ANALYSIS OF PHYTOCHEMICAL COMPOUNDS, ANTIOXIDANT AND ANTI-INFLAMMATORY ACTIVITIES OF METHANOLIC EXTRACT OF RUTHANA DATE FRUITS *(PHOENIX DACTYLIFERA)*

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2023

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by

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Thesis submitted in fulfilment of the requirements
for the degree of
Master of Science

March 2023

ACKNOWLEDGEMENT

I would like to express my gratitude to Allah SWT for helping me and giving me the ability to complete this study. Moreover, very special gratitude goes to my supervisor, Dr. Eshaifol Omar Bin Azam for his precious guidance, collaboration, and support throughout my study, I was fortunate to have him chosen as my supervisor.

I am thankful to my co-supervisors, Dr. Sa'ud Al-Sanad, from Imam Muhammad Ibn Sa'ud University, and Dr. Hasni Bin Arsad for their advice, collaboration, and support in my research project.

I also extend my sincere thanks to USM University and its faculty members. In addition to the team members of the Institute of Postgraduate Studies (IPS) for their assistance, and updated advice regarding the USM Guidelines.

Great appreciation for my husband "Dr. Yazan A. Alsariera" who gave me full support and never failed to help me throughout the study period, and for my daughter, "Raheeq" whose presence gives me the strength to reach my goal.

Nosiba. A. Al-Sarayrah

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LIST OF SYMBOLS

G	Gram
Kcal	Kilocalories
M	Molar
Mg	Milligram
mg GAE	Milligram Of Gallic Acid Equivalent
mg QE	Milligram Of Quercetin Equivalent
mM	Molar Mass
Nm	Nanometer
Rpm	Round Per Minute
SD	Standard Deviation
Mg	Microgram
Mmol	Micro Mol

LIST OF ABBREVIATIONS

ABTS+	(2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid))
AGS	Adenocarcinoma Gastric Cell Line
$AlCl_3$	Aluminium Chloride
Caco-2	Colon Cancer
COX	Cyclooxygenase Enzymes
COX-1	Cyclooxygenase-1
COX-2	Cyclooxygenase-2
DDE	Digested Date Extract
DF	Dietry Fiber
DFME	Date Fruit Methanolic Extract
DNA	Deoxyribonucleic Acid
DPE	Date Polyphenols Extract
DPPH	2,2-Diphenyl-1-Picrylhydrazyl
FAO	Food and Agriculture Organization
Fe^{2+}	Ferrous Ion
Fe^{3+}	Ferric Ion
$FeCl_3$	Ferric Chloride
FRAP	Ferric Reducing Antioxidant Power Assay
GPx	Glutathione Peroxidase
HCC	Hepatocellular Carcinoma
IC50	Half-Maximal Inhibitory Concentration
IPS	Institute of Postgraduate Studies
$K_2S_2O_8$	Potassium Persulfate
LNCaP	Lymph Node Carcinoma of The Prostate
LPO	Lipid peroxidation
Min	Minute
MTT	Methylthiazolyl Tetrazolium Assay
$Na_2[Fe(CN)5NO] \cdot 2H_2O$	Sodium Nitroprusside
Na_2CO_3	Sodium Carbonate
$NaNO_2$	Sodium Nitrite
NaOH	Sodium Hydroxide

NCI-$H_4$60	A cell line derived from lung cancer cells
NFκB	Nuclear Factor kappa B
NO	Nitric oxide
NO_2	Nitrite
NO_3	Nitrate
NSAIDs	Non-Steroidal Anti-Inflammatory Drugs
PC3	Prostate Cancer
pH	Potential Of Hydrogen,
RBCs	Red Blood Cells
RNS	Reactive Nitrogen Species
ROS	Reactive Oxygen Species
TEAC	Trolox Equivalent Antioxidant Capacity
TFC	Total Flavonoid Content
TPC	Total Phenol Content
TPTZ	(2,4,6-Tris-(2'-Pyridyl)-S-Triazine)
USM	University of Science Malaysia
UV	Ultraviolet
Vitamin A	Retinol
Vitamin B1	Thiamine
Vitamin B2	Riboflavin
Vitamin B3	Niacin
Vitamin C	Ascorbic Acid

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ANALISIS SEBATIAN FITOKIMIA, AKTIVITI ANTIOKSIDA DAN ANTI RADANG DARIPADA EKSTRAK METANOLIK BUAH KURMA RUTHANA (*PHEONIX DACTYLIFERA*)

ABSTRAK

Buah kurma Ruthana merupakan, salah satu jenis kurma terbaik, yang mengandungi pelbagai khasiat dan sebatian fitokimia yang penting untuk kesihatan manusia. Oleh itu, adalah penting untuk mengetengahkan analisis nilai perubatan buah-buahan ini dalam kajian berkaitan pemakanan klinikal. Dalam kajian ini, kandungan fitokimia fenol dan flavonoid, dalam ekstrak metanol buah kurma (DFME) diukur kerana kepentingannya dalam aktiviti menghalang radikal bebas. Aktiviti antioksida ekstrak tersebut telah dinilai dengan beberapa kaedah analisis, termasuk ujian penghapusan radikal 2,2-Diphenyl-1-Picrylhydrazyl (DPPH), kuasa antioksida penurun ferik (FRAP), ujian penghapusan radikal 2,2'-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid), ($ABTS^{+}$), dan ujian penghapusan radikal nitrik oksida (NO). Di samping itu, kesan anti-radang DFME telah dikaji secara *in vitro* menggunakan kaedah denaturasi albumin, dan ujian penstabilan membran sel darah merah. Keputusan menunjukkan bahawa DFME mengandungi nilai sebatian polifenol yang tinggi. Kandungan fenol ialah 119.2 mg GAE/100gm berat kering. Sebaliknya, kandungan flavonoid ialah 55.6 mg QE/100 gm berat kering. DFME juga mempamerkan aktiviti antioksida terhadap radikal DPPH, FRAP, $ABTS^{+}$ dan NO dengan nilai IC_{50} masing-masing adalah 3.97±0.261 mg of Trolox/ g of plant dry weight, 4.08±0.269 mg Trolox/g of plant dry wight, 4.18±0.262 mg Trolox/g of plant dry wight, dan 22.173± 0.380 μg/ml. Ekstrak DFME juga menunjukkan aktiviti anti-radang yang ketara, kerana ia menyumbang kepada pencegahan denaturasi albumin

sebanyak 58% pada kepekatan 50 µg/ml, dengan nilai IC_{50} 36.049±0.238 µg/ml. DFME mengekalkan membran sel darah merah yang terdedah kepada haba pada kadar maksimum 53% pada kepekatan 500 µg/ml, dengan IC_{50} sebanyak 403.877±0.253µg/ml. Ekstrak buah kurma Ruthana mewakili sumber semulajadi yang memberangsangkan dan berpotensi untuk dijadikan sumber pengasingan sebatian baharu dengan nilai kesihatan dan perubatan, serta dalam pembuatan suplemen pemakanan.

