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THEME

Phytochemical profile and Anti-inflammatory activity of date seeds (*Phoenix dactylifera* L. *)* variety (Ghars) "in vitro"

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بسم الله الرحمن الرحيم

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Abstract

The aim of this study is to evaluate the phytochemical properties and anti-inflammatory activity of date seeds from the "Ghars" variety, *Phoenix dactylifera.L*. The extraction yield by soaking in a methanol/water solution (80/20) was estimated at 11.425% for the date pit sample. Total polyphenol content was determined using Folin-Ciocalteu reagent, resulting in a concentration of 290.5 mg/mL. The flavonoid content was estimated to be 12.2 mg/mL by aluminum chloride (AlCl3) analysis. The extract showed a condensed tannin content of 118.6 mg/mL, with a hydrolyzable tannin content of 68.81 mg/mL. The antioxidant activity was evaluated using DPPH and FRAP assays, demonstrating its activity against free radicals attributed to its antioxidant components. However, date kernel extract showed lower antiradical activity compared to ascorbic acid, with the highest IC50 value recorded at 0.04 mg/mL. In terms of anti-inflammatory activity, the extract showed an inhibition rate of 18.29%, while diclofenac showed an inhibition of 69%. As for the qualitative analysis of the studied extracts, which was conducted using HPLC, there were quantitative and qualitative differences in the presence of some reference phenolic compounds, which differed depending on the plant extract. These results confirm the therapeutic importance of *Phoenix dactylifera*. L as a natural repository of bioactive compounds for disease management. It is recommended that further research be conducted to isolate potential compounds and verify their effectiveness through clinical trials.

Keywords: antioxidant activity, anti-inflammatory activity, Ghars , *Phoenix dactylifera. l*

Résume

Le but de cette étude est d'évaluer les propriétés phytochimiques et l'activité anti-inflammatoire des graines de dattes de la variété « Ghars », *Phoenix dactylifera*.L. Le rendement d'extraction par trempage dans une solution méthanol/eau (80/20) a été estimé à 11,425 % pour l'échantillon de fosse de dattes. La teneur totale en polyphénols a été déterminée à l'aide du réactif Folin-Ciocalteu, ce qui a donné une concentration de 290,5 mg/mL. La teneur en flavonoïdes a été estimée à 12,2 mg/mL par analyse du chlorure d'aluminium (AlCl3). L'extrait a montré une teneur en tanins condensés de 118,6 mg/mL, avec une teneur en tanins hydrolysables de 68,81 mg/mL. L'activité antioxydante a été évaluée à l'aide des tests DPPH et FRAP, démontrant son activité contre les radicaux libres attribuée à ses composants antioxydants. Cependant, l'extrait de noyau de datte a montré une activité antiradicalaire inférieure à celle de l'acide ascorbique, la valeur IC50 la plus élevée étant enregistrée à 0,04 mg/mL. En termes d'activité anti-inflammatoire, l'extrait a montré un taux d'inhibition de 18,29 %, tandis que le diclofénac a montré une inhibition de 69 %. Quant à l'analyse qualitative des extraits étudiés, réalisée par HPLC, il existe des différences quantitatives et qualitatives en présence de certains composés phénoliques de référence, qui diffèrent selon l'extrait végétal. Ces résultats confirment l'importance thérapeutique de *Phoenix dactylifera.* L comme dépôt naturel de composés bioactifs pour la gestion des maladies. Il est recommandé que des recherches supplémentaires soient menées pour isoler les composés potentiels et vérifier leur efficacité par le biais d'essais cliniques.

Mots clés : activité antioxydante, activité anti-inflammatoire, Ghars , *Phoenix dactylifera. l* ,

ملخص

Abstract

الهدف من هذه الدراسة هو تقييم الخصائص الكيميائية النباتية والنشاط المضاد للالتهابات في نواة التمر من صنف غرس من فصيلة (Phoenix dactylifera. L) قدر مردود الاستخلاص بطريقة النقع في محلول ميثانول/ماء (20/80) ب 11.425 % لعينة نوى التمر. تم تحديد إجمالي محتوى البوليفينول باستخدام كاشف Folin-Ciocalteu ، مما أدى إلى تركيز 290.5 ملغ/ مل. تم تقدير محتوى الفلافونويد بـ 12.2 ملغ/مل من خلال تحليل كلوريد الألومنيوم (AlCl3). أظهر المستخلص محتوى تانين مكثف قدره 118.6 ملغ/مل، مع محتوى تانين قابل للتحلل المائي قدره 68.81 ملغ/مل. تم تقييم نشاط مضادات الأكسدة باستخدام فحوصات DPPH وFRAP، مما يدل على فعاليته ضد الجذور الحرة المنسوبة إلى مكوناته المضادة للأكسدة. ومع ذلك، أظهر مستخلص نواة التمر نشاطًا مضادًا للجذور أقل مقارنة بحمض الأسكوربيك، حيث سجلت أعلى قيمة IC50 عند 0.04 ملغ/مل. من حيث الفعالية المضادة للالتهابات، أظهر المستخلص معدل تثبيط قدره 18.29%، بينما أظهر ديكلوفيناك تثبيطًا بنسبة 69%. أما بالنسبة للتحليل النوعي للمستخلصات المدروسة والذي أجري باستخدامHPLC ، فقد كانت هناك فروق كمية ونوعية في وجود بعض المركبات الفينولية المرجعية والتي اختلفت باختلاف المستخلص النباتي. تؤكد هذه النتائج على الأهمية العلاجية لنوى (Phoenix dactylifera. L) باعتبارها مستودعًا طبيعيًا للمركبات النشطة بيولوجيًا لإدارة الأمراض .يوصى بإجراء مزيد من الأبحاث لعزل المركبات المحتملة والتحقق من فعاليتها من خلال التجارب السريرية

الكلمات المفتاحية : نشاط مضاد للأكسدة، نشاط مضاد للالتهابات، الغرس *Phoenix dactylifera. L*

Summary

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Abreviation List

Abreviations List

% : Percentage

°C : Degree Celsius

AA : Ascorbic Acid

AAR : Antiradical Activity

Abs Contrôle négatif : Absorbance of negative control

Abs test: Sample absorbance

ACN: acetonitrile

BSA : Bovine serum albumin

Cm : Centimeter

DM : Dry matter

DPPH: 2,2-phényl-1-picrylhydrazyl

EA: Extrait aquatique

EAL: Extrait alcoolique

Fe : Iron.

Fe2+ : Ferrous ion

Fe3+ : Ferric ion

FRAP : Ferric Reducing Antioxidant Power

g : gramme

H2O : water

HPLC : High performance liquid chromatography

HRBC : Human Red Blood cells.

I % : percentage inhibition

IC50 : Inhibitory concentration 50%.

Abreviation List

MeOH : Methanol

mg : Milligramme

min : Minute

ml : Milliliter.

mm : Millimeter.

mM : Millimolar

nm : Nanometer

PA : dry plant weight in grams.

PE : extract weight in grams.

PH : Potential hydrogen

R : yield (%)

rpm : ro**u**nd per minute

TAE : Tannic acid equivalents

TCA : Trichloroacetic acid

TPTZ : 2,4,6-tris(2-pyridyl) -1,3,5-s-triazine

UV : Ultra-Violet

µg : Microgramme

µL : Microlitre

Introduction

Introduction

Inflammation is the immune system's response to harmful stimuli, such as pathogens, damaged cells, toxic compounds, or irradiation **(Medzhitov, R.2010)** The inflammatory pathway consists of inducers, sensors, mediators, and target tissues. and acts by removing injurious stimuli and initiating the healing process **(Ferrero-Miliani L and al .2007)** Inflammation is therefore a defense mechanism that is vital to health **(Nathan, C and Ding ,A.2010)** Usually, during acute inflammatory responses, cellular and molecular events and interactions efficiently minimize impending injury or infection. This mitigation process contributes to restoration of tissue homeostasis and resolution of the acute inflammation. However, uncontrolled acute inflammation may become chronic, contributing to a variety of chronic inflammatory diseases **(Zhou, Y and al .2016)**

Important microcirculatory events that occur during the inflammatory process include vascular permeability changes, leukocyte recruitment and accumulation, and inflammatory mediator release (**Ferrero-Miliani ,L and al .2007; Chertov ,O and al . 2000).**

In spite of the fact that now we have at our command a number of modern drugs, it is still genuinely urgent to discover and develop new therapeutic agents. It has been estimated that the acceptable therapy is available only for one third of the known human ailments. Therefore, the fight against diseases must be carried on relentlessly. Traditional plant medicines still enjoy significant position in the modern-day drug industries due to the minor side effects as well as the synergistic action of the combination of compounds. Most of the important drugs of the past 50 years, which have revolutionized modern medicinal practice, have been isolated/derivatized from plants. These chemical ingredients exhibit therapeutic properties of plant and animal drugs.

