the patients that are affected with evil spells in which the patients will be bathed with the mixture of water and Bidara leaves (Ahmad, 2012). Furthermore, during the funeral of deceased Muslim, the Bidara leaves is used in the cleansing rituals as it helps soften the dead body, strengthen the skin and delays the decaying process (Muhammad Yusri *et. al.*, 2017). Accordingly, the Bidara leaves had showed anti-inflammatory and antibacterial activities due to the

phytochemicals constituents present in which can become a potential source of topical treatment for wound healing (Sumanth and Bhargavi, 2014). Meanwhile, Bidara fruit is commonly being consumed fresh, dried or pickled among Malaysian. Pickled Bidara is only known to be produced by the locals in Kelantan which the fresh fruit being mostly imported from Thailand (Ismail, 2015). In other part of the world, for example in Nigeria, the fruit is being consumed during drought season in order to replenish their nutrients and energy (Lockett *et. al.*, 2000). Thus, *Z. mauritiana* should be considered as one of the Crop for Future (CFF) that can serve as an important food source due to their nutritive value.

The underutilized plant carries many potentials due to their ethnomedicinal properties that can help preventing chronic diseases related to the present of phytochemical constituents that have physiological role as antioxidant. Antioxidant is an important chemical compound that helps in reducing the adverse effect of free radicals related to degenerative diseases such as aging, cancer, asthma and diabetes. Current studies on *Z. mauritiana* showed that in the leaves, it has numerous properties including antioxidant, antiviral, antibacterial, anti-inflammatory actions (Batool *et. al.*, 2018; Jain *et. al.*, 2019). Meanwhile, the fruit of *Z. mauritiana* showed an antioxidant, antibacterial and anticancer potential which can be used for therapeutic application (Okala *et. al.*, 2014; Beg *et. al.*, 2016). The seed extract of *Z. mauritiana* had shown a hypnotic effect that can be used for insomnia treatment and it also carries anticancer potential against cancer cell lines and carcinoma cells (Mishra *et. al.*, 2010; Moh *et. al.*, 2013). Thus, it was hypothesized that the phytochemical compound present in leaves, fruit and seed of *Z. mauritiana* have associated with their potential as nutraceutical product.

1.2 Problem Statement

Nowadays, synthetic antioxidants and drugs had been known to be used in modern medicine that resulted in many harmful long-term side effects. This problem had arisen a concern and the need for the alternative natural resource. Underutilized plant of *Z. mauritiana* was used to treat common diseases such as constipation (Khan *et. al.*, 2015), asthma (Jan *et. al.*, 2018) and wound (Patel *et. al.*, 2018) that proven to possess medicinal benefits. Moreover, this plant can be found easily grown in Malaysian tropical climate and soil which has the potential to be cultivated on a large scale. Although the awareness of many potential health benefits of plants are increasing, the availability of scientific evidence associated with nutraceutical value of different plant parts and extracts from underutilized *Z. mauritiana* are still lacking and in ascent stage, especially in Malaysia.

1.3 Objectives

The main objective of this present study was to investigate the nutritional composition and physicochemical value of *Z. mauritiana* extracts associated with antioxidant capacity of the different extraction solvents. Thus, the study was performed corresponding to the following specific approaches:

1. To determine the nutritional composition and the physicochemical properties of *Z. mauritiana* leaves, fruit and seed.
2. To investigate the phenolic (TPC) and flavonoid (TFC) contents of different plant parts of *Z. mauritiana*.
3. To evaluate the antioxidant activity of different plant parts of *Z. mauritiana* and their significant correlation using Principal Component Analysis (PCA).

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