ANALYSIS OF PHYTOCHEMICAL COMPOUNDS, ANTIOXIDANT AND ANTI-INFLAMMATORY ACTIVITIES OF METHANOLIC EXTRACT OF RUTHANA DATE FRUITS (*PHOENIX DACTYLIFERA*)

ABSTRACT

Ruthana date fruit, one of the finest types of dates, contains various nutrients and phytochemical compounds important for human health. Therefore, it is important to highlight the analysis of the medicinal value of these fruits in studies related to clinical nutrition. In this study, phenols, and flavonoid contents of date fruit methanolic extract (DFME) were measured due to their importance in inhibiting activities of free radicals. The antioxidant activities of the extract were evaluated by several analytical methods, which include 2,2-Diphenyl-1-Picrylhydrazyl radical scavenging assay (DPPH), Ferric reducing antioxidant power (FRAP), 2,2'-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) radical scavenging assay ($ABTS^{+}$), and nitric oxide radical scavenging assay (NO). In addition, the anti-inflammatory effects of DFME were studied *in vitro* using albumin denaturation, and red blood cell membrane stabilization assays. The results showed that DFME contains a high value of polyphenol compounds. The phenols content was 119.2 mg GAE/100gm of dry weight. On the other hand, the flavonoids content was 55.6 mg QE/100 gm of dry weight. DFME also exhibited antioxidant activity against DPPH, FRAP, $ABTS^{+}$ and NO radicals with IC_{50} values of 3.97±0.261 mg of Trolox/ g of plant dry weight, 4.08±0.269 mg of Trolox/ g of plant dry wight, 4.18±0.262 mg of Trolox /g plant dry wight, and 22.173± 0.380 µg/ml, respectively. The extract also showed significant anti-inflammatory activity, as it contributed to preventing denaturation of albumin by 58% at a concentration of 50 µg/ml, with an IC_{50} value of 36.049±0.238 µg/ml. DFME

maintained the red blood cell membrane exposed to heat at a maximum rate of 53% at a concentration of 500 µg/ml, with IC_{50} of 403.877±0.253 µg/ml. Ruthana date fruit extract represents a promising and potential source for the isolation of new compounds with health and medicinal value, as well as in the manufacture of nutritional supplements.

CHAPTER 1

INTRODUCTION

1.1 Overview

A random and unhealthy lifestyle can cause several health risks that lead to various diseases that may be serious, such as obesity, diabetes, heart disease, cancers, infections, and malnutrition. The spread of diseases increases the financial burden, either on individuals or on countries. Health care costs have been estimated at approximately 17.3 trillion US dollars between 2011 and 2030 (Mozaffarian, 2016). As a result, the scientific community seeks through research and exploration to reach effective strategies in controlling illness, as well as avoiding negative effects resulting from the use of manufactured drugs (Greenwell and Rahman, 2015), by investigating natural sources, such as various plant products, as a source of treatment (Siddiqui, 2011). Existing menus advocate the consumption of beneficial nutrients based on their main content of bioactive compounds, which provide significant protection against many diseases (Micha et al., 2017). No doubt that vegetables and fruits of all kinds are a major component of the human diet, and it's very important to include in daily diet based on their components of vitamins, minerals, carbohydrates, fibers, and other elements that contribute to promoting health (Khalid et al., 2017).

One of the recommended natural products is the date fruit. It has been described as the most important type of trees that grow in semi-arid and arid areas, and its production of dates represents an agricultural and economic resource for many countries. Dates are considered as a basic fruit in the Kingdom of Saudi Arabia and the countries of the Middle East and North Africa (El-Mergawi et al., 2019). This fruit provides an excellent source of natural sugars (44–88%), fiber (6.4–11.5%), and minerals that are involved in many vital metabolic processes in the body, such as

potassium and magnesium. It is also rich in vitamins indispensable to the exercise of life, such as vitamin A, thiamine (B1), riboflavin (B2), niacin (B3), pantothenic (B5), pyridoxine (B6), folate (B9), and vitamin C (Harasym and Oledzki, 2014, Eoin, 2016, Arshad et al., 2019). Date fruit contains high-quality proteins ranging between 2.3 to 5.6%, making it a good source of energy and supporting the immune system (Alla and Jithendran, 2018). In addition, its seeds which represent beyond 10% of the fruit mass, contain fatty acids (palmitic, oleic, and myristic). These carboxylic acids can be extracted and introduced into the manufacturing of nutritional health products or the beauty and care products industry (Nehdi et al., 2010) or as fertilizers and animal feeds (Radfar et al., 2019). According to Chao and Krueger (2007), date fruits are a highly nutritious source and possess great health and medicinal value, either used alone or in addition to herbs. The health properties of date fruits are attributed to the active compounds, such as phenols and carotenoids, that support eating them as a functional food (El-Mergawi et al., 2019). Flavanols are the predominant polyphenols in dates. Polyphenols (phenols and flavonoids) have anti-free radical-suppressing properties and reduce the severity of non-communicable disorders such as obesity, cancer, and heart disease. The effectiveness of polyphenols as an antioxidant has been proven *in vitro* and is still under research in terms of biological activity in preventing the development of inflammation in terms of activity in preventing protein breakdown and membrane damage (Habib et al., 2022).

The focus of this study is to investigate a species of date fruit, namely *Ruthana*, which is consumed as food in many middle eastern countries. *Ruthana* is also being used in traditional medicine to support the body's energy, strengthen the immune system, and maintain physical health. Due to its richness in various nutrients, particularly vitamins, minerals, and fibers, including bioactive compounds such as

polyphenols, it may be effective in curbing free radicals and controlling oxidative stress in the body.

1.2 Problem Statement

Researchers in alternative medicine and pharmaceutical manufacturing indicated that two-thirds of plants worldwide carry medicinal values. However, a large proportion of these plants have not yet been evaluated, and the exact mechanisms for the effectiveness of plant extracts are still not completely clear and need further study and exploration. Date fruit is a potential source for improving diet quality, strengthening the immune system, protecting public health, and thus preventing or treating diseases. However, Phoenix dactylifera date fruit species, such as *Ruthana*, are hardly known for their health benefits due to the lack of scientific data despite their abundance in the region of Saudi Arabia.

1.3 Research Hypothesis

The hypothesis of this study suggests that *Ruthana* date fruits, which are consumed as fruit and traditional medicine, are able to prevent or treat various health disorders and support the immune system. This may be due to the high levels of vitamins and minerals found in date fruits, as well as bioactive compounds such as phenols and flavonoids. These compounds have been found to effectively reduce free radicals, control oxidative reactions, and reduce inflammation, making date fruits a valuable ingredient in the production of functional foods and nutritional supplements for pharmaceutical use.

1.4 Aims and Objectives

This study aims to evaluate the nutritional values of one type of date fruit grown in the Kingdom of Saudi Arabia, namely *Ruthana*; therefore, this study was initiated to achieve the following objectives:

1. Measure the content of phenols and flavonoids in *Ruthana* date fruit using colorimetric methods.
2. Measure the antioxidant activity of *Ruthana* date fruit through selected colorimetric radical scavenging assays.
3. Evaluate the anti-inflammatory activity of *Ruthana* date fruit using selected screening methods.

1.5 Significance of the Study

This study aims to expand the literature on the evaluation of the antioxidant and anti-inflammatory activities of *Ruthana* date fruits *in vitro*, specifically in relation to their phenolic and flavonoid content, which will be evaluated for the first time for this variety. Furthermore, the study aims to evaluate the ability of *Ruthana* date fruit in preventing the development of inflammation, which was not addressed in previous literature. The data gathered from this study may support the adoption of *Ruthana* date fruit as a functional food and its use as a nutritional supplement in the food and nutraceutical industries.