Studies by the World Health Organisation (WHO) on traditional medicine have been concerned with the development of traditional medicine as an alternative approach to health in the developing world **(WHO , 2002 ;WHO, 2001).**

Since ancient times, remedial plants are used in medicines. Many drugs such as antitumor, antimicrobial and anti-hepatotoxic compounds are made with the aid of therapeutic plants. Lesser complications have been observed from the use of pharmaceutical drugs derived from medicinal plants **(Howes and al., 2020)**. These phytomedicines are cheap, secure, efficient and feasible. Compounds of folk medicines are acquired through therapeutic plants. Folk medicines are used by

Introduction

80 % population of developed countries. The properties and effectiveness of plants should be explored properly for better use of them **(Yadav and Agarwala, 2011).**

Thus, the research of pharmacologically/ biologically active agents obtained by screening natural sources such as plant extracts had led to the detection of many pharmaceutically valuable drugs that play a key role in the treatment of human diseases **(FAO, STAT.2005)** The phytochemical-pharmacological research work has recently yielded effective solutions to certain diseases which synthetic drug industry has failed to afford. For this reason we chose our study on Date palm tree (*Phoenix dactylifera L.*) is grown extensively in arid and semi-arid regions of the world; like northern Africa, the Arabian Peninsula and Iran **(Ahmed, et al., 1995)**. Date palm constitutes the principal source of remuneration and the basis of economy for the people living in the Sahara **(FAO, STAT.2005).**

Phenolic compounds of fruit seeds mainly phenolic acids and flavonoids, have been shown to possess such benefits as antioxidant **(Peterson and Dwyer, 1998)**, anti-carcinogenic **(Bailey and Williams,1993; Block, 1992)**, antimicrobial (Takechi, Tanaka, Nonaka, & Nishioka, 1985), anti-mutagenic **(Liverio and al 1994)** anti-inflammatory activities **(Landolfi and al 1984)** as well as reduction of cardiovascular diseases **(Diplock and al., 1998; Halliwell, 1997).**

"The objective of this study is to evaluate the phytochemical profile and in vitro anti-inflammatory activity of date seeds (*Phoenix dactylifera L*)."

First part

Bibliographic synthesis

Chapter I

Inflammations

1. Définition :

In the first centuries of the current era, the concept of inflammation was known and characterized by five distinct qualities: rubor (redness), calor (heat), tumor (swelling), dolor (pain), and functio laesa (loss of function) **(Stephen, A. 2015).**

Inflammation is a normal response within the body, as an integral part of the innate immune system. The initiation of inflammation aims to eliminate pathogens that may cause tissue damage **(Feghali and Wright.1997)** .

Inflammation is the first line of defense in invertebrates and vertebrates as it helps in fight against tissues injury or foreign invaders. Uncontrolled or excessive inflammation is destructive for normal homeostatic processes of body**(Ayesha, M. 2021).**

Inflammation is essential in the immune response as it allows for many types of immune cells, such as neutrophils, to migrate to an infected area and recognize a pathogen they need to attack. While this beneficial reaction is meant to be acute, some people can experience chronic inflammation as well. This chronic inflammation can cause tissue damage and, if left untreated, organ damage. This may seem counterintuitive due to the fact that inflammation is a mechanism of the immune system, used to protect the body, but a response is not always warranted **(Bauman. 2017).**

Inflammation is a severe response by living tissue to any kind of injury. There can be four primary indicators of inflammation: pain, redness, heat or warmness and swelling. When there is injury to any part of the human body, the arterioles in the encircling tissue dilate. This gives a raised blood circulation towards the area (redness) **(Burke and al. 1958).** Vasoactive chemicals also increase the permeability (increase pore size) of these arterioles which allows blood cells, chemical substance, blood proteins and fluid to accumulate in that region. This fluid accumulation causes swelling and may compress nerves in the area resulting in pain. In addition, prostaglandins, that might also result in 'irritation' of the nerves and further contribute to pain **(Ward, P. 2010)** .

2. Types of inflammation:

2.1. Acute inflammation:

Acute inflammation is the first step of the inflammatory cascade. The cascade begins with primarily a vascular response after infection or damage to sterile tissue. The main processes that occur during this phase of inflammation include a brief localized constriction of arterioles

stimulated by the contractions in smooth muscles around these arterioles. Subsequent, dilatation of the arterioles that supply blood to the damaged region causes increased localized blood flow causing hyperemia. Increases in the permeability of outer walls of blood vessels lead to extravasation of plasma proteins and fluid into the affected area. The acute inflammation episode is very rapid and happens within minutes of the infection or damage to cells **(Cotran and al. 1999; Lawrence and al. 2002)**.

The cells involved in acute inflammation are mainly mast cells, neutrophils, eosinophils, macrophages and monocytes. These leucocytes in the presence of infection or damaged tissue interact with the endothelial cells of capillaries. The interaction is mediated by selectins on the leucocytes (L selectins) and the endothelial cells (E and P selectins) **(Petri and al. 2008).** This interaction leads to further interaction with chemotractant factors such as eotaxin/CCL11 and leukotriene on the endothelium which leads to up regulation of integrins which promote further binding of leucocyte to the endothelial cells.

Consequently, leukocytes move on vascular endothelial walls where they find suitable openings and they migrate into the interstitial spaces **(Phillipson and al. 2006).**

The migrated leucocytes in tissue provide a wide array of inflammatory mediators which range from vasoactive amines such as histamines and serotonin, lipid 3 mediators (eicosanoids), cytokines, chemokines, and reactive oxygen species which propagate the inflammatory process. A component of the acute inflammatory response sometimes manifests as allergy. An allergic reaction which is life-threatening is termed anaphylaxis. Most anaphylactic reactions are immunologic in nature and mediated by the action of immunoglobulin E (IgE) on mast cells. The mast cell is the main effector cell among other cells such as the basophil, neutrophils and oesinophils that mediate allergic responses as well as fatal anaphylactic reactions **(Amira and al. 2011).**

The activation of mast cells causes a series of signalling mechanisms resulting in an initial degranulation causing the expulsion of mediators stored in vesicles such as histamine, tryptase heparin and chymase. This is followed by the breakdown of membrane phospholipids to form arachidonic acid which is metabolised by cyclooxygenases and lipoxygenases to form prostaglandins, thromboxanes and leukotrienes and later the gene expression of a range of cytokines and chemokines **(Ogawa and Grant. 2007)**. These locally generated mediators lead to a local increase in vascular permeability which is one of the characteristic features of allergic inflammation **(Burke and Miles. 1958).** The mediators are paracrine in nature and as such initiate, recruit and activate other cells and mediators which contribute to the advancement of the symptoms observed

in anaphylactic reaction **(Ogawa and Grant. 2007).** As reported by **(Hallett and al. 2008)** acute inflammation predominantly protects the host organism, is self-regulatory and as such advances to the point where the inflammation is resolved. **(Alessandri and al. 2013)** stated that the process of resolving inflammation is an active one encompassing various inflammatory cells/mediators and mechanisms that control the cessation of the reaction by reducing the recruitment of various leukocytes and the reversal of 4 the dilation of blood vessels as well as vascular permeability.

