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Intitulé

Phytochemical analysis and antioxidant activities of leaves extracts of two varieties of date palm (*Deglet Nour* and *Ghars*) grown in Algeria

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To **humanity** for we hope they benefit from our work;

To our **parents** and, our families;

To all **warriors** of corona virus (**COVID-19**) in whole the world;

This humble piece of work is lovingly dedicated to every **researcher** and **student** who serves as inspiration for us.

Assia & Maram

Abstract

Abstract

Ph. dactylifera L. (date palm) is well known for its innumerable health benefits and nutritional values. Therefore, the present work aimed to determine the total phenolic content in two varieties of date palm leaves, Deglet Nour, Ghars from three regions in Algeria, extracted with two types of solvents methanol and aqueous methanol (80%) and to evaluate in vitro their antioxidative properties by chemical assay (test DPPH° assay), and compare the results based on geographical region, variety.

Consequently, the total phenolic contents (TPC) of these extracts were measured using Folin Ciocalteu spectrophotometric method. Total phenolic content in date palm leaves extracts ranged between (2131.61-13993.55) mg GAE/100g DW, thereafter all extracts show antioxidant activity witch interpreted by effective scavenging concentration (IC_{50}) and ranged between (0.0018-0.0085) g/l. The positive correlation between TPC and antioxidant activity for date palm extracts was confirmed statistically. Finally, the use of date palm varieties leaves extract for protection against oxidative stress has been recommended.

Key words: *Ph. dactylifera L.,* Total phenolic content, DPPH°, Variety, Antioxidant activity.

Résumé

Ph. dactylifera L. (palmier dattier) est bien connu pour ses innombrables bienfaits pour la santé et ses valeurs nutritionnelles. Par conséquent, le présent travail visait à déterminer la teneur totale en phénols de deux variétés de feuilles de palmier dattier, Deglet Nour, Ghars de trois régions d'Algérie, extraites avec deux types de solvants méthanol et méthanol aqueux (80%) et à évaluer in vitro leur propriétés anti-oxydantes par dosage chimique (test DPPH °), et comparer les résultats en fonction de la région géographique, de la variété.

Par conséquent, le contenu phénolique total (TPC) de ces extraits a été mesuré en utilisant la méthode spectrophotométrique Folin Ciocalteu. Le contenu phénolique total dans les extraits de feuilles de palmier dattier se situait entre (2131,61-13993,55) mg GAE / 100g DW, ensuite tous les extraits présentent une activité antioxydant interprétée par une concentration de piégeage efficace (IC_{50}) et se situaient entre (0,0018-0,0085) g / l. La corrélation positive entre le TPC et l'activité antioxydant pour les extraits de palmier dattier a été confirmée statistiquement. Enfin, l'utilisation d'extraits de feuilles de palmier dattier pour la protection contre le stress oxydatif a été recommandée.

Mots clés: *Ph. dactylifera* L., Teneur phénolique totale, DPPH °, Variété, Activité antioxydant.

الملخص

يُعْرِف نخيل التّمر بفوائدِه الصِّحيّة وقيمته الغذائيِّة التي لا حصر لها، لِذلك يهدف هذَا العمل إلى تحديد المحتوى الفينُولي الكلي (TPC) لصِنفين من أوراق نخيل التَّمرِ، دقلة نور والغرس في ثلاث مناطق بالجَزائِر، مُستخلصة بنوعين مِن المذيبات؛ الميثانول والميثانول المائي (80%)، وقُيمت خصائصِها المضادة للأكسدة مخبرياً عن طريق الفحص الكيميائي (اختبار الـDPPH°)، وتمت مُقارنة النَّتائِج عَلى أساس المنطقة الجغرافِيّة، الصِّنف، حيثُ تم قياس المحتوى الفِينُولي الكلِّي لهذِه المستخلصَات باستخدام طريقة Folin Ciocalteu الطَّيفِية، إذ تراوح المحتوى الفِينُولي الكلِّي في مستخلصات أوراق نخيل التَّمرِ بين GAE/100g DW (2131.61-13993.55).

وقد أظْهرَت جميع مستخلصات أوراق نخيل التَّمرِ نشاطًا مضادًّا للأكسدة والذِّي تم تَفسيرُه بتركيز الكَسح الفعَّال (IC_{50}) حيث تراوح بين g/l (0.0018-0.0085)، كما تم التوصل لعلاقة ارتباط ايجابية إحصائيا بين المحتوى الفِينُولي الكلِّي والنشاط المضاد للأكسدة لمستخلصات أوراق نخيل التَّمرِ وعليه نوصي باستخدام مستخلصات أوراق نخِيل التَّمر للوِقاية مِن الإجهَاد التأكسُدي.

الكلمات المفتاحية: نخيل التَّمر، المحتوى الفِينُولي الكلي، اختبار الـDPPH°، الصِّنف، النَّشاط المضاد للأكسدة.

List of Abbreviations

BHA	Butylated hydroxy anisole.
BHT	Butylated hydroxy toluene.
CAT	Catalase.
CNS	Central Nervous System.
DN	Deglet Nour.
DNL	Deglet Nour Laghouat.
DNM	Deglet Nour El Meghaïer.
DNT	Deglet Nour Tolga.
DPPH	2,2-Diphenyl-1-picrylhydrazyl.
DW	Dry Weight.
eNOS	Endothelial nitric oxide synthase.
FAOSTAT	Food and Agriculture Organization Corporate Statistical Database.
GAE	Gallic acid equivalent.
GH	Ghars.
GPX	Glutathion peroxidase.
GR	Glutathion reductase.
I%	The percentage of inhibition.
IC_{50}	The concentration (g/l) or the extract that inhibited the formation of radical by 50%.
iNOS	Inducible nitric oxide synthase.
MeOH / H_2O system	Aqueous Methanolic extracts (mixture of 8/2).
MeOH system	Methanolic extracts (absolute methanol).
NADPH	Nicotinamide adenine dinucleotide phosphate.
nNOS	Neuronal nitric oxide synthase.
pH	Potential of hydrogen.
Ph.	*Phoenix*.
PUFAs	Polyunsaturated fatty acids.
RNS	Reactive nitrogen species.
ROS	Reactive oxygen species.
SOD	Superoxide dismutas.
TBHQ	Tert-Butylhydroquinone.
TPC	Total phenolic content.
UV	Ultraviolet.

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Introduction

Introduction

The date palm (*Phoenix dactylifera* L.) to early civilizations is well documented and it is among the earliest cultivated fruit trees **(Mohamoud *et al.*, 2019)**. In Algeria, the date palm is an important tree for people and it plays principal roles in social, environmental, and economic sectors. Besides, it has biological and pharmacological activities such as antioxidant activity due to its phytochemical composition **(Laouini *et al.*, 2012)**.

Due to the importance of date palm (*Ph. dactylifera* L.), our study focuses on her biological activity through our study entitled: Phytochemical analysis and antioxidant activities of leaves extracts of two varieties of date palm (Deglet Nour and Ghars) grown in Algeria.

The study Problematic was focused on the following main question: Is there a difference between the antioxidant activity values of date palm leaves in Daglet Nour and Ghars varieties?

In order to treat and analyze this problematic and to understand it clearly, the following **sub-questions** were asked:

1. Does the difference in the geographical area affect the value of the antioxidant activity of Deglet Nour variety?

2. Does the difference of variety affect the value of the antioxidant activity of Deglet Nour and Ghars varieties?

3. Does the extraction system affect the antioxidant activity of date palm leaves varieties?

4. Is there a relationship between the total phenolic content (TPC) and the antioxidant activity in date palm leaves?

In purpose to answer the previous questions asked and hence answer the problematic of the study, the following **hypotheses** were formulated in the same previous order:

1. Yes, the difference in the geographical area affects the value of the antioxidant activity of Deglet Nour variety.

2. Yes, the difference of variety affects the value of the antioxidant activity of Deglet Nour and Ghars varieties.

3. Yes, the extraction system affects the antioxidant activity of date palm leaves varieties.

4. Yes, there is a relationship between the total phenolic content (TPC) and the antioxidant activity in date palm leaves.

The **importance** of this study appears through the following notes: Firstly, the importance of this study stems from the novelty of its topic in the context of the study of plant species biological activity, which today is of great importance and the antioxidant activity represent one of their most important divisions. Secondly, this research has a specific

importance stems from the comparative fields studied about the affect of the factors: geographical area and variety to the antioxidant activity. Thirdly, it represents an addition to the scientific knowledge in this domain. Finally, exploiting the results that we hope to reach which can contribute effectively to opening the horizon to study the different varieties of the date palm in Algeria and to estimate their biological activity and their pharmaceutical proprieties.

The principal **objective** of this study is to measure the antioxidant activity of the date palm leaves varieties; Deglet Nour and Ghars; in addition to several sub-goals which are: A review of the specific theoretical frames and concepts on free radical and oxidative stress, highlight the influence of some factors specific to date palm on their antioxidant activity, quantification of the phenolic compounds of the date palm leaves extracts.

We can brief the **reasons** of choosing this topic in following; generally, the topic of biological activity and especially the antioxidant activity is among topics posed on the scientific scene and interest of the medical and pharmaceutical studies recently and, our desire to study the local varieties of date palm that didn't have many studies at the botanical and biological levels. Besides, deficiency of the referenced studies about date palm leaves nationally and internationally. Then, the date palm is an economic wealth that didn't have the great interest.

To answer our problematic, and for check the hypotheses, then devise the study into parts preceded by an introduction and followed by a conclusion comprises a general summary about the subject and followed by the most important results and several of suggestions.

The first part "**bibliographic part"** contains two chapters; the first one includes general concepts of date palm, its botanical classification and its chemical composition, while the second one contains free radical, oxidative stress and its effect and the antioxidant's role. While the second part **"the experimental part"** which consists also of two chapters; materials and methods and results and discussion; treats the photochemical screening, the extraction yield, determination of total phenolics content (TPC) and finally determination of the antioxidant activity of date palm leaves varieties.

Introductory chapter

In order to achieve this study we used a methodology that is appropriate to the nature of the subject of our study through:

- **Study limits**

The limits of the study represent in the following:

a) Processing limits: the subject was treated in the theory side depending on paper and electronic books, accredited scientific sites, magazines and articles.

b) Temporal and spatial limits: the experimental part of the study was conducted during the period of February and March 2020.

- **Study Approach**

In line with the nature of the topic and in order to respond to the problematic posed, we have chosen the experimental approach compatible with studies in the natural science field, and else the comparative approach suitable to the nature and feature of the topic during results discussion.

- **Study materials**

Convenient materials were used to accomplish the experimental purpose, they were laboratory equipments and instruments, and preview the results statistically using the computer program Excel, SPSS V 22.0 software, and the Mapinfo V8.0 for drawing the maps.

- **Previous studies**

To continue the bibliographic part and in order to consolidate the study concept and its hypotheses, the researcher's previous efforts have been viewed in this domain and to benefit from some of them in this current study, below is a presentation of some studies which are historically arranged from recent to ancient.

01- Dia *et al.* study in 2018	
Study title	Solvent and techniques extraction influence on phytochemical profile and free radicals inhibition of *phoenix dactylifera* L.
Study type	Scientific article.
Study objective	Comparison of the extraction efficiency of different methods od date palm leaves extracts and evaluation of its phenolic content and its antioxidant activity.
Study results	Date palm leaves extract considered as rich source of natural bioactive compounds with high capacity of antioxidant activity.
Using in our stady	Comparison of its results with ours.

02- Laouini study in 2014	
Study title	Etude photochimique et activité biologique d'extrait des feuilles de *Phoenix dactylifera* L. dans la région du sud d'Algérie (la région d'Oued souf).
Study type	PhD thesis on industrial chemistry sciences.
Study objective	Evaluation of date palm leaf extracts from the perspective of their phytochemical composition and their effectiveness as antioxidant and as anti-inflammatory.
Study results	Date palm extracts rich in phytochemical composition and they have an effective activity as antioxidant and anti-inflammatory.
Using in our stady	Comparison of its results with ours.
03- Laouini *et al*. in 2014	
Study title	Scavenging effect, anti-inflammatory and diabetes related enzyme inhibition properties of leaves extract from selected varieties of *phoenix dactylifera* L.
Study type	Scientific article.
Study objective	Investigate the antioxidant, anti-inflammatory and antidiabetic activities of three varieties of date palm.
Study results	The studied varieties are natural source of antioxidant and anti-inflammatory drugs and antidiabetic medicine.
Using in our stady	Comparison of its results with ours.
04- Tirichine study in 2010	
Study title	Etude ethnobotanique, activité antioxydante et analyse phytochimique de quelques cultivars de palmier dattier (*Poenix dactylifera* L.) du Sud-Est algérien.
Study type	Magister's thesis in biology.
Study objective	Ethno-botanical survey, phytochemical analysis and evaluation of antioxidant potential of twenty varieties of date palm leaflets.
Study results	The studied varieties have a various uses, and they have a high antioxidant activity.
Using in our stady	Comparison of its results with ours.

- **Study difficulties**

Among the most important obstacles we faced while carrying out our study were:

✓ The restricted period of laboratory research due to the quarantine arising from the outbreak of the Corona-virus and consequently the removal of numerous experimental steps as well as the elimination of some study samples.

✓ Poorly of laboratory equipment and scarcity of materials and solutions.

Bibliographic part

Chapter I

General concepts of date palm

I.1. Background

The date palm (*Ph. dactylifera* L.) is considered a symbol of life in the desert, it is one of the oldest trees from which man has derived benefit, and it has been cultivated since ancient times (**Meddich *et al.*, 2018)**.

The heavenly religions honored the date palm, but no other religion has stressed the regard for dates and the date palm as much as Islam. The Holy Koran mentions dates and the date palm in many suras (chapters) and verses. Prophet Muhammad (peace be upon him) is reported to have said that the best property is the date palm **(El-juhany, 2010)**.

I.2. Origin of date palms

The origins of the date palm are still unknown and controversial **(Raid, 1996)**, many researchers agree that date palm originated in Babel (Iraq). However, others believe that it originated in the Arab Gulf and was afterward spread to Babel **(Al-abdoulahadi *et al.*, 2012)**.

The origin and domestication of the date palm is still problematic mainly because its wild ancestor has yet not been clearly identified. On the one hand, it has been suggested that the cultivated date palm may have its ancestor from a wild species of the *Phoenix* genus, different from *dactylifera*. *Ph. sylvestris* L. *Roxb.*, the sugar date palm, has been considered as a possible ancestor because of its proximity in morphological and genetic characters with the cultivated date palm. On the other hand, it has been suggested than date palm resulted from the hybridization of wild *Ph. dactylifera* species **(Hernández *et al.*, 2014)**.

I.3. Botanical name

The scientific name of the date palm put by Linné in 1734, *Phoenix dactylifera* L. is presumably derived from a *Phoenician* "*Phoenix*", which means date palm, and "*dactylifera*" derived from a Greek word "*daktulos*" meaning a finger **(Achoura and Belhamra, 2010; Benamor *et al.*, 2014)**.

I.4. Botanical Classification

Date palm (*Ph. dactylifera* L.) belongs to the family *Arecaceae* (previously known as *Palmaceae* or *Palmae*) **(Table I)**. *Phoenix* palms are monocotyledonous and dioecious, in which that the male and female parts are on separate plants, thus pollination is required for fruit-bearing **(Diaz *et al.*, 2003; Lobo *et al.*, 2014)**. This family is divided around 2.400 species in 183 genera **(Brokamp, 2015)**, while the genus comprises 14 recognized species **(Gros-Balthazard *et al.*, 2018)**. *Phoenix* species are morphologically very close and sometimes hardly distinguishable **(Pintaud *et al.*, 2010)**.

The date palm is the only domesticated species in the genus but the other species are also extensively used for ornamental purposes (*Ph. canariensis* and *Ph. roebelenii*), food for instance *Ph. sylvestris sap* **(Newton *et al.*, 2013)**.

In date palms, most of the female cultivars are recognized by their fruit characteristics such as size, shape, color, and taste. Morphologic characters of the tree are also taken into consideration for cultivar identification. For the male trees, cultivar identification is a cumbersome process because they mostly are seed-borne and are hardly identical to any female cultivar. However, farmers dealing with date palms can identify some male cultivars from their experience **(Simaozrag *et al.*, 2016)**.

Table I. Taxonomy of date palm **(Lobo *et al.*, 2014; Mirza *et al.*, 2019)**.

Classification units	**English name**	**Latin name**
Kingdom	Plants	*Plantae*
Subkingdom	Vascular plants	*Tracheobionta*
Super-division	Seed plants	*Spermatophyta*
Division	Flowering plants	*Magnoliophyta*
Class	Monocots	*Liliopsida*
Subclass	-	*Arecidae*
Order	-	*Arecales*
Family	Palm family	*Arecaceae*
Genus	date palm P.	*Phoenix* L.
Species	date palm P.	*Phoenix dactylifera* L.

I.5. Botanical description of date palm

Date palm tree consists of three parts, the roots system, vegetative organs and reproductive organs **(Figure 01)**.

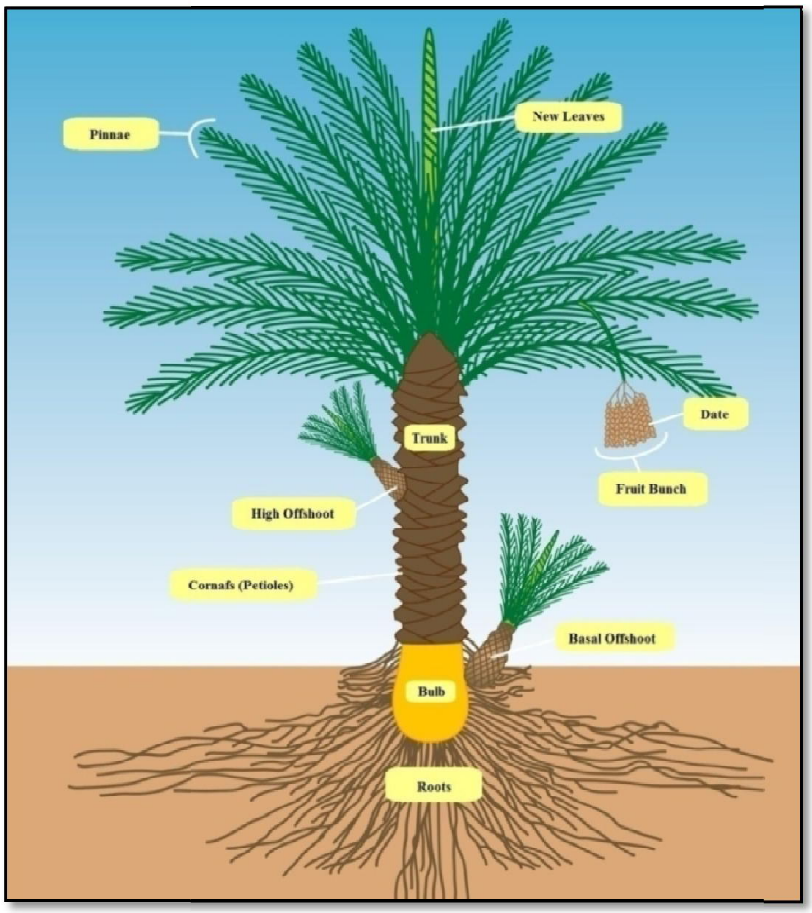


Figure 01. Schimatic diagram showing the morphology of the date palm **(Lisan, 2012)**.

I.5.1. Roots system

Date palm has a fasciculated fibrous root system that originates from a bulb at the trunk base. Primary roots give rise to secondary roots that further branch to form tertiary roots that are shorter in length and diameter **(Table II)**. Primary roots originate from seeds **(Figure 02)** but may also continue to grow if date palm is grown from an offshoot or a tissue-cultured seedling **(Al-Yahyai and Manickavasagan, 2012)**. The date palm root system is divided into four zones **(Figure 03) (Peyron, 2000)**:

Zone I: The respiratory roots are used for gas exchange. They sometimes develop very high at the base of the trunk, or stipe, growing under the petiolar bases of palms, kornafs or cornafs. They are then aerial roots. The underground respiratory roots have few rootlets. This system plays an important role, and necessary for the palm tree, in gas exchanges with the air of the soil atmosphere;

Zone II: The roots of nutrition constitute the highest proportion of the roots of the system. They are very long, oblique or horizontal. They have many rootlets and can develop well beyond the projection area of the foliage of an adult palm;

Zone III: The absorption roots have the function of seeking water. The area of these roots is more or less developed depending on the type of culture and on the depth of underground water;

Zone IV: The roots of the pivoting beam, the pivot of absorption roots is almost nonexistent if the culture line allows sufficient absorption at the level of the nutrition and absorption roots. It is reduced if the water table is at a shallow depth. But if necessary, this true root pivot can reach water up to a depth of 17 meters.

Table II. Morphological description and distribution of date palm root system **(Zaid and Wet, 2002)**.

Roots Order	Origin	Form	Average length (m)	Average diameter (mm)	Characteristics
Primary	Trunk base	Cylinder	4 (up to 10)	9.5 (7-12.5)	- Vertical. - Adventitious. - No root hair. - Conic tip. - Called auxirhyzes and also main roots.
Secondary	Primary roots	Similar to primary roots	0.20 - 0.25	3.5	- Called mesorhyzes.
Tertiary	Secondary roots	Similar to secondary roots but thin	0.02-0.1	0.3 - 1.5	- Low growth. - Short and abundant. - Called brachyrhizes.

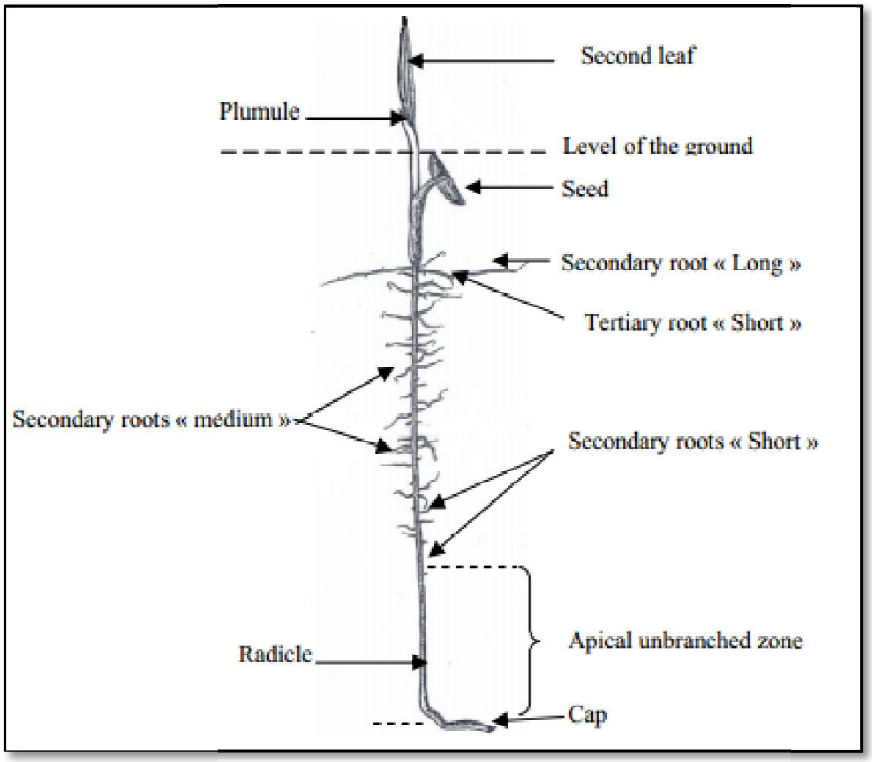


Figure 02. Root system of the date palm seedling, 64 days after germination **(Jrad and Ben salah, 2014)**.

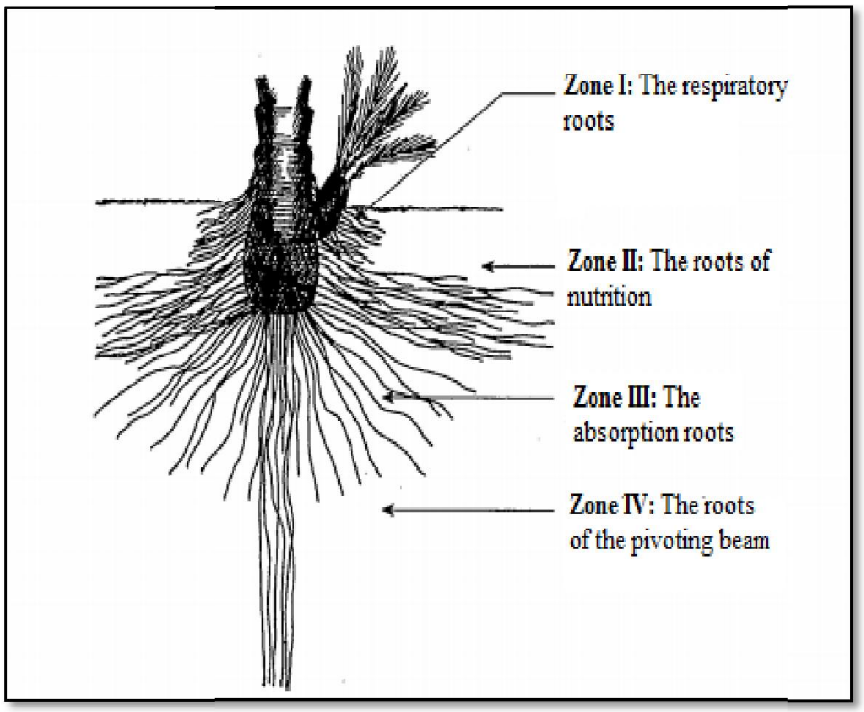


Figure 03. The four types of roots **(Dupont, 2018)**.

I.5.2. Vegetative organs

I.5.2.1. Trunk

The date palm trunk, also called stem or stipe, it is an erect columnar trunk, 40 to 50 cm in diameter (without leaf sheaths) that may reach a height of 20 to 28 m or more. The trunk may have one to several suckering offshoots at or near the base **(Zaid and Wet, 2002; Janick, 2008)**.

The trunk is covered with leaf bases which called corns that are enclosed in fiber, an evolutionary mechanism to protect the trunk from herbivorous insects and animals, as well as an insulation to reduce water loss **(Peyron, 2000; Al-yahyai and Manickavasagan, 2012)**. The trunk has only a single terminal bud (phyllophor), and like nearly all other monocotyledons, lacks a cambium cylinder, sometimes date palms show a branching phenomenon which is a result of either dichotomy, axillary bud development, polyembryony or attack by a disease **(Nixon, 1951; Zaid and Wet, 2002)**.

I.5.2.2. Leaves

Date palm leaves, called fronds, are pinnate, compound leaves spirally arranged around the trunk **(Dransfield and Uhl, 1998) (Figure 04)**. These leaves are subtended by a cylindrical sheath of rough reticulate mass and fibrous material at its base which forms a firm protective covering for terminal bud **(Ghori *et al.*, 2018)**.

The leaves of the date palm have a lifespan of 3 to 7 years, the color ranges from light to dark shades of green. The leaves are glaucous in different degrees depending on the variety

and age of the leaves. The leaves are 3 to 7 m in length, leaves with a blade length less than 335 cm are considered short, from 335 to 427 cm as medium, and long if more than 427 cm **(Zaid and Wet, 2002; Janick, 2008)**.