1.6 Scope of the Study

This study focuses on determining the contents of phytochemicals, particularly polyphenols, which are known to contribute to the health benefits and nutritional value of date fruits. Additionally, the study aims to evaluate the capacity of date fruits to inhibit free radicals by using various measurement methods to assess antioxidant activity, as well as to determine the extent of anti-inflammatory activity through the use of two specialized *in vitro* tests.

CHAPTER 2

LITERATURE REVIEW

2.1 Origin and Growing Regions of the Date Palm

The date palm has the scientific name *Phoenix dactylifera L*, and is categorized under the *Arecaceae* family (Angiosperms, monocotyledon), as shown in Table 2.1.

Table 2.1 Botanical description (Uddin and Nuri, 2021).

Kingdom	Plantae
Subkingdom	Tracheobionta
Superdivision	Spermatophyta
Division	Magnoliophyta
Class	Liliopsida
Subclass	Arecidae
Order	Arecales
Family	Arecaceae
Genus	Phoenix
Species	*P. dactylifera*

The nomenclature of its types varies according to the country of origin, and its Arabic names, for example Tamr, Balah, and Rutab (Al-Alawi et al., 2017). The name "dactylifera" comes from the Greek word "dactylus," which means finger holder, and this indicates the number of fruits that the tree produces (Ashraf and Hamidi-Esfahani, 2011). The date palm flowers are described as small and yellow in colour, as shown in Figure 2.1., attached directly to spikelets, and then develop through the stages of growth to the stage of maturity, to fruits of different sizes and qualities according to the variety (Kamalika and Dobhal, 2022). (Figure

Figure 2.2) shows the fruitful date palm tree.

Figure 2.1 Date Palm Flowers (Nature Middle East, 2018).

Figure 2.2 Date Palm Tree (The National Centre for Palms and Dates, 2021).

According to agricultural reports, the date palm is one of the oldest perennial trees that have been cultivated and pollinated globally (Sharabasy and Ghazzawy, 2022). The agricultural production of dates is a major source within the economy of some countries in the Middle East, South America, and North Africa. It also holds great historical and religious value for the Arab peoples. In addition, date palm trees are planted due to their strength in resisting weather conditions such as strong winds and

rain and giving sufficient shade from the sun's heat, thus providing a protective cover for delicate trees such as citrus (Farag et al., 2021).

The beginnings of date production are attributed to Iraq, specifically in the southern regions, more than 5000 years ago. Then its cultivation began to spread in many countries due to its ability to adapt and grow in dry and semi-arid environmental conditions (Rambabu et al., 2020).

Currently, date palms cultivate and grow in 35 countries distributed around the world with an area of approximately one million acres of land, including North African and Southern European countries, as well as the Middle East, Pakistan, India, and Australia (Chao and Krueger, 2007).

The demand for consuming dates began to increase, since it produced over 3 million metric tons in 1990 at the global level ;in 2000, production exceeded 6.5 million metric tons, and approximately7.5 million metric tons by 2014 (Al-Alawi et al., 2017). Egypt and Iran accounted for the highest production of dates in the world in that year, with a production quantity of 17% and 14%, respectively, according to the Food and Agriculture Organization (2014), as shown in Figure 2.3.

In the Kingdom of Saudi Arabia, the date palm is relied upon as one of the most important sources of primary agriculture (Oladipupo Kareem et al., 2019), where Saudi Arabia's global production of date fruits is approximately 1.3 million tons annually. The huge production of dates corresponds to a large number of palm trees planted, which exceed 28 million trees. (KSA General Authority for Statistics, 2015), while Algeria outputs about one million tons, and that of Oman 0.4 million tons annually (Siddiqi et al., 2020). In general, the annual global production of dates is between 6 to 8 million tons, and this figure represents a market value of more than one billion US dollars (El Hadrami and Al-Khayri, 2012).

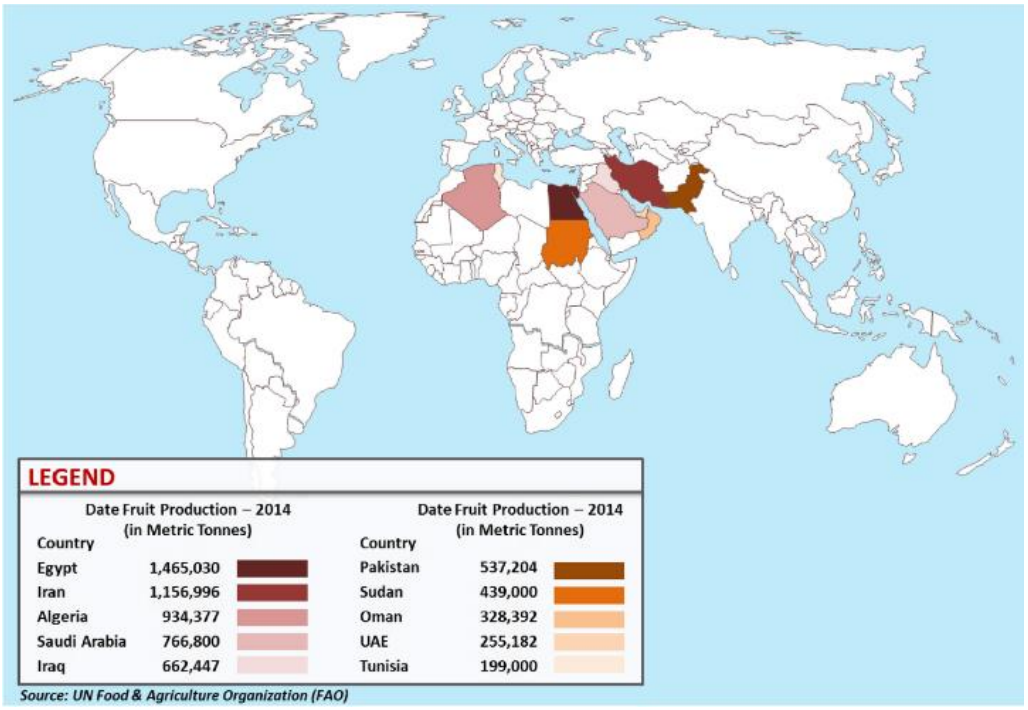

Figure 2.3 World Map of The Top Ten Date Fruit Producing Countries In 2014, (Al-Alawi et al., 2017).

2.2 Botanical Description of the Palm Tree

The date palm is a dicotyledonous perennial tree, propagated by planting seeds or tissues and branches (Alla et al., 2022).

2.2.1 Root System

Root growth starts from the seed, and the roots extend approximately 75%, and spread as branches around the palm tree, while another 25% of the root system extends downward toward the soil (Al udhaib, 2015). The primary roots help the date palm resist the water stress the tree is exposed to due to water scarcity and drought by utilizing the underground moisture (Cherif et al., 2015).

2.2.2 Trunk

The trunk of the cylindrical date palm extends to a height of about 20 meters, depending on the conditions and characteristics of growth (Shaaban, 2012). The stem consists of 23% hemicellulose, 32% lignin, and 45% cellulose (Al udhaib, 2015). .

2.2.3 Leaves

The leaves of the date palm are characterized by being thin and long (Figure 2.4). Their length varies between 2 to 6 m and between 10 to 20 new leaves are formed annually (Amroune et al., 2015).

Figure 2.4 The Date Palm Leaves (Sproule, 2022).

2.2.4 Inflorescence

The date palm tree is classified according to the flowering system as dioecious, meaning that the male flowers grow on a male tree and the female flowers grow on a female tree. The flowering stage begins four to five years after planting when a distinction is made between the male and female trees (Hanieh et al., 2020) . (Figure 2.5) shows the difference between female and male inflorescence.

.

Figure 2.5 Morphological description of Phoenix dactylifera L inflorescences in female tree (right) and male tree (left) (Intha and Chaiprasart, 2018).

2.2.5 Fruits

The flesh represents 85-90% of the weight of the fruit (Elleuch et al., 2008). It is a rich source of sugars , where fructose is the primary sugar (81-88%), in addition to sucrose, and glucose (Zhang et al., 2015, Assirey, 2015) . Lauric acid is the basic acid in date fruit from saturated fatty acids, with concentrations ranging between 15.0 to 17.8% (Bentrad et al., 2017).

2.2.6 Seeds

The seeds represent approximately 15% of the fruit's weight. It contains between 4 to 13% of the oil, and the percentage differs based on the type of dates (Ben-Youssef et al., 2017). The seed is described as oblong in shape and grooved ventrally with a small embryo formed from a cellulose deposit (Hajian and Hamidi-Esfahani, 2015). It contains 6.46% moisture, 5.22% protein, 16.20% fiber, 62.51% carbohydrates, and 1.12% ash. As in previous studies, these concentrations vary relatively according to the date variety (Gibson et al., 2015).

2.3 The Classification and Fruit Maturity Stages

Date fruits go through five stages until they ripen, each stage bears a different name according to the country of origin's culture (Hemmateenejad et al., 2015).

2.3.1 Hababouk Stage

The first stage of fruit formation after fertilization is in the first 4 to 5 weeks of growing and is called “Talea”. The fruit is immature with green colour, and it weighs less than 1 g, almost covered by the calyx, and shows only one direction of the ovary (Hussain et al., 2020), as shown in Figure 2.6.

2.3.2 Kimiri Stage

This stage is called the green stage and considered the longest stage in the stages of the development of date fruits that lasts between 9 to 14 weeks, due to the different varieties, but the fruit is still inedible (Figure 2.6). The average weight of the date fruit in the Kamiri stage is approximately 6 g, fruit length of 27.5 mm, and its diameter of 7.8 mm (Shahdadi et al., 2015)

2.3.3 Khalal Stage

In the Khalal stage, the colour of the fruit begins to turn from green to yellow and red, as shown in Figure 2.6. It lasts from 3 to 6 weeks, the average length of the fruit reaches 32.5 mm, and the diameter continues to increase to reach 21 mm. The nutritional content of the fruit at this point is 2.7% protein, 0.3% fat, and 2.8% ash. Some varieties can be eaten at this stage (Shahdadi et al., 2015).

2.3.4 Rutab Stage

This stage is called the soft ripe stage, its duration ranges from between two to four weeks, and the fruit begins to ripen from the top part. The texture becomes soft, and the colour begins to turn brown or black (Figure 2.6), depending on the type. The

protein and ash concentration content decreases during this stage to 2.6%. The percentage of fat remains constant (0.3%) while the content of sugars increases, so it becomes sweet in taste, and this is the appropriate stage for harvesting and storage (Haider et al., 2018).

2.3.5 Tamr Stage

It is the stage of full maturity or the final stage of ripeness. The fruits of dates are fully ripened and take a black or brown colour, as illustrated in Figure 2.6, however, not all types of dates develop to this stage (Khodabakhshian and Khojastehpour, 2021). The texture is hard in dry dates and soft in rutab dates (Al udhaib, 2015). The fruit loses a large percentage of moisture, which leads to continued low weight and prevention of corruption or fermentation, and the content of sucrose in semi-dry and dry dates is approximately 50% (Baliga et al., 2011). Table 2.2 shows different varieties of dates in the tamr stage.

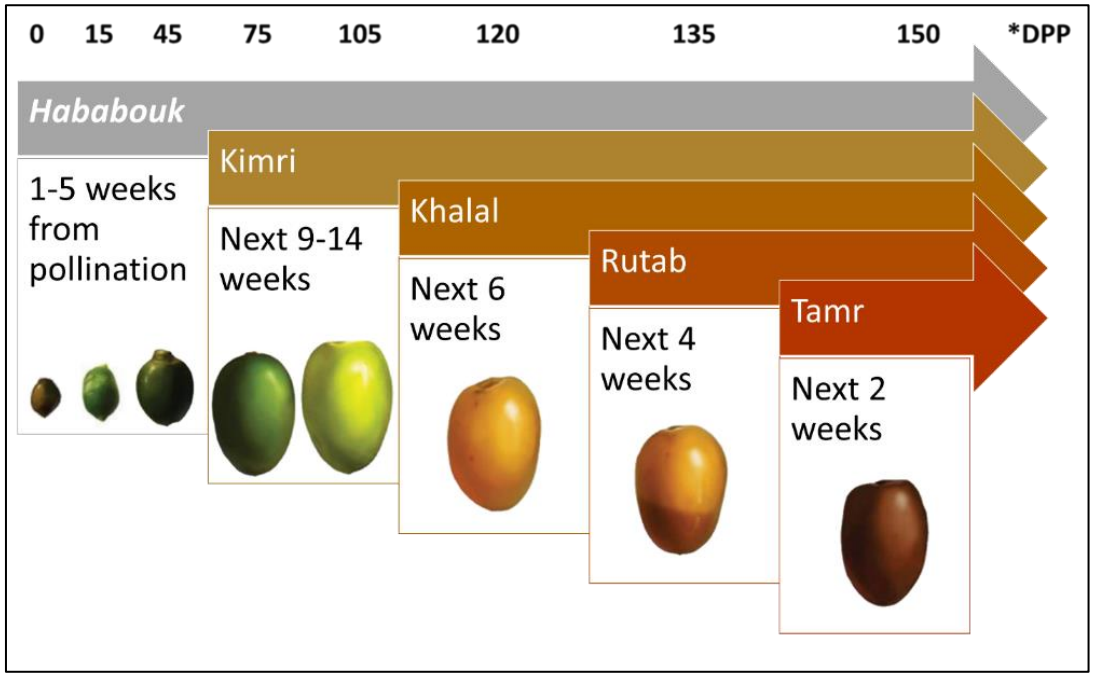

Figure 2.6 The Growth Stage of Date Fruit (Ghnimi et al., 2017).

Table 2.2 The Varieties of Dates in the Tamr Stage (Ministry of Agriculture in the Kingdom of Saudi Arabia, 2010).

Date Variety	**Illustration**
Majhoul	
Sukkari	
Zaghloul	
Halawi	
Ruthana	
Ajwa	

2.3.6 Date Fruit Parts

The main parts of the date fruit include endocarp mesocarp, pericarp, and one seed is called the kernel, pit, or pyrene as shown in Figure 2.7, (Ghnimi et al., 2017).