Alessandri and his team further stated that other pathways include the shift from the generation of proinflammatory mediators to anti-inflammatory ones; the termination of signals linked with cytokine generation and leukocyte survival, apoptosis of recruited inflammatory cells and their subsequent clearance. The fate of acute inflammation as documented by (**Freire and Van, D. 2013)** dwells on the balance between mediators/cells that augment or control the inflammatory reaction. Deregulation or incessant triggering of acute inflammation, tend to be detrimental to the cells/tissue and subsequently leads to progression from an acute to a more chronic situation that is termed inflammatory disease which results in necrosis and fibrosis **(Alessandri and al. 2013; Freire and Van, D. 2013).**

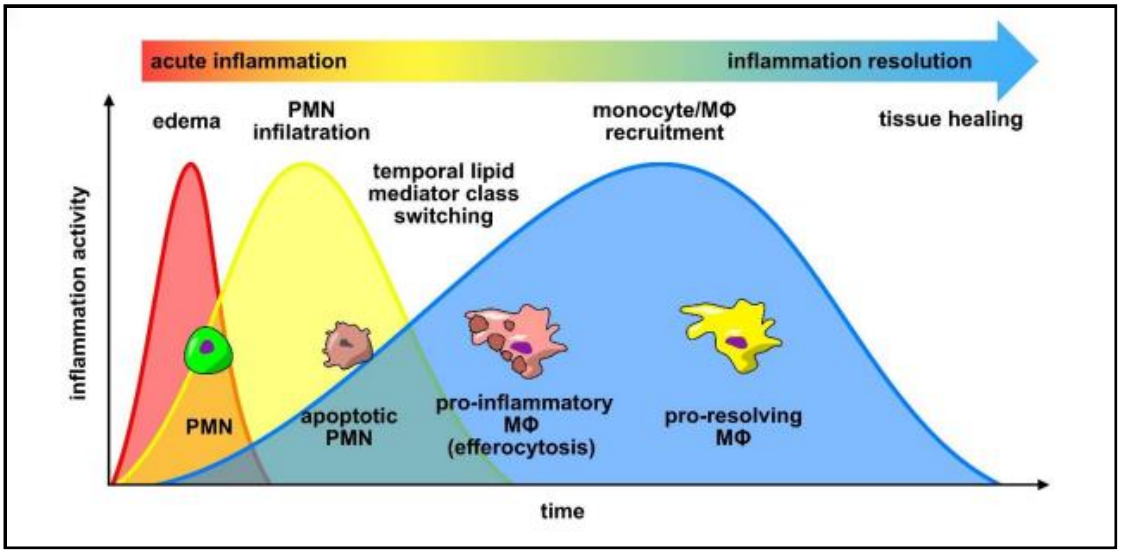

Figure n (01): The onset and resolution of acute inflammation. **(Cotran ,R .S and al 1999)**

2.2. Chronic inflammation :

The causes of chronic inflammation include persistent infections, prolonged exposure to potentially toxic agent's and autoimmunity. Persistent infection and prolonged exposure to toxic substances lead to delayed hypersensitivity reaction which prevents acute inflammation from

resolving leading to granulomatous reactions. Auto-antigens and common environmental substances also incite immune responses to an organisms own cells/tissues leading to chronic inflammatory diseases like rheumatoid arthritis, multiple sclerosis and chronic allergic diseases, such as bronchial asthma. Chronic inflammation is implicated in the development of several degenerative diseases such as atherosclerosis, arthritis, inflammatory bowel disease, aging and other neurodegenerative central nervous system depression **(O'Byrne and Dalgleish. 2001; Dalgleish and O'Byrne. 2002)**.

A typical protracted inflammation episode is characterized by infiltration with numerous leukocytes such as monocytes, macrophages, lymphocytes and plasma cells as a result of 5 persistent infections, prolonged exposure to potentially toxic agent's and efforts at restoring homeostasis or normal condition leading to replacement of damaged tissue with necrotic and fibrous tissue. According to **Akbar and Salmon., 1997**, the resolution phase in chronic inflammation is disordered, and leads to the continuous presence of inflammatory exudate, tissue damage and scarring. Chronic inflammation is primarily mediated by cells and chemical mediators that perpetuate the pro-inflammatory response. There is an interplay of mast cells, monocytes, macrophages, T and B lymphocytes and chemical mediators, including histamine, thrombin, cysteinyl leukotrienes, oxidants, IL-1, TNF-alpha, and eicosanoids. Monocytes mature into macrophages on entering tissues, phagocytize bacteria, foreign substances, and also discharge various mediators that perpetuate the pro-inflammatory response. Infected cells are destroyed by T lymphocytes whilst antibodies are produced by B lymphocytes against attacking microbes for destruction at the later stages of chronic inflammation. The persistence of a large number of these cells and mediators in the tissue propagate the chronic inflammatory episode. **(Akbar, A.N and Salmon ,M . 1997)**

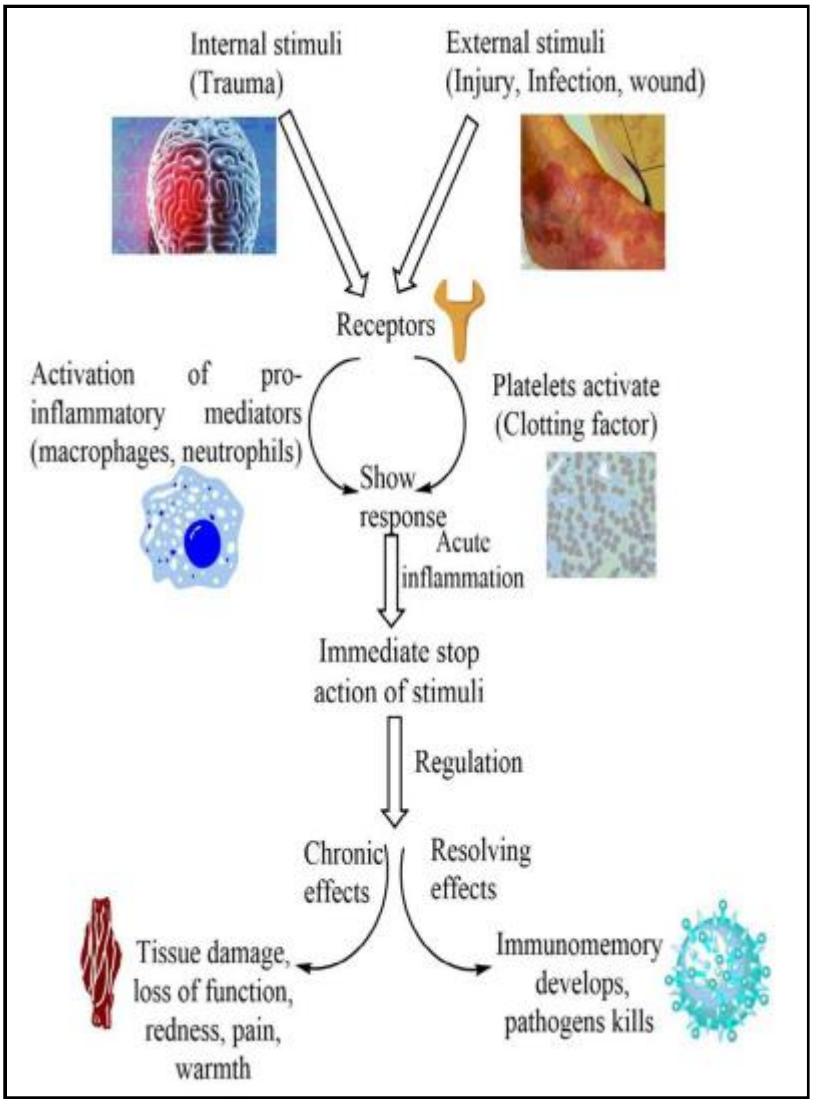

Figure n (02): Acute and chronic Inflammation in mammals.**(Ayesha ,M. 2021)**

3. Mechanism of inflammation:

Inflammation is a complex biochemical mechanism which leads to the stimulation of infectious agents and promotes injury. It causes tissue damage and pain monitored through treatment **(Del ,G and Gangestad. 2018)**. Chemical agents are secreted by immune cells like cytokines, chemokine and reactive oxygen species at injury site to eliminate pathogens **(Diakos and al. 2014).** A major constituent of inflammatory process is arachidonic acid which is a by product of fast acting cell membrane. Arachidonic acid is transformed into prostaglandins and thromboxane enzyme by cyclooxygenase (COX) **(Jayesh and al. 2020).**