The midrib or petiole is triangular in cross-section, with its greatest width at the base, tapering rapidly towards the apex, are vertical in orientation, and are armed with rows of pinnae (also referred to as leaflets) on each side **(Janick, 2008)**.

The pinnae are long, narrow and folded upward and lengthwise. Varieties differ in the number, thickness, length, breadth and relative stiffness of the pinnae and how they droop or curve down. The leaves have terminal leaflets, and the basal leaflets are modified into spines **(Janick, 2008)**.

Spines, also called thorns, vary from a few cm to 24 cm in length and from a few mm to 1 cm in thickness. They are differentially arranged on the two outer edges of the fronds while their number varies from 10 to about 60. Spines can be single, in groups of two, or groups of three **(Zaid and Wet, 2002)**.

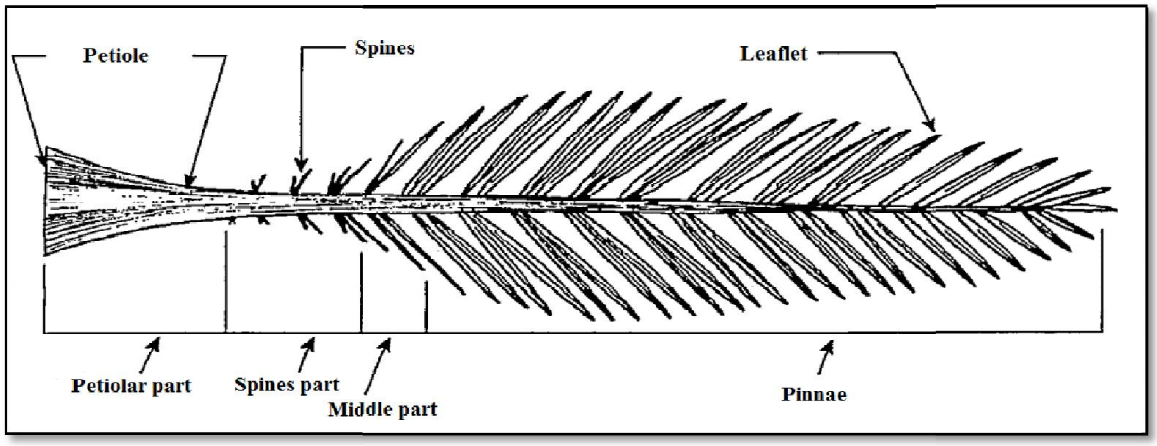


Figure 04. Date palm leaves **(Peyron, 2000)**.

I.5.3. Reproductive organs

The date palm is dioecious with separate male and female trees **(Mohmoud *et al.*, 2019)**.

I.5.3.1. Inflorescences

The unisexual flowers are borne in a big cluster (inflorescence) called spadix or spike, which consists of central stem called rachis and several strands or spikelets (usually 50-150 lateral branches) **(Gammodi *et al.*, 2016)**.

The inflorescence, also called flower cluster, in its early stages is enclosed in a hard covering/envelope known as spathe which splits open as the flower mature exposing the entire inflorescence for pollination purposes **(Gammodi *et al.*, 2016) (Figure 05)**.

I.5.3.2. Flowers

The flowers are unisexual with a very short peduncle. They are ivory in color, yellow-greenish depending on the sex and the cultivar or variety **(Sedra, 2012) (Figure 06)**.

Both female and male flowers usually have three sepals and three petals. Female flowers **(Figure 7)** usually are yellowish green, rounded, it has a diameter of about 3 to 4 mm and has rudimentary stamens and three carpels closely pressed together and the ovary is superior (hypogynous). Whereas the male flowers **(Figure 07)** are usually waxy white, flat, glabrous with a sweet scented, normally has six stamens and each stamen is composed of two little yellowish pollen sacs **(Zaid and Wet, 2002; Qureshi *et al.*, 2006; Chao and Krueger, 2007)**.

Throughout the years, there have been various reports of hermaphroditic trees developing, or male trees developing female characteristics **(Chao and Krueger, 2007)**. The male inflorescences bear atypical "bisexual" flowers. The shape of the bisexual flower inflorescence is conical and generally found at the base of the inflorescence. They are persistent while the normal male flowers are deciduous **(Dahr *et al.*, 2015)**.

Pollen from the male flower must be collected by hand and transported to the females mechanically. In nature, some pollination takes place by wind, but it does not facilitate the production of commercial crops **(Robinson, 2002)**.

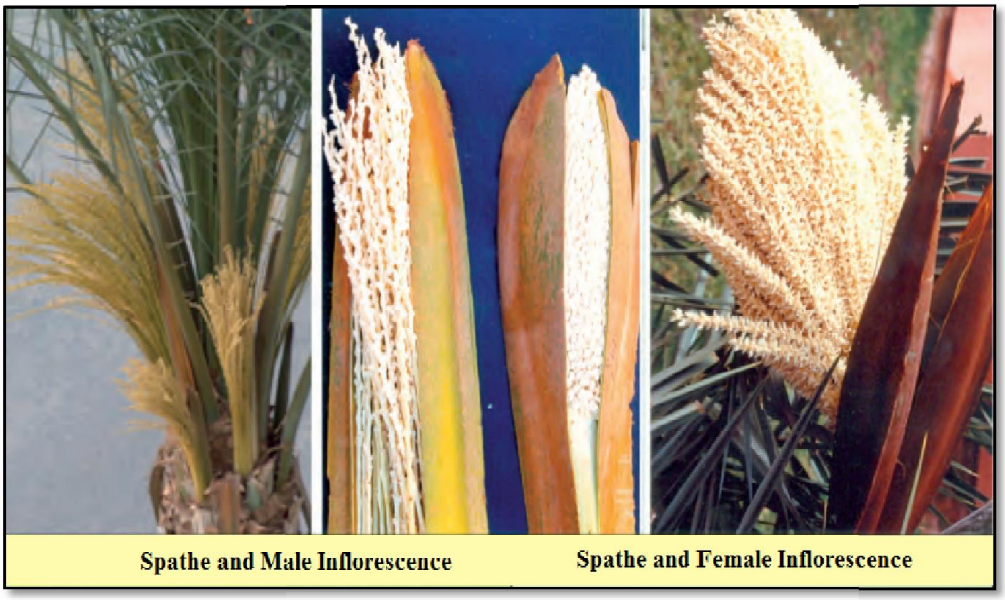


Figure 05. Spathes and inflorescences of date palm **(Sedra, 2012)**.

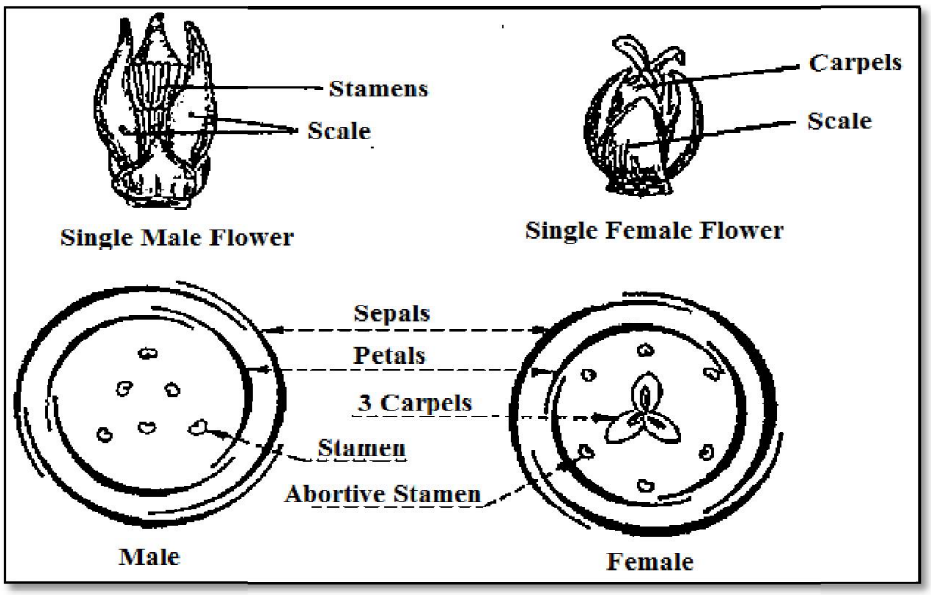


Figure 06. Date palm flowers (Male and Female) **(Zaid and Wet, 2002)**.

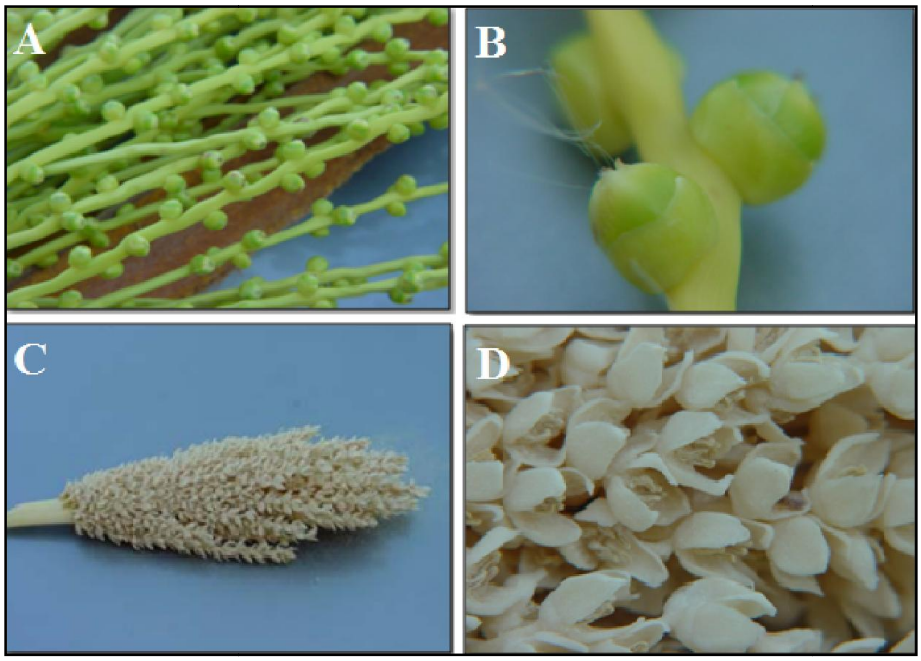


Figure 07. Male Female flowers of date palm:(A) Female flowers, (B) Female flowers close up, (C) Male flowers, (D) Male flowers close up **(Robinson, 2002)**.

I.5.3.3. Fruits (Dates)

The fruit of date palm, is a drupe known as a date **(Figure 08, 09)**, develop from one fertilized ovule forming one carpel, while the other two ovules are aborted but remain visible at the fruit calyx. If no fertilization occurs, three or more carpels develop simultaneously. Dates are large with a thick layer of fruit pulp, edible, rich in sugars. They are oval or cylindrical, 3-7 cm long and 2-3cm in diameter. Each date contains a single seed about 2-3 cm and 6-8 mm in thickness. The skin is thin and papery **(Figure 10)**. When unripe, they are green, and change to yellow, golden brown, mahogany red or black as they ripen, depending on the variety **(Al-yahyai and Manickavasagan, 2012; Khan and Khan, 2016)**.

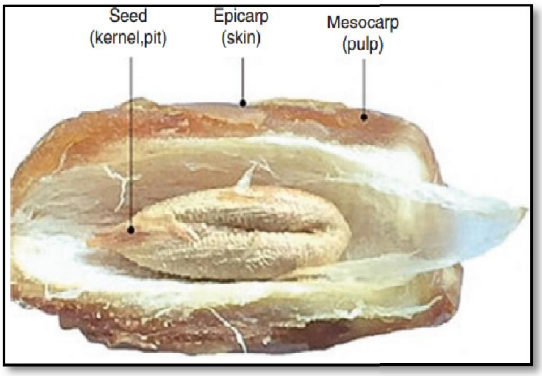


Figure 08. The anatomy of the date fruit at Tamar stage **(Ramadan, 2019)**.

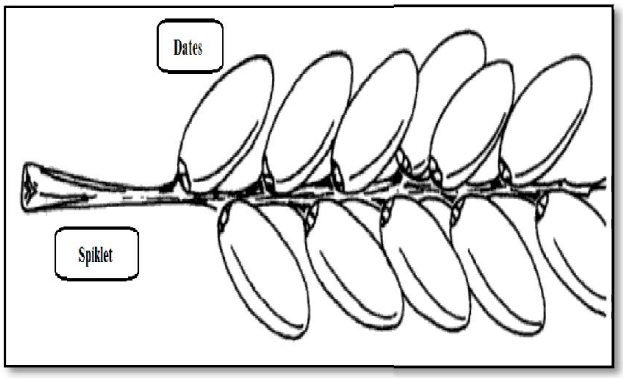


Figure 09. Date palm fruit and spiklet **(Peyron, 2000)**.

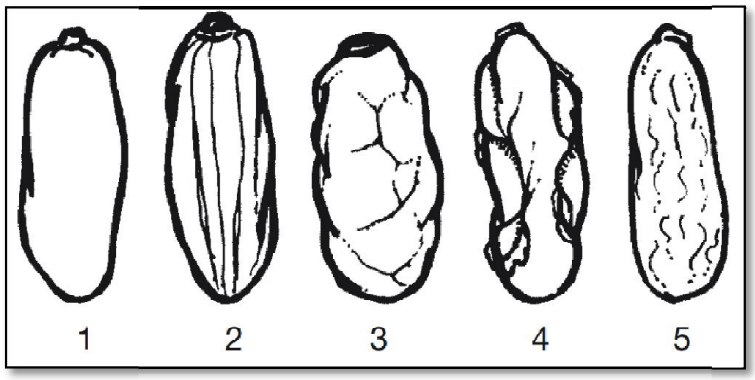


Figure10. Forms of the date Epicarp: 1.Smooth, 2. Pleated, 3. Embossed, 4. Blistered, 5. Tattooed **(IPGR, 2005)**.

I.5.3.3.1. Seeds

Seed characteristics vary according to variety, environmental and growing conditions. A seed's weight could range from less than 0.5 g to about 4 g, in length from about 12 to 36 mm and in breadth from 6 to 13 mm. The seed is usually oblong, ventrally grooved, with a small embryo and with a hard endosperm made of a cellulose deposit on the inside of the cell walls **(Figure 11) (Ziad and Wet, 2002)**.

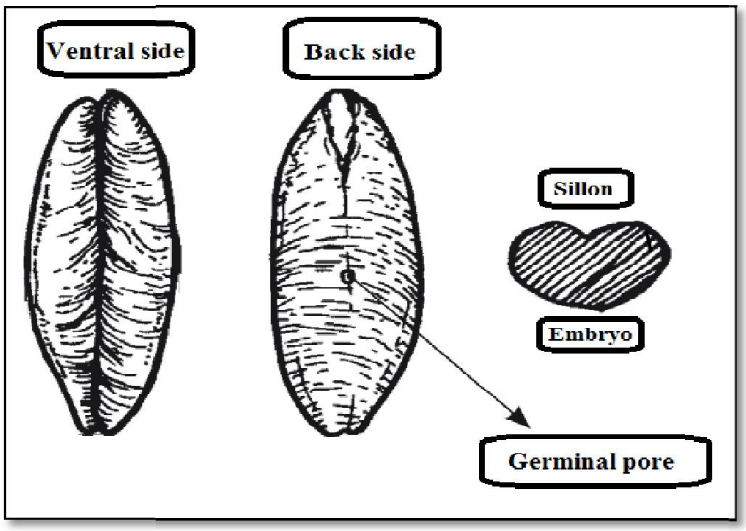


Figure 11. Longitudinal and cross section of date palm seed **(IPGR, 2005)**.

I.5.3.3.2. The growth stages of a date fruit

The stages of pre-maturation, maturation and ripening of date are: Hababauk, Kimri, Khalal, Rutab, and Tamer **(Figure 12) (Al-Mssallem *et al.*, 2013)**.

Depending on the maturity stages during growth and development of the date, different external and internal changes are observed with color, sweetness, texture and chemical composition **(Al-Alawi *et al.*, 2017)**.

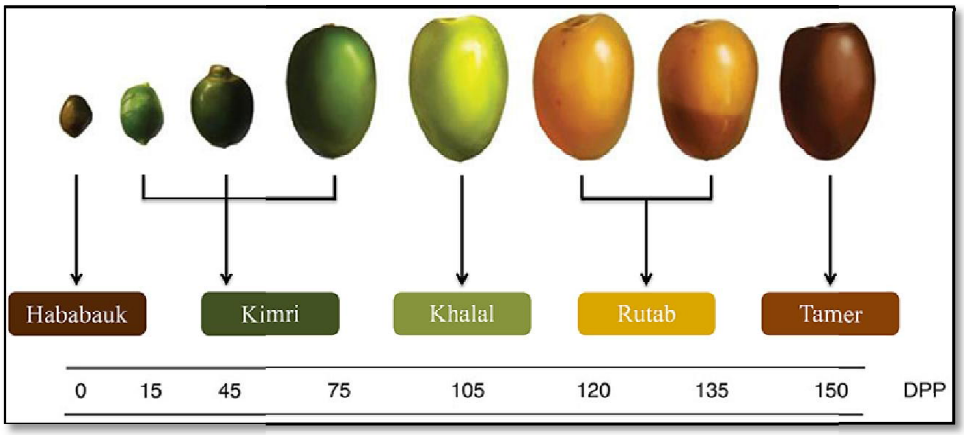


Figure 12. The five growth stages of a date fruit by days post pollination (DPP) **(Al-Mssallem *et al.*, 2013)**.

I.5.3.3.3. Types of date fruits

There are three main types of date fruits which are known as soft, semi-dry and dry **(Table III)**. The classification of date palm fruits based on the texture of the ripe fruit, is related to the moisture content and different forms of sugar as well as sugar acidity. The differences between the soft, semi-dry and dry types of dates are also in phenotype and post-harvest treatment to climatic requirements **(Al-Khayri *et al.*, 2015; Diboun *et al.*, 2015)**.

Table III. Classification of date palm fruits based on the texture and sugar content **(Lim, 2012; Ramadan, 2019)**.

Date taypes	Moisture %	Sucrose sugar %	Redusing sugar %
Soft	> 30	Low	> 70
Semi-dry	20-30	18-30	45-54
Dry	< 20	33-46	33-46

I.6. Date palm propagation

The techniques used to propagate date palm are seed propagation, offshoot propagation and tissue culture (micro-propagation). Seed propagation is by far the easiest and quickest method; however, half of the seed progeny are male and half are female, with no easy and reliable way of determining sexuality at an early stage. Furthermore, female plants originating from seedlings usually produce inferior quality fruits which are of limited value commercially **(Zaid *et al.*, 2011)**.

Offshoots develop from axillary buds on the trunk of the mother plant; consequently the fruits produced are of the same quality as the mother tree. A limited number of offshoots are produced during the lifetime of a date palm. Before transplanting, the offshoots usually must remain attached to the parent tree until an adequate root system develops **(Tisserat, 1979)**. The final method of propagation, and most widely used is tissue culture or in vitro techniques (micro-propagation), the use of these techniques allows for large-scale production of healthy, disease-free, true-to-type plants **(Al-Khayri and Naik, 2017)**.

I.7. Development cycle of date palm

The development cycle the date palm generally comprises four phases **(Benzohra, 2019)**:

a) Young phase: growth and development (5 - 7 years);

b) Juvenile phase: period of entry into production (30 years);

c) Adult phase: beginning of production decline (60 years);

d) Senescence phase: fall in production (80 years and over).

I.8. Vegetative cycle of date palm

It is possible to distinguish five vegetative phases of palm growth **(Table IV) (Bounaga, 1993)**.

Table IV. Vegetative phases of date palm growth.

Phases	Description of phases
Seed	The seedat maturity, the ripe embryo is limited to a cotyledon, where the seedling is formed.
Germinative phase	There are two phases during germination: preparation phase which marks the end of the maturation of the embryo and germination phase proper.
Plant construction	The establishment phase, at which the plant becomes autotrophic.
The vegetative adult phase	The date palm will build its trunk and acquire its "palm port" by a continuous extension of the vegetative axis.
The adult reproductive phase	Only at this stage that one can recognize its sex, resulting from seedlings and also It is from this phase that the date palm returns to production.

I.9. Geographical distribution

I.9.1. Geographical distribution in the world

Date palm is found in n the arid and semi-arid hot areas of the Old World, at latitude between 15° N and 35° N north and south of Africa and south of Spain, the Middle East, Pakistan and North West of India. On the other hand, in the XXI e century, it has been introduced by seeds in Australia and the United States, in particular in Southern California **(Benzohra, 2019) (Figure 13)**. It should be noted that to have a clear picture on the

geographical distribution of date palm, it is worth looking at it from the following aspects; distribution according to latitude, distribution according to altitude and number of date palms in the world **(Zaid and Wet, 2002)**.

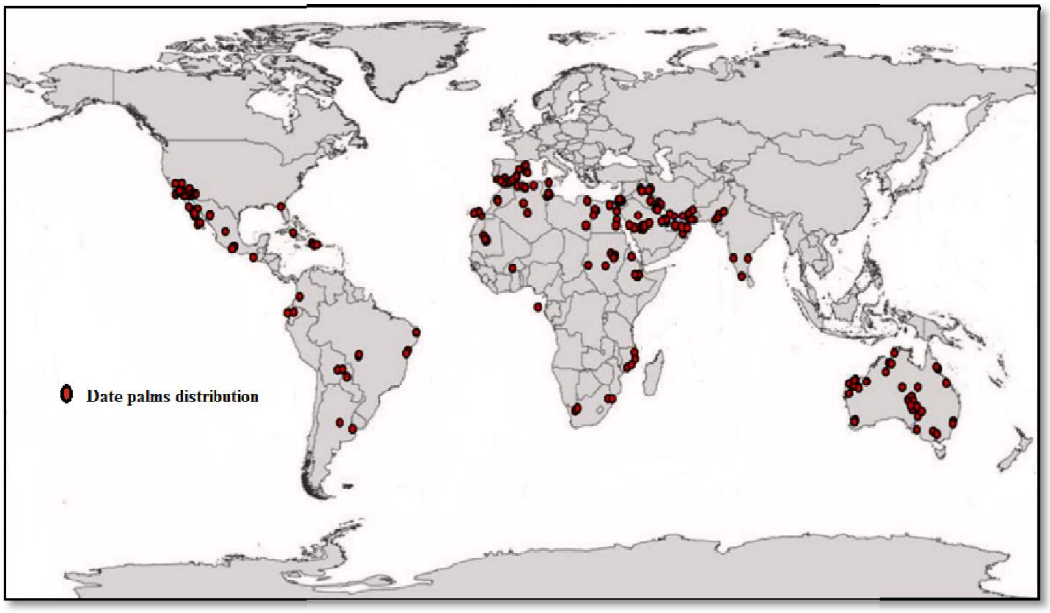


Figure 13. Global map distribution of date palm **(Shabani *et al.*, 2012)**.

I.9.2. Geographical distribution in Algeria

The Algerian palm grove has a rich and diverse phoenicultural heritage made up of 940 different cultivars **(Guettouchi *et al.*, 2015) (Table V)**, it occupies an area estimated at 167.000 hectares for an estimated number of palm trees over 18.6 million units and a date production of nearly 990.000 tonnes **(Atallaoui *et al.*, 2017)**. This palm grove is mainly located in the areas of the south-eastern part of the country **(Achoura and Belhamra, 2010)**.

Table V. Cultivar of the three Algerian date palm areas **(Bouguedoura *et al.*, 2015)**.

Area	Region	Location	Number of cultivars identified/Reported	Some Names of culivars identified
East	Ziban	Biskra, Tolga	9/140	Arechti, Deglet Noor,Ghars, Ghazi.
	Oued Souf	El Oued El Meghaier Djamaa	37/70	Tantboucht, Tinicine, Zoggar,Moggar, Tachlikt, Tarmount, Zaghraya, Zehdi,
	Oued Righ	El Arfiane Ouargla Touggourt	22/200	Aliyane, Beidh H'mam, Bentqbala, Bouldjib, Hamraya, Tafezwin, Akermoust,
	Aures	Khenchela	3/200	Buzrur, 'Alig, Buhles, Mech Degla, Tanghimen, Tabanist
	Tassili	Batna	3/180	
Center	M'zab	Ghardaia Berriane Guerrara Zelfana	26/140	Tamezouaret, Tanaguarout, Tanetboucht, Adham Bent Q'bala, Ajujil, Baydir, Bent Q'bala,
West	Touat	Adrar Timimoun	8/190	Bamekhlouf, Feggus, Hmira, Ouarglia, Takerbucht Hamra, Taqerbucht Safra
	Saoura	Bechar Béni Abbes	14/180	Adham Boula, Adham Tirnou, Cherka, Deglet Talmine, Feggus, Hmira, Hartan,
	Tidikelt	-	4/60	Tgazza, Taqerbucht, Cheddakh, Agaz

I.10. Ecological requirements

I.10.1. Climate requirements

The date palm belongs to the arid semi-arid regions, it grows luxuriantly in a wide range of warm climates **(Ighbareyeh *et al.*, 2015)**, it is cultivation adapted only to region s marked by high temperature combined with low humidity. The optimal temperatures of date palms ranged between 10 °C and 40 °C **(Figure 14)**. In addition, humidity is an important factor with many varieties **(Peyron, 2000; Sonakke, 2016)**.

The date palm cultivation area is in a very bright area; this last factor is essential for the production of dates. The date palm withstands wind well; however, the latter can determine various accidents. If they are light in spring they promote pollination but on the other hand, they carry pollens when they are violent and also cause fruit to fall. But the most dangerous are the hot and drying winds that cause dates to scald **(Tourern, 1967)**.

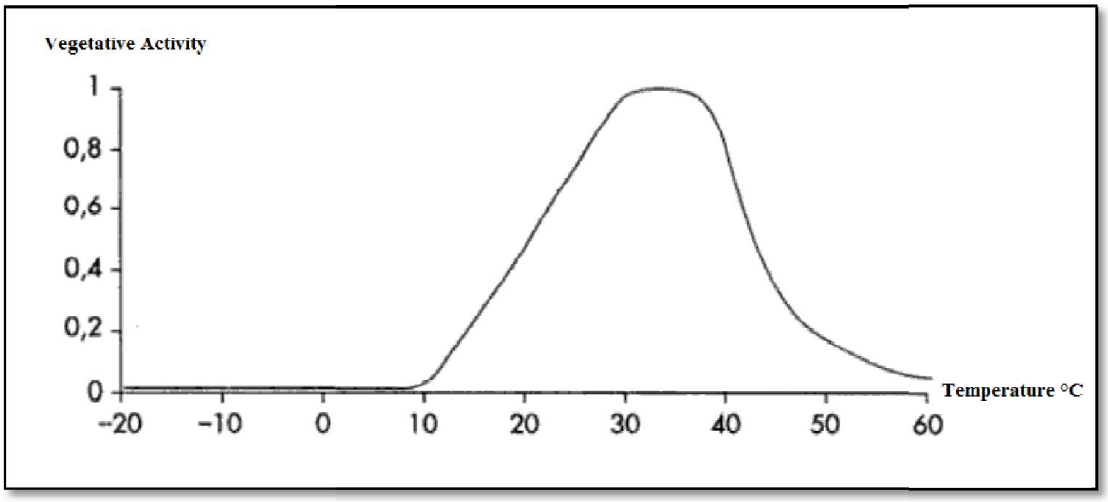


Figure 14. Vegetative activity as a function of temperature **(Peyron, 2000)**.

I.10.2. Hydrique requirements

For maximum growth and fruit production, the date palm requires an abundance of water. The finest dates of the Sahara Desert are those grown in oases which receive a continuous and dependable supply of water either by gravity from high bodering mountains, or from artesian sources **(Nixon, 1951)**.

Although fruit production requires an adequate supply of water, the date palm, because of its unique structure, is able to survive long periods of drought. For this reason it is widely grown in desert areas where irregular and erratic supplies of water are insufficient to sustain other fruit trees **(Nixon, 1951)**.

I.10.3. Edafique requirements

Date palms are grown in a wide variety of soil **(Janick, 2008)**, but soil with maximum water-holding capacity consistent with good drainage is desirable **(Fauchier, 2003)**. The palm

clearly shows its preference for light soils with low clay content. the soil pH generally varies between 7.5 and 8. Similarly, saline soils are fearsome because they lead to a lower yield **(Mimoun and Bennaceur, 2019)**.

I.10.4. Nutrient requirements and fertilisation

Date palms have deep root systems capable of searching for nutrients in depth. Date palm can growth and form fruit for several years in moderately fertile soil without fertilization additional. However, The application of organic manure is recommended for poorly fertile soils **(Table VI) (Alaoui, 2005)**.

It has been shown that there is a stronger effect of nitrogen in terms of yield and quality than Phosphorous and Potassium. The effect of Phosphorous and Potassium on yield and quality is not proven **(Augstburger, 2002)**.

Table VI. Average manure to be brought to the date palm **(Touren, 1967)**.

Date palm age	**Manure (kg)**	**N Annual dose (g)**	**P_2O_5 Annual dose (g)**	**K_2O**
Plantation	Background manure			Generally, soils contain sufficient quantities. To be verified by analyzes.
6 years	40	250	50	
9 years	60	350	60	
12 years	80	450	70	
15 years	90	475	75	
21 years	100	500	80	

I.11. Importance of date palms

Date palm is a multi-purpose tree, being highly regarded as a national heritage in many countries. its tolerance to harsh environmental desert conditions and provides food, timber products and all parts of the palm can be used **(Mahmoudi *et al.*, 2008)**.

I.11.1. Ecological benefits

There are many environmental advantages of planting palm trees, it has an important role in enhancing ecological balance and reducing desertification, besides palm trees have a big potential in absorbing CO_2 from the atmosphere. Compared to other plants of a comparable size, the palm tree needs a minimal amount of water **(Sharif *et al.*, 2010)**. Date palm reduces macro-climate and micro-climate temperatures allowing another shade-loving crop (sciophytes) to flourish and conserves soil from all types of erosion **(Ibrahim, 2010)**.

I.11.2. Nutritional and midicinal value of date palm

Ph. dactylifera is a useful traditional medicinal plant, all its products such as fruits, seeds, pollen, leaves, and syrup have beneficial uses for human and animals (**El-Far *et al.*,**

2018). The pharmacological studies conducted on *Ph. dactylifera* indicate the immense potential of this plant in the treatment of conditions such as gastric ulcer, cardiovascular disorder, inflammatory ailments.. etc **(Vyawahare *et al.*, 2008)**.

The date fruit and its by-products, have both nutritional (macro- and micronutrients) and medicinal value **(Maqsood *et al.*, 2019; Hussain$_{(a)}$ *et al.*, 2019)**, including activities; anti-mutagenic, antioxidant, anti-microbial, anti-inflammatory and anti-cancer **(Baliga *et al.*, 2010; Khalid *et al.*, 2017)**.

I.11.3. Economic importance of date palm

The date palm is considered one of the main fruit crops In the world **(Figure 16)** and specially in Arab countries **(Figure 17)**, because it possesses the majority of the world's date palms and produces the major portion of the world's total date crop **(Baloch *et al.*, 2014)**. Multiple usages and economic importance in ecological improvement of the deserts make date palm a good choice for farmers **(Hassan *et al.*, 2006)**. It is one of the fruit trees with the highest production per hectare **(Ata *et al.*, 2012)**. Date palm leaves are often used in insulating board, given their richness in fiber. They also represent a source of fuel and raw material **(Al- Hadrami and El Khayri, 2012)**.

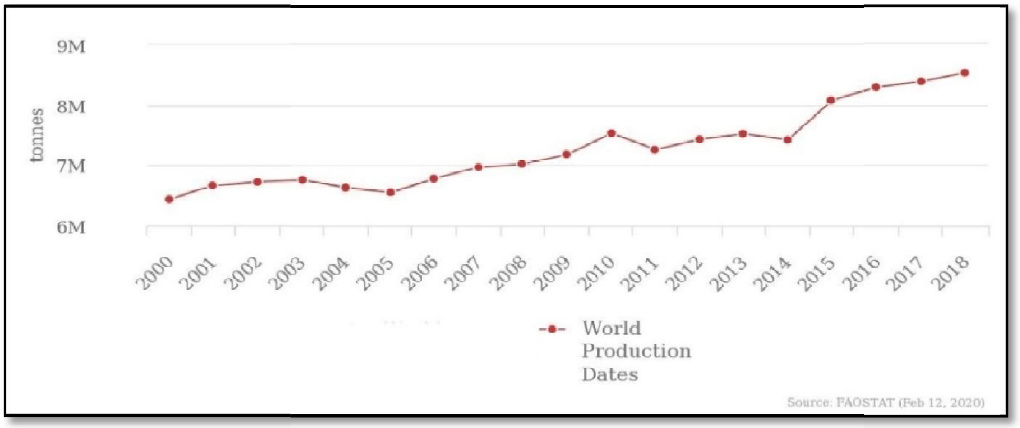


Figure 15. Development of production quantities of dates in world **(FAOSTAT, 2020)**.

I.12. Phytochemical and secondary metabolism

The organism's life depends on photosynthesis process to obtaining the organic materials to be used for metabolic processes **(Nimir and Guisheng, 2018)** which considered all of the biochemical processes enabling cells to produce metabolites and the energy that is necessary for their life, through the degradation of complex organic materials, metabolism divided to: **primary metabolism** which is all catabolic and anabolic pathways supplying the body with the energy and molecules required for physiological structures and activity (respiration, transport, assimilation, growth, etc), the most important are amino acids, carbohydrates, organic acids, nucleotides and vitamins **(Marouf and Reynaud, 2007)**, while

secondary metabolite is a metabolic intermediate or product, found as a differentiation product in restricted taxonomic groups, not essential to growth and life of the producing organism **(Verpoorte and Alfermann, 2013)**, but important and can protect plants against a wide variety of microorganisms (viruses, bacteria, and fungi) and herbivores (arthropods, vertebrates) and serve survival functions for the organisms producing them **(Tiwari and Rana, 2015)**.

I.12.1. Classification

Secondary metabolites can be classified on the basis of chemical structure (for example, having rings, containing a sugar), composition (containing nitrogen or not), their solubility in various solvents, or the pathway by which they are synthesized (e.g., phenylpropanoid, which produces tannins) **(Tiwari and Rana, 2015)**. Based on their biosynthetic origin, they can be divided into three major groups: alkaloids, terpenoids, and polyphenols **(Crozier *et al.*, 2006)**.

I.12.1.1. Alkaloids

Alkaloids are a group of molecules with a relatively large occurrence in nature around the Globe. They are very diverse chemicals and biomolecules, but they are all secondary compounds and they are derived from amino acids or from the transamination process **(Aniszewski, 2007)**. Alkaloids are low-molecular-weight nitrogen-containing compounds and, due to the presence of a heterocyclic ring containing a nitrogen atom, are typically alkaline **(Matsuura and Fett-Neto, 2017)**.

Alkaloids produced by a large variety of organisms, including bacteria, fungi, plants, and animals. Many alkaloids can be purified from crude extracts by acid-base extraction, and many of them are toxic to other organisms **(Kakhia, 2011)**.

Alkaloids classification based on chemical structures to two broad divisions **(Evan, 1996)**:

a) Non-heterocyclic or Atypical alkaloids, sometimes called ‘protoalkaloids’ or biological amines, such as: Colchicine, Mescaline, Erythromycin … etc.

b) Heterocyclic or Typical alkaloids, divided into 14 groups according to their ring structure like: Pyrrolizidine, Quinolin, Isoquinoline… etc.

Alkaloids are important for the well-being of the organism that produces them. One of the main functions is that of chemical defense against herbivores or predators. Some alkaloids are antibacterial, antifungal, and antiviral; and these properties may extend to toxicity towards animals. Alkaloids can also be used by plants as herbicides against competing plants **(Fattorusso and Taglialatela-Scafati, 2008)**.

I.12.1.2. Terpenoids

Terpenoids are modified class of terpenes (simple hydrocarbons) with different functional groups and oxidized methyl group moved or removed at various positions **(Perveen, 2018)**. Most natural terpenoids hydrocarbon have the general formula $(C_5H_8)_n$. They can be classified based on the value of "n" or the number of carbon atoms present in the structure **(Table VII) (Yadav *et al.*, 2014)**.

Terpenoids are vital for the growth and survival of photosynthetic organisms, since they play an essential role in the conversion of light into chemical energy and for assembly and function of photosynthetic reaction centers (chlorophylls, bacteriochlorophylls, rhodopsins, and carotenoids). Other known functions of plant terpenoids include important roles in stress response or in defense mechanisms, with such a wide range of biological functions, terpenoids have extensive applications in the fields of pharmaceuticals, cosmetics, colorants, disinfectants, fragrances, flavorings and agrichemicals **(Pattanaik and Lindberg, 2015)**.

Table VII. Classification of Terpenoids **(Yadav *et al.*, 2014)**.

Number of carbon atoms	Value of n	Class
10	2	Monoterpenoids (C_{10} H_{16})
15	3	Sesquiterpenoids (C_{15} H_{24})
20	4	Diterpenoids (C_{20} H_{32})
25	5	Sesterpenoids (C_{25} H_{40})
30	6	Troterpenoids (C_{30} H_{48})
35	7	Tetraterpenoids (C_{40} H_{64})
>40	>8	Polyterpenoids (C_5 H_8)n

I.12.1.3. Polyphenols

Phenolic or polyphenol compounds are secondary metabolites characterized by the presence of an aromatic ring carrying free or engaged hydroxyl groups with a carbohydrate. They are present in all parts of higher plants (roots, stems, leaves, flowers, pollens, fruits, seeds, and wood), and are involved in many physiological processes such as cell growth, rhizogenesis, seed germination or maturation fruits **(Boizot and Charpentier, 2006)**.

Over 8000 phenolic compounds have been isolated from different natural products, including flavonoids, phenolic acids, coumarins, and tannins. Each group is further divided into subgroups based on chemical structure **(Erdman *et al.*, 2007)**.

Natural polyphenols showed several of various biological activities and the preventive and curative health benefits of such diseases especially those related to age **(Li *et al.*, 2014)**. The following **Table VIII** expresses the most important biological activities of polyphenols.

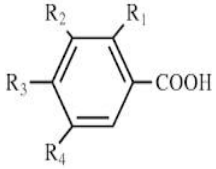
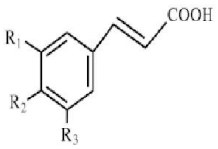
Table VIII. Polyphenols biological activity.

Biological activity	Role	Reference
Anti-oxidant Activity	Polyphenol action is to inhibit the activity of enzymes which participate in the creation of reactive forms of oxygen.	**(Kurek-Górecka *et al.*, 2014).**
Anti-cancer Activity	Polyphenols influence the metabolism of procarcinogens by modulating the expression of cytochrome P450 enzymes involved in their activation to carcinogens (in smouth, stomach...etc).	**(Johnson *et al.*, 1994; Scalbert *et al.*, 2005).**
Anti-diabetic Activity	Polyphenols modulate and maintenance of insulin secretion and reduce the activity of enzymes, like α-amylase and α- glucosidase, involved in the release of glucose.	**(Abbas *et al.*, 2017).**
Anti-inflammatory Activity	Polyphenols can inhibit the pro-inflammatory enzymes, and inhibit some cellular signals of inflammatory process.	**(Santangelo *et al.*, 2008; Li *et al.*, 2014).**

I.12.1.3.1. Phenolic acids

Phenolic acids constitue one of the major parts of phenolics, they account for about one-third of dietary phenolics. Phenolic acids are the phenols possessing one carboxylic acid, they can be in free or bound form in plant cell, but are mostly in the bounded form. Two classes of phenolic acids can be distinguished **(Table IX)**: derivatives of benzoic acid and derivatives of cinnamic acid; hydroxybenzoic and hydroxycinnamic **(Yildiz, 2009)**.

Table IX. Different types of phenolic acids **(Macheix, 2005; Bruneton, 2009)**.

Class	General characteristics	Basic chemical structure	Types	
Hydroxybenzoic Acids	-Derived from benzoic acid. - C_6-C_1 -Free more than bounded -Founded in ester or heteroside state		R_1=R_2=R_3=R_4=H R_1=R_2=R_4=H ,R_3=OH R_1=R_4=H, R_2=R_3=OH R_1=R_4=H,R_2=OCH_3, R_3=OH R_1=H, R_2=R_3=R_4=OH R_1=H, R_2=R_4=OCH_3,R_3=OH R_1=OH, R_2=R_3=R_4=H R_1=R_4=OH, R_2=R_3=H	Benzoic acid P-hydroxybenzoic acid Protocatechic acid Vanillic acid Gallic acid Syringic acid Salicylic acid Gentisic acid
Hydroxy-cinnamic Acids	-Dirived from cinnamic acid -C_6-C_3 Rarely free -Often esterified		R_1=R_2=R_3= H R_1= R_3= H, R_2=OH R_1=R_2=OH, R_3= H R_1= OCH_3,R_2=OH,R_3= H R_1= R_3= OCH_3, R_2=OH	Cinnamic acid P-coumaric acid Caffeic acid Ferulic acid Sinapic acid

I.12.1.3.2. Tannins

Tannins are water-soluble phenolic compounds having molecular weights between 500 and 3000 and besides giving the usual phenolic reactions, having special properties such as the ability to precipitate alkaloids, gelatin, and other proteins **(Moreno-Arribas and Polo, 2009)**. It can be classified into three broad groups according to the solubility; hydrolysable tannins, condensed tannins, and pseudo-tannins **(Table X)**, also they can be classified into four groups according to the structural characteristics (**Table XI) (Hussain** $_{(b)}$ ***et al.*, 2019)**.

Table X. Classes of tannins according to solubility **(Ashok and Upadhyaya, 2012)**.

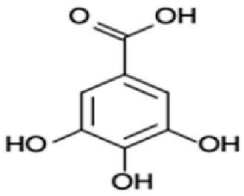
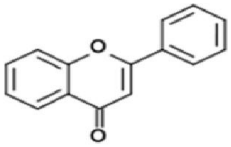
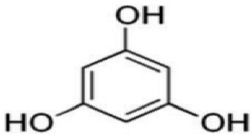
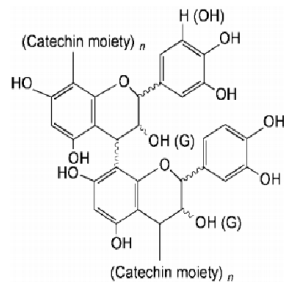
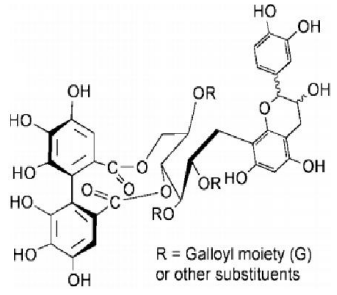
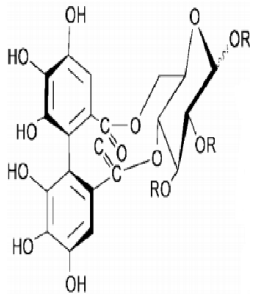
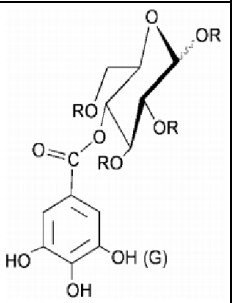
Class	Properties	Basic unit
Hydrolyzable Tannins	- At the center there is a carbohydrate (usually D-glucose) partially or totally esterified with phenolic groups. - Water soluble.	
Condensed Tannins	- Polymers of 2 to 50 (or more) flavonoid units. - Insoluble in water.	
Pseudotannins	- Sub group with low molecular weight compounds.	

Table XI. Classes of tannin according to structural characteristics **(Khanbabaee and van Ree, 2001)**.

Condensed Tannins	Complex Tannins	Ellogitannins	Gallotannins
			

I.12.1.3.3. Coumarins

Coumarins, a class of natural products with a characteristic fragrant odor, are common to numerous plants. Coumarin is the lactone derived from cis-ortho-hydroxycinnamic acid, known as benzo-α-pyrone or benzo-1,2-pyron **(Figure 16) (Xu *et al.*, 2011)**.

There are different classifications for the coumarin derivatives. Generally, they can be chemically classified according to the most common cores: simple coumarins, complex coumarins, and various coumarins. Therefore, they can be classified as: simple coumarins,

furocoumarins, dihydrofurocoumarins, pyranocoumarins (linear and angular), phenylcoumarins, and biscoumarins **(Matos *et al.*, 2015)**.

Coumarins have different therapeutic role: malignant melanoma, renal cell carcinoma, prostate cancer, leukemia, breast cancer, cervical carcinoma, Skin disorders, Anticoagulant **(Rohini and Srikumar, 2014)**.

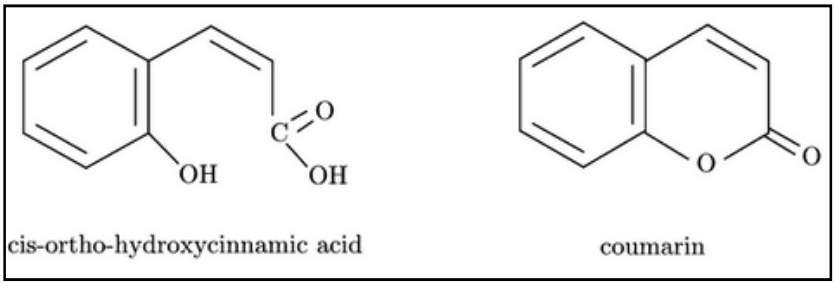


Figure 16. Chemical structure of coumarin and cis-ortho-hydroxycinnamic **(Xu *et al.*, 2011)**.

I.12.1.3.4. Flavonoids

Flavonoids are natural pigments that give plants their colors **(Causse, 2004)**, they are found in all its parts (roots, stems, flowers, and leaves) **(Cazaubon, 2005)**. Chemically flavonoids are based upon a fifteen-carbon skeleton consisting of two benzene rings (A and B as shown in **Figure 17** linked via a heterocyclic pyrane ring (C) **(Kumar and Pandey, 2013)**. Flavonoids occur both in the free-state and as glycosides; they are products of both the shikimic acid and acetate pathways, being formed by the condensation of a phenyl- propanoid precursor with three malonyl coenzyme A units **(Pengelly, 2004)**.

Flavonoids can be subdivided into different subgroups depending on the carbon of the C ring on which the B ring is attached and the degree of unsaturation and oxidation of the C ring, these subgroups are: flavones, flavonols, flavanones, flavanonols, flavanols or catechins, anthocyanins and chalcones **(Figure 17) (Panche *et al.*, 2016)**.

Flavonoids are well known for their many beneficial biological and pharmacological functions **(Table IXX)**. Structural modifications, for example, sulphation, methylation, and glycosylation usually change their solubility, stability, and biological activities **(Teles and Souza, 2018)**.

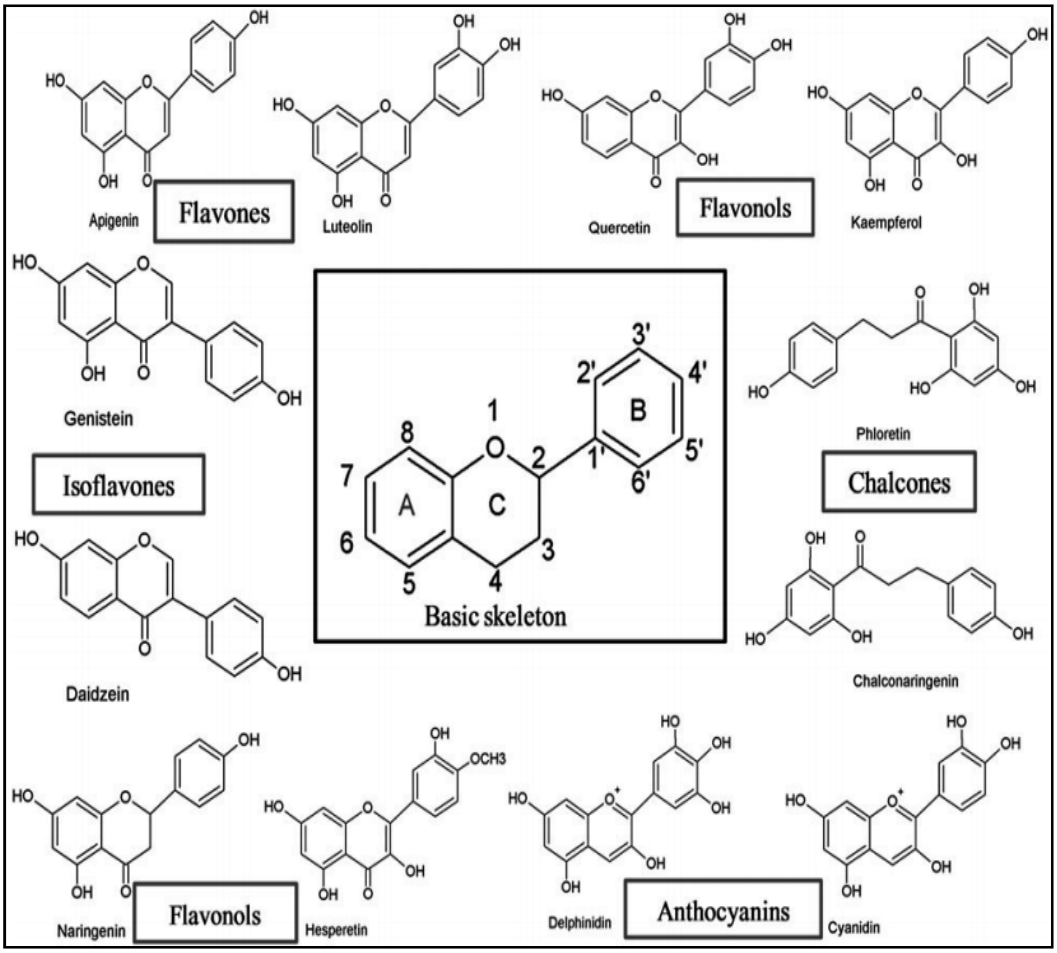


Figure 17. Basic skeleton structure of flavonoids and their classes **(Panche *et al.*, 2016)**.

Table XII. Flavonoids biological activity.

Biological activity	Role	Reference
Anti-oxidant Activity	Flavonoids suppress the ROS formation by inhibition of enzymes and regulation of antioxidant defenses.	**(Tiwari and Husain, 2017)**
Anti-bacterial Activity	Flavonoids are very effective antimicrobial substances against a wide array of micro-organisms by disrupt microbial membranes.	**(Tiwari and Husain, 2017)**
Anti-viral Activity	Flavonoids also displayed antiviral, including anti-HIV activity and similar immunodeficiency virus infections.	**(Narayana *et al.*, 2001)**
Anti-cancer Activity	Flavonoids inhibit many cancer-triggering enzymes.	**(Tiwari and Husain, 2017)**
Anti-diabetic Activity	Flavonoids, especially quercetin, bring about the regeneration of pancreatic islets and proprably increases insulin.	**(Jain *et al.*, 2010).**

Chapter II

Oxidative stress and Antioxidants

II.1. Background

Oxygen is a molecule essential for life, when cells use it to generate energy; free radicals are created as by-products which are generally reactive oxygen species (ROS) as well as reactive nitrogen species (RNS) that result from the cellular redox process. These species play a dual role as both toxic and beneficial compounds **(Haleng, 2007; Pham-Huy and Pham-Huy, 2008)**.

Free radicals and other reactive species are constantly generated *in vivo*; its accumulation coupled with an increase in oxidative stress implicated in the pathogenesis of several disease states. A process held in check by the existence of multiple antioxidant and repair systems, biological systems was adapted with the coexistence of damaging free radicals and also was developed mechanisms of using them to their advantage. **(Dhawan, 2014; Manual, 2016)**.

II.2. Oxidative stress (OS)

Oxidative stress is defined as the imbalance between generations and clearances of oxidants **(Palipoch and Koomhin, 2015)**; this imbalance results from excessive generation of free radicals, particularly oxygen free radicals (ROS) and/or a disturbance of the antioxidant environment **(Pal *et al.*, 2019; Jackson *et al.*, 2020)**. It leads to oxidative damage to proteins, lipids, and DNA **(Schäfer and Wener, 2011)**. However under normal physiological condition is considered as oxidative eustress **(Mu and Chen, 2020)**.

II.2.1. Free radicals

A free radical is a chemical species, atom, or molecule, containing an unpaired electron in an atomic orbital which means any species capable of independent existence **(Lobo *et al.*, 2010; Benlemlih and Ghanem, 2012)**. The odd number of electron(s) of a free radical makes it unstable, short lived and highly reactive. Because of their high reactivity, it can abstract electrons from other compounds to attain stability **(Phaniendra *et al.*, 2015)**. Free radicals can have positive, negative or neutral charge **(Khanna and Shiloh, 2009)**.

Free radicals can be formed by homolytic cleavage of covalent bond of normal molecule, with each fragment retaining one of paired electrons or by redox reactions **(Kumar, 2011; Radi, 2018)**.

Two types of free radicals can be distinguished: firstly, the radical compounds, include; primary reactive species; molecules derived directly from oxygen and which play a particular role in physiology, including the hydroxyl radical (OH°). The other contains the secondary radicals formed by the interaction of these primary radicals with the biochemical

compounds of the cell (proteins, sugars and lipids), rendering it a radical capable of propagating a chain reaction such as the peroxyl (ROO°) and alkoxyl (RO°) radicals **(Perl-Treves and Perl, 2002; Favier, 2003)**. Secondly, non-radical species, which includes oxygen derivatives such as H_2O_2, it can play the role of oxidizing agents "radical precursors", as they are capable of converting to a radical **(Favier, 2003; Vamecq, 2004)**.

The term reactive oxygen species (ROS) is a collective descript that includes not only the oxygen radicals but also some non-radical derivatives of O_2, The term reactive species has been expanded to include reactive nitrogen, chlorine, and bromine species **(Table XIII) (Halliwell, 2006)**.

As a result of an energy transfer or through transmission of electron reactions, ground-state oxygen may be changed to a much more reactive type of ROS. It can be produced in a living system by a variety of processes, including biological metabolism, enzymatic processes, and as by-products of biological reactions. Additionally, different types of chemical reactions can be responsible for the production of ROS. The prominent pathways of ROS production are presented in **Figure 18 (Klotz, 2002; Mitra *et al.*, 2019)**.

Reactive nitrogen species (RNS) is often linked to Reactive oxygen species (ROS), e.g., in the formation of peroxynitrite causing nitrosative stress. Oxidative and nitrosative stress have been etiologically implicated in a wide variety of disease processes and states **(Bhattacharyya *et al.*, 2014)**.

From a biological viewpoint, various oxygen derived free radicals have been attracting attention because various active oxygen species are generated in the body during the process of utilizing of oxygen. For that most studies focus on the oxygen radicals (reactive oxygen species ROS) **(Yoshikawa and Naito, 2002; Halliwell, 2006)**.

Table XIII. Some reactive species and their diversity of chemical nature **(Halliwell, 2006; Sies *et al.*, 2017)**.

Free radicals	Nonradicals
Reactive oxygen species (ROS)	
Superoxide ($O_2^{\circ-}$) Hydroxyl (OH°) Peroxyl (RO_2°) Alkoxyl (RO°)	Hypochlorous aid (HOCl) Peroxynitrite ($ONOO^-$) Peroxynitrate (O_2NOO^-) Peroxomoocarbonate ($HOOCO_2^-$) Ozone (O_3°) Organic peroxides (ROOH) Peroxynitrous acid (ONOOH)
Reactive chlorine species	
Atomic chlorine (Cl°)	Hypochlorous acid (HOCl) Chlorine gas (Cl_2) Chlorine dioxide (ClO_2) Nitryl chloride (NO_2Cl)
Reactive bromine species	
Atomic bromine (Br°)	Hypobromous acid (HOBr) Bromine gas (Br_2) Bromine chloride (BrCl)
Reactive nitrogen species (RNS)	
Nitric oxide (NO°) Nitrogen dioxide (NO_2°) Nitrate radical (NO_3°)	Nitrosyl cation (NO^+) Nitrosyl anion (NO^-) Peroxynitrite ($ONOO^-$) Dinitrogen tetroxide (N_2O_4) Dinitrogen trioxide (N_2O_3) Nitrous acid (HNO_2) Alkyl peroxynitrites (ROONO) Alkyl peroxynitrates (RO_2ONO)
Reactive sulfur species	
Thiyl radical (RS°)	Sulfenate (RSO^-) Sulfonate (RSO_3^-) Hydrogen sulfide (H_2S)

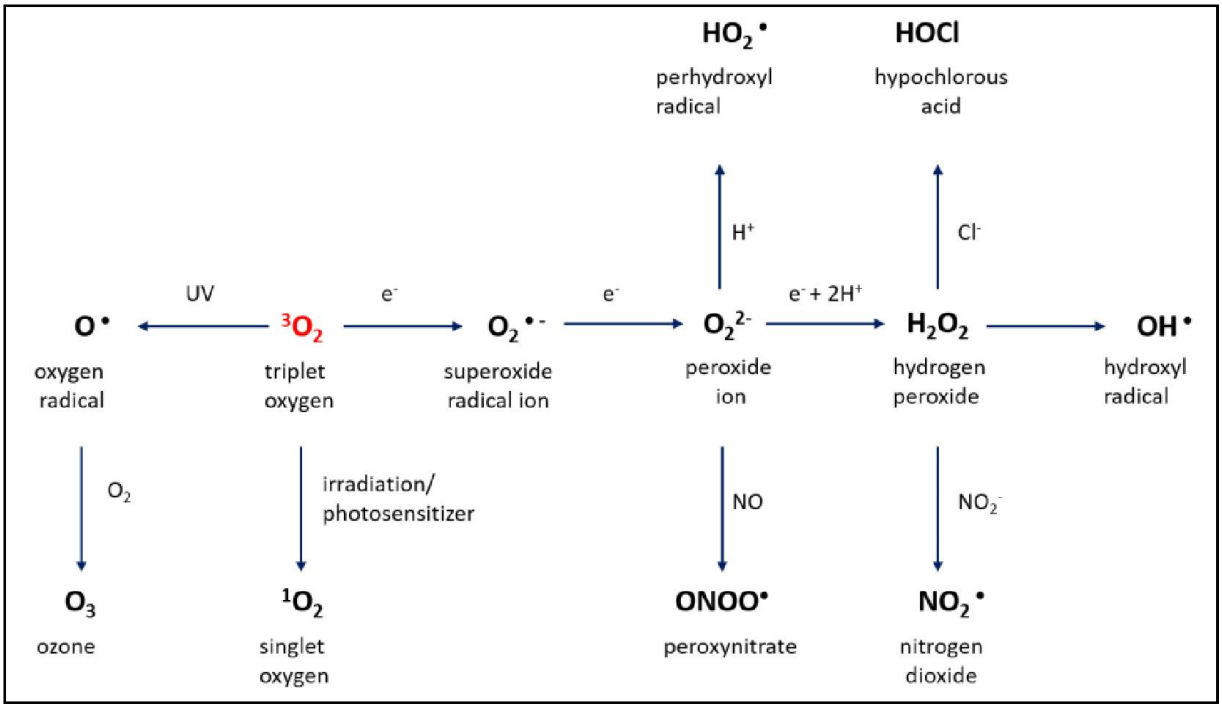


Figure 18. Schematic of primary ROS production mechanism **(Mitra *et al.*, 2019)**.

II.2.1.1. Sources and generation of free radicals

The free radicals are derived from both endogenous sources and exogenous sources, chain reactions involving free radicals can usually be divided into three distinct processes; initiation, propagation, and termination **(Phaniendra *et al.*, 2015; Khan *et al.*, 2018)**:

a) Initiation reactions: are those, which result in a net increase in the number of free radicals. They may involve the formation of free radicals from stable species or they may involve reactions of free radicals with stable species to form more free radicals.

b) Propagation reactions: involve free radicals in which the total number of free radicals remains the same.

c) Termination reactions: are those reactions resulting in a net decrease in the number of free radicals. Typically two free radicals combine to form a more stable species, such as the reaction below.

$$Cl\cdot + Cl\cdot \rightarrow 2Cl$$

II.2.1.1.1. Endogenous sources

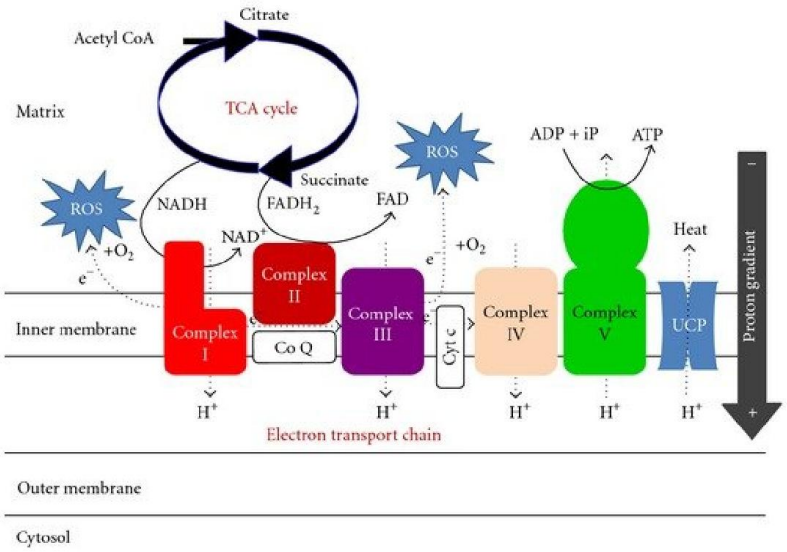
Free radical formation occurs continuously in the cells as a consequence of both enzymatic and non-enzymatic reactions. Enzymatic reactions, which serve as source of free radicals, include those involved in the respiratory chain; free radicals can also be formed in non-enzymatic reactions **(Lobo *et al.*, 2010)**.

• **Enzymatic sources**

ROS essentially produced by enzymes and are from different origins; mainly from the cytoplasmic membrane NADPH oxidase and the enzyme complex of the mitochondrial respiratory chain, but also sources of other organelles such as xanthine oxidase (XO) **(Collin, 2019)**. Those are discussed as follows.

✓ **Mitochondrial electron transport chain**

Most intracellular ROS are derived from superoxide ($O_2^{\bullet -}$), which is generated by the one electron reduction of O_2 **(Sena and Chandel, 2012)**. It has been estimated that about 4-5% of molecular oxygen is converted to reactive oxygen species during aerobic respiration **(Salman and Ashraf, 2013)**, complexes I (NADH-ubiquinone oxidoreductase) and III (ubiquinol-cytochrome c oxidoreductase) of the respiration chain are the major sources of cellular ROS. However, complex II (succinate-ubiquinone oxidoreductase) has been suggested as an under-studied source of cellular ROS **(Figure 19) (Anderson *et al.*, 2014)**.



TCA: tricarboxylic acid, NADH & NAD^+: nicotinamide adenine dinucleotide reduced form & oxidized form, FADH2 & FAD; flavin adenine dinucleotide reduced form and oxidized form, ADP: adenosine diphosphate, ATP: adenosine triphosphate, Co Q: coenzyme Q, Cyt c: cytochrome c, UCP: uncoupling protein.

Figure 19. The electron transport chain and ROS production in mitochondria **(Shinmura, 2013)**.

✓ **NADPH Oxidase**

The NADPH oxidases (Nox) comprise a family of 7 structurally related transmembrane NADPH-dependent oxidoreductases that include the Nox enzymes (Nox1–5) as well as the Duoxes (Duox1 and 2) **(Fulton *et al.*, 2017)**. It was originally considered to be expressed only in phagocytic cells. It is now evident that there is a family of NADPH oxidases, based on homologues of the catalytic subunit gp91phox that are functionally active in non-phagocytic cells **(Figure 20) (Lambeth, 2004)**.

NADPH oxidase is the major enzymatic source of ROS generation in cells but the other enzymes produce ROS only as by-products. It catalyzes the production of superoxide by the reduction of oxygen, using NADPH as the electron donor such as the action below **(Ozcan and Ogun, 2015)**.

$$2O_2 + NADPH \longrightarrow 2O_2^{\circ-} + 2NADP^+ + H^+$$

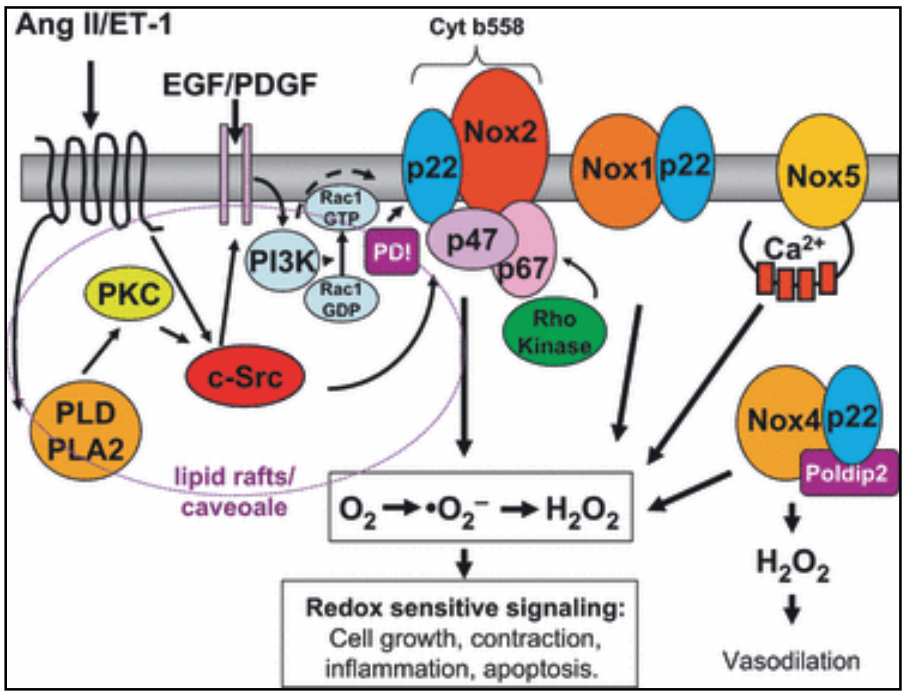


Figure 20. Endothelial cells Nox isoforms: Nox1, Nox2, Nox4 and Nox5 **(Montezano and Touyz, 2012)**.

✓ **Xanthine Oxidase (XO)**

XO enzyme is an important source of ROS especially $O_2^{\circ-}$ and H_2O_2 through the oxidation of hypoxanthine and xanthine to uric acid during inflammatory diseases **(Kalley *et al.*, 2010)**, the process of ischemia may lead to the formation of xanthine oxidase from xanthine dehydrogenase via activation of calcium-dependent protease and also to a breakdown of ATP with the generation of AMP via the adenylate kinase reaction **(Figure 21) (Sen *et al.*, 2000)**.

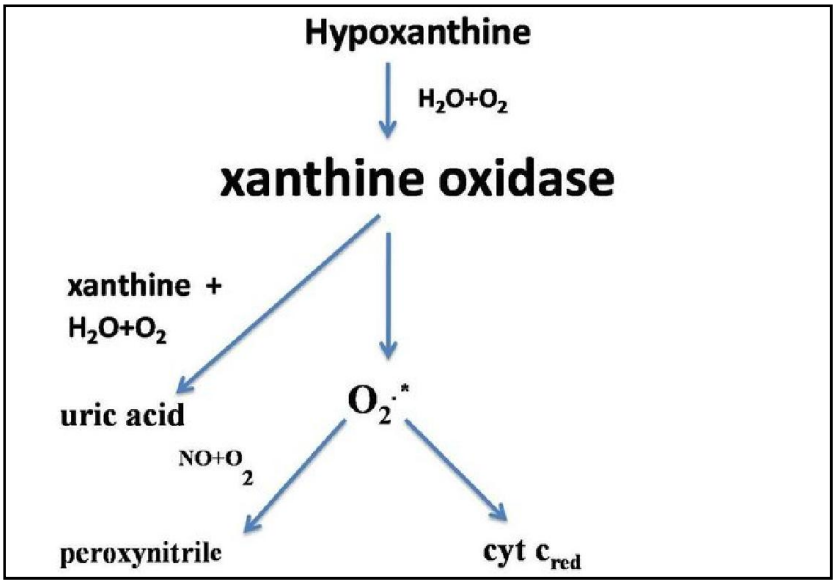


Figure 21. The pathways catalyzed by Xanthine oxidase **(Pathak *et al.*, 2017)**.

✓ **Myeloperoxidase (MPO)**

Myeloperoxidase is a hemoprotein expressed in neutrophils and monocytes, which is secreted during activation of these cells **(Wassman *et al.*, 2004)**. As seen in the reaction below, this enzyme can convert H_2O_2 to another free radical HOCl by reacting with chloride (Cl^-) ions **(Ifeanyi, 2018)**.

$$H_2O_2 + Cl^- \longrightarrow HClO + OH°$$

✓ **Lipoxygenases (LOX) and Cyclooxygenases (COX)**

Various cell types may generate ROS as by-products during the metabolism of arachidonic acid (AA) by enzymes: lipoxygenases (LOX) and cyclooxygenases (COX) **(Cho *et al.*, 2011)**.

COXs enzymes have three isoforms: the constitutive isoform COX-1, and the inducible isoforms COX-2 and COX-3 which catalyze the conversion of AA to prostanoids, whereas LOX enzymes are classified according to the position of the insertion of oxygen in AA to many isoforms **(Cho *et al.*, 2011; Villaverde *et al.*, 2019)**.

✓ **Nitric Oxide Synthase (NOS)**

The NOS family of enzymes is responsible for the synthesis of Nitric oxide (NO°), the three NOS isoforms have been identified as the product of three distinct genes with specific profiles of expression **(Figure 22)**: the neuronal Nos (nNos, Nos1, NosI), macrophage inducible Nos (iNos, Nos2, NosII) and the endothelial Nos (eNos, Nos3, NosIII) **(Andreakis *et al.*, 2011)**.

The nNOS and eNOS are constitutively expressed and require the formation of Ca^{2+}-calmodulin complexes for their activation, whereas iNOS exerts its activity in a Ca^{2+}-

independent manner, in general all three NOS isoforms need co‑factors, such as haem **(Calabrese *et al.*, 2007; Pitsikas, 2016)**.

Reactive nitrogen species (RNS) are toxic molecules that are produced when nitric oxide (NO°) encounters reactive oxygen species (ROS) **(Auger *et al.*, 2011)**. NO° is the main RNS generated in living systems and is the primary ingredient for the production of other RNS **(Martinez and Andriantsitohaina, 2009) (Figure 23).**

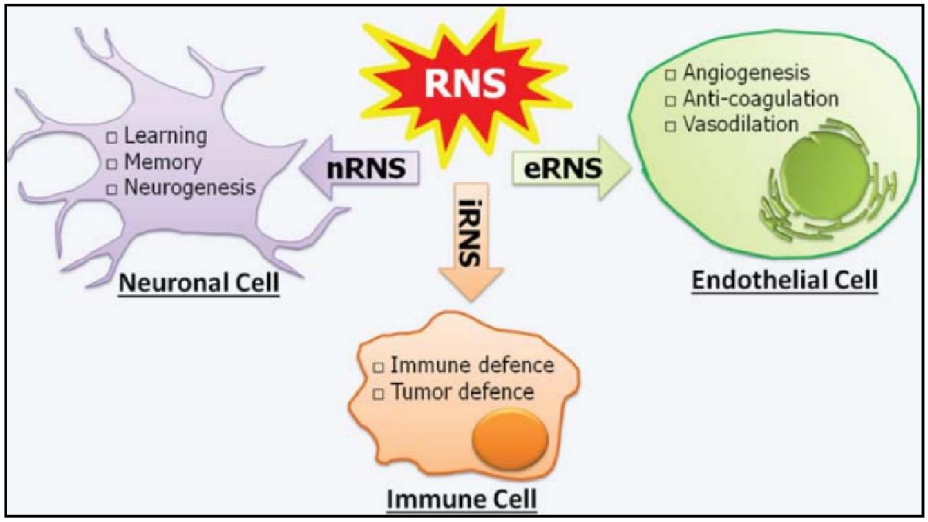


Figure 22. NOS isoforms and their biological functions **(Tharmalingam *et al.*, 2017)**.

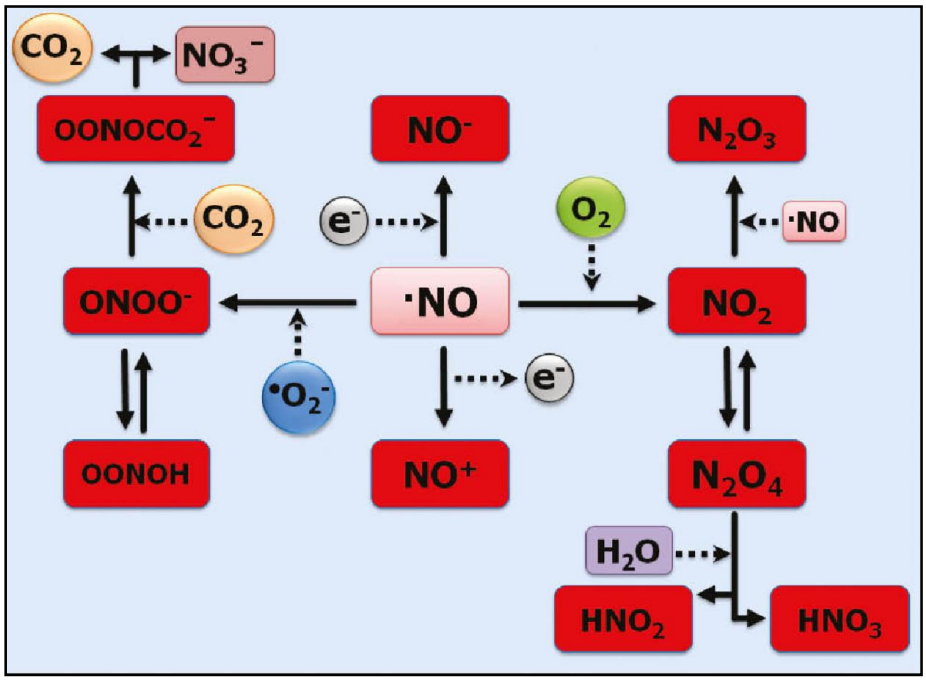


Figure 23. Production of RNS from Nitric oxide (NO°) **(Tharmalingam *et al.*, 2017)**.

- **Non enzymatic sources**

Non enzymatic sources of ROS consist of transition metal ions and auto-oxidation discussed below.

✓ **Transition metal ions**

Metals are important inducers of formation of reactive oxygen species through two mechanisms; redox-active metals generate reactive oxygen species through redox cycling, while metals without redox potential impair antioxidant defences **(Sevcikova *et al.*, 2011)**.

Redox-active metals can enhance the formation of ROS through Fenton reactions, Fenton-like reactions, or the Haber Weiss cycle reaction, all yielding hydroxyl radicals **(Figure 24) (Fu *et al.*, 2014; Iwabuchi *et al.*, 2015)**.

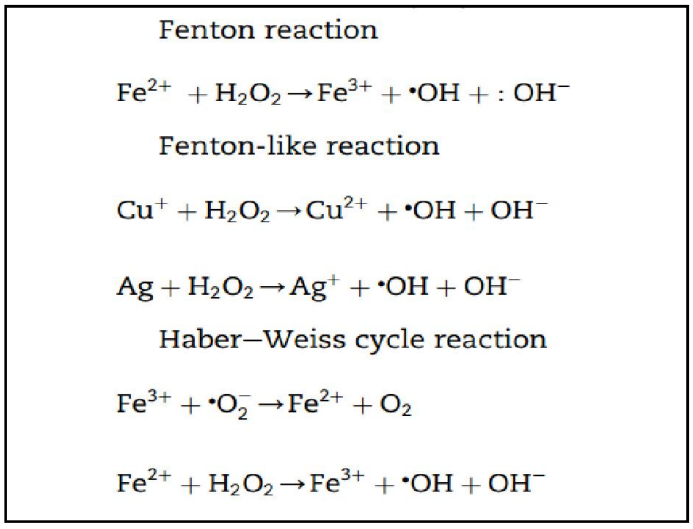


Figure 24. Fenton reactions, Fenton-like reactions and Haber Weiss cycle reaction **(Fu *et al.*, 2014)**.

✓ **Auto-oxidation**

Many molecules can be oxidized by O_2 to produce $O_2^{\circ-}$; they include, tetrahydrobiopterin, flavins, quinols and dopamine (redox cycling of dopamine metabolites) **(Figure 25)**. Since O_2 is poorly reactive the reaction need transition metal ions, especially manganese ions **(Aldini *et al.*, 2011; Halliwell and Gutteridj, 2015)**.

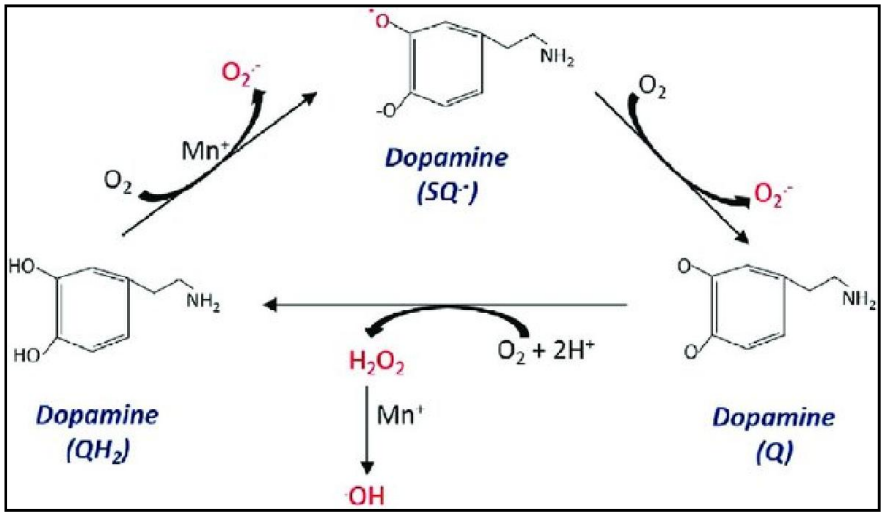


Figure 25. Auto-oxidation of dopamine **(Cobley *et al.*, 2018)**.

II.2.1.1.2. Exogenous Source

The free radical formation in the body is continuous and unavoidable process of human body. It can be generated from exogenous sources such as; dietary factors (coffee), toxins (carbon tetrachloride CCl_4), drugs (adriamycin), which accelerates free radical formation process **(Qazi and Molvi, 2018)**. In addition the environment factors like air pollutants (tobacco smoke), chemical solvents (chlorine), radiations (x-rays, ultraviolet) and heavy metal ions are all potent generator of free radicals **(Qazi and Molvi, 2018; Birben *et al.*, 2012)**.

II.2.1.2.The roles of free radicals

ROS are produced by living organisms at low to moderate concentrations **(Birden *et al.*, 2012)**, this lower concentrations exert beneficial effects, whereas the higher concentration and long-time exposure of ROS may lead to various disorders and can cause exclusively toxic effects which are associated with various pathologies **(Di Meo *et al.*, 2016; Singh *et al.*, 2019)**.

Over the past two decades, ROS has undergone a shift from being molecules that invoke damage into beneficial molecules. For example; regulating signaling pathways, cellular differentiation, tissue regeneration, and prevention of aging **(Schieber *et al.*, 2014; Taverne *et al.*, 2020)**.

The evolution of oxygen photosynthesis was one of the most important steps in Earth's history. The accumulation of O_2 in surface environments completely reshaped the biogeochemical properties of the planet and dramatically affected the trajectory of subsequent evolution and facilitated the diversification of complex life **(Schmitt and Allakhverdiev, 2017; Taverne *et al.*, 2020)**.

II.2.2. Oxidative damage to biomolecules

Oxidative stress can cause cellular dysfunction and induce much pathology by damaging lipids, proteins, and DNA **(Figure 26) (Sajjad, 2018)**.

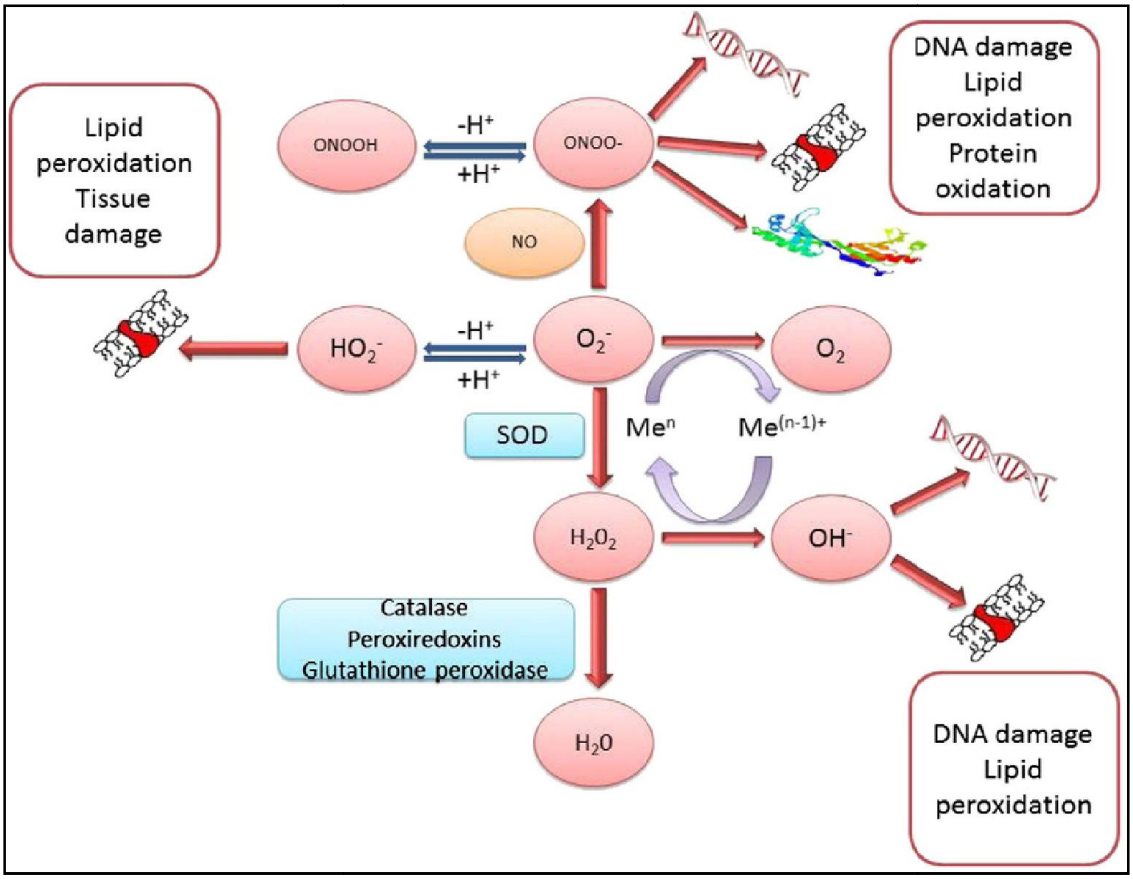


Figure 26. Reactive oxygen species (ROS)-induced oxidative damage **(Shah *et al.*, 2013)**.

II.2.2.1. Lipid peroxidation (LPO)

Lipid peroxidation can generally be described as a process in which oxidants such as free radicals attack lipids containing carbon-carbon double bonds, in particular polyunsaturated fatty acids (PUFAs) **(Ayala *et al.*, 2014)**. Peroxidation of lipids may interfere with the assembly of the membrane, causing changes in fluidity and permeability, changes in ion transport and inhibition of metabolic processes. In addition, it has been involved in a wide range of tissue injuries and diseases, as well as in the aging process **(Desai *et al.*, 2014; Azouzi *et al.*, 2017)**.

The peroxidation of lipids involves three distinct steps: initiation, propagation and termination **(Figure 27) (Hanaa, 2012)**. During peroxidation pathway via reactive intermediates, several end products are formed such as 4-hydroxy-2-nonenal (HNE), malondialehyde (MDA) (**Repetto *et al.*, 2012; Ghosh, 2015)**, this by-products can be considered as biomarkers of oxidative stress **(Tsikas, 2017)**.

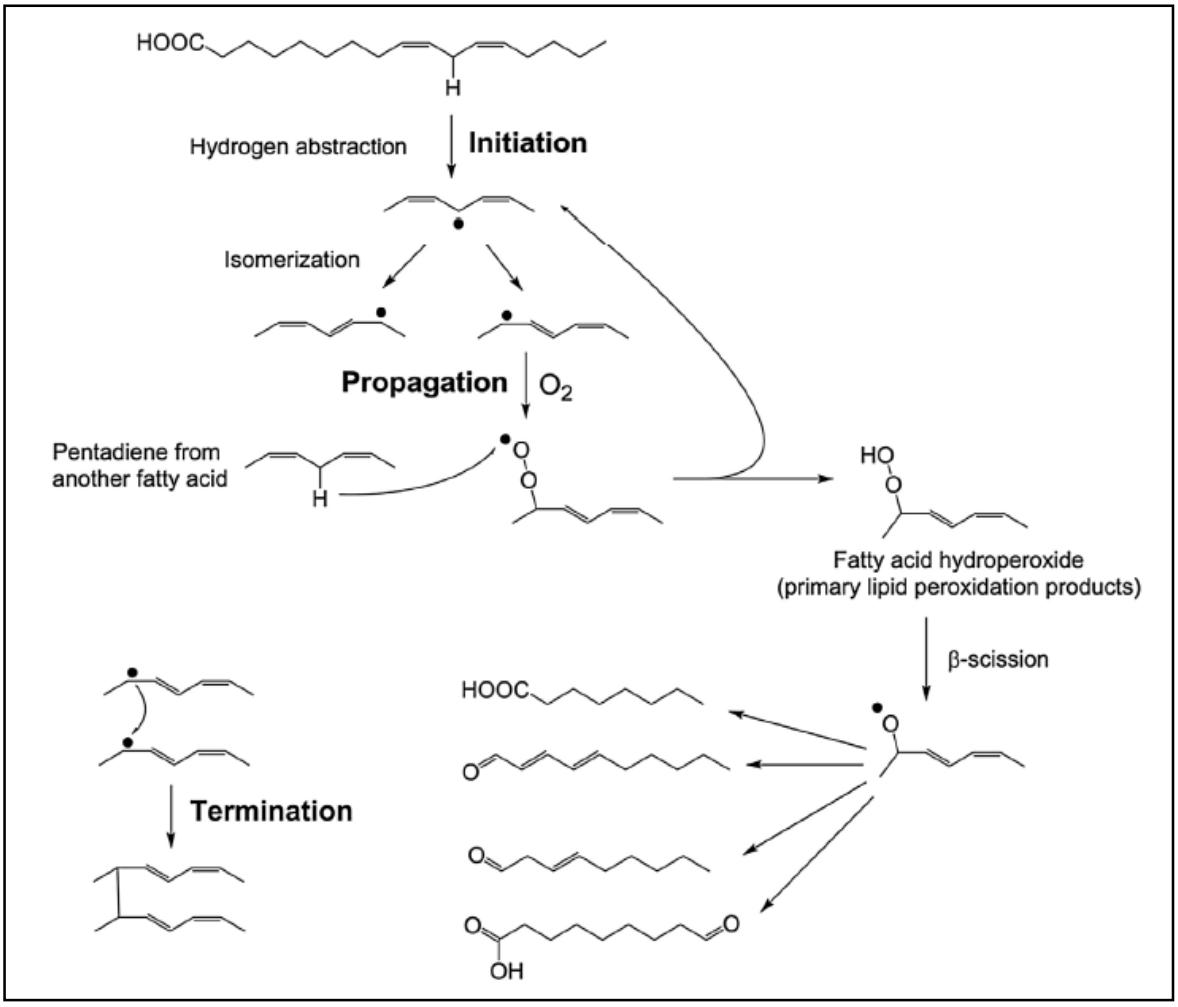


Figure 27. A simplified scheme of lipid peroxidation **(Wang *et al.*, 2017)**.

II.2.2.2. Proteins oxidation

Proteins are major targets for radicals in biological systems **(Davies, 2016)**. Protein oxidation induced by ROS can cause the modification of backbones and side chains of proteins. These modifications lead to the structural changes at the levels of primary, secondary, and tertiary structure of proteins. These structural changes can induce conformational and functional modifications of proteins including enzyme activity **(Zhang *et al.*, 2013)**. The process of protein oxidation produces a group of compounds that can be considered as biological biomarkers of oxidative stress such as carbonyl groups **(Marroco *et al.*, 2017)**.

II.2.2.3. DNA oxidation

Reactive species may induce oxidation, nitration, or base methylation. These changes thus interrupt transcription and translation, resulting in truncated and/or non-functional protein formation. Which often lead to mutagenesis, cancer, or even premature aging phenomena **(Valko *et al.*, 2006)**.

Oxidation of the bases DNA in particular of the guanine bases generates a modified base such as 8-hydroxyguanine (8-OH-G) **(Figure 28)**, which is a good biomarker of the oxidative stress of the organism **(Lagadu *et al.*, 2010)**.

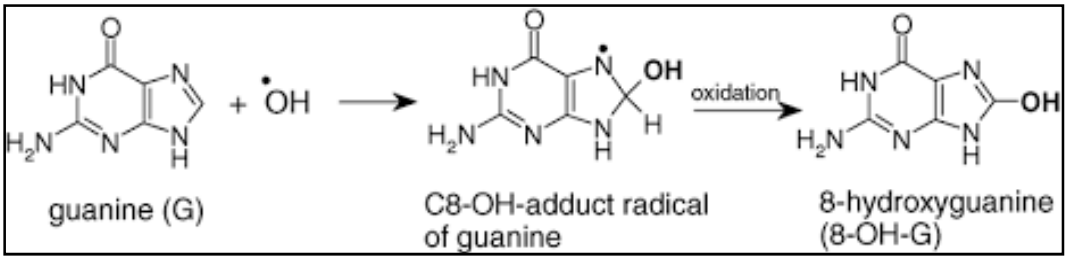


Figure 28. Reaction of guanine with hydroxyl radical **(Valko, 2006)**.

II.2.3. Oxidative stress and diseases

Oxidative stress plays a key role in the pathophysiology of many different diseases **(Ghezzi *et al.*, 2020)**. The following is an explanation of the major diseases associated with oxidative stress **(Table XIV)**.

Table XIV. Diseases related with oxidative stress.

Diseases	The role of oxidative stress	Reference
Cardiovascular disease (CVD)	- ROS plays a vital role in the pathogenesis of various forms of CVD. - Most clearly defined is the role of ROS in endothelial dysfunction.	**(Wood, 2020)**
Aging	- The exact mechanism of oxidative stress-induced aging is still not clear. - Probably increased ROS levels lead to cellular senescence (stops cell proliferation in response to replication damage).	**(Liguori *et al.*, 2018)**
Neurodegenrative Diseases	- Due to high oxygen consumption and low levels of antioxidant enzymes, the CNS is particularly susceptible to oxidants. - Oxidative stress has also been associated with several neurodegenerative diseases such as Alzheimer's and Parkinson's.	**(Uttara *et al.*, 2009 ; Phaniendra *et al.*, 2015)**
Cancer	- Free radicals could be mutagenic and carcinogenesis. - ROS by altering the growth signals and gene expression cause continuous proliferation of cancer cells.	**(Phaniendra *et al.*, 2015 ; Murad *et al.*, 2019)**
Type 2 diabetes	-Free radicals are essential for transducing the insulin signal. However, free radicals can be involved in insulin resistance (decrease in insulin action).	**(Bonnard *et al.* , 2008)**

II.3. Antioxidants

The term antioxidant refers to "any substance that delays, prevents or reverses the oxidative damage in a target molecule" **(Reyes-Fermín *et al.*, 2020)** that slows down free radical damage **(Tortora and Derrickson, 2018)**, thereby maintaining normal cell morphology, promoting immunity and preventing various diseases **(Mackness *et al.*, 2007)**.

II.3.1. Mechanism of Antioxidant Activity

There are mainly three types of mechanism known for the antioxidant activity; chain breaking, preventive, and synergetic mechanism **(Misra *et al.*, 2014)**:

a) Chain breaking mechanism; inhibiting oxidation processes and producing less reactive product so that no additional reaction occurs.

b) Preventive process; antioxidant chelates the transition metal or scavenges the free radical species and breaks down into non-substance and thus prevents the initiation of the chain.

c) Synergetic process; pairing one antioxidant with another and functioning in synergy. They work together more efficiently than the single antioxidant alone.

II.3.2. Classification

Antioxidants can be classified into two major types based on their source to natural and synthetic antioxidants.

II.3.2.1. Natural antioxidants

Natural antioxidants either are synthesized in human body through metabolic process or are supplemented from other natural sources; their activity very much depends upon their physical and chemical properties and mechanism of action. This can be further divided into two categories; enzymatic antioxidants and non-enzymatic antioxidants **(Misra *et al.*, 2014)**.

II.3.2.1.1. Enzymatic antioxidants

Enzymatic antioxidants are uniquely produced in the human body and can be subdivided into:

- **Primary Antioxidants**

Include three main enzymes: Superoxide dismutase (SOD), Catalase (CAT), and Glutathione peroxidase (GPx).

✓ **Superoxide dismutase (SOD)**

SOD is a metalloprotein whose enzymatic activity consists in making the dismutation of ($O_2^{\circ-}$) radical into (H_2O_2) radical **(Santelli, 2012)** as shown in the interaction below **(Sheng *et al.*, 2014)**, founded in all cells and the extracellular fluid and it has three different types:

cytosolic SOD (Cu-Zn SOD), mitochondrial SOD (Mn-SOD) and extracellular SOD **(Yen *et al.*, 2009; Durand and Beaudeux, 2011)**.

$$2H^+ + 2O_2^{\circ-} \xrightarrow{SOD} H_2O_2 + O_2$$

✓ **Catalase (CAT)**

CAT is a tetrameric enzyme, each of its four subunits contains a heme-group in its active site and one tightly bound molecule of NADPH, it is founded in all major body organs especially in liver, kidneys, erythrocytes and even in brain heart and skeleton muscle. Catalase is located inside peroxisomes, it can transform (H_2O_2) radical into (O_2) molecule and water as it shown in the next interaction **(Elsner and Maibach, 2005; Babuty *et al.*, 2007; Battelo, 2016)**.

$$2H_2O_2 \xrightarrow{CAT} 2H_2O + O_2$$

✓ **Glutathione Peroxidase (GPx)**

This enzyme is selenium-dependent and essentially cytosolic, it catalyzes the reduction of mineral hydroperoxides (H_2O_2) or organic hydroperoxides (LOOH) into a water molecule (**Reaction1**) or alcohol (**Reaction2**) using reduced glutathione (GSH) as a hydrogen donor **(Amarenco *et al.*, 2001; Durand and Beaudeux, 2011, Lubos *et al.*, 2011)**.

$$H_2O_2 + 2GSH \xrightarrow{GPX} 2H_2O + GSSG \quad (1)$$

$$ROOH + 2GSH \xrightarrow{GPX} ROH + GSSG + H_2O \quad (2)$$

- **Secondary antioxidant**

The secondary enzymatic defense includes: Glutathione reductase (GR) which reduces glutathione (antioxidant) from its oxidized to its reduced form, and by this recycling to continue neutralizing more free radicals, and Glucose-6-phosphate dehydrogenase (G-6-PD) who regenerates NADPH **(Shebis *et al.*, 2013)**.

The following diagram (**Figure 29**) illustrates the complementarity between the action of enzymatic antioxidants (primary and secondary).

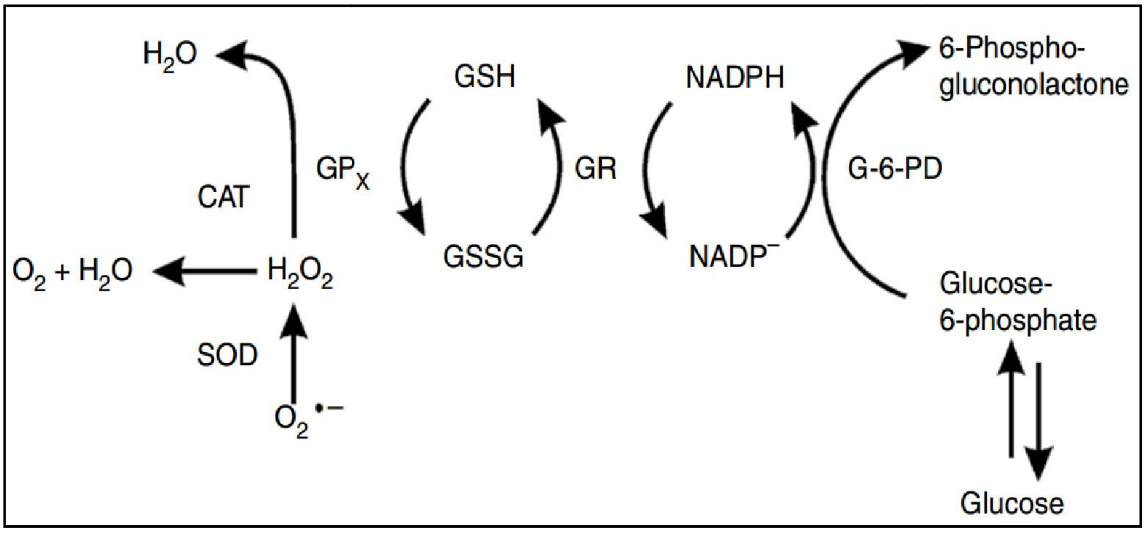


Figure 29. Diagram illustrates the complementarity between the actions of enzymatic antioxidants **(Weydert and Cullen, 2010)**.

II.3.2.1.2.Nonenzymatic antioxidants

They are a class of the antioxidants which most of them are not found in the body naturally but are required to be supplemented for the proper metabolism, the main ones being: peptides (glutathione), vitamins, carotenoids, polyphenols and minerals **(Shebis *et al.*, 2013; Misra *et al.*, 2014)**.

✓ **Glutathione**

Glutathione, an important water-soluble antioxidant, is synthesized from the amino acids glycine, glutamate, and cysteine. Glutathione directly quenches ROS such as lipid peroxides, and also plays a major role in xenobiotic metabolism. Exposure the liver to xenobiotic substances induces oxidative reactions through the up-regulation of detoxification enzymes **(Kumar, 2014)**.

✓ **Vitamins**

Vitamins are organic compounds that can't be synthesized by humans but required as nutrients in minute amounts **(Ferri, 2019)**, it's catalysts for all reactions using proteins, fats, and carbohydrates for energy, growth and cell maintenance **(Kizior and Hodgson, 2019)**. Vitamins divided according to solubility into: water-soluble vitamins; circulate freely in the blood, in the watery fluids between cells, and in the fluids inside cells. Fat-soluble vitamins; found in the liver and the fatty tissues of the body where they are stored and used **(Blake, 2008)**. The folowing **Table XV** shows vitamins's rule which have an antioxidant effect.

Table XV. Types of vitamins with antioxidant effect **(Baskin and Salem, 1997; Botham *et al.*, 2017; Weber *et al.*, 2019)**.

Vitamin	Solubility	Function
A (β-Carotene)	In fats	Inhibition of lipid peroxidation.
E (α-Tocopherol)	In fats	Scavenge free radicals and act as a chain-breaking radical.
C (Ascorbic acid)	In water	Reduce ($O_2^{\circ-}$) radical and (OH°) radical. Maintenance of reduced vitamin E.

✓ **Polyphenols**

Antioxidant activity of polyphenol refers to both the ability of this compounds to prevent damage from reactive oxygen species (ROS) (such as through radical scavenging) or to prevent generation of these species thus reducing the rate of oxidation by inhibiting the formation of or deactivating the active species and precursors of free radicals (by binding iron) **(Perron and Brumaghim, 2009; Tsao, 2010)**. The role of polyphenols as natural antioxidants is in the prevention and treatment of cancer, inflammatory and cardiovascular diseases **(Bougandoura and Bendimerad, 2013)**, and that though to quench ($^1O_2$) radical and reactive oxygen species; such as ($O_2^{\circ-}$) radicals, (OH°) radicals, (ROOH) radicals and (ROO^-) radicals **(Draelos, 2009)**, it also inhibits enzymes with oxidative stress **(Kumar, 2014)**.

Polyphenols are the most abundant antioxidants in the diet. Their total dietary intake could be as high as 1g/d, which is much higher than that of all other classes of phytochemicals and known dietary antioxidants **(Scalbert *et al.*, 2005)**.

While polyphenols are strong antioxidants, it should be pointed out that when a phenolic molecule loses an electron or when it acts as a reducing agent, the molecule itself becomes a radical, albeit a relatively stable one; its oxidized intermediates can also become pro-oxidants. Interaction between polyphenols and transition metal ions can result in pro-oxidant formation **(Zhang and Tsao, 2016)**.

✓ **Carotenoids (CAR)**

The mechanism and rate of scavenging is dependent on the nature of the oxidizing species and less important to the structure of the carotenoid molecule. Carotenoids interact with free radicals in three main ways: electron transfer (**Reaction1**), hydrogen abstraction (**Reaction 2**) and addition of a radical species (**Reaction 3**) **(Sies *et al.*, 2004)**.

$$CAR + ROO^{\circ} \longrightarrow CAR^{\circ+} + ROO^- \quad (1)$$

$$CAR + ROO^{\circ} \longrightarrow CAR^{\circ} + ROOH \quad (2)$$

$$CAR + ROO^{\circ} \longrightarrow ROOCAR^{\circ} \quad (3)$$

✓ **Minerals**

Minerals are present in body tissues and body fluids, they are required for several enzymatic reactions and some of them act as antioxidants include: selenium Se (a component of GPX enzyme), iron Fe (a component of hem enzymes such as CAT), zinc Zn (a component of SOD enzyme) and manganese Mn (component SOD enzyme) **(Chemouny, 2012; Atta *et al.*, 2017)**.

✓ **Other Antioxidants**

Metal binding proteins such as ferritin, lactoferrin, albumin and ceruloplasmine which sequester free iron and copper ions which can catalyze oxidative reactions **(Kumar, 2014)**, and non-protein antioxidants such as bilirubin, uric acids, and ubiquinol that inhibit oxidation by free radicals scavenging **(Misra *et al.*, 2014)**.

II.3.2.2. Synthetic antioxidants

Synthetic antioxidants are synthesized chemically, since they are not present in nature. They are added as preservatives to food which help to prevent lipid oxidation **(Atta *et al.*, 2017)**, and are used in edible vegetable oil, and cosmetics **(Anbudhasan *et al.*, 2014)**. The following are among the forms of synthetic antioxidants which are derived mainly from phenols **(Reynal and Multon, 2009)**:

- **Monophenolic,** e.g.**,** BHA، BHT ،Tocopherol.
- **Diphenolic,** e.g., TBHQ.
- **Triphenolic,** e.g., Gallates.

The antioxidants usually act as one strengthened community against ROS and RNS in different ways (**Figure 30**), i.e. the combined effect of the antioxidants is stronger than each individual, and this is one of the causes of its synergistic effects **(Wang *et al.*, 2011)**.

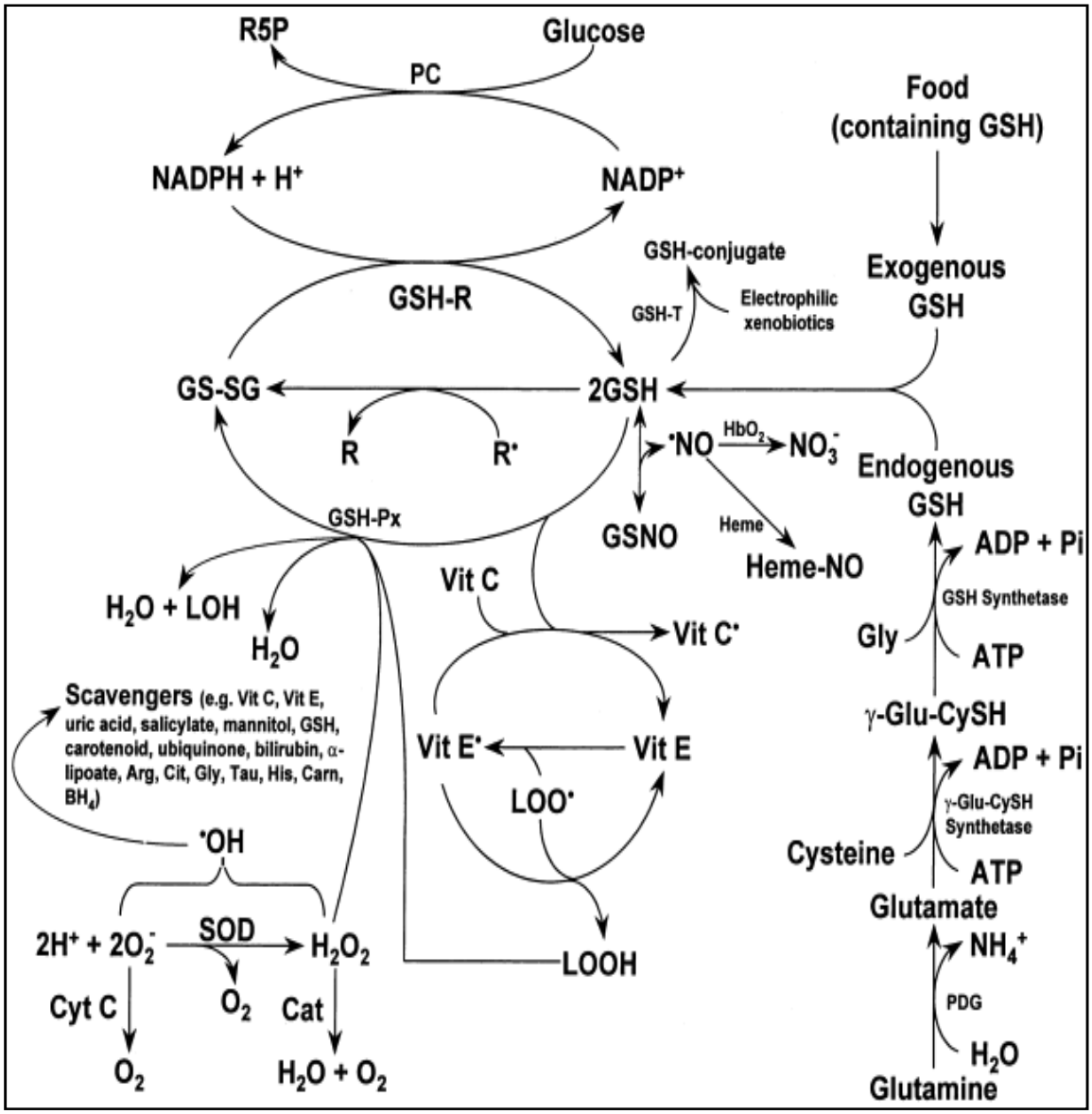


Figure 30. Removal of oxygen and nitrogen free radicals and other reactive species **(Fang *et al.*, 2002)**.

Experimental part

Chapter III

Materials and Methods

III.1. Background

This chapter includes an explanation of how to collect samples and prepare them for study. Then, screening some phytochemical compounds of date palm leaf, estimating the amount of phenolic compounds for date palm leaf extracts in two different solvent systems, then determined of antioxidant activity using the DPPH° radical scavenging activity. To accomplish this work, chemicals reagents and equipment located in the biology laboratory (SNV) of university Mohamed Boudiaf M'sila were used.

III.2. Chemicals and reagents

All chemicals and reagent used were obtained locally and were of high purity as showing in **Table XVI**.

Table XVI. Chemical substances and solutions used in the study.

Chemicals and reagents	**Molecular formula**
Ascorbic acid	$C_6H_8O_6$
Chloroform	$CHCl_3$
Copper sulfate	$CuSO_4$
Distilled water	H_2O
DPPH	$C_{18}H_{12}N_5O6$
Fehling's solution A (copper (II) sulphate)	$CuSO_4 \cdot 5H_2O$
Fehling's solution B (potassium sodium tartrate)	$KNaC_4H_4O_6 \cdot 4H_2O$
Ferric chloride	$FeCl_3$
Folin - Ciocalteu	$H_2PM_{12}O_2$ -$H_3PW_{12}O_{40}$
Gallic acid	$C_7H_6O_5$
Hydrochloric acid	HCl
Mercuric iodide	$HgCl_2$
Methanol	CH_3OH
Potassium Iodide	KI
Sodium carbonate	Na_2CO_3
Sodium hydroxide	NaOH
Sulfuric acid	H_2SO_4

III.3. Equipment

Glassware used in the laboratory was used in addition to: Electronic scale (KERN ALS 160-4A), incubator, Rotary evaporator (BÜCHI R- 210) **(Figure31)**, and Spectrophotometer (Shimadzu UVmini-1240) **(Figure 32)**.

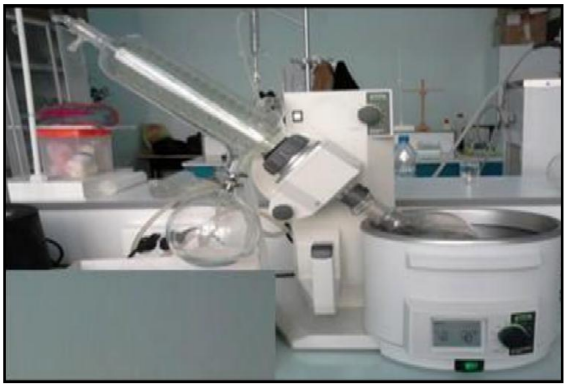


Figure 31. Rotary evaporator (BÜCHI R - 210).

Figure 32. Spectrophotometer (Shimadzu UVmini-1240).

III.4. Preparation of sample material

III.4.1. Sample collection

The leaves of the two varieties from female cultivars of *Phoenix dactylifera* L. were collected from three regions in Algeria in December 2018 and January 2019. The **Table XVII** below presents the samples, their names, and region collection.

Table XVII. Samples of date palm leaf varieties.

Samples and varieties	Date palm leaves
Deglet Nour - Laghouat (DNL)	
Deglet Nour - El Meghaïer (DNM)	
Deglet Nour - Tolga (DNT)	
Ghars - Tolga (GH)	

III.4.2. Sample preparation

Sample preparation is of primary importance for any reliable analysis. Sample preparation procedures vary widely depending on the nature of the compounds to be analyzed.

The most commonly described assay methods include several steps for sample preparation, each of them aims to increase the sensitivity and selectivity of the assay.

After the samples were dried in an incubator at 37 °C, the dry leaves were crunched in an electric mill to become powder **(Figure 33)**, and then the samples were kept out of moisture and light **(Figure 34)**.

Figure 33. Crunching of samples in electric mill.

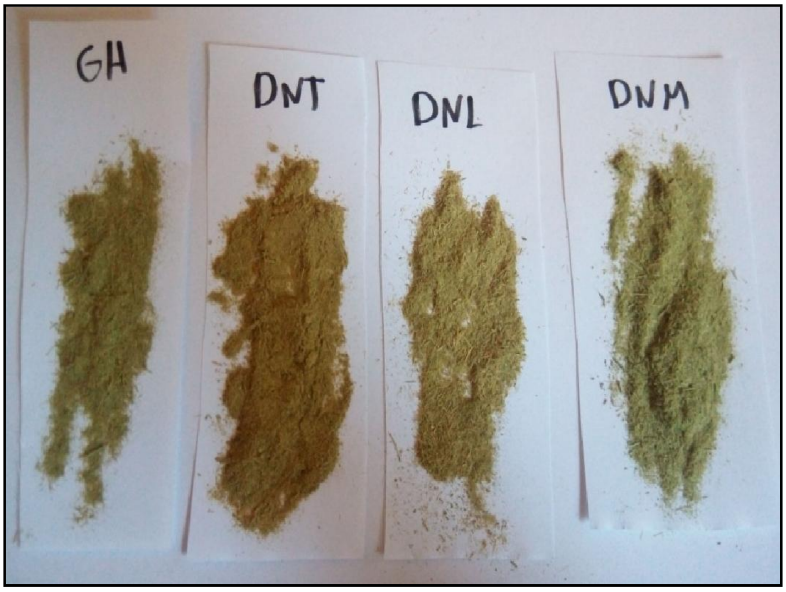


Figure 34. Date palm leaves samples after crunching.

III.4.3. Presentation of the study area

Location of the study area and the geographical distribution of all the samples collected within the limit of the study area were presented in **Figure 35**.

The study area extends over three regions spread over three different states. Which are: Tolga, Laghouat, and El Meghaïer. Tolga province (wilaya of Biskra) is located at the southeast while Laghouat (wilaya of Laghouat) is a province in the south of Algeria, El Meghaïer (wilaya of El-Oued) lies in the southeast of Algeria.

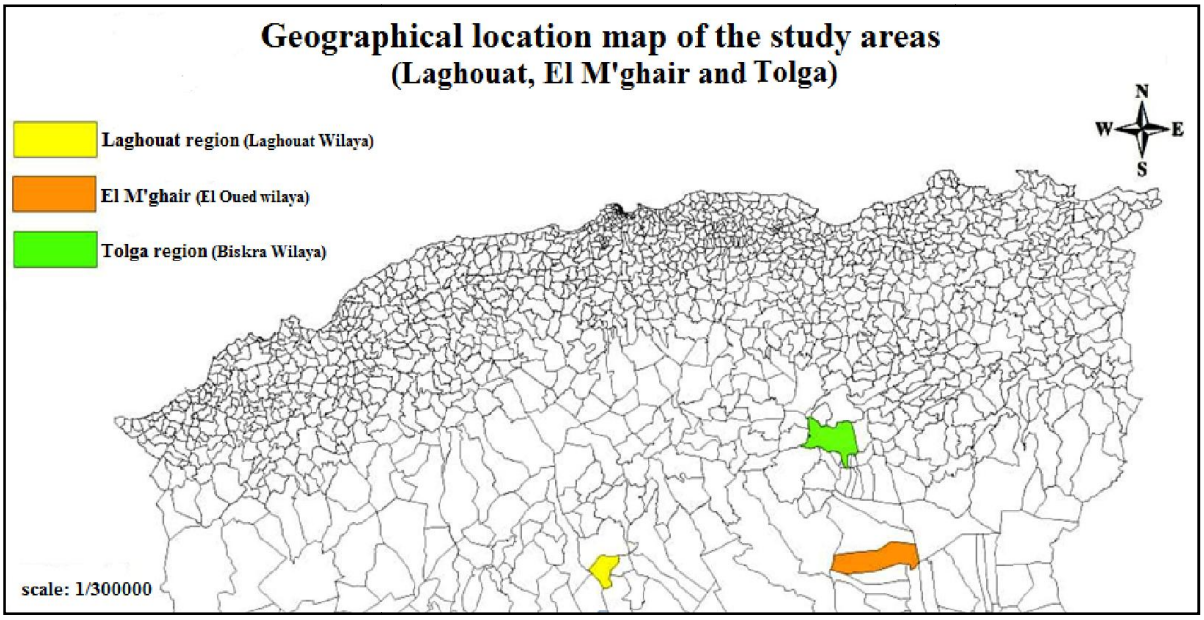


Figure 35. The geographical distribution map of the study areas **(Mapinfo v8.0)**.

III.5. Phytochemical screening

Phytochemical analysis of all the samples was determined using two types of extracts which prepare as below (**Figure 36, 37**) **(EL-Haoud *et al.*, 2018)**.

a). Aqueous extract

Approximately 2 g of dried powder of leaves added to boil distilled water. This soaked up was placed aside for 24 hours then filtered. The filtrate obtain used for the phytochemical screening.

b). Methanolic extract

Approximately 2 g of dried powder of leaves added to 20 ml of methanol. This soaked up was placed aside for 24 hours then filtered. The filtrate obtain used for the phytochemical screening.

III.5.1. Test for Glucosides (Fehling's test)

To 2 ml of each extract of the equal volumes of Fehling's solutions A and B was added to the aqueous extrait then boiled for few minutes. A red or brick red precipitate was developed. It is the standard test for reducing sugars **(Bhuiyan *et al.*, 2015)**.

III.5.2. Test for Proteins (Biuret test)

5-6 drops of 5% NaOH and 5-7 drops of 1% $CuSO_4$ were added in 2 ml aqueous extracts. Violet color indicated the presence of proteins **(Samejo *et al.*, 2013)**.

III.5.3. Test for Phenols (Ferric chloride test)

Each Methanolic extract from the leaves of the selected varieties was treated with few drops of neutral ferric chloride solution ($FeCl_3$) 5%, intense color developed indicates the presence of phenols **(Pandith, 2012)**.

III.5.4. Test for Steroids

Aqueous extracts were mixed with 2 ml of chloroform and concentrated H_2SO_4 was added sidewise. The development of a greenish coloration indicated the presence of steroids **(Yadav and Agarwala, 2011)**.

III.5.5. Test for Alkaloids

Preparation of Meyer's reagent

This was prepared by measuring 1.36 g of mercuric iodide ($HgCl_2$) on a weighing balance and 5 g of KI (Potassium Iodide) and dissolving both in distilled water, made up to 100 ml **(Udeozo *et al.*, 2013)**.

Method of screening

2 ml of the Methanolic extrait was mixed with 5 ml of 1% aqueous HCl. 1 ml of mixture was taken separately in test tube. Few drops of Mayer reagent was added and occurrence of orange-red precipitate was taken as positive **(Al-Daihan *et al.*, 2013)**.

III.5.6. Test for Terpenoids

5 ml of aqueous extracts were mixed with 2 ml of chloroform and few drops concentrated H_2SO_4 was carefully added to form a layer. Blue/green rings indicated the terpenoids are present **(Samejo *et al.*, 2013)**.

III.5.7. Test for Coumarins (NaOH test)

10% NaOH was added to the aqueous extracts, and chloroform was added. Formation of yellow color shows the presence of coumarins **(Vimalkumar *et al.*, 2014)**.

III.5.8. Test for Saponins

One gram of powdered sample was boiled in 10 ml of distilled water and then filtered. 3 ml of distilled water was added to the filtrate and shaken vigorously for about 5 min. formation of foam after shaking was taken as a confirmation for the presence of saponins **(Al-Daihan *et al.*, 2013)**.

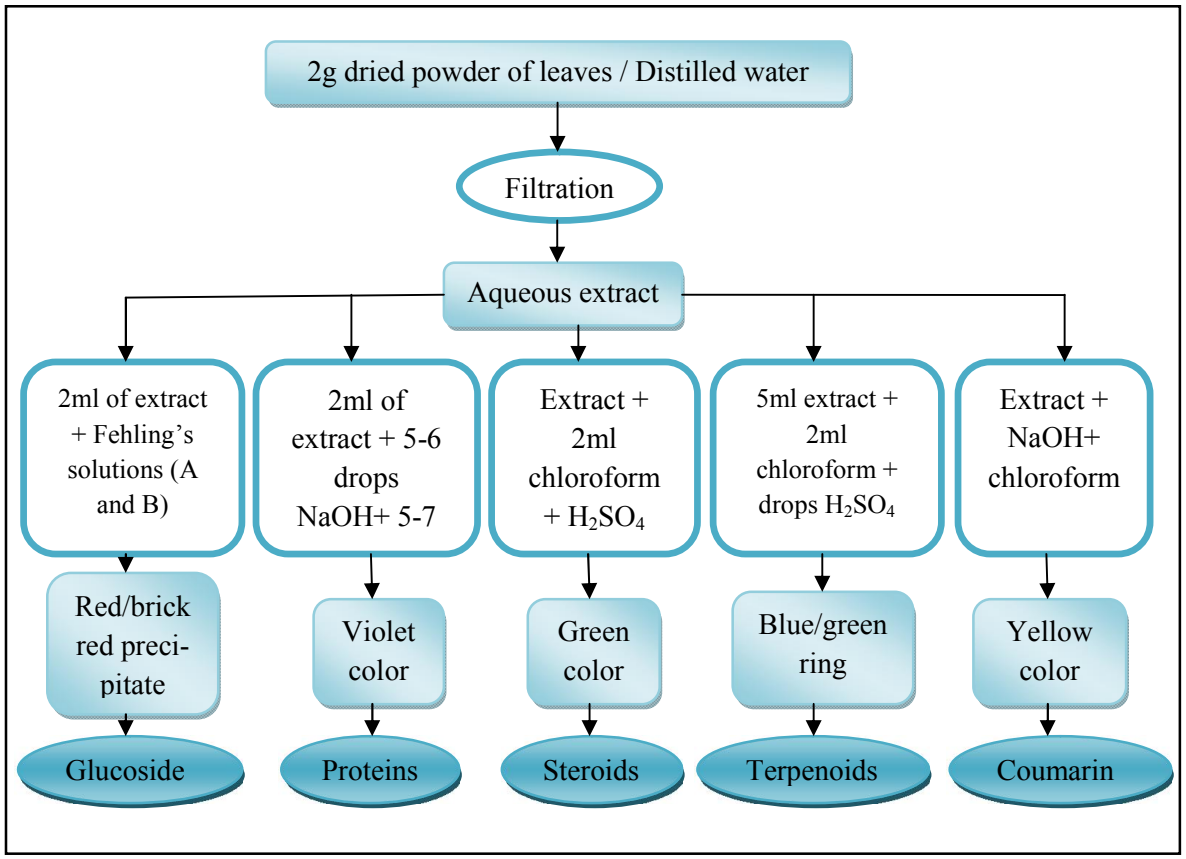


Figure 36. Phytochemical analysis tests (1)

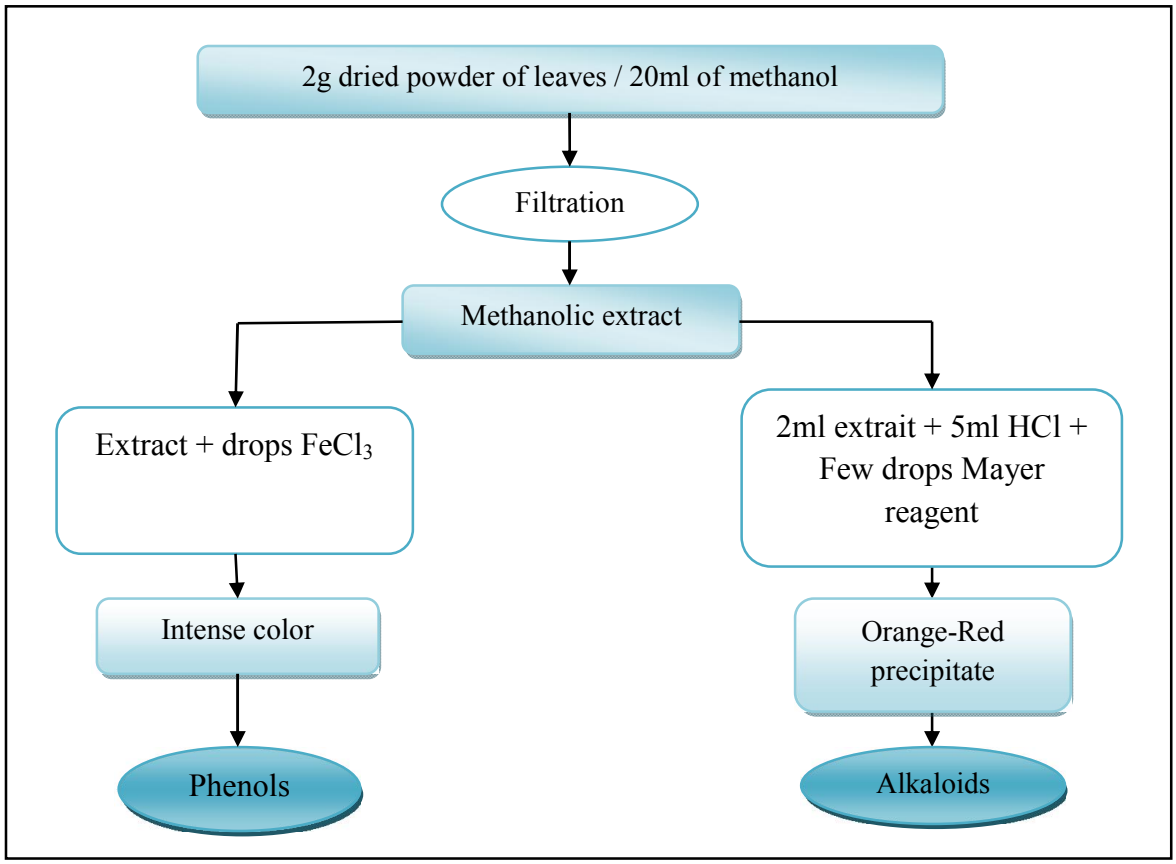


Figure 37. Phytochemical analysis tests (2).

III.6. Extraction

The extraction of phenolic compounds is generally based on solvents and is time-consuming. There are traditional and modern extraction techniques for polyphenols, such as solid-liquid extraction (SLE), pressurized liquid extraction (PLE), supercritical extraction (SCE), ultrasonic-assisted extraction (UAE), and microwave-assisted extraction (MAE) **(Ajila *et al.*, 2011)**.

III.6.1. Preparation of extract

There are many traditional methods to extract phenolic compounds and the method that we chose is a solid-liquid technique using maceration method (cold-soaking) according to **Benhammou *et al.*, (2008)**.

The principle of maceration extraction is based on the prevalence of phenolic compounds in the medium of a suitable extraction of organic solvents or a mixture of alcohol and water according to the method described by **Jovanovic *et al.* (2017)**.

III.6.2. The method of work

The dry plant material was macerated after weighing 1g of palm leaf powder for each variety in 50ml of the solvent, where two solvent systems were used; the first system contains absolute methanol (MeOH) and the second mixture consists of (8/2) (MeOH / H_2O), the mix leaving for 48h in the dark and at room temperature. After filtering the samples, the solvents were disposed of using a rotary evaporator, and finally the crude extracts obtained in the methanol were dissolved and stored in the refrigerator until use **(Figure 38-42)**.

Figure 38. Extraction operation by rotary evaporator.

Figure 39. Samples extract obtained.

Figure 40. MeOH and MeOH / H_2O extracts of date palm leaves.

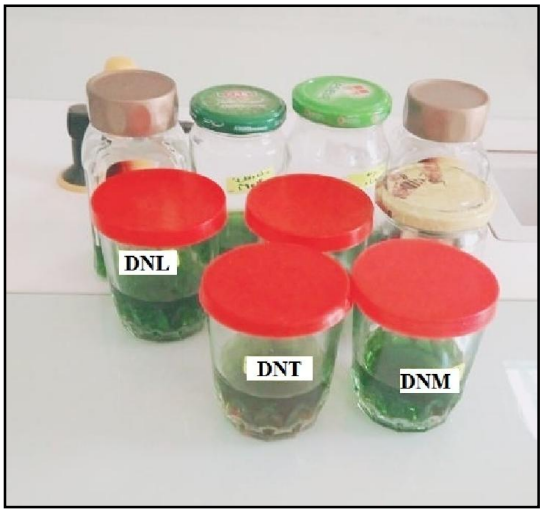


Figure 41. Samples after filtering.

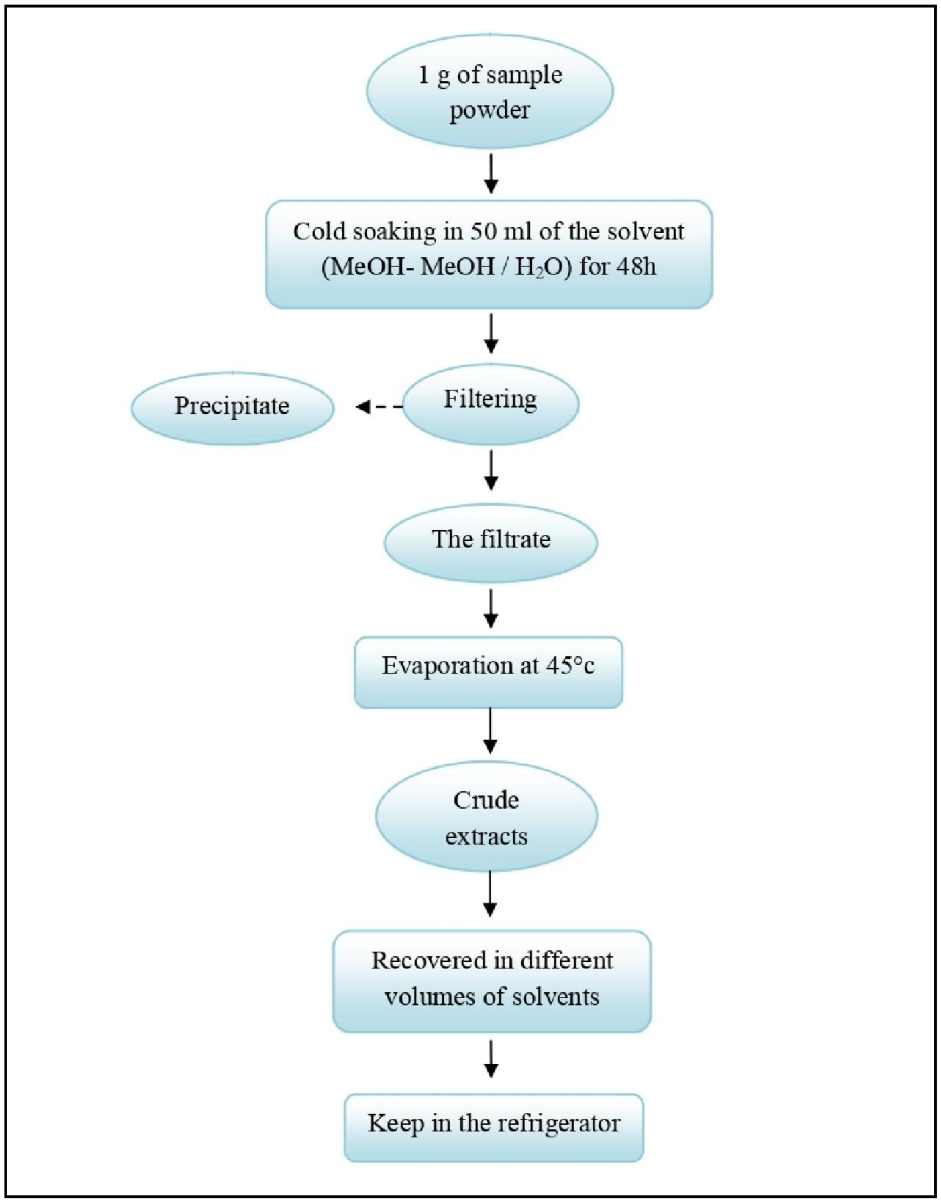


Figure 42. The method of extraction of total phenolic compounds.

III.6.3. Extraction yield

Calculation of extraction yield for the extracts of date palm leaves in the two solvent systems (MeOH) and (MeOH / H_2O) using the following equation.

$$\textbf{Yield (\%)} = (W_1 \times 100)/W_2$$

Where:

W_1: Weight of the extract residue obtained after solvent removal.

W_2: Weight of the plant powder.

III.7. Determination of total phenolic content (TPC) (Colorimetric method)

The total phenolic content was determined quantitatively (spectrophotometrically) using the Folin-Ciocalteu reagent, as described by **Dia *et al.* (2017)** with some modifications.

The reagent used is the "Folin-Ciocalteu" reagent; it is a mixture of complexes of phosphotungstate acids ($H_3PW_{12}O_{40}$) and phosphomolybdate ($H_3PMo_{12}O_{40}$) of yellow color. The principle of the method is based on the oxidation of phenolic compounds by this reagent; it leads to the formation of a new molybdenum-tungsten complex (MO_8O_{23})/ (W_8O_{23}) of blue color which absorbs at 760 nm **(Laouini, 2014)**.

III.7.1. Prepare the standard solution

Starting with a standard solution of gallic acid (GA) at a concentration of 0.3 g/l, a series of standard extensions were prepared at concentrations of 0.03-0.3 (g/l), 100 l were taken from each standard solution, and 500l of the Folin-Ciocalteu reagent was added at a concentration of 10% was mixed, and leave it for 5 minutes, then add 2 ml of Na_2CO_3 sodium carbonate solution at a concentration of 5%, the result was kept in the dark, and at room temperature for 30 minutes, then the absorbance was measured by the Spectrophotometer at the wavelength of 760 nm.

III.7.2. Sample preparation

An extended concentration was prepared for each extract and treated the same way that the GA standard series was treated; the concentration of total polyphenols in the extracts was expressed as mg gallic acid equivalent (GAE) per 100 g of dry weight, using the following equation.

$$\textbf{TPC (mg / 100g)} = \left(\frac{A}{K} \times d \times \frac{V}{m}\right) \times 100)$$

With:

A: Absorbance at 760 nm.

K: The standard slope of the gallic acid GA.

d: Coefficient of expansion relative to extracts.

TPC: Total phenolic content (mg/100g).

V: Volume of the extract solution in ml.

m: Primary weight of the dry sample in g.

III.8. Determination of antioxidant activity

III.8.1. DPPH radical scavenging activity

Various analytical methods for measuring the antioxidant capacity in biological samples have been developed, including the 2,2-azino-bis-3-ethylbenzthiazoline-6-sulfonic acid (ABTS) radical-scavenging assay, 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical-scavenging assay, ferric reducing antioxidant power (FRAP) assay, and oxygen radical absorbance capacity (ORAC) assay. Those methods have been conventionally used owing to their easy and fast characteristics. These assay methods are based on the radical-scavenging activity and redox potential of antioxidants. Those methods for antioxidant activity measurement were originally developed to measure this in foods and nutrients **(Chedea and Pop, 2019)**.

The DPPH° is a stable free radical which has an unpaired valence electron at one atom of nitrogen bridge **(Sharma and Bhat, 2009)**, reacts with compounds that can donate hydrogen atoms. The method is based on the scavenging of DPPH° by antioxidants which upon a reduction reaction, decolorizes the deep purple DPPH° methanol solution; when it mixed with a substance to a yellow color **(Figure 43) (Martinez *et al.*, 2006; Ndhlala *et al.*, 2010)**.

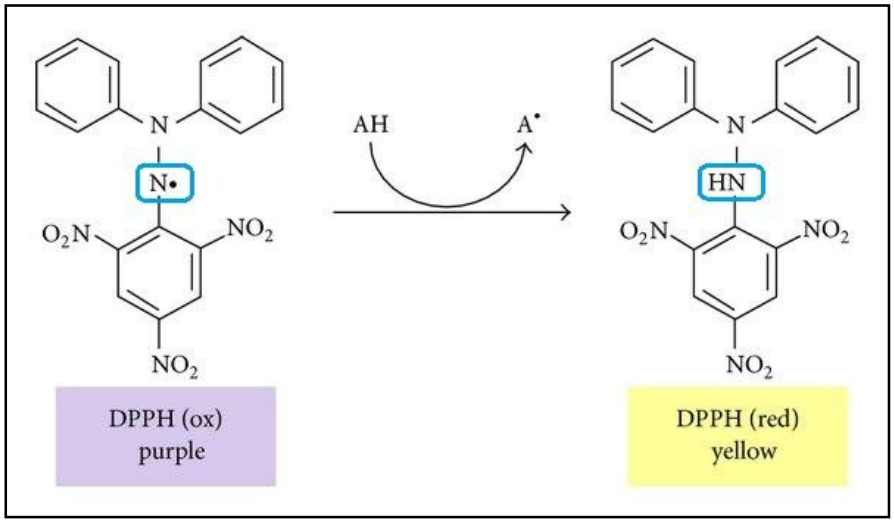


Figure 43. Principle of DPPH radical scavenging capacity assay **(Teixeira *et al.*, 2013)**.

III.8.2. Preparation of standard solutions

A standard solution of ascorbic acid was prepared at a concentration of 0.1 g/l, and then a standard chain of it was prepared at concentrations (0.01-0.1) g/l. 500 l of the methanol

solution was added to the radical of the DPPH° at a concentration of 250 µM, the mixture was shaken well and then kept in the dark at room temperature for 30 min, absorbance was finally measured at 517 nm wavelength.

III.8.3. Preparing the reference witness

A control solution containing the same volume of methanol was prepared in the location of the standard solution to measure the maximum absorbance of DPPH°.

III.8.4. Sampling preparation

A mother's solution was prepared for each extract with a specific concentration, and then extended concentrations were prepared from it, and treated in the same way as the standard solutions (**Figure 44, 45**).

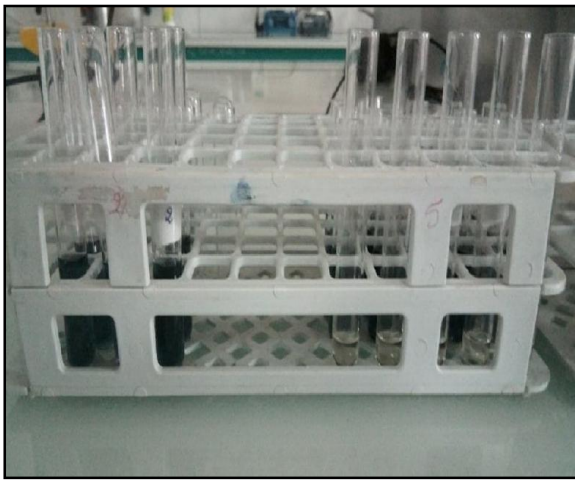


Figure 44. Mother's solution dilution of each extract.

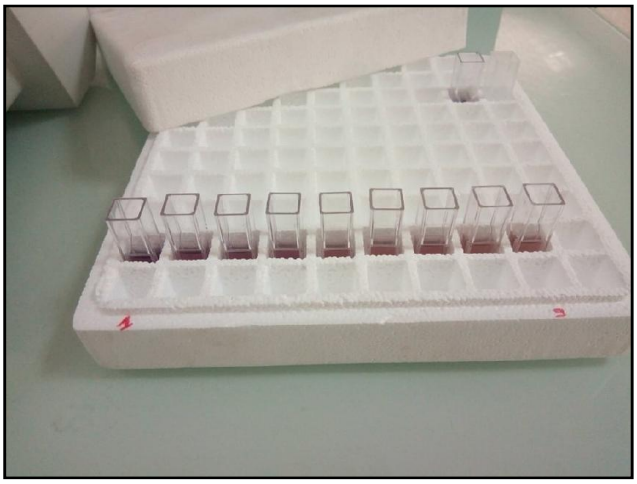


Figure 45. Testing of DPPH radical scavenging.

The inhibitory power of the extracts was determined by calculating the percentage of inhibition (I %) of DPPH° using the following equation:

$$(I\ \%) = (A_0 - A_i) / A_0 \times 100$$

Where:

I: DPPH scavenging activity (%).

A_0: The absorbance of control at 30 min.

A_i: The absorbance of the sample extract at 30 min.

III.9. Statistical Analysis

Three repetitions of every sample were analyzed. Data are expressed as means ± standard deviation (± SD). **Microsoft Excel Office 2007** used to process the statistics. While Pearson correlation coefficients were calculated using **SPSS** version 22.0 software.

Chapter IV

Results and Discussion

IV.1. Background

This chapter reviews the all of results obtained for extraction yield, TPC, and antioxidant activity. It were compared with previous studies, as well as their comparison based on three basic criteria, which are the varieties differences, the variance in the geographical area, as well as the difference of the extraction systems, which is an experimental factor that is implicit in the other criteria.

IV.2. Phytochemical screening

The preliminary evaluation of the phytochemical composition of the extracts of leaves of two selected varieties of *Ph. dactylifera* L. gives the results represented in **Table XVIII**, qualitative results are expressed as (+) for the obvious result and (++) for the more obvious whereas (−) for the absence of phytochemicals.

Table XVIII. Phytochemical analysis of the date palm leaves extracts.

Phytochemical test	DNL	DNM	DNT	GH
Glucosides	+	+	+	+
Proteins	+	+	+	+
Polyphenols	+	+	+	++
Alkaloids	+	+	+	+
Coumarins	+	+	+	+
Steroids	−	−	−	+
Saponins	+	+	+	+

From these results, it is noted that the extracts of the leaves of *Ph. dactylifera* L. DNL, DNM, DNT, and GH studied contain glucosides, proteins, polyphenols, alkaloids, coumarins, and saponins. This results somewhat correspond to the results obtained by **Al-zeiny and Abbas (2017)**.

The results of phytochemical analysis comprehensively validate the presence of therapeutically important and valuable secondary in date palm leaves extract. These secondary metabolites are important for plants, and therefore are able to protect plants from pathogens. Besides, they constitute important UV absorbing compounds, thus preventing serious leaf damage from the light **(Hussein and El-Anssary, 2018)**.

These being an integral part of the basic metabolism also have an ecological role and are often involved in plant protection against biotic or abiotic stresses. Moreover; plant

secondary metabolites have pharmaceutical properties effective for human health **(Jain *et al.*, 2019)**.

IV.3. Extraction yield

The sticky extracts with a green hue concerning the date palm leaves were obtained **(Figure 46)**, and then the yield ratio was calculated for each variety of date palm leaves in both extraction systems. All of the results are listed in **Table XIX**.

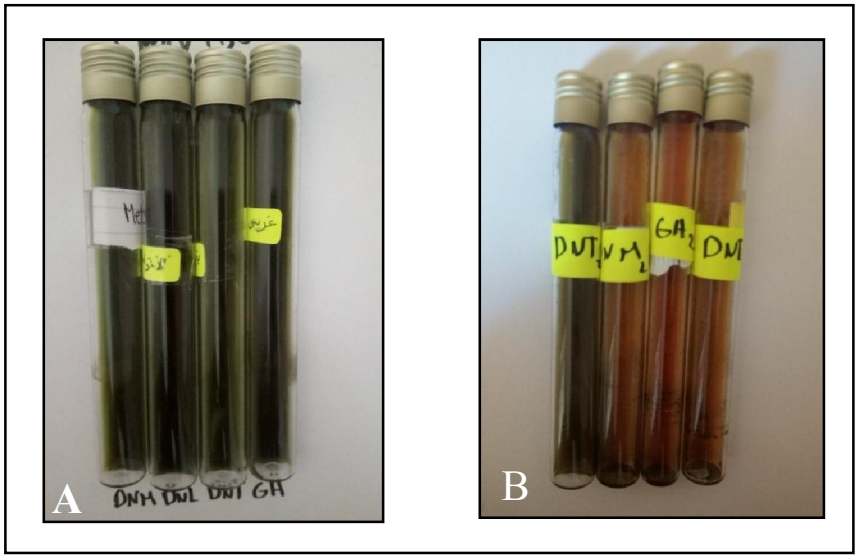


Figure 46. The extracts of date palm leaves: **A:** MeOH extracts, **B:** MeOH/H_2O extracts.

Table XIX. Extraction yield of *Phoenix dactylifera* L. leaves.

Variety	Region	Yield (%)	
		MeOH	**MeOH/H_2O**
DN	Laghouat	13.87	12.60
	El Meghaïer	10.67	11.90
	Tolga	12.60	18.40
GH		13.80	30.60

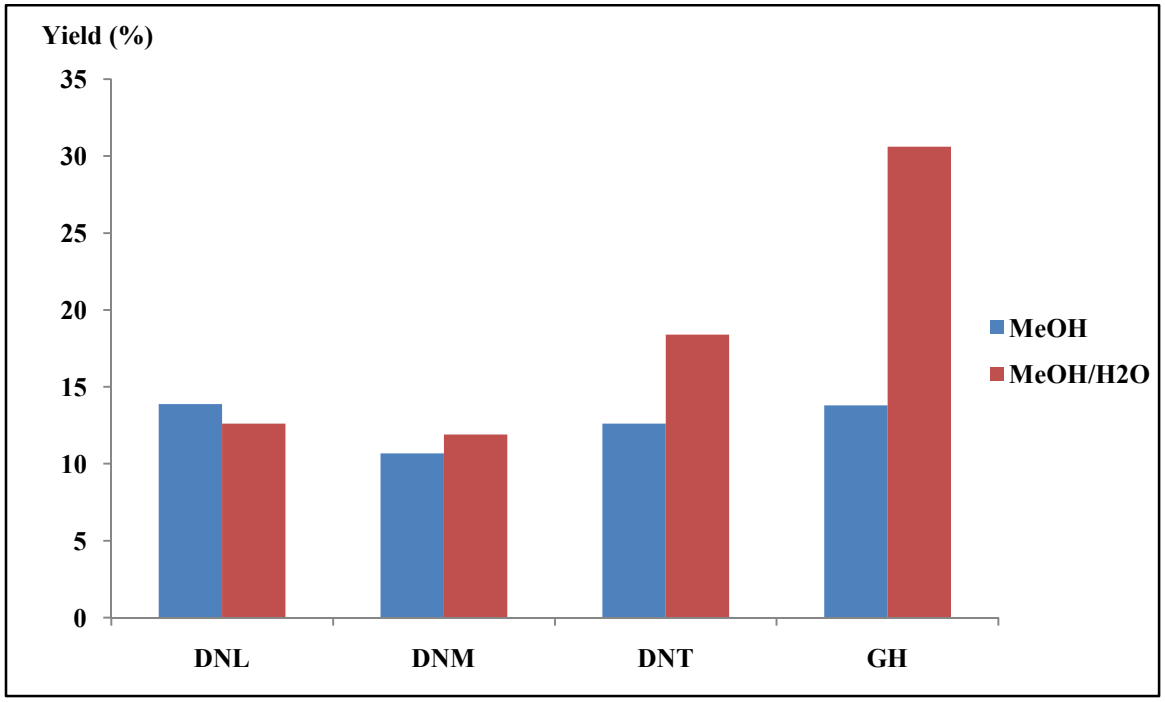


Figure 47: Extraction yield of *Ph. dactylifera* L. leaves in MeOH and MeOH/H_2O solvents.

Through the results obtained in **Table XIX**, we notice that the yield of extraction ranges between 10.67% and 30.60% in date palm leaves; the highest value obtained in the methanol system is 13.87% for the DNL variety, whereas in the MeOH / H_2O system, the highest value is for the GH variety with 30.60%.

We also note that the yield values were affected by the change in the geographical area, where the best yield of DN was recorded in the methanol system for the Laghouat sample, whereas in the MeOH / H_2O system, the best yield was for the Tolga region sample.

It is also worth noting that yield values differed in the Tolga region; where in the booth systems (MeOH and MeOH / H_2O) the yield value of GH variety was better than DNT.

The most effective extraction solvent for samples DNT and DNM of DN variety, and GH variety is the methanol system (MeOH) while for DNL sample the most effective extraction solvent is the MeOH / H_2O as **Figure 47** shows.

In these study, the range of yield extraction is highest than the study of **Badereddine and Moussaoui (2014)** about three varieties of *Ph. dactylifera* L. leaves (Deglet Nour, Ghars, Hamraya) were collected from the region of El-Oued with three types of extraction (maceration, ultrasound, soxhlet) and using MeOH/H_2O (70 % - 30 %) as a solvent which varied from 6.89 % to 13.60 % (maceration), 5.24 % to 8.58 % (ultrasound) and 12.59 % to 22.55 % for soxhlet method.

Whereas, the results of the study of **Kriaa *et al.* (2012)** are in close agreement with own results by 6.6 % to 30 % range, this study is about three Tunisian varieties of

Ph. dactylifera L. leaves (Deglet Nour, Medjhoul, and Barhee) with maceration method of extraction and using four types of solvents; hexane, ethyl acetate, methanol, and water.

In the study of **Laouini *et al.* (2012)** about three varieties of *Ph. dactylifera* L. leaves (Deglet Nour, Ghars, Hamraya) were collected from the region of Djedeida (El-Oued). The extraction yields of leaves obtained by methanol 80% ($MeOH/H_2O$) solvent are in the range of 15.64 % - 19.12 %. The yield value of Deglet Nour (DN) is 19.12 %, this value is the highest among the values of own results for Deglet Nour samples (DNT, DNL, DNM) whereas, about Ghars variety our yield value (30.60 %) is much higher than the sample of **Laouini *et al.* (2012)** study (16.50%), Bearing in mind that the solvent is identical ($MeOH/H_2O$). Another study of **Laouini *et al.* $(2013)_a$** about the same varieties mentioned above and collected from the same region the difference just in the method of extraction (Soxhlet) and using 70% ethanol as solvent, the yield ranged between 16.20 % and 18.43 %. Similarly **Laouini *et al.* $(2013)_b$** reported yield ranged between 15.64 % and 19.12 % for the same samples also, and maceration as technique of extraction and 80 % MeOH as solvent.

A study of **Dia *et al.* (2018)** estimated the yields of crude extracts from the leaves of *Ph. dactylifera* L., obtained by diverse methods (classical, ultrasound, and soxhlet extraction) using a different type of aqueous solvents (methanol, ethanol, and acetone). The recovery percentage of extractable compounds of the extracts is ranged from 10.66 % to 25.73%. This is in close agreement with our study.

Many techniques using to obtaining phytochemicals from plants such as milling, grinding, homogenization, and extraction. Among these steps, extraction is the main step for recovering and isolating phytochemicals from plant materials **(Stalikas, 2007)**.

The variations in yield extract might be explained by the polarity of extracted component and solvent applied solubility of endogenous compounds, the extraction method used, sample particle size, and amount of extractable material presented in variety **(Mujtaba *et al.*, 2016)**. The extractive yield directly affected by the longer extraction time values allow for the longer amount of time the solute and solvent were in contact, so it makes the systems have been successfully mass transfer. This generally led to a higher percentage yield. The yield of extraction also depends on the pH and temperature **(Tambunan *et al.*, 2017; Dia *et al.*, 2018)**.

IV.4. Total Phenolic Content (TPC)

Calibration curve for the determination of Total Phenolic Content (TPC) is established using commercial gallic acid and the results are expressed in mg gallic acid equivalent per gram of dry matter (mg GAE/100g DW). The calibration curve is established with a correlation coefficient $R^2 = 0.991$ **(Figure 48)**.

The results of Total Phenolic Content (TPC) in *Ph. dactylifera* L. leaves are represented in the **Table XX** for MeOH and MeOH / H_2O solvents.

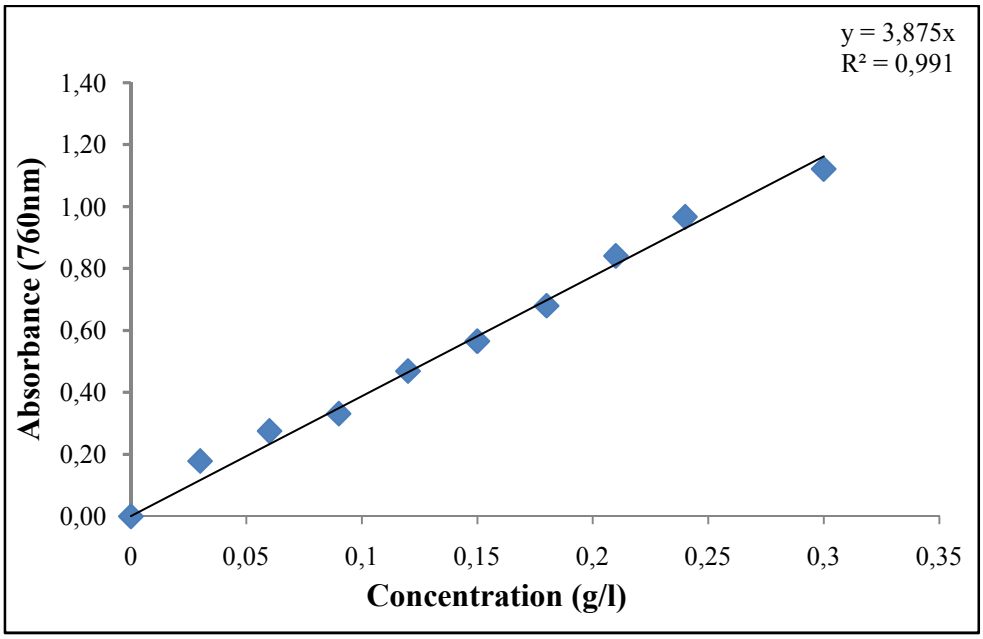


Figure 48. Calibration curve of gallic acid.

Table XX. Total phenolic content (TPC) in different *Ph. dactylifera* L. leaves samples.

Variety	Region	TPC (mg GAE/100g DW)	
		MeOH	MeOH/H_2O
DN	Laghouat	2131.61±65.69	3791.61±73.90
	El Meghaïer	4232.26±350.36	5455.48±983.56
	Tolga	2696.13±95.80	5032.26±131.39
GH		13993.55±246.35	6750.97±43.80

The amount of TPC varied widely in the date palm leaves extracts investigated and ranged from 2131.61 to 13993.55 mg GAE/100g DW. The higher amount of these compounds found in MeOH extract of GH variety 2131.61 mg GAE/100g DW and the lowest concentration obtained from MeOH extract of DNL variety 2131.61 mg GAE/100g DW.

These concentrations are lower if are compared to the results obtained by those studies; **Laouini (2014)** has reported the total phenolics compounds from 15646 to 21524 mg GAE/100g DW, this study was about three varieties of *Ph. dactylifera* L. leaves (Deglet Nour,

Ghars, Hamraya) with extraction by soxhlet and using 80% ($MeOH/H_2O$) solvent. In addition, study of **Laouini *et al.* (2013)$_a$** about the same varieties mentioned above and collected from the same region, the difference is just in the method of extraction (Soxhlet) and using 70% ethanol as solvent, the yield ranged between 18027 and 34245 mg GAE/100g DW.

Another two studies showed the highest amount of total phenolics, the first study is of **Dia *et al.* (2017)** about *Ph. dactylifera* L. leaf extract recovered by ultrasonic-assisted extraction (UAE), soxhlet extraction (SE) and maceration extraction (CE), using 70 % acetone solvent. The results ranged from 48444 to 62517 mg GAE/100g DW. The second study is for **Dia *et al.* (2018)** estimated the total phenolics of crude extracts from the leaves of *Ph. dactylifera* L., obtained by diverse methods (classical, ultrasound, and soxhlet extraction) using a different type of aqueous solvents (methanol, ethanol, and acetone). The results obtained are in the range of 17264 - 62517 mg GAE/100g DW. The same applies to the results obtained in the study of **Tirichine (2010)** which ranged between 8577 and 24456 mg GAE/100g DW.

The present results were in close agreement with to those reported by **Badereddine and Moussaoui (2014)** about three varieties of *Ph. dactylifera* L. leaves (Deglet Nour, Ghars, Hamraya) with three types of extraction (maceration, ultrasound, soxhlet) and using $MeOH/H_2O$ (70 % - 30 %) as a solvent. This reported that TPC values were ranged from 3210 to 13145.6 mg GAE/100g DW.

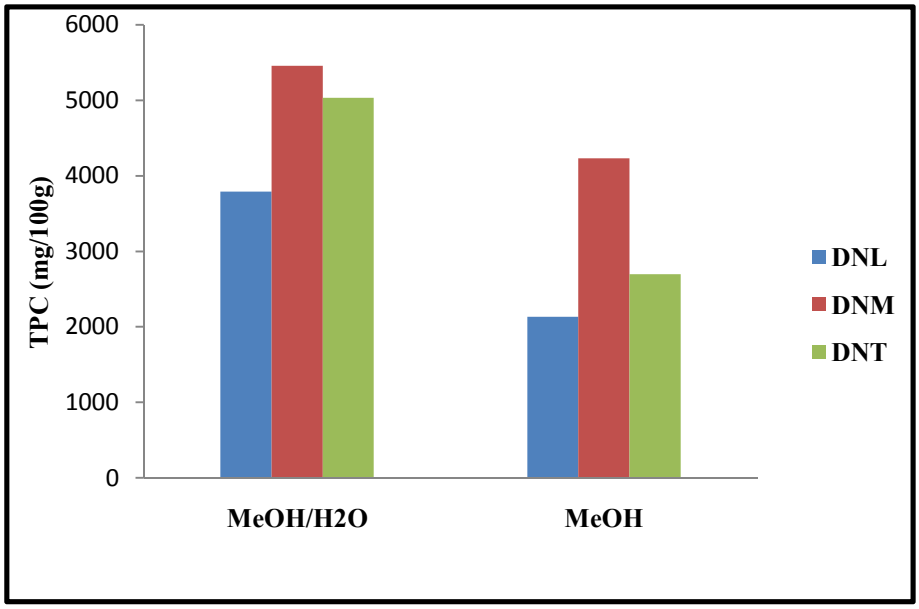


Figure 49. Comparative columns for the TPC based on the geographical area of date palm leaves.

We can observe from **Figure 49** that the amount of phenolics compounds of the DN variety samples differs from one geographical area to another, and their amount varies with the change of the extraction system.

The arrangement of the quantity of phenolics compounds of Deglet Nour (DN) variety leaves in the three regions was the same in the two systems of solvent, and arranged as follows: **DNM > DNT > DNL**, thus it can be considered that the MeOH / H_2O solvent is the most effective to extract the largest amount of phenolics compounds concerning the variety of DN leaves.

The observed differences may mainly be attributed to the cultivars and extraction conditions such as solvent and ratio material/solvent **(Ghiaba *et al.*, 2014)**. There are also many other hypotheses such as growing conditions, maturity, seasons, geographic origin, fertilizer, soil type, and storage conditions. The amount of sunlight received may also be critical in this respect **(Bouhlali *et al.*, 2017)**.

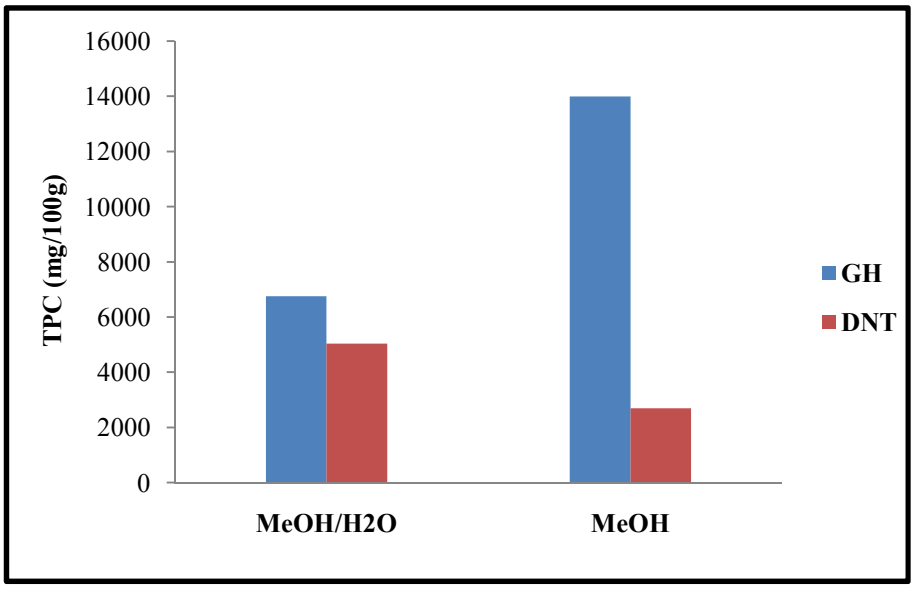


Figure 50. Comparative columns for the TPC based on the varieties differences of date palm leaves.

We notice from **Figure 50** that the amount of phenolics compounds of the booth varieties Deglet Nour and Ghars was affected by the varieties differences, and their amount varies with the change of the extraction system.

For the Methanolic extracts the amount of phenolics compounds in the GH variety is higher than the DN variety and it is the same for MeOH / H_2O extracts. Taking into account the extent of contrast in the Methanolic extracts is much higher.

It can be mentioned also that's the most effective solvent for GH variety is the MeOH, while for the DN variety is the MeOH / H_2O solvent.

This difference in the results obtained is mainly due to the difference in variety, maturity, and genetic diversity **(Ghiaba *et al.*, 2012; Ghiaba *et al.*, 2014)**.

IV.5. Correlation between Extraction yield and TPC

Pearson correlation coefficients were calculated in order to measure the linear correlation between variables. All statistical calculations were performed by using **SPSS** (Statistical Product and Service Solutions) software version 22.0. The graphical expressions of the results are shown in **Figure 51** and **Figure 52** while different statistical significance is shown in **Table XXI**.

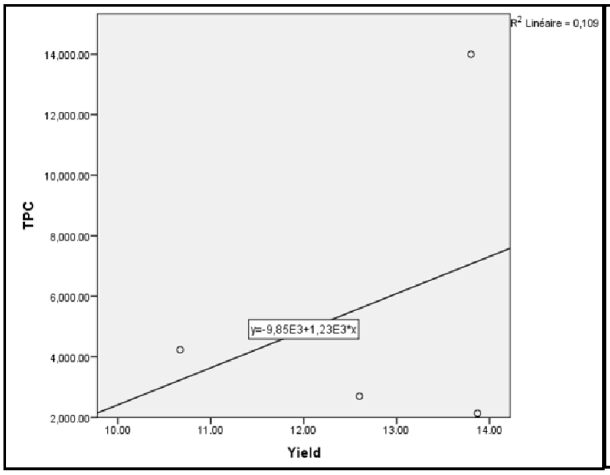


Figure 51. Correlation between Yield (%) and TPC mg GAE/100g DW in date palm leaves (MeOH extracts).

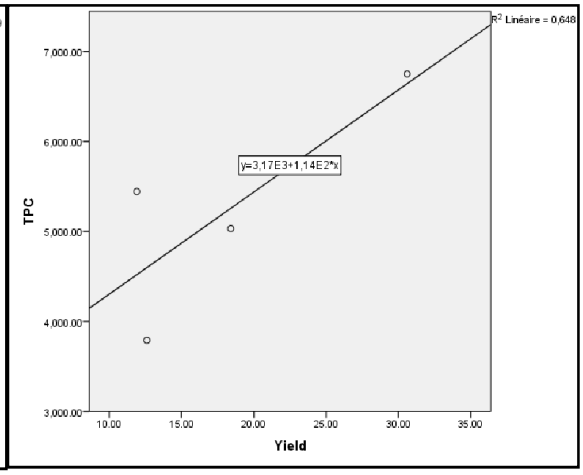


Figure 52. Correlation between Yield (%) and TPC mg GAE/100g DW in date palm leaves (MeOH/H_2O extracts).

Table XXI. Different statistical significances values.

Statistical significance	Linear regressions (β)	Pearson's correlation coefficient (r)	Coefficient of determination (R^2)
Yield vs.TPC MeOH extracts	1.23×10^3	0.330	0.109
Yield vs. TPC MeOH/H_2O extracts	1.14×10^2	0.805	0.648

Depending on **Figure 51, 52** and **Table XXI** we can notice positive linear regressions in both systems of extraction (MeOH and MeOH/H_2O). The positive linear regressions were attributed to the fact that there is a positive relationship between yield and TPC.

Extraction yield and total phenolics content (TPC) were significantly correlated in MeOH/H_2O extracts of date palm leaves ($r = 0.805$). This implies a strong positive correlation between extraction yield and TPC (R^2 %= 64.8 %).

Whereas, for MeOH extracts the extraction yield and total phenolics content values show weak correlated (r = 0.33). This implies a weak positive correlation between extraction yield and TPC (R^2 %= 10.9 %).

Correlation analysis shown that there is correlation between yield and total phenolics content, where it is very much higher in MeOH extracts than in MeOH/H_2O extracts which suggests that MeOH was a better extraction solvent (efficiency) for phenolics.

Extraction and isolation are the first important steps for separation, characterization, and quantification of phenolics content from plant material. Phenolics content are often most soluble in organic solvents less polar than water. Solubility depends on the polar properties of the phenolics content **(Haminiuk *et al.*, 2014)**. Therefore type of extraction solvent as well as the isolation procedures may have a significant impact on the yield of extraction phenolics content from plants material of date palm leaves. It is also suggested that it was also possible to obtain higher correlation in MeOH extracts if it was conducted the isolation and purification processes.

IV.7. DPPH radical scavenging activity

The results obtained from the determination of DPPH° scavenging activity of the studied extracts and the calibration curve of the ascorbic acid is expressed in **Figures 53-55**.

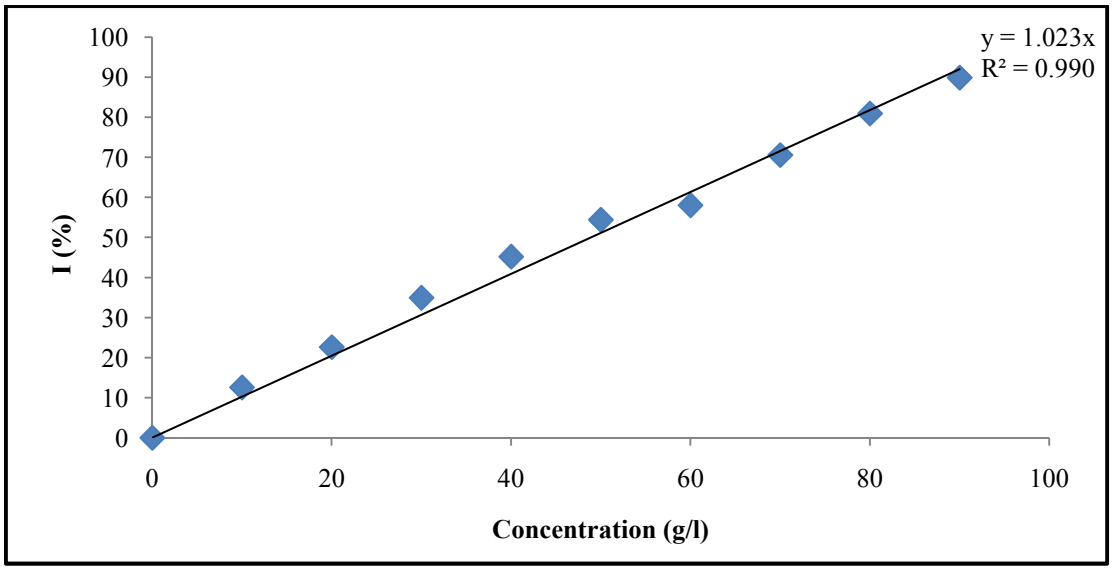


Figure 53. Calibration curve of DPPH ° assay of Ascorbic acid.

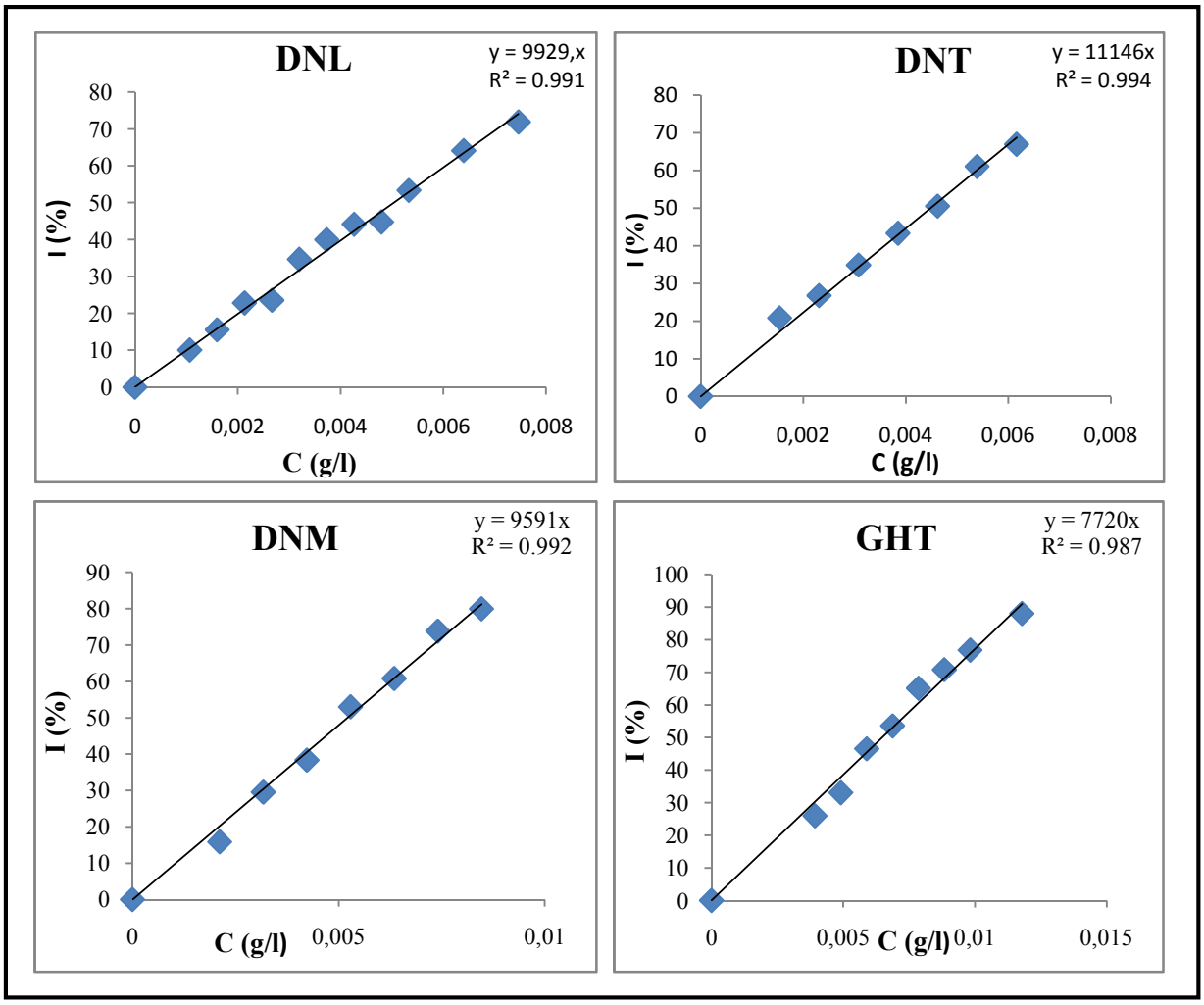


Figure 54. DPPH° assay curves of leaves varieties in MeOH system.

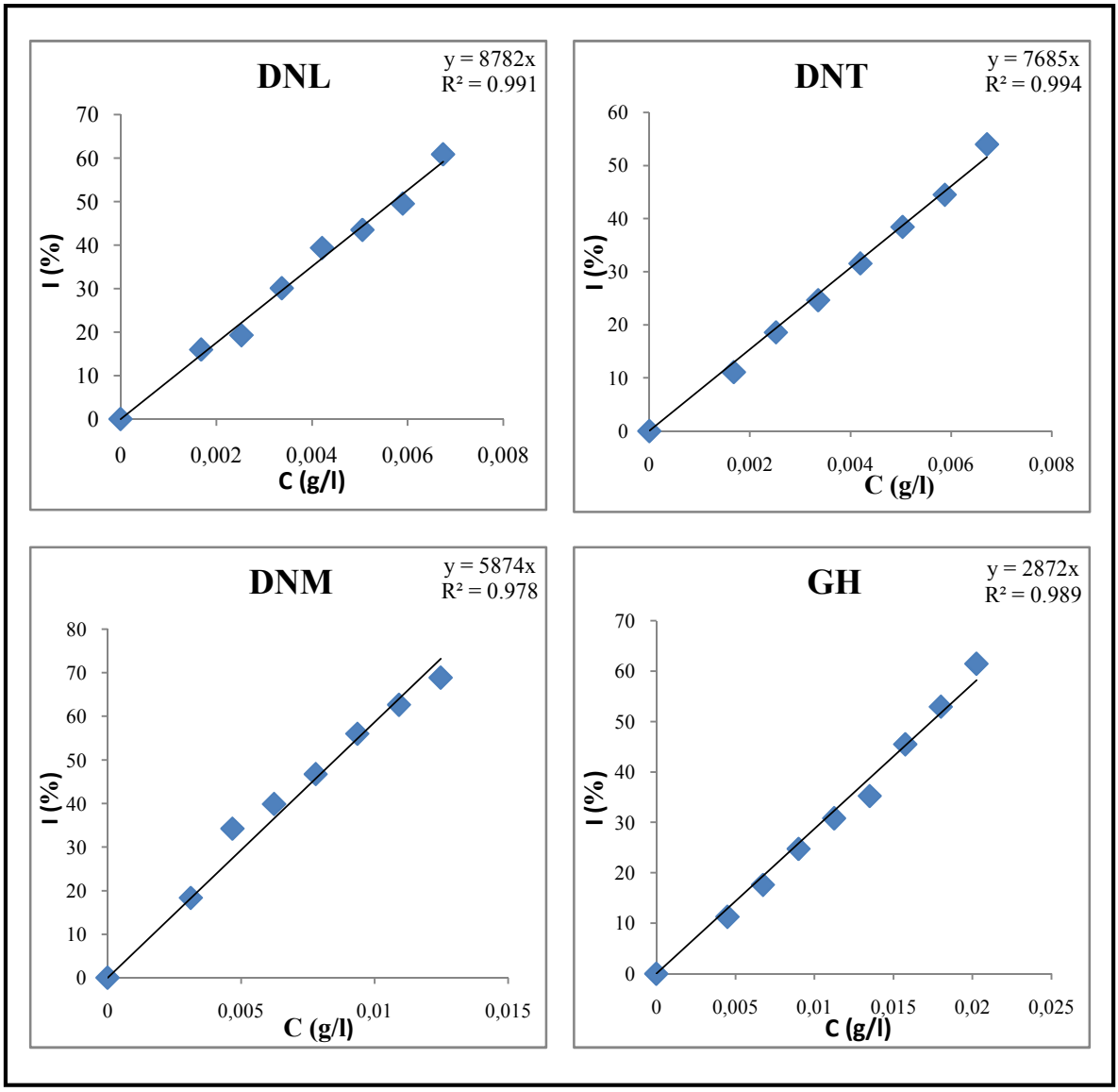


Figure 55. DPPH° assay curves of date palm leaves varieties in MeOH/H_2O system.

We notice through **Figure 54** and **Figure 55** that there is a direct relationship between the extracts concentration and the rate of inhibition that whenever the concentration increases, the inhibition of the different samples of date palm leaves studied increases and this in both of extraction systems.

According to **Ghiaba (2012)** and **Trabzuni *et al.* (2014)**, the ability of test samples to scavenge DPPH° radical was assessed on the basis of their IC_{50} values, defined as less concentration inhibits 50% of DPPH° radical activity, so it is the concentration of extract to reduce 50% of the initial concentration of the DPPH° radical, it calculated from the formula below which determines the inhibition rate in terms of the inhibitor concentration, whene the IC_{50} is lower the ability to scavenge the radicals is better.

$$\mathbf{IC_{50} = 50/K}$$

With: **K:** The standard slope of extracts.

Table XXII. IC_{50} values of date palm leaves extracts**.**

Variety	Region	IC_{50} (g/l)*	
		MeOH	**MeOH/H_2O**
DN	Laghouat	0.0050±5.29	0.0057±3.28
	El Meghaïer	0.0052±1.54	0.0085±4.16
	Tolga	0.0044±6.45	0.0065±3.76
GH		0.0018±9.07	0.0043±8.01
Ascorbic Acid		0.0048±3.20918	

* SD $\pm\times10^{-5}$

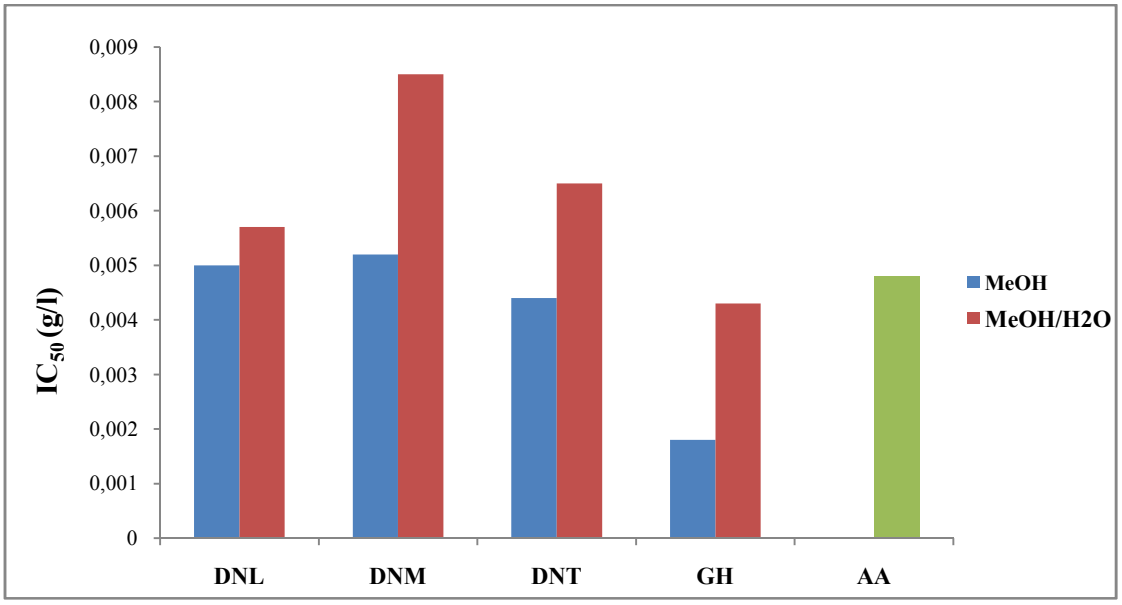


Figure 56. Graph of IC_{50} values of date palm leaves extracts and ascorbic acid.

We observe from the values obtained and mentioned in **Table XXII** that all the extracts studied have a capacity of DPPH° radical inhibition that it is in the range of (0.0018-0.0085) g/l, the highest value is MeOH/H_2O extract of DNM variety and the lowest value is MeOH extract of GH variety.

These results are bigger than those obtained in **Laouini (2014)** and **Laouini *et al.* $(2013)_a$** studies which are mentioned previously and they are in the range of (0.00298-0.00483) g/l. However, the results are smaller than those obtained in **Tirichine (2010)** study about twenty leaves varieties of date palm which are in the range of (0.0006-0.02847) g/l and **Laouini *et al.* (2014)** study that is in the range of (0.00744-0.01261) g/l.

According to these results, we can also observe that all the studied extracts have an approximately antioxidant activity to the ascorbic acid which is one of the effective antioxidant used in the food industry **(Figure 56)**, the GH variety of the MeOH extract

showed an activity better three times than the ascorbic acid, while DNM sample of the MeOH/H_2O extract showed an activity nearly less twice than the standard.

By changing the geographical area **(Figure 56)**, we observe a different antioxidant activity to DN variety leaves and also different between the extraction systems, the arrangement of IC_{50} values in Methanolic system was as follows: **DNM > DNL > DNT**, while in the MeOH / H_2O system it was arranged as **DNM > DNT > DNL**.

Generally, the antioxidant activity of date palm leaves was more effective in the Methanolic extraction system.

This difference in antioxidant activity is due to geographical and agricultural factors changing. Also, sunlight effects on the antioxidant activity in the same variety; leaves are exposed to the sunlight that influences the biosynthesis of phenolic compounds, especially in the genetic expression of these compounds genes **(Allaith, 2019)**.

The results differ between varieties leaves DNT and GH harvested from the same region (Tolga) **(Figure 56)**, as well as between the extraction systems. The IC_{50} value of DN variety is bigger than the IC_{50} value of GH variety in both systems, that mains the GH variety has better antioxidant activity.

The difference of results can be explained because of variation of varieties and the distribution of phenolic compounds in plant parts of varieties, and else the effect of watering with wastewater which increases the antioxidant activity, but the most important factor is the genetic factor **(Ghiaba, 2012; Allaith, 2019)**.

The antioxidant activity of date palm leaves extracts were also expressed as the ascorbic acid equivalent antioxidant capacity (AEAC) as g AAE/100 g DW. The AEAC was calculated with the equitation below **(Velde *et al.*, 2013)**. The results of antioxidant activity AEAC expressed as graph is shown in **Figure 57**.

$$\mathbf{AEAC = IC_{50\ (AA)} / IC_{50\ (Sample)} \times 10^3}$$

$IC_{50\ (AA)}$: was determined with an ascorbic acid calibration curve and its value was 0.0048 (g/l).

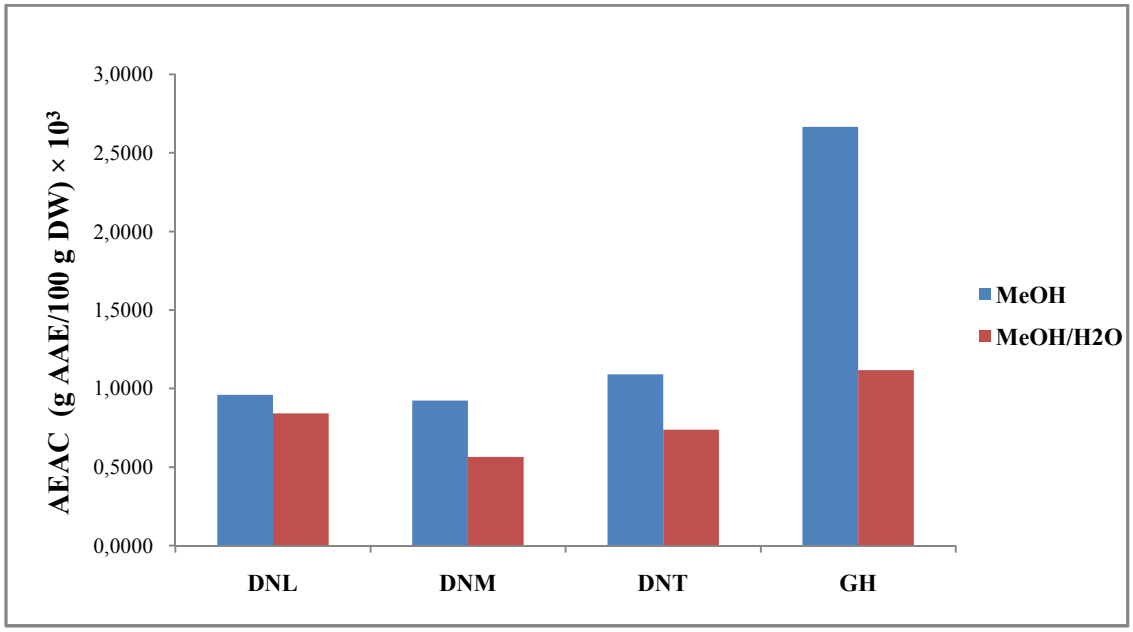


Figure 57. The antioxidant activity (AEAC) of date palm leaves extracts (MeOH-MeOH / H_2O).

The antioxidant activity (AEAC) of date palm leaves extracts ranged from 560 to 2660 (g AAE/100 g DW). The results of comparison depend on the geographical area, varieties, and solvents were compatible and homogeneous with the antioxidant activity results expressed by IC_{50} values described before.

IV.8. Correlation between TPC and antioxidant activity

Phenolics molecules are important antioxidant components that are responsible for deactivating free radicals based on their ability to donate hydrogen atoms to free radicals. Different literature reports indicate a linear correlation of total phenolics content with antioxidant activity **(Aryal *et al.*, 2019)**.

Pearson correlation coefficients were calculated in order to measure the linear correlation between variables. All statistical calculations were performed by using **SPSS** (Statistical Product and Service Solutions) software version 22.0. The graphical expressions of the results are shown in **Figure 58** and **Figure 59** while different statistical significance is shown in **Table XXIII**.

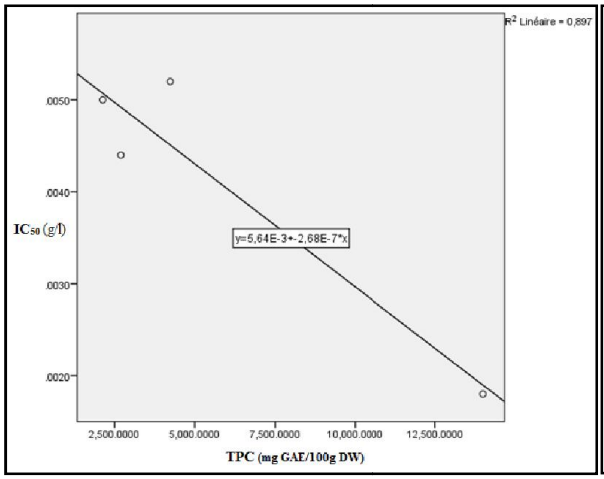


Figure 58. Correlation between TPC and IC_{50} values in date palm leaves (MeOH extracts).

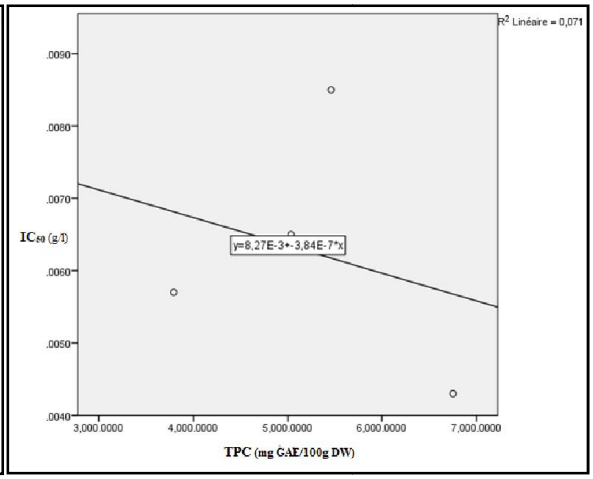


Figure 59. Correlation between TPC and IC_{50} values in date palm leaves ($MeOH/H_2O$ extracts).

Table XXIII: Different statistical significances values in date palm leaves.

Statistical significance	Linear regressions (β)	Pearson's correlation coefficient (r)	Coefficient of determination (R^2)
TPC vs. IC_{50} (DPPH) MeOH extracts	-2.68×10^{-7}	-0.947	0.897
TPC vs. IC_{50} (DPPH) $MeOH/H_2O$ extracts	-3.84×10^{-7}	-0.0267	0.071

Depending on **Figure 58, 59** and **Table XXIII** we can notice negative linear regressions in both systems of extraction (MeOH and $MeOH/H_2O$). The negative linear regressions were attributed to the fact that the lower value of IC_{50} (DPPH assays), means higher inhibition of the antioxidant activity.

Total Phenolics Content (TPC) and IC_{50} were significantly anti-correlated in MeOH extracts of date palm leaves (r = -0.947). This implies a strong positive correlation between TPC and antioxidant activity (R^2 %= 89.7 %) because lower IC_{50} value correspond with higher antioxidant activity.

From these results we can suggest that the presence of phenolics compounds in date palm leaves extract may be responsible for its antioxidant activities. However phenolics compounds present in the extracts are a good source of electron donors **(Singh *et al.*, 2016)**.

Thus, the total phenolics contents in the extracts can be used to predict their antioxidant activities with reasonable accuracy **(Alara *et al.*, 2016)**.

Whereas, for MeOH/H_2O extracts the total phenolics content (TPC) and IC_{50} values show weak anti-correlated (r = -0. 0267). This implies a weak positive correlation between TPC and antioxidant activity (R^2 % = 7.1 %).

Although aqueous methanol (MeOH/H_2O) is known to be an efficient and broadly used solvent system to extract natural anti-oxidative compounds, especially the phenolics. This is thus greater efficacy towards the extraction of polar phytochemical but it can also extract non-phenol compounds such as carbohydrates and terpenes **(Mujtaba *et al.*, 2016)**.

The TPC is the sum of reaction potential (including radical scavenging and metal chelation) of the phenolics compounds. It is, however, not the sum of amounts of phenolic compounds due to variability in the reaction potentials of different phenolic compounds. Antioxidant activity, on the other hand, is the measure of the consequences of radical scavenging components including polyphenols and other non-phenolics antioxidants **(Niroula *et al.*, 2019)**.

Besides, the synergistic effects of the different compounds, experimental conditions and mechanisms of antioxidant reactions method used may affect the association between the bioactive compounds and antioxidant activity **(Jayaprakasha and Patil 2007)**. In addition, most of the methods have their limitations in the determination of antioxidant activity such as the different reaction mechanisms involved in these assays **(Tamuly *et al.*, 2013)**.

As a result of all data, it can be assumed that the antioxidant activity of extracts is greatly dependent on the composition of the polyphenol complex as well as the amount and structure of the compounds. The number and amount of components, which possess high radical scavenging potential, to a high extent, predetermines the activity of the extracts. It is of great importance to choose the proper solvent which is able to extract as much as possible of the components from the polyphenol complex with simultaneously high yields and appropriate structure for analysis or medicinal use **(Dagnon *et al.*, 2018)**.

Conclusion

Conclusion

This study belongs to the biological studies framework, about the biological activity of phytochemical secondary metabolites, which depend on laboratory work to obtain results that reflect the extent of the existence of these biological activities and their effectiveness in plants. Our study is a ring in the chain of biological activity of date palm mainly about the antioxidant activity through the quantification of phenolic compounds in two solvent systems and measuring the value of this activity using the DPPH° scavenging assay in Deglet Nour and Ghars varieties.

Through the quantification of phenolic compounds results we reached to: Firstly, the phenolic compound quantity in date palm leaves varieties is affected by the geographical area, that we have found the highest value of phenolic compounds in MeOH/H_2O extract of DNM sample (DN variety). Secondly, the amount of phenolic compounds in date palm leaves varieties is affected by the variety; we have found the highest value of phenolic compounds in the GH variety of the Methanolic extract. Finely, the amount of phenolic compounds differs according to the extraction system in all varieties.

Through the determination of antioxidant activity we reached: Firstly, the both varieties of date palm showed an antioxidant activity. Secondly, the value of the antioxidant activity of date palm leaves varieties is affected by the geographical area, we have reported the highest value in the Methanolic extract of DNT sample (DN variety), and this confirms the first hypothesis. Thirdly, the value of the antioxidant activity of date palm leaves varieties is affected by the variety where we have found the highest one in the GH variety of the Methanolic extract, and this confirms the second hypothesis. Then, the extraction system effects of the antioxidant activity in all studied varieties, and this confirms the third hypothesis. Finally, there are relationship and correlation between the total phenolic content and antioxidant activity in date palm leaves, and this confirms the fourth hypothesis.

Through our study, we have established a set of recommendations concerning both the academic and the applied side, as follow:

- Concentrate on the clarification of chemical concepts related to biological activities.
- The botanical study of local date palm varieties.
- Interest in study the biological activity of date palm in its various botanical parts.
- Insertion of date palm leaves into alternative medicinal recipes.
- Attention of updating methods for separating and purifying phenolic compounds to precisely determine the most effective compounds in the antioxidant activity.

We propose for the future studies some prospects and topics which came as an extension of our study and to compensate for the deficiencies of it and repair it:

Conclusion

- Quantification of flavonoid and tannic compounds in date palm varieties.
- Comparative study of phytochemical composition of local date palm varieties.
- Study of biological activities of date palm (anti-microbial activity, anti-inflammatory activity …etc).
- Study of antioxidant activity of the various plant parts of date palm.

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