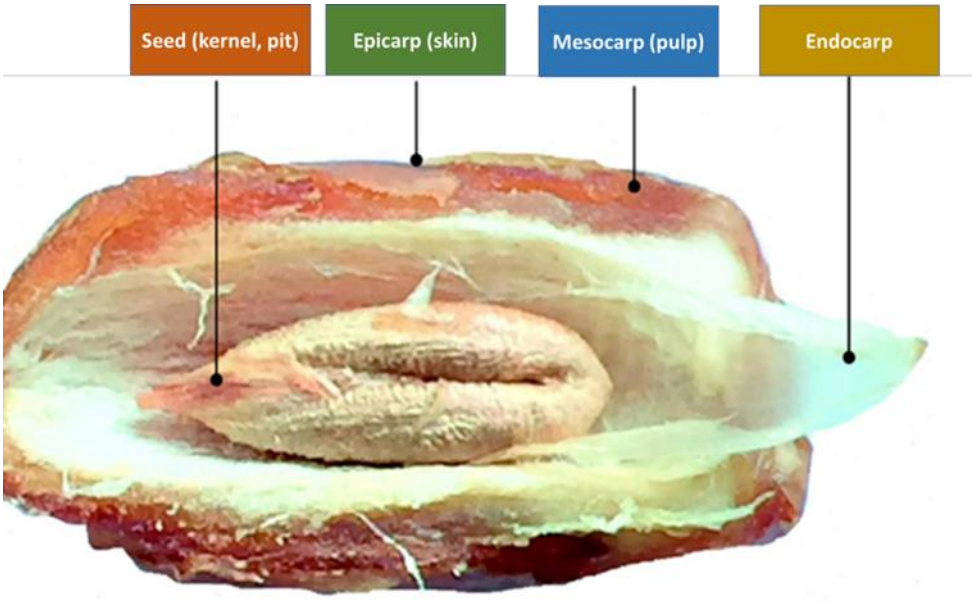

Figure 2.7 The Date Fruit Parts (Ghnimi et al., 2017).

2.4 Date Fruit and Body Health

The value and importance of dates were mentioned 20 times in the Surahs of Holy Qur'an, among them Surat Maryam, Yassin, Al-Rahman, Al-Kahf, and Al-Isra. It was also mentioned as one of the fruits of Paradise (The Qur'an). In the honorable Sunnah of the Prophet, the Prophet Muhammad, peace be upon him, advised to eat the fruits of dates every morning, and indicated it may act as a curative agent for more than one disease.

Everyone is now looking for healthy food options that maintain the quality of health and provide energy and physical strength, such as vegetables and fruits, as well as natural healthy drinks that improve nutritional intake and increase life span. Furthermore, people who follow a diet program to lose weight would certainly focus on

choosing alternative foods with low calories but high nutritional value (Mohammadi et al., 2018).

The compositional analysis showed that date seeds contain dietary fiber, which helps in promoting the health of the intestines and the digestive system in general (Uddin and Nuri, 2021, Niazi et al., 2017a). It is rich in many minerals and in good proportions, such as potassium 255.43 mg / 100 g of oil, which acts as an electrolyte inside the body and is mainly linked to sodium, thus controlling nerves and heartbeat. It also contains magnesium (62.78 mg / 100 g of oil), phosphorous (41.33 mg / 100 g of oil), and calcium (48.56 mg / 100 g of oil), all of them are mainly involved in the vital processes in the body and the maintenance of its balance (Nehdi et al., 2010).

It was pointed out that the role of polyphenols, fibers, and micronutrients contained in dates is able to reduce the incidence of vascular diseases in human when consumed as whole dates or their extracts, because it modifies vascular health signals, such as the level of cholesterol and triglycerides in the blood, as well as oxidative stress mediators (Al-Dashti et al., 2021).

The date-producing countries use the date fruit to produce natural sweetening drinks and alcohol. In addition, it is used in folk medicine as an alternative therapy in cases of stomach ulcers. Healers believe that date extracts preserve the stomach mucosa from the impact of hydrochloric acid, therefore, avoiding the development or expansion of stomach ulcers that may consequently cause bleeding. It also strengthens immunity and raises fertility, especially in women (Ebrahimi F et al., 2017). Additionally, its role was reported as an effective agent against microbial resistance (Eid et al., 2014) . It has also recently been demonstrated to play an important role in controlling diabetes (Chaari et al., 2020).

From the pharmaceutical point of view, the fruits of dates provide an excellent source of medicines. They contain sterols, anthocyanins, flavonoids, carotene, and procyanidins, which are very useful phytochemical compounds to fight cancer, including acting as antimicrobial, anti-mutagenic, anti-inflammation of the stomach, liver, and kidneys, and provides important support to the immune system(Abd Elgadir, 2021), as summarized in Figure 2.8.

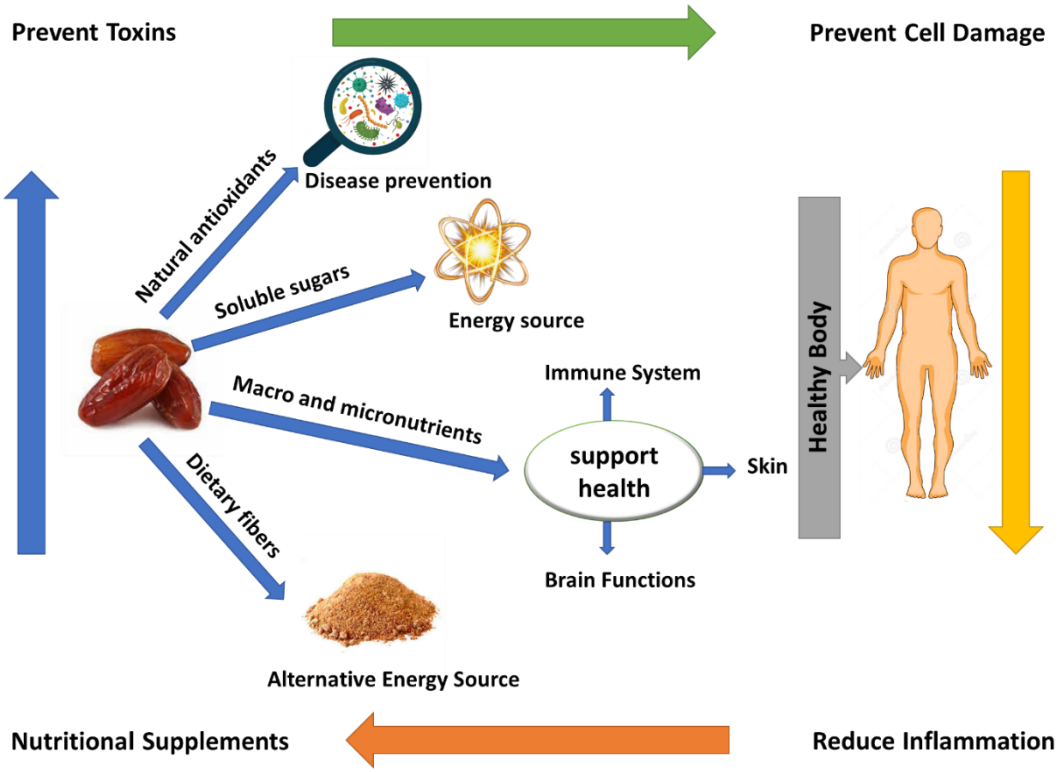

Figure 2.8 The Role of Dates in Disease Prevention Via Antioxidant, Anti-Microbial, and Anti-Inflammatory Activity (Siddiqi et al., 2020).

2.5 Nutritional Components of Date Fruit

Date fruit is described as a manufacturer to produce beneficial nutrients that protects the human body from various health setbacks. In this section, the nutritional content of date fruit will be presented according to several published studies.

2.5.1 Phytochemical Component (Phenolic and Flavonoid Content)

Polyphenols are known as natural metabolites that offer many benefits to the human body. From the chemistry point of view, they are organic compounds composed of aromatic hydroxyl rings (Tsao, 2010). Its nature, formulation, and concentration in date fruit are dependent on the environmental conditions of growing and extraction techniques (Ghnimi et al., 2017) . Phenolic (Figure 2.9), and flavonoid (Figure 2.10) compounds are described as powerful inhibitors of free radicals or also known as antioxidants, and they are being extensively researched for their ability to reduce the risk of various diseases (e.g., infections, heart disease, Alzheimer's disease, and various form of cancers). Examples of individual compounds that are classified under polyphenolic components includes, coumaric acid, ferulic acid, and sinapic acids, which represent phenolic acid group of compounds, and flavonoid compounds include luteolin, quercetin, and apigenin(Vlase et al., 2014) .

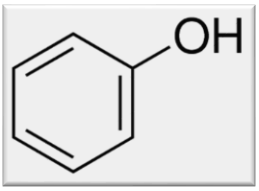

Figure 2.9 The Phenol Basic Structure (National Center for Biotechnology Information, 2022).

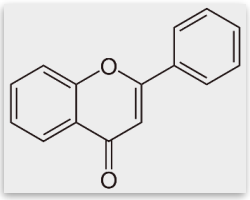

Figure 2.10 The Flavonoid Basic Structure (Panche et al., 2016)

Many studies have demonstrated that the concentration of polyphenols is directly related to antioxidant activity. The *Ajwa* date variety is considered to be rich in phenolic compounds, amounting up to 3.82 g gallic acid equivalent (GAE) kg^{-1} , while *Sukkari* and *Ruthana* dates show similar ratios of phenolic content of 2.48 g (GAE) kg^{-1}, and 2.67 g (GAE) kg^{-1}, respectively (El-Mergawi et al., 2019). According to Razali et al. (2019), it was reported that the phenolic content of *Medjool* dates was 53 mg GAE/100 g sample, which averages the contents of different types of studied dates such as *Deglet Noor* and *Khalas* (Razali et al., 2019). Based on these results, date fruits contain more phenolic compounds than other types of fruits, such as berries (0.06-2.28 g/kg^{-1} of fresh weight (FW)), strawberries (0.04-0.33 g/kg^{1} fresh weight (FW)), apples (0.01-0.48 g kg^{-1} fresh weight (FW)), and tomatoes (0.01-0.30 g kg^{-1} fresh weight (FW)) (Proteggente et al., 2002). The flavonoid content of *Sukkari* dates was 4.86 mg quercetin equivalent (QE)/100g extract (Trabzuni et al., 2014), *Ajwa* dates 172 mg QE/100g extract, and that of *Majdool* dates was 8.97 mg (QE)/100 g extract (Batool et al., 2020, Khan et al., 2016). Currently, there has been no indication of the flavonoid content of *Ruthana* from available literature search, at the time this thesis was being prepared.

2.5.2 Other Nutritional Components

2.5.2(a) Moisture and Sugar Content

Studies have indicated that date fruits contain high levels of moisture and good content of carbohydrates. According to Adwas et al. (2019), the proportions differ from one species to another (Adwas et al., 2019). Among the dates that grow in Saudi Arabia, the *Sukkari* dates shows the highest concentration of sugars ranging between 78 to 80%, where glucose, fructose and sucrose composed of 52%, 48%, 3% of the total sugar content, respectively, with a moisture content of 21%, (Siddeeg et al., 2019, Batool et al., 2020). As for *Ruthana* dates, it is depicted by the low moisture content of about

10%, and total sugar content of 24%. This makes the *Ruthana* type suitable for storage and is not exposed to mold contamination due to its low moisture content (Perveen and Bokahri, 2020). The sugar content of dates helps to provide the best source of energy for the human body, as well as promote fat metabolism and reduce protein losses (Jamil et al., 2010).

2.5.2(b) Minerals

Date fruit provides great nutritional value, reinforced by the fact that it contains minerals of different types and proportions, depending on the type of dates, or whether it is dried or fresh. Some types of dates contain about 15 types of minerals, among them calcium, magnesium, zinc, potassium, and iron (Nadeem et al., 2019). Minerals mainly contribute to many vital processes in the body and are also considered as catalysts for some reactions, such as metabolism and the generation of nerve signals (Perveen and Bokahri, 2020).

2.5.2(c) Protein and Fiber Content

Dates contain proteins in proportions ranging from 1% to 7%, including essential amino acids that the human body cannot manufacture that must be available from the healthy diet (Parvin et al., 2015) . Some dates are superior in protein content compared to different types of fruits, and some amino acids are not available in certain fruits, such as bananas, oranges, and apples (Ardekani et al., 2010). Date proteins include many types of amino acids, including histidine and arginine, which are essential in maintaining the physiological performance of the human body. (Ayad et al., 2020).

In addition, date fruit provides a good percentage of dietary fiber, which offers several health benefits such as lowering blood cholesterol by reducing low-density lipoprotein, as well as controlling the risks of obesity, hemorrhoidal diseases, and improving bowel health (Maqsood et al., 2020).

2.5.2(d) Vitamins

Date fruits in the Khalal stage had the highest concentration of vitamins, like beta-carotene and ascorbic acid . Some essential vitamins that date fruits contain include niacin, thiamine, riboflavin, folic acid, and vitamin C (El-Sohaimy and Hafez, 2010). Table 2.3 shows some of the vitamin content of date fruit. These vitamins give dates a high nutritional value for different age groups.

Table 2.3 The Vitamin Content of Date Fruits.

Vitamin	**Concentration (%)/ gm**
Vitamin C	0.77 - 2.7
Vitamin A	4.8 - 6
Folic acid	0.004 - 0.007
Vitamin B1	0.07 - 0.1
Vitamin B2	0.03 - 0.05
Vitamin B3	0.33 - 2.2

2.6 The impact of Oxidative Stress and Inflammation

Oxidative stress is associated with a wide range of degenerative conditions, such as chronic inflammation, and its progression to severe diseases such as cancers, nervous system diseases, diabetes, cardiovascular disease, tissue damage and aging. Oxidative stress causes the activation of nuclear factor-κB (NFκB), which is a transcription factor for certain genes that promotes various cytokine production. Cytokine production in turn, stimulates inflammatory responses across endothelial cells in various organs. Reactive oxygen species (ROS) and reactive nitrogen species (RNS) are generated by neutrophils that reach distant organs and interact with molecular components that consequently damage the cells by activating the inflammatory process and cascades. In chronic cases and with the persistence of inflammation, the affected cells are prepared to turn into tumors by stimulating mutations in the DNA of the affected cell through

nitrosative or oxidative stress. Hence, oxidative stress is a primary objective of current research to control the occurrence of diseases associated with it.

2.6.1 Albumin denaturation

Proteins are basic molecules in the structure of living organisms, made up of long chains of small molecules of amino acids. Proteins play an important role in speeding up some reactions in the body, as well as responding to signals, moving other molecules, and replication of DNA. If protein loses its normal shape and structure, it loses its stability and becomes ineffective. This status is called protein denaturation s. It is important to study the relationship between protein denaturation and the occurrence of inflammation in order to overcome the progression of diseases related to these pathologies.

There is a close relationship between protein denaturation caused by injury to living tissues and the consequent process of inflammation. For example, burns, fractures, bruises, and animal bites, cause denaturation of protein chemistry, both intracellular and the intercellular substance, that involves the breaking of bonds such as the hydrogen bonds in a protein so that it turns into a random biological structure and loses its normal function (Ricciotti and FitzGerald, 2011). This situation leads to the stimulation of inflammatory signals, and the transmission of white blood cells to the site of the injury as an immune response, and to help restore tissue homeostasis (Zhou et al., 2016). Uncontrolled inflammation leads to several types of disease-related conditions, such as arthritis, stroke, and some forms of cancer (Osman et al., 2016). Therefore, it is necessary to search for protective agents that may contribute to a resistant and stable protein structure against potential toxicants or injuries, and towards the stimuli that cause denaturation such as heat.

2.6.2 RBC's membrane stabilization

The damage of the erythrocyte (RBC) membrane is an indication of inflammation. The cell membrane may rupture, due to the accumulation of excess fluid inside it, thus damaging the cell. The main factor of erythrocyte membrane damage is lipid peroxidation, which is produced by free radicals (Bukhari et al., 2013). Anti-inflammatories work through the inhibition of cyclooxygenase, where chronic or acute inflammatory conditions are caused by the activity of these enzymes outside the cell (Mounnissamy et al., 2007a), therefore, reducing membrane permeability caused by inflammatory mediators may prevent fluid or serum protein leakage into the tissue. Some plant extracts may help increase membrane resistance and stability by blocking the secretion of inflammatory mediators and lysosomal enzymes or by stabilizing the lysosomal membrane (Chaitanya et al., 2011).

2.7 Antioxidant and anti-inflammatory Activities of Date Fruit

Recently, many studies targeted oxidative stress, as the imbalance between the ratio of reactive free radicals and antioxidant materials causes the oxidation of proteins, fats, nucleic acids, as well as carbohydrates. This leads to the development of many degenerative diseases, vascular and heart diseases, Alzheimer's disease, cancers, neurological and inflammatory diseases (Phaniendra et al., 2015). Some oxidation reactions occur because of metabolism and result in reactive oxygen radicals (superoxide radicals, hydroxyl radicals, and peroxyl radicals). One of the most important solutions to control free radicals is to supplement the diet with antioxidant compounds from natural sources. Traditional medicine has used various types of plant extracts to combat and prevent diseases, given that the principle of antioxidant action is not only inhibiting free radicals but also regulating antioxidant enzymes as well as detoxification and modulation of redox signalling and genetic expressions (López-

Alarcón and Denicola, 2013). Various studies have shown the role of date fruits as antioxidants, enhancing the immunity of the human body and fighting tumors by stimulating programmed cell death and inhibiting telomerase activities (Azab and Albasha, 2018).

Animal studies have indicated the role of date extracts in inhibiting free radicals (superoxide and hydroxyl radicals). These studies exhibited the effectiveness of date extracts in reducing iron-induced lipid and protein oxidation which showed significant correlation between the effects with the concentrations used in the study, conducted on the rat brain.(Alyahya et al., 2022)., and this study is consistent with the results of studies conducted on different types of dates (Adeosun et al., 2016, Fallahi et al., 2015). Oral feeding of experimental rats with coumaric acid, which is one of the components of date fruits, led to an increase expression of the genes that regulate the antioxidant enzymes. In addition, the antioxidant activity was also indicated with the presence of flavonoids, procyanidins and anthocyanins (Yeh et al., 2009). In another study, the Tunisian dates, *Korkobbi,* and *Rotbi* dates showed significant values in the fight against hydroxyl radicals and lipopyrroxyl, respectively, compared to the equivalent value of Trolox (Chaira et al., 2009).

The antioxidant effect of dates is affected by the stage of maturity. It is known that as they mature, the antioxidant effects would be less (Mohamed Lemine et al., 2014). Moreover, the method of sun drying may also affect the concentration of antioxidants negatively and thus, reduces the effectiveness of the date extracts fighting free radicals. Date fruit also contains a trace compound called selenium, which has been shown to have efficacy in combating free radicals that plays a basic role in the antioxidant process, in the form of selenocysteine residues that are an integral

constituent of ROS-detoxifying selenoenzyme (GPx), and thioredoxin reductases (El-Far et al., 2019, Gümüşay et al., 2015).

In the same context, a study that included 17 types of Saudi dates showed antioxidant values of significant differences. Among them *Ruthana* dates antioxidant activity was 3.37 ± 0.22 μmol Trolox g^{-1}, *Sukkari* variety 4.12 ± 0.18 μmol Trolox g^{-1}, *Majdool* variety 4.66 ± 0.23 μmol Trolox g^{-1}, and the *Ajwa* variety showed the highest value of 6.14. ± 0.22 μmol Trolox g^{-1}, as per DPPH assay. Compared to the FRAP assay, the studied dates showed higher significant values that reached 10.50 ± 0.34 μmol Trolox g^{-1} of *Ruthana* cultivar, 10.53 ± 0.27 μmol Trolox g^{-1} of *Majdool* cultivar, 10.89 ± 0.37 μmol Trolox g^{-1} of *Sukkari* cultivar, and *Ajwa* cultivar outperformed all other dates with a value of 14.45 ± 0.23 μmol Trolox g^{-1} (El-Mergawi et al., 2019). These results are consistent with the reported results by (Zhang et al., 2017), where a comparison was carried out between 30 types of aqueous and methanolic extracts of dates; among them are the varieties of *Luban, Ruthana, Khashram, Sukkari, Majdool*, and *Ajwa*, using two methods. The first was LPO method, which indicates the type of compounds with antioxidant activity, and the second was MTT method, which targets the ability of these compounds in the antioxidant activity. The study concluded that the methanolic extracts of dates show significantly higher values than the aqueous extracts.

The antioxidant activity shown by date fruit extracts of all kinds is due to the diversity of phytochemicals such as phenols, flavonoids, and carotenoids, and this activity offers great hope in the field of nutrition, alternative medicine, and pharmaceutical industries, as the fight against free radicals may provide protection from the development of various chronic diseases and conditions (Hafzan et al., 2017). The principle of antioxidants involves the activity of antioxidant enzymes, thus regulating

the detoxification mechanisms, redox processes, and gene expression of these enzymes (López-Alarcón and Denicola, 2013).

Inflammation is defined as an automatic response of the immune system to a threat or the response of the host against microbial or chemical factors (Thompson et al., 2015). Clinically, inflammation is manifested as fever, redness, pain, and loss of normal function (Jajam et al., 2017) . Acute inflammation conditions are usually treated with steroids and non-steroidal drugs, but they are not effective in treating chronic inflammatory conditions such as rheumatoid arthritis because its mechanisms of development and causes that lead to critical stages are not sufficiently understood. Accordingly, there is an urgent need to obtain new agents that are effective and safe, and plant compounds used in traditional medicine are some of the potential candidates (Patel and Savjani, 2015). Furthermore, the introduction of these components to a balanced diet greatly helps in the prevention of chronic inflammation and its related diseases.

Various parts of the date palm *(Phoenix dactylifera L)* have been studied for their anti-inflammatory efficacy based on their use in folk medical practices. Since a long time ago, it is believed that dates can treat bronchitis, inflammation of the intestines and stomach, and relieve fever. The extracts of this plant were mixed with paracetamol and ibuprofen, and was shown to improve its effectiveness in relieving pain, giving rest, and the ability to sleep (Taleb et al., 2016).

The ability of three types of date extracts (*Ruthana, Sukkari,* and *Ajwa*) has been demonstrated to inhibit the cyclooxygenase enzymes (COX-1 and COX-2), with an inhibition rate ranging between 32 to 40% (Zhang et al., 2017). The presence of cyclooxygenase stimulates the production of prostaglandins, and levoglandins, which are the mediators that involved in pathophysiological processes of inflammation, and it

was also found that they interact reversibly with membrane-coupled receptors. Therefore, there are evidences to conclude the importance of non-steroidal anti-inflammatory drugs (NSAIDs) to prevent activation of COX enzymes, thus inhibiting the inflammatory process (Utzeri and Usai, 2017).

One of the important results that support the role of date components is that date leaf extracts offer anti-inflammatory activity and are rich in antioxidants (Eddine et al., 2013). Also, date fruit extracts have been shown to reduce foot swelling and production of plasma fibrinogen (Niazi et al., 2017b). The results of an animal model study indicated the role of dates pollen as a potential protective agent in modulating cytokine expression and efficacious in reducing induced atypical prostatic hyperplasia in experimental mice (Elberry et al., 2011). It was shown that the phenolic compounds in six types of dates grown in Moroccan, namely *Boufgous, Bouskri, Bousrdon, Bousthammi, Jihl,* and *Majhoul*, have an obvious effect in suppressing the inflammation, swelling, and oedema of the experimental mice caused by the carrageenan injection. Additionally, caffeic, ferulic, and gallic acids, which are polyphenolic compounds in dates, showed the highest potency in preventing red blood cell membrane damage and inhibiting protein denaturation *in vitro* (El Hilaly et al., 2018).

2.8 Functional Food Applications of the Date Palm

Awareness among the public has increased towards eating healthy and beneficial food. Therefore, industries are currently seeking to meet the needs and desires of consumers to provide healthy, safe, and risk-free food products. In addition it is crucial to clarify the compounds contain in the food product, its specific dosage, and describe the teleological elements it may contain. (Abdul-Hamid et al., 2020). There are

a number of products that have been manufactured using dates as the main ingredient, including date syrup, date sugar, dried date powder, date candy products, and date-mixed high fructose liquids (Al-Otaibi et al., 2016). In addition, several products, such as date jam and jelly, have been widely used in the Arab countries. Commercial date vinegar has also been noted for widespread use in Malaysia, and some studies have provided an analysis of the date fruit to produce fruit sugar, which is considered a healthy and nutritious alternative to refined sugar (Ashraf and Hamidi-Esfahani, 2011).

The date palm fruits are consumed not only as food for humans but as by-products, such as seeds, pollen, leaves, and buds, that are also used in many applications. For example, the seeds are used in animal feed or roasted, ground, and used as decaffeinated Arabia coffee or a healthy alternative to flour. Pollen is also used in many practical scientific applications (Al-Farsi and Lee, 2011). The pit constitutes about 18% of the weight of the fruit, and it is rich in fiber, carbohydrates, protein, and fats. The oil extracted from dates possesses an antioxidant activity like that of olive oil. The fruits also contain polyphenol, essential oils, steroids, saponins, and serranoids, which enhances their potential to be considered a potential functional food (Baliga et al., 2011).

CHAPTER 3

MATERIALS AND METHODOLOGY

The flowchart in Figure 3.1 illustrates the study workflow, which entails the collection and extraction of plant samples, analysis of phytochemical components, such as phenols and flavonoids, and evaluation of the antioxidant effect of the extract using four selected methods. Additionally, the anti-inflammatory effects of the extract are evaluated through two selected methods.

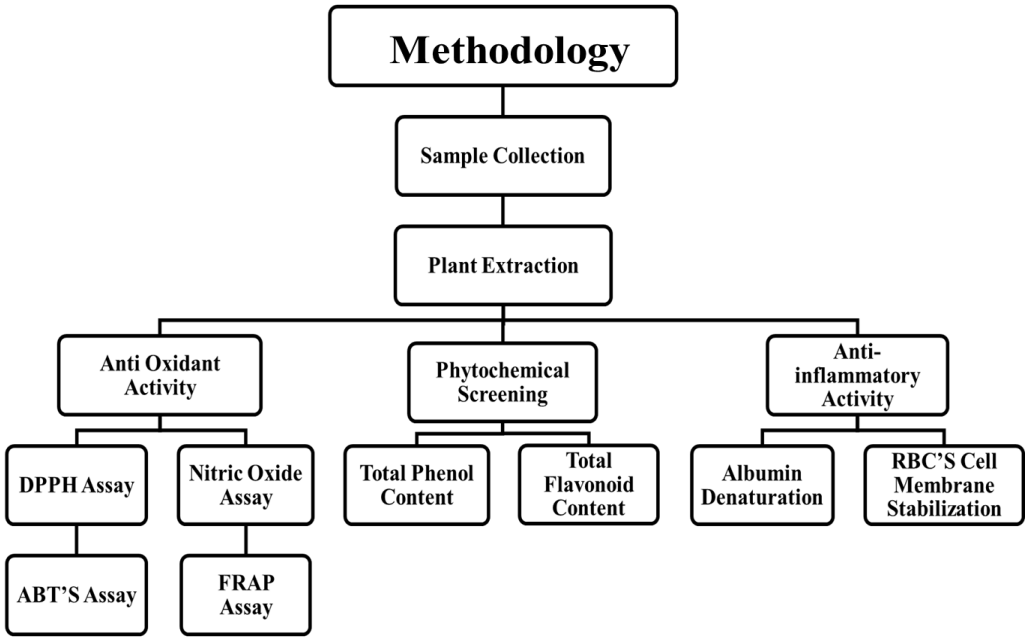

Figure 3.1 The workflow of the study methods.

3.1 Sample Collection

Fresh *Ruthana* fruits were obtained directly from the palm farm in the Al-Madinah Al-Munawwarah region in Saudi Arabia at the time of maturity in May 2021 (Table 3.1). The fruits were picked manually, with the help of a farmer, after removing the cover that protects the harvest from insects. The ripe and intact fruits were obtained for use in the research. Then the fruits were kept in a dark room until extraction.