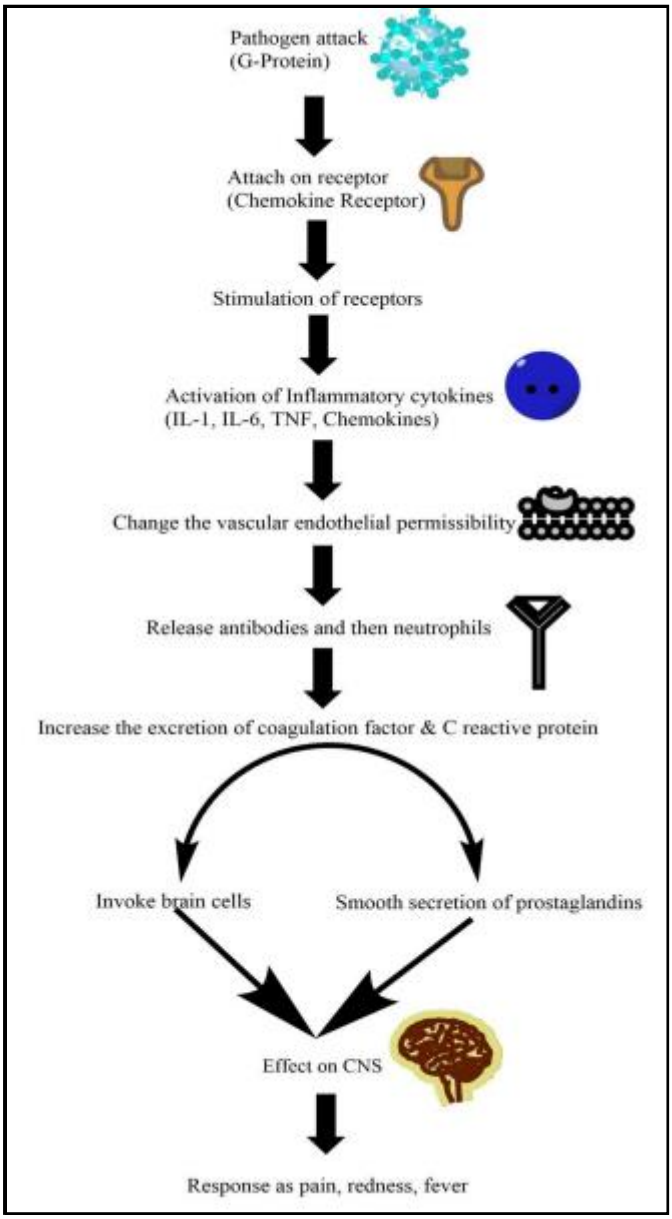

Figure n (03): Steps during inflammatory process **(Ayesha ,M. 2021).**

Neutrophils begin to attach strongly to the endothelium by using carbohydrate ligands to show symptoms of inflammation. Endothelial cells in their stimulated form are responsible for the production of surface bonded and soluble particles. They produce a strong adhesion between neutrophils and endothelium. Neutrophils leave the bloodstream and travels across endothelium **(Merritt. 2019).** Production of particular cells like cell adhesion molecules (CAMs), their activators and chemical stimulus is responsible for the neutrophils emigration **(Yoshizaki and al. 2010).** Molecular or cellular actions of infectious response tend to increase blood movement, capillary damage, leukocytes access and the creation of chemical agents **(Valacchi and al. 2018).** Stimulation of these chemical agents initiates the formation of inflammatory cytokines including TNF, IL-1, chemokines and IL-6 that causes tissue damage. Due to phagocytic activity of cells,

migratory neutrophils are eventually removed from inflammatory site through apoptosis and produce antiinflammatory cytokines **(Ansar and Ghosh. 2016; Brod. 2017).**

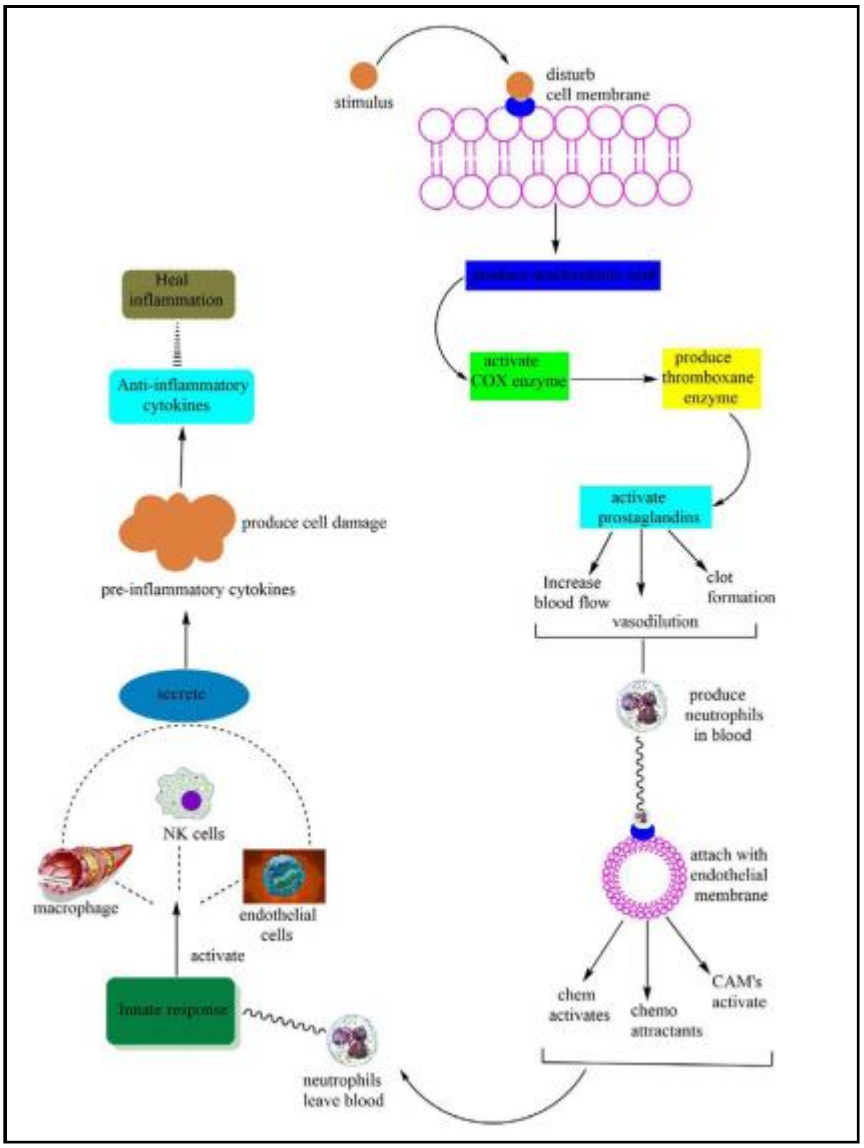

Figure n (04): Mechanism of inflammation.**(Ayesha ,M. 2021)**

4. Anti-inflammatory cytokines:

4.1. Interleukin-4 (IL-4) :

Interleukin-4 (IL-4) is a highly functional cytokine emitted by mast cells, basophils, T-helper 2 cells, eosinophils, stromal cells and epithelial cells (**Gadani and al. 2012; Pulendran and Artis. 2012; May and Fung. 2015).** When IL-4 interacts with the IL-4 receptor, IL-4 signaling initiates a

response that prompts to genes transcription of B and T cell activation **(Goenka and Kaplan. 2011; Zeng and al. 2014).**

IL-4 applies an anti-inflammatory activity by decreasing the activity and production of proinflammatory cytokines. It almost completely inhibits the production and synthesis of TNF-α and IL-1β. It suppresses the production of IL-6 and its activity when activated by endothelial cells **(Shiau and al. 2019).** It also regulates the discharge of Th1 cytokines and restrains the production of chemokines that hire Th-1 cells in the area of infection **(Gordon and Martinez. 2010; Lazarski and al. 2013)**

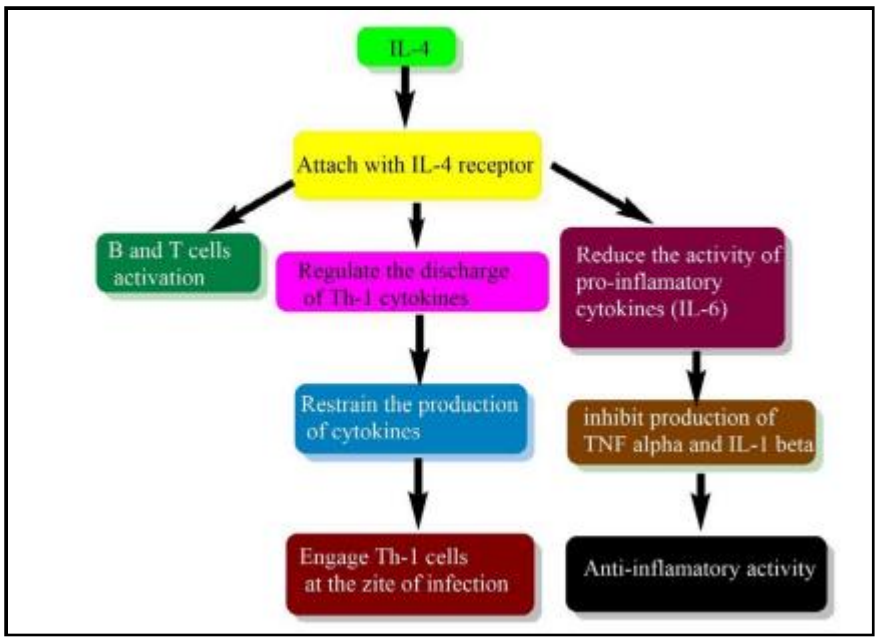

Figure n (05): Role of IL-4 in anti-inflammation **(Shiau and al. 2019).**

4.2.Interleukin-10 (IL-10):

IL-10 is considered to be a strong anti-inflammatory cytokine. Initially, it was called as cytokine amalgamation inhibitory factor (CSIF) as it can repress the production of IFN-c (proinflammatory cytokines) and TNFα **(Ge and al. 2020).** Like many anti-inflammatory cytokines, IL-10 targets various (innate and adaptive) leukocytes to suppress their activity and function, while suppressing inflammatory cytokine bursts, preventing injury to the helper, and maintaining functional tissue integrity **(Ng and al., 2013)**. A wide range of cell types include dendritic cells (DC's), CD4 T cells, mast cells, macrophages, CD8 T cells, neutrophils, eosinophils, B cells, (cNKs) conventional natural killer cells and few (ILCs) innate lymphoid cells have been reported to produce IL-10 **(Fang and Zhu. 2020).**

IL-10 attaches to IL-10R1 and then IL-10R2 **(Yoon and al. 2006)**. At the point, when IL-10 attaches to IL-10R1 it causes structural changes in IL-10 that causes its adhesion with IL-10R2 and IL-10/IL-10R complex. This complex suppresses the immune reactions **(Dagvadorj and al. 2008; Thibodeau and al. 2008)** and represses proliferation and activation of T cells **(Saraiva and al. 2020).** It also promotes the multiplication and production of mast cells, thymocytes and B cells antibodies **(Ragheb and al. 2011).**

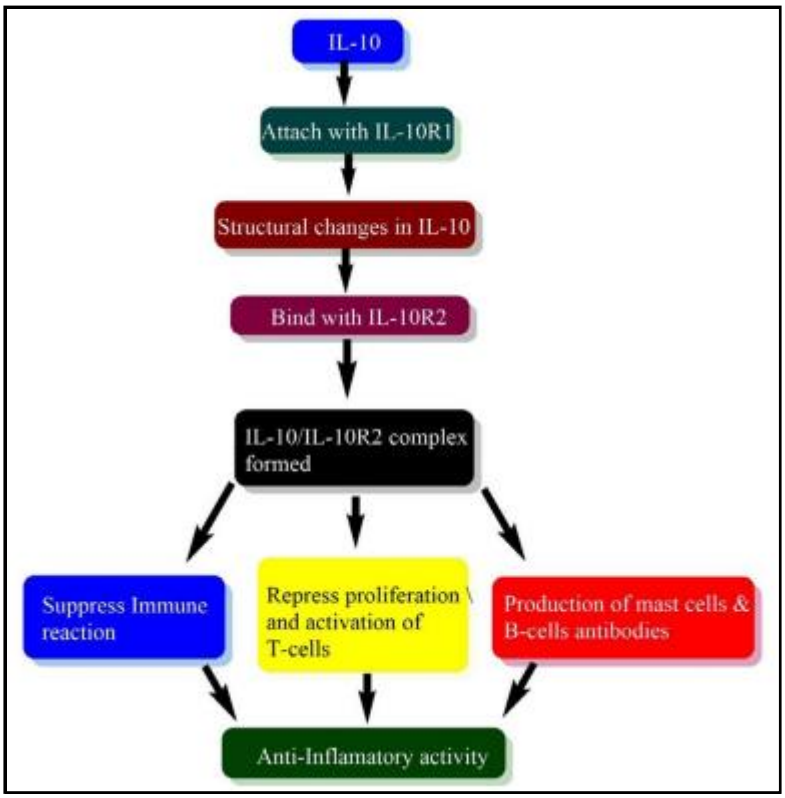

Figure n (06): Role of IL-10 in anti-inflammation**(Shiau and al. 2019).**

5. Treatment of inflammation:

5.1. Anti-histaminic:

Inflammation can be cured by using anti-histaminic, corticosteroidal **(Akhtar and Shabbir. 2019)** , diuretics **(Uroos and al. 2017)** and NSAID's (non-steroidal antiinflammatory drugs) such as aspirin **(Satani and al. 2019)**, ketoprofen, diclofenac **(Gupta and al. 2019)**, clinoril, naproxen **(Haley and Recum. 2019)**, sodium salicylates **(Duron and al. 2020)**, ibuprofen **(Rifai and al. 2019)**,indomethacin **(Munjal and Allam.2020)**. These drugs are effective in their action because they reduce inflammation and pain **(Joseph and Raj. 2012; Pahwa and Jialal. 2019)**. They are different in their structural form yet all drugs have similar antipyretic, analgesic and anti-inflammatory properties **(Abbate and al. 2016; Lundgren and al. 2017)**.

5.2. NSAIDs:

NSAIDs mitigate pain by lowering neighboring inflammatory reactions by the suppression of prostaglandin synthesis **(Lucas. 2016).** Corticosteroids and statins are likewise used to decrease inflammation **(Pahwa and Jialal. 2019).** Dyslipidemia and low-grade inflammation is treated by using metformin. It diminishes circulating IL-1beta, TNF-alpha, fibrinogen and hsCRP (High sensitivity C-reactive protein) **(Pahwa and Jialal. 2019).**

5.3. Indomethacin:

Indomethacin appeared as one of the most highly intense pain relieving and anti-inflammatory medicine through non-particular prohibition of COX (cyclo-oxygenase) enzyme which answerable for the conversion of arachidonic acid into prostaglandins **(Abbate and al. 2016; Lucas. 2016).** Anti-inflammatory reactions follow after inflammation arrives at its peak and represents to its preventive response **(Stankov. 2012)**. Indomethacin was first drug among all NSAIDs that was utilized for curing various inflammatory diseases **(Mishra and al. 2019; Lopez,C and al. 2020)** but was noted to cause disastrous health issues during its longterm use and was considered a hepatotoxic (Abatan et al., 2006), hematotoxic, gastric ulcerogenic **(Akpamu and al. 2016)** and nephrotoxic agents **(Olusegun and Lawal. 2008).** Consequently, finding of new and natural medicinal plants and their secondary metabolites with strong antiinflammatory activity and insignificant detrimental impacts, is demanded **(Malathi and al. 2012).**

Diseases caused by NSAID's Artificially adapted drugs cause high risk of stroke, kidney problem, heart attack especially uses in higher doses or may cause ulcers, upset stomach or bleeding in intestine or stomach (**Tambewagh and al. 2017; Bensman. 2019; Bradley. 2020)**. It may cause hypersensitivity and symptoms like fever, conjunctivitis, renal failure, dizziness, blurred vision, pancreatitis etc. Nonetheless, there is a limitation on the use of certain medications because of their detrimental effects like ulcer of gastrointestinal tract, renal damage, cardiac abnormalities and bronchospasm **(Gosavi and al. 2011; Shah and Alagawadi.2011; Gupta and al. 2019).** Unawareness of the side effects of NSAID's may cause death **(Hoxha and al. 2020).**

5.4. Natural products and medicinal plants for treatment:

Due to the adverse effects of NSAIDs it is a dire need of hour to move towards natural anti-inflammatory products. New natural medicinal plants and their metabolites with strong anti-inflammatory activity and insignificant detrimental impacts has been exploring by the world scientific community due to its bioavailability and cost efficiency **(Malathi and al. 2012).** Many

plants have been identified which consists of compounds and molecules with anti-inflammatory properties. Their study gained more interest due to their active potential and beneficial effects. These active molecules help the body to defend against inflammatory related pathogens **(Tili and Michaille. 2016)**.

5.5. The cytokine network in inflammation :

The cytokine network consists of soluble mediators, i.e. cytokines, which are essential to the communication network between key cellular players in an inflammatory response. The term cytokine is derived from the Greek words kyttaro (cell) and kines (movement) and represents a broad and loose category of small proteins (~5–20 kDa) which play an important role in the communication between various immunocompetent cells and between immunocompetent cells and other cell types (e.g. endothelial cells) involved in inflammation **(Turner ,M.D and al. 2014)**. Cytokines act via their specific receptors and regulate maturation, growth, and responsiveness of different cell populations.

They may affect the cytokine releasing cell directly (i.e. autocrine effects) or neighbouring cells in the common microenvironment, thereby creating a paracrine signalling loop. There is a terminology overlap between cytokines and growth factors, but less so between cytokines and hormones. It is possible for a given cytokine to be released by many different cells, whereas hormones tend to be secreted by specific cells and act on distant organs and effector cells. However, cytokines can also be detected outside the microenvironment of their releasing cells (e.g. serum or plasma) and hence can also exert distant effects.

Specialized immunocompetent cells express pattern recognition receptors (PRRs) which recognize different harmful injuries known as damage-associated molecular patterns (DAMPs) or pathogen-associated patterns (PAMPs), thereby initiating a cascade of immune reactions **(Guo ,H and al . 2015;Latz ,E and al. 2013)**.

The main effector molecules in these immune reactions are pro-inflammatory cytokines that are activated and released through the inflammasome (a multiprotein oligomer including specialized innate immune system receptors and sensors) **(Guo ,H and al . 2015; Latz ,E and al. 2013)**.

The biological effects of pro-inflammatory cytokines are further modulated through the interplay between the various pro-inflammatory mediators and their interactions with anti-inflammatory cytokines and other soluble mediators like soluble adhesion molecules, proteases, and protease inhibitors. All these soluble mediators, together with a wide range of immunocompetent and tissue

cells, form a highly dynamic network, which often renders it difficult to predict and explain in detail the pathogenesis of inflammatory diseases in clinical models **(Chu ,D and al . 2003).**

Cytokines, adhesion molecules, and matrix metalloproteases (MMPs) are key components involved in inflammation **(Turner ,M.D and al. 2014;Nissinen ,L and Kahari VM. 2014 ;Nourshargh ,S and Alon ,R. 2014).**

Cytokines are important for cell-cell communication during inflammation, and they are highly heterogeneous and can be classified based on their function or structure (**Turner ,M.D and al. 2014**). Adhesion molecules are important mediators of cellular adhesion between leucocytes and endothelial cells and can exist in the membrane-bound as well as the biologically active soluble forms (**Nourshargh, S and Alon ,R. 2014**).

Finally, MMPs have emerged not only as molecules involved in modelling extracellular tissue, but also as important regulators of inflammatory responses, e.g. through their activation and modulation of pro-inflammatory cytokines (**Nissinen ,L and Kahari, V.M. 2014**). In addition to its roles in autoimmune and infectious diseases, inflammation is also involved in the pathogenesis of several disorders. Thromboembolic disease has been shown to be associated with local inflammatory response **(Roumen-Klappe ,E.M. 2002),** and inflammation has been recognized as an important player in the pathogenesis of both venous and arterial thromboses **(Von Bruhl ,M.L . 2012; Furie ,B and Furie ,B,C. 2008)**.

Furthermore, sepsis is in itself and as a major complication of surgical interventions and intensive anticancer treatment, associated with a high mortality rate, and thus septic response to severe infection is probably the most extensively studied inflammatory process. Recent studies of biomarker profiles have suggested that treatment targeting combinations of pro-inflammatory mediators should be investigated further in patients with sepsis **(Dellinger ,R.P and al.2008; Singer, M and al .2016)**.

Treatment specifically targeting individual cytokines, e.g. tumour necrosis factor (TNF)-Į LQKLELWRUV targeting TNF-Į, is now a powerful therapy option, especially in autoimmune inflammatory diseases **(Biggioggero ,M and Favalli ,E.G.2014),** whereas as yet cytokine-directed therapy has not become part of routine treatment for patients with severe infections, e.g. sepsis **(Giamarellos-Bourboulis EJ.2013)**. In note, biomarker studies have suggested that diagnostic or therapeutic procedures, e.g. peripheral blood stem cell apheresis, may also have pro-inflammatory consequences **(Akkok ,C.A and al .2011)**.

6. Management of inflammation :

Body defense mechanism, commonly known as inflammation, is a response to many physiological conditions such as infection and thermal and/or physical injuries. Inflammatory response is necessary for the survival against environmental pathogens and harms **(Das and Kanodia.2011)**. Inflammation is categorized into five cardinal signs which are known as redness, swelling, heat, pain and loss of function **(Purnima and al.2010).**

Prostaglandins are produced by the cells which are involved in the production of pain, fever and inflammation. Several enzymes such as cyclooxygenase including COX-1, COX-2, and COX-3 are responsible for the production of prostaglandin. COX-2 is responsible for promoting pain, inflammation and fever by producing the prostaglandin. Hence by inhibiting the cyclooxygenase enzyme, prostaglandin production can be blocked. Non-steroidal anti-inflammatory drugs (NSAIDs) are usually indicated in order to relieve the symptoms.

Use of NSAIDs and other opioids, many side effects can occur such as gastric lesion; so the uses of these drugs are not successful. The use of non-steroidal anti-inflammatory drug (NSAID) medication is still the mainstay of most classically taught clinicians for joint and spine related inflammatory pain, despite their commonly known side effects. Non-steroidal anti-inflammatory drug mechanisms are primarily through interaction with pro-inflammatory cytokines interleukin (IL)-1a, IL-1b, IL-6 and tumor necrosis factor (TNF-α). Increased concentrations of TNF-α is believed to cause the cardinal signs of inflammation to occur . These pro-inflammatory cytokines result in chemo-attractant for neutrophils and help them to stick to the endothelial cells for migration. They also stimulate white cell phagocytosis and the production of inflammatory lipid prostaglandin E2 (PGE2). NSAID ability to interfere with the production of prostaglandin during the inflammatory cascade is the major mechanism cited for the anti-inflammatory success of these medications **(Talalay P.2001).**

Chapter II

Traditional medicine

1. Definition of traditional medicine :

WHO defines traditional medicine as the sum total of knowledge or practices, whether explicable or inexplicable, used in diagnosing, preventing or eliminating a physical, mental or social disease, which may rely exclusively on past experience or observation handed down from generation to generation, verbally or in writing **(WHO. 2008)**. It includes diverse health practices, approaches, knowledge and beliefs incorporating plant, animal, and/or mineral based medicines, spiritual therapies, manual techniques and exercises applied singularly or in combination to maintain wellbeing, as well as to treat, diagnose or prevent illness**(WHO. 2003; WHO.2002; WHO.2001)** .

Traditional medicine is the broad term for the diverse range of health practices of communities across the world, defined as the "sum total of the knowledge, skill, and practices based on the theories, beliefs, and experiences indigenous to different cultures, whether explicable or not, used in the maintenance of health as well as in the prevention, diagnosis, improvement or treatment of physical and mental illness" **(WHO.2019).** The term is often interchanged with "indigenous medicine," "folk medicine," or "traditional healing system," all of which are correct and point to the native origins of a particular healing system. Ultimately, traditional, indigenous, or folk medicine is to be understood as a healing system that is situated within the context of a particular environment and unique history, whose interactions with the individual's body and ethnic group's physiological dispositions lend itself to the creation of "a set of beliefs that has a shared social dimension" that guide what people do when they become ill. **(Wing.1998**). Some examples of traditional medicinal practices that are well known across the world are Ayurveda, traditional Chinese medicine, Jamu (Indonesia), and unani.

2. Phytotherapy:

Phytotherapy is one of the common approaches of integrative medicine that is practised by different cultures based on traditional knowledge. In the era of evidence-based medicine, it is essential to investigate the specific clinical safety and efficacy information of herbal medicines based on scientific experimental and clinical approaches **(Mamta ,S and al .,2013).**

3. Medicinal plants:

Traditional medicinal plants are a therapeutic resource used by the population of the African continent specifically for health care, which may also serve as starting materials for drugs **(Yi-Feng ,C and Elmira ,S . 2014)** infectious diseases account one-half of all deaths in the tropical countries. As a result, people of all continents have long applied poultice and imbibed infusions of indigenous plants dating back to prehistory for health purposes (**Theophine ,Ch .O and Emeka**

,K. O. 2014) It comprises of therapeutic practices in existence for hundreds of years before the development of modern scientific medicine and is still in use today without any documented evidence of adverse effects.Medicinal plants represent as an alternative treatment to various diseases, and their use is increasingly prevalent throughout the world. Due to the large number of plant species with medicinal properties **(Unnamed. 2021).**

Medicinal plants are priceless sources of bioactive compounds, and despite the contemporary progresses in pharmaceuticals and drug developments they remain a major source of medicine for a large proportion of our world**. (Refaz, A .D and al. 2017).**

3.1 Anti-inflammatory medicinal plants :

Several natural substances are being used for the treatment of inflammation including bark of yellow willow tree **(Klessig and al. 2016)**, white mulberry, black mulberry, curcumin or turmeric **(Patidar and al. 2014; Kocaadam and Sanlier. 2017)**, ginger, hyssop, resveratrol, quercetin, caffeic acid phenethyl ester, Harpagophytum procumbens, piceatannol (PIC), green tea, propolis, cannabis, fibroin and sericin **(Pahwa and Jalal. 2019).**

The Oryeongsan expressed anti-inflammatory potency by suppressing various mediators of inflammation e.g., IL6, NO, IL-1β and TNF-α. Besides this effect, it remarkably repressed the synthesizes of iNOS and COX-2 enzymes and inhibited the activation of NF-κB signaling pathway **(Arulselvan and al. 2016)**. Edible red algae has porphyran which act as major bioactive compound in this plant. It acts as mediator of anti-inflammation by preventing the activation of NF-κB **(Isaka and al. 2015)**. Cassia occidentalis have bioactive compounds i.e. emodin and chrysophanol which help in prohibiting proinflammatory cytokines IL1β and TNF-α. These compounds have their effective role for the natural treatment and therapy of inflammation **(Patel and al. 2014)**. Sericin influenced the production of few anti-inflammatory cytokines to reduce allergy and skin inflammation **(Aramwit and al. 2009)**. It can suppress the enzyme activity such as COX-2 and iNOS and start healing process.

3.2. Phytochemistry of medicinal plants:

The biochemicals in the plant are called phytochemicals , which got their name from the Greek word Phyto (Plant). The growth of plants and safety from pathogens and predators is due to the presence of these phytochemicals. Many pharmacological properties are present in phytochemicals but they are not considered important diet components and are not required for survival **(Thakur and Sharma. 2018).**provide health benefits for humans further than those attributed to macronutrients and micronutrients**(Hasler ,C.M and al. 1999)** They protect plants from disease and

damage and contribute to the plant's color, aroma and flavor. In general, the plant chemicals that protect plant cells from environmental hazards such as pollution, stress, drought, UV exposure and pathogenic attack are called as phytochemicals**(Gibson ,E.L and al 1998; Mathai, K. 2000)** .

Bioactive agents present in the therapeutic plants provide health care defense to the human body and these include carbohydrates, flavonoids, steroids, tannins, alkaloids and terpenoids. These are produced in plants by both primary and secondary metabolic procedures **(Jan and al.2021)** .

Primary and secondary metabolites are obtained through plants in the form of compounds that have a long-lasting positive effect on one's health and also assist to cure ailments meritoriously. Especially, the tonic role in human beings is played by secondary compounds as they are produced in abundance by plants **(Fardiyah and al. 2020).** More than 4,000 phytochemicals have been cataloged **(American Cancer Society. 2000)** and are classified by protective function, physical characteristics and chemical characteristics Meagher E ., 1999 and About 150 phytochemicals have been studied in detail **(American Cancer Society.2000).**

In wide-ranging dietary phytochemicals are found in fruits, vegetables, legumes, whole grains, nuts, seeds, fungi, herbs and spices **(Mathai ,K. 2000).** Broccoli, cabbage, carrots, onions, garlic, whole wheat bread, tomatoes, grapes, cherries, strawberries, raspberries, beans, legumes, and soy foods are common sources**(Moorachian ,M.E. 2000)** Phytochemicals accumulate in different parts of the plants, such as in the roots, stems, leaves, flowers, fruits or seeds**(Costa ,M.A. 1999). .** Levels vary from plant to plant depending upon the variety, processing, cooking and growing conditions**(King ,A. 1999)** Phytochemicals are also available in supplementary forms**(American Cancer Society. 2000).**

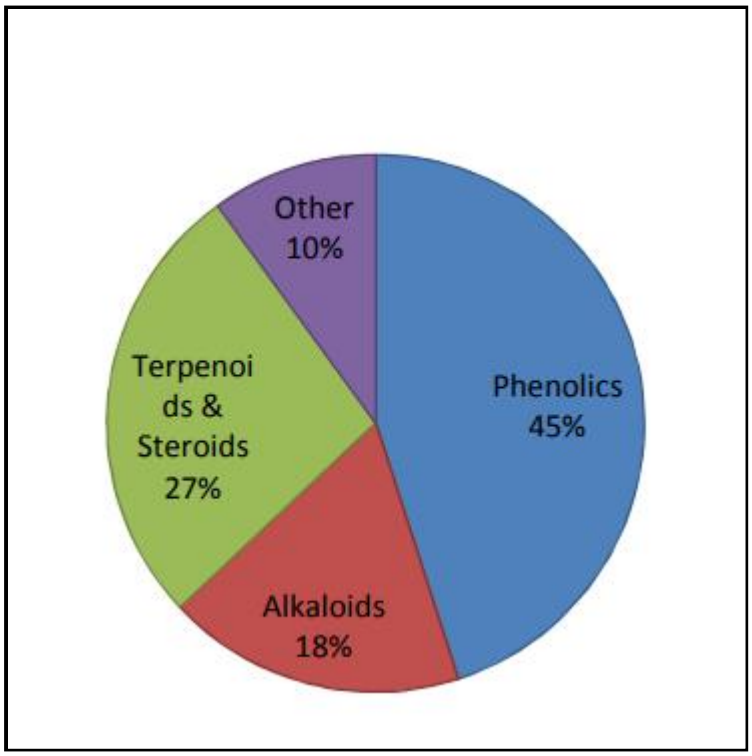

Figure n (07) : A proportional circle representing the division of phytochemicals**(Mamta ,S and al . 2013).**

3.2.1.Phenolics :

Phenolic phytochemicals are the largest category of phytochemicals and the most widely distributed in the plant kingdom. The three most important groups of dietary phenolics are flavonoids, phenolic acids, and polyphenols. Phenolic are hydroxyl group (-OH) containing class of chemical compounds where the (-OH) bonded directly to an aromatic hydrocarbon group. Phenol (C6H5OH) is considered the simplest class of this group of natural compounds. Phenolic compounds are a large and complex group of chemical constituents found in plants**(Walton ,N.J and al . 2003)** . They are plant secondary metabolites, and they have an important role as defence compounds. phenolics exhibit several properties beneficial to humans and its antioxidant properties are important in determining their role as protecting agents against free radical-mediated disease processes. Flavonoids are the largest group of plant phenols and the most studied**(Dai ,J and al . 2010) .**

Table n(01): The Major Classes of Phenolic Compounds in Plants**(Mamta ,S and al .2013).**

S.N.	Number of carbon atom	Basic skeleton	Class
1.	6	C_6	Simple phenols Benzoquinones
2.	7	C_6-C_1	Phenolic acids
3.	8	C_6-C_2	Acetophenones Tyrosine derivatives
4.	9	C_6-C_3	Hydroxycinnamic acid, Coumarins
5.	10	C_6-C_4	Naphthoquinones
6.	13	C_6- C_1-C_6	Xanthones
7.	14	C_6- C_2-C_6	Stilbenes
8.	15	C_6- C_3-C_6	Flavonoids
9.	18	$(C_6$- $C_3)_2$	Lignans
10.	30	$(C_6$- C_3-$C_6)_2$	Bioflavonoids
11.	N	$(C_6$- C_3-$C_6)_n$	Condensed tannins

3.2.1.1. Flavonoids:

Which are a major group of bioactive substances. Many compounds such as flavanones, flavones, and flavonols of class flavonoids belong to common secondary metabolites present in different types of vegetables, fruits, and other therapeutic plants. Anesthetic and strong antioxidant properties are shown by these compounds **(Adamczak and al. 2019).** Foods obtained from plant origin have flavonoids (dietary polyphenols) and phenolic acids which are expended in large quantities. These have a major role in the curing of prolonged and degenerative ailments besides many other positive effects **(Afzal , I and al . 2023)** Many other activities such as anti-diabetic, antitumor, antiproliferative, and immune stimulatory activities along with anesthetic and antioxidant **(Kumar and al. 2019).**It is also stated in recent data that flavonoids have a role in wound healing and also have defensive potential against cutaneous inflaming reactions **(Chuang and al. 2017).**

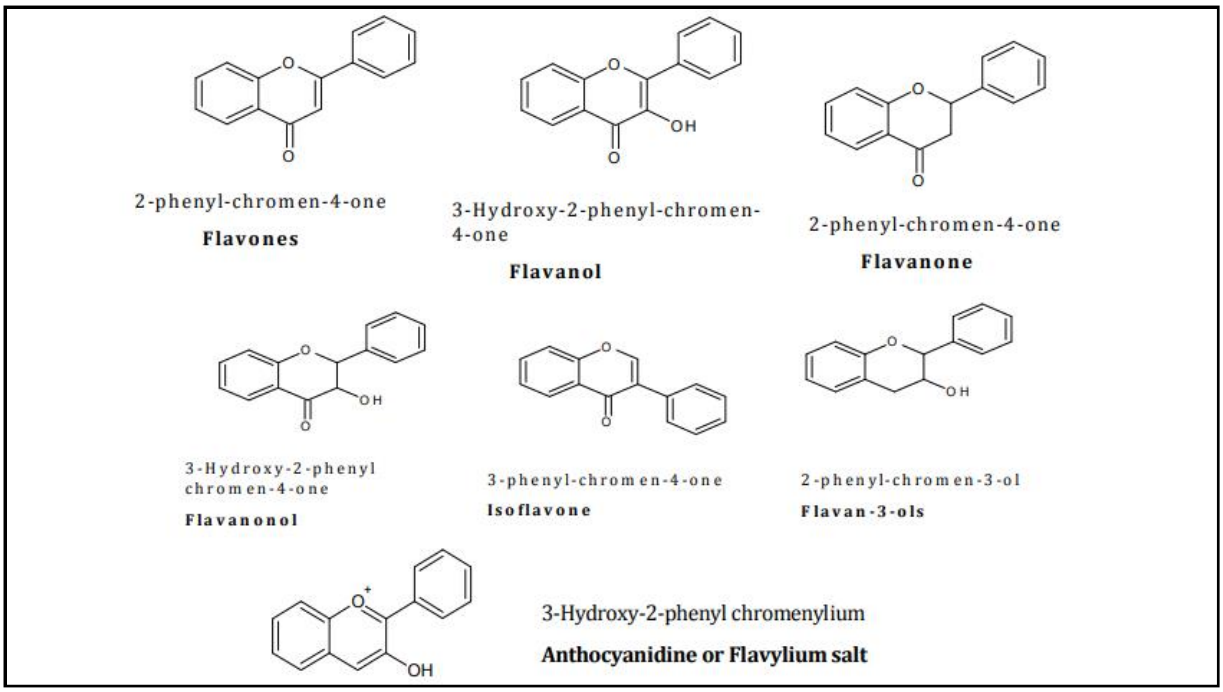

Figure n(08): Chemical structures of some representative flavonoids**(Afzal, I and al . 2023)**.

3.2.1.2.Tannins:

From a chemical point of view it is difficult to define tannins since the term encompasses some very diverse oligomers and polymers**(Halliwell ,B and Gutteridge ,J.M.C. 1990; Harborne ,J.B .1999)** . It might be said that the tannins are a heterogeneous group of high molecular weight polyphenolic compounds with the capacity to form reversible and irreversible complexes with proteins (mainly), polysaccharides (cellulose, hemicellulose, pectin, etc.), alkaloids, nucleic acids and minerals, etc**(Schofield ,P and al .2001; Mueller-harvey ,I and Mcallan ,A.B. 1992).** On the basis of their structural characteristics it is therefore possible to divide the tannins into four major groups: Gallotannins, ellagitannins, complex tannins, and condensed tannins**(Mc-Leod ,M.N.1974)**

(1) Gallotannins are all those tannins in which galloyl units or their meta-depsidic derivatives are bound to diverse polyol-, catechin-, or triterpenoid units.

(2) Ellagitannins are those tannins in which at least two galloyl units are C–C coupled to each other, and do not contain a glycosidically linked catechin unit.

(3) Complex tannins are tannins in which a catechin unit is bound glycosidically to a gallotannin or an ellagitannin unit.

(4) Condensed tannins are all oligomeric and polymeric proanthocyanidins formed by linkage of C-4 of one catechin with C-8 or C-6 of the next monomeric catechin. Tannins are found commonly in

fruits such as grapes, persimmon, blueberry, tea, chocolate, legume forages, legume trees like Acacia spp., Sesbania spp., in grasses i.e; sorghum, corn, etc**(Giner-Chavez, B.I.1996)**. Several health benefits have been recognized for the intake of tannins and some epidemiological associations with the decreased frequency of chronic diseases have been established**(Serrano, J and al .2009).**

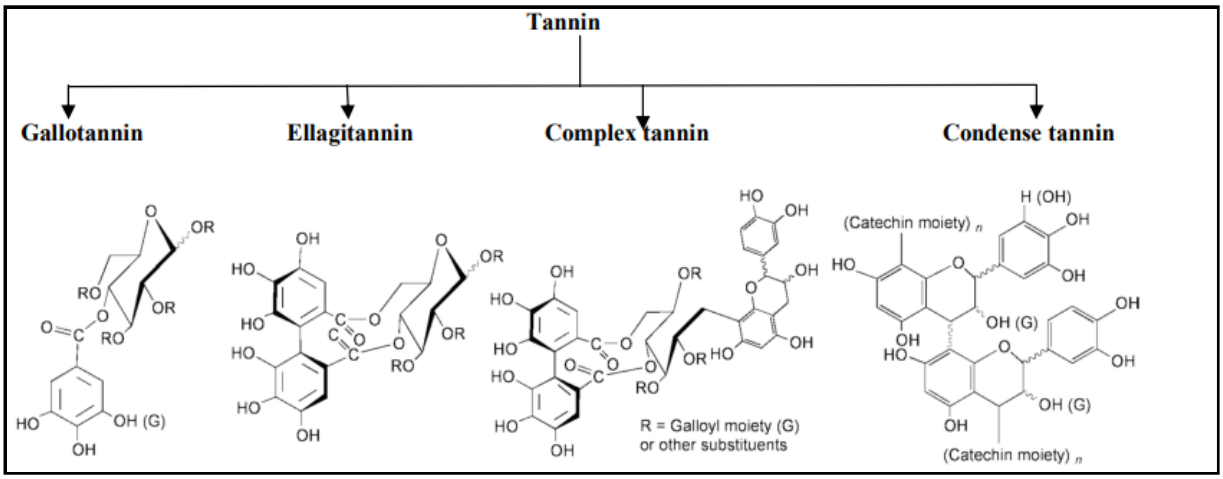

Figure n(09): Classification of tannins**(Giner-Chavez ,B.I.1996)**.

3.2.1.3.Quinones:

As a result of secondary metabolism in plants, quinones are produced. Based on several benzene rings, quinones are divided into 4 types namely benzoquinone, naphthoquinone, anthraquinone and phenanththrenequinone. The initiation of inflammatory mediators is obstructed by quinones. Cytokine expression is slowed down, oxidation is suppressed and inflammation is reduced by quinines Thymoquinone, a 1,4-benzoquinone isolated from Nigella sativa have shown to exhibit antiproliferative, anti-inflammatory and antimalarial activities **(Patel and al. 2021).** Dunnione 44 is a 1,2-napthoquinone-based natural product isolated from the leaves of Streptocarpus dunnii (Gesneriaceae) and used as a substrate for quinone-reductases that may be associated with its antimalarial properties **(Bian and al.2015).** Bioactive quