



UNIVERSITI PUTRA MALAYSIA

***EXTRACTION OPTIMIZATION, CHARACTERIZATION AND
EVALUATION OF ANTIOXIDANT AND NUTRACEUTICAL PROPERTIES
OF DATE PALM (*Phoenix dactylifera* L., VARIETY AJWA) KERNEL***

SUZZIYANA HOSNI

IPPH 2020 3



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(*Phoenix dactylifera* L., VARIETY AJWA) KERNEL**

By

SUZZIYANA HOSNI

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

June 2020

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DEDICATION

I would like to dedicate this thesis and all of my academic achievements to my loving mother and all my respected family members.

This is for all of you

My Mom

&

My Siblings



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

**EXTRACTION OPTIMIZATION, CHARACTERIZATION AND EVALUATION OF
ANTIOXIDANT AND NUTRACEUTICAL PROPERTIES OF DATE PALM
(*Phoenix dactylifera* L., VARIETY AJWA) KERNEL**

By

SUZZIYANA HOSNI

June 2020

Chair : Siti Salwa Abd Gani, PhD
Faculty : Halal Products Research Institute

Several studies have advocated that date palm *Phoenix dactylifera* L. kernels, generally regarded as unwanted agricultural waste, is of high potential as a nutraceutical product. The present study was conducted on variety Ajwa to investigate kernels' potential by determining the optimum conditions for antioxidant extraction thereby increasing efficiency in subsequent processes. Response surface methodology (RSM) was employed using four independent variables viz. roasting temperature (180-220°C), roasting time (30-120 minutes), extraction temperature (80-160°C), and extraction time (30-120 minutes). The results demonstrated that roasting at 200°C for 74 minutes and aqueous extraction at 123°C for 79 minutes had effectively resulted in optimum 2,2-diphenyl-1-picrylhydrazyl (DPPH) (93.03 % inhibition), total flavonoid content (TFC) (14.341mg QE/100mL), total phenolic content (TPC) (987.4mg GAE/100mL) and ferric reducing antioxidant power (FRAP) (90.63mM Fe²⁺/100mL). The coefficients of determination (R² values) for the same four parameters were recorded at 0.962, 0.935, 0.946, and 0.924 respectively. The study put forward efficient process parameters with the objective of increasing production efficiency while exploiting the potentialities of date palm kernels as a nutraceutical product. Proximate analyses were done by methods described by Association of Official Analytical Chemists (AOAC) and mineral nutrients were assessed using inductively coupled plasma mass spectrometry (ICP-MS). Proximate analysis further showed that the kernels contained 65.0±2.6% crude fibre, 6.0±0.20% crude protein, 5.00±0.20% moisture, 8.05±0.40% crude fat and 1.20±0.05% ash. Among the mineral nutrients, potassium recorded maximum amount at 376.39±12.3mg/100g followed by phosphorus 126.46±5.04mg/100g, magnesium 78.16±2.14mg/100g, calcium 19.24±1.02mg/100g and sodium 14.91±0.58mg/100g of kernels. The high chromatogram peak of the phenolic

compound that available inside kernels was confirmed using ultra-high-performance liquid chromatography-quadrupole time-of-flight mass spectrometry (UPLC-QTOF / MS) while evaluation for their potentials in α -glucosidase and cancer inhibition activity was conducted using standard in vitro inhibition assay. The outcome showed that date palm kernels had a potent inhibitory activity of α -glucosidase up to 96.62% at a concentration of 34.7533 μ g/mL and inhibited cancer cells to 57.76 and 53.47% in HT-29 and CRL1739 cells, respectively, at a concentration of 500 μ g/mL. In agreement with previous studies on nutritional benefits of date kernels, the Ajwa date is potentially a promising candidate for pharmaceutical applications or functional food impacting healthcare system in numerous positive ways based on their bioactive constituents and antioxidant profiles.



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

**PENGOPTIMUMAN PENGEKSTRAKAN, PENCIRIAN DAN PENILAIAN
ANTIOKSIDAN DAN NUTRASUTIKAL BIJI KURMA (*Phoenix dactylifera* L.,
JENIS AJWA)**

Oleh

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Pengerusi : Siti Salwa Abd Gani, PhD
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Beberapa kajian telah mengemukakan bahawa biji kurma (*Phoenix dactylifera* L.) yang pada umumnya dianggap sebagai sisa pertanian yang tidak diingini, mempunyai potensi yang tinggi sebagai produk nutraseutikal. Kajian ini dijalankan ke atas kurma jenis Ajwa untuk menyelidiki potensi biji dengan menentukan keadaan optimum dalam pengekstrakan antioksidan bagi meningkatkan kecekapan dalam proses selanjutnya. Kaedah Response Surface (RSM) telah digunakan melalui empat parameter bebas: suhu pemanggang ($180\text{-}220^{\circ}\text{C}$), jangkamasa pemanggangan ($30\text{-}120$ minit), suhu pengekstrakan ($80\text{-}160^{\circ}\text{C}$), dan jangkamasa pengekstrakan ($30\text{-}120$ minit). Keputusan menunjukkan bahawa pemanggangan pada 200°C selama 74 minit dan pengekstrakan menggunakan air pada 123°C selama 79 minit berkesan menghasilkan 2,2-diphenyl-1-picrylhydrazyl (DPPH) (93.03%), total flavonoid content (TFC) ($14.34\text{mg QE}/100\text{mL}$), total phenolic content (TPC) ($987.4\text{mg GAE}/100\text{mL}$) dan ferric reducing antioxidant power (FRAP) ($90.63\text{mM Fe}^{2+}/100\text{mL}$). Koefisien penentuan (nilai R^2) bagi empat parameter yang sama dicatatkan masing-masing pada 0.962, 0.935, 0.946, dan 0.924. Kajian ini mengemukakan proses yang cekap bagi tujuan meningkatkan kecekapan pengeluaran serta mengeksplorasi potensi biji kurma sebagai produk nutraseutikal. Analisis proksimat telah dilakukan melalui kaedah yang disyorkan oleh Association of Official Analytical Chemists (AOAC) dan mineral dinilai dengan menggunakan Plasma Mass Spectrometry (ICP-MS). Analisis proksimat menunjukkan bahawa biji kurma mengandungi $65.0 \pm 2.6\%$ serat mentah, $6.0 \pm 0.20\%$ protein mentah, $5.00 \pm 0.20\%$ kelembapan, $8.05 \pm 0.40\%$ lemak mentah dan abu $1.20 \pm 0.05\%$. Antara mineral, potassium mencatatkan jumlah maksimum pada $376.39 \pm 12.3\text{mg}/100\text{g}$ diikuti oleh fosforus $126.46 \pm 5.04\text{mg}/100\text{g}$, magnesium $78.16 \pm 2.14\text{mg}/100\text{g}$, kalsium $19.24 \pm 1.02\text{mg}/100\text{g}$ dan natrium $14.91 \pm 0.58\text{mg}/100\text{g}$

biji kurma. Puncak kromatogram yang tinggi bagi kompaun fenolik yang terdapat pada biji kurma telah disahkan dengan menggunakan ultra-high-performance liquid chromatography-quadrupole time-of-flight mass spectrometry (UPLC-QTOF / MS) sementara potensi perencatan aktiviti α -glucosidase dan kanser dijalankan dengan menggunakan piawai ujian in vitro. Keputusan menunjukkan bahawa biji kurma mempunyai aktiviti perencatan yang mantap terhadap α -glucosidase sehingga 96.62% pada kepekatan 34.7533 μ g/mL dan merencatkan sel kanser sel HT-29 dan CRL1739, masing-masing pada 57.76 dan 53.47% dengan kepekatan 500 μ g/mL. Kajian ini selaras dengan kajian sebelumnya mengenai faedah biji kurma, dan merupakan calon yang berpotensi tinggi dalam kegunaan farmaseutikal atau makanan berfungsi. Ia berupaya memberi kesan yang positif terhadap kesihatan berdasarkan kepada kandungan bioaktif dan profil antioksidan.



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I end these dry words of appreciation with a deep feeling of gratitude and obligation, and with a hope to reciprocate in due course. I remain

Sincerely,

Suzziyana Hosni
Serdang.

February 6th, 2020

This thesis was submitted to the Senate of 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

CAT	Catalase
DPPH	2,2-diphenyl-1-picrylhydrazyl
FR	Free radicals
FRAP	Ferric reducing antioxidant power
GAE	Gallic acid equivalent
GPx	Glutathione peroxidase
ICP-MS	Inductively coupled plasma mass spectrometry
QE	Quercetin equivalent
QTOF MS	Quadrupole time-of-flight mass spectrometry
ROS	Reactive oxygen species
RSD	Relative standard deviation
RSM	Response surface methodology
SOD	Superoxide dismutase
TFC	Total flavonoid content
TPC	Total phenolic content

CHAPTER 1

INTRODUCTION

1.1 Background of study

Date palm (*Phoenix dactylifera*) is the oldest plant cultivated by man. The species is widely distributed in the Middle East, Arabian Peninsula and North Africa. Fruits from date palms or just dates are popular among children and adults. This is because dates have a distinctive sweetness, with many health benefits such as preventing blood clotting, anti-inflammation, stroke, help in bone growth, strengthen nerves and many others (Elberry, 2011). The species is not only scientifically proven of their health benefits, it is also mentioned many times in the Holy Qur'an and Hadith and was said to be well-liked by Prophet Muhammad SAW.

Date palms, especially the Ajwa variety, have also been honored as a blessing of Jannah. Rasulullah SAW said, "Ajwa date is from paradise, and it contains a cure for poison." Tirmidhi (2068). Ajwa dates also have amazing benefits to cure chronic diseases. It was narrated by al-Bukhari in his Sahih (5779) that Rasulullah SAW had said, "If somebody takes seven Ajwa dates in the morning, neither magic nor poison will hurt him that day". There have been reports on nutritional and therapeutic values of the Ajwa dates with significant antioxidant, antibacterial, antifungal, and anti-proliferative properties and said to have health-promoting benefits.

For the people in Medina, they consume dates as their staple food much like wheat in other countries. In some countries including Malaysia, there are food industries processing dates as the main ingredient in date palm products such as date palm oils, dried dates, chocolate dates, healthy drinks, and others. During product processing, its kernel is removed. Date palm kernel constitutes about 10% - 20% of the fruit and is generated as by-product and often regarded as waste.

Recent investigations on phytochemical compositions of skin, shell, husk and even other parts such as leaf, bark, seed and root of date palms have shown that they contain potentially beneficial compounds, opening up new possibilities for adding value to agricultural by-products of date palm kernels (Ali *et al.*, 2019).

The date kernels are available in concentrated quantities when pitted dates are produced in packing plants or in industrial date processing juice. Finding ways to make value-added products and to increase utilization of date kernels other than pulverized date seeds used traditionally on small scale in animal feed, would be beneficial to both date farmers and processors.

The presence of large quantities of fiber and substantial amount of tannins, resistant starch, anabolic agents as well as selenium in date kernels have been reported by several investigators (Habibi *et al.*, 2016), suggesting that they have numerous health benefits, making it relevant for evaluation as an excellent source of functional foods components. Approximate analysis of date kernels as well as the latest finding on bioactive constituents with antioxidants, anticancer and other health promoting activities are discussed in subsequent sections.

Ajwa date kernel extract is full of great benefits. It is loaded with all essential elements such as iron, calcium, potassium, zinc, sulfur, phosphorus, manganese, copper and magnesium. The kernels are also rich in natural fiber along with oil. The present study was conducted to further strengthen evidences of compounds found in date kernels with the objective of determining their efficacy.

Hippocrates, the famous physician of ancient times (460-377 BC) once said: "Make the food your medicine, and the medicine is your food". Although it was revealed thousands of years ago, it is still relevant at present. A balanced diet can provide daily energy, providing essential nutrients for the maintenance of physiological system, avoiding illness and improving immunity.

In this modern era, people opt for dietary supplements to maintain good health and decrease risks of chronic diseases. Some natural compounds are used as supplements as a means to increase important nutrients in the diet. These compounds are known as nutraceuticals. The majority of bioactive compounds characterized as nutraceuticals are derived from plant materials. Numerous bioactive compounds have been isolated from inedible plant parts and have been shown to be efficacious in reducing lipids and cholesterol levels, as antioxidants, and in increasing bone calcium density or status, and they are also said to possess anticancer properties (Yin & Linda, 2014).

Extraction methods influence antioxidant activity in a sample (Mojtaba *et al.*, 2015). Extraction procedure is the first important step to recover bioactive compounds from plants in natural product research. Christina *et al.* (2009) stated that many factors significantly influence extraction efficacy such as extraction temperature and extraction time.

Extraction conditions are important factors in the processes of obtaining bioactive compounds from plant matrix. These compounds differ structurally. Structures of phyto-compounds and their interactions with other food ingredients are not fully known, while these two aspects play significant roles in extranets choice and determination of extraction process.

Extraction yield is also affected by extraction technique and solvent used (Tushar *et al.*, 2012). Barchan *et al.* (2014) indicated that polar solvents were important for obtaining fractions with high antioxidant activity and total phenolics content. High temperatures increased solubility and diffusion (Ionnis *et al.*, 2018). Based on these facts and other studies, extraction method was performed using aqueous and heat treatment

which were more effective and efficient to draw out maximum yield of antioxidants of date palm kernels. This was in line with the concept of “*halalan toyyiban*”, environment friendly as well as both cost and time effective.

The extraction-optimization methodology enabled the present study to develop an appropriate extraction process with a time-efficient execution of experiments. The experimental values agreed with those predicted, thus indicating suitability of the proposed model. The conditions for extraction of bioactive compounds were optimized and determined the antioxidant properties by an empirical or statistical method. However, empirical methods have limitations in perfect optimization (Liyana & Shahidi, 2005). Response surface methodology (RSM) is a powerful tool for improving and optimizing extraction process variables. (Youssef *et al.*, 2012).

The study was undertaken with the key objectives of optimizing conditions for high yield of antioxidant activity by inhibition of 2,2-diphenyl-1-picrylhydrazyl (DPPH), total flavonoid content (TFC), total phenolic content (TPC), and ferric reducing antioxidant power (FRAP) of Ajwa variety of date palm kernels to benefit antioxidants contained within in food model system.

This research project was to evaluate nutritional and pharmaceutical properties of products derived from optimized date kernels extract with a view of determining those with beneficial effects that go beyond basic nutritional functions.

The requisite information when accessible will improve knowledge on optimization procedures and discover the potential of underutilized date palm kernels. Therefore, the utilization of date kernel could be an economical way of reducing environmental waste, providing added value to date palm kernel and increasing revenue to the date palm industry sector.

1.2 Problem Statement

The utilization of natural ingredients as traditional or alternative medicines has received tremendous interest world-wide. In Malaysia, a number of traditional medicines have been manufactured in large-scale fabrications with products reaching local and foreign markets. Traditional medicines, known to have no or insignificant side effects, are much preferred compared to drugs derived from chemicals. Utilizing raw materials derived from commonly available source plants, traditional medicines are relatively cheaper and more affordable which have caused most people to turn to for treatments of illnesses or maintenance of well-being.

Dates is one of the most popular superfoods and has been used as a source for alternative medicine. However, only its flesh is generally being used. The kernels are removed and regarded as a waste product. Literature has it that the kernels contain high-value components as much as its flesh (Hosain *et al.*, 2014). It has been used in many

applications such as food products formulations, cosmetics, and supplements (Golshan *et al.*, 2017).

The general aim of this research is to explore effective extraction procedure to enhance bioactive constituents of date kernels as food and food products with a view of utilizing it in the production of alternative medicines. The development of highly potential date kernels-based products would give a high-return benefit for consumer's health.

1.3 Scope and Limitation of study

The present study focuses on effects of extraction process on antioxidant activities of date palm variety Ajwa kernels. It covers qualitative screenings on presence of bioactive compounds and related components in attempt to utilize palm kernel to more useful products than waste. Data collections were conducted using statistical techniques in developing simpler, efficient, effective and accurate model.

1.4 Research hypotheses

Based on the formulation of the research problem, the hypotheses in this study include the following:

- i. Response surface methodology (RSM) is an efficient technique for optimization process to extract high concentration of antioxidants;
- ii. High concentration of phenolic compounds of date palm kernels extract can be found based on the results of the analysis using chemical analytical techniques;
- iii. The properties of date palm kernels can be characterized through proximate analysis, antioxidant properties, and nutraceutical properties.

1.5 Research Objectives

The main objective of the present study was to evaluate the health benefits of date palm kernels extract. Specifically, the research objectives were as follows:

1. To determine the optimum conditions for extraction of antioxidants from date seeds using response surface methodology (RSM);
- ii. To characterize composition of date kernel using chemical analytical techniques (ICP-MS, and QTOF);
- iii. To evaluate the properties of date seeds through proximate analysis and nutraceutical properties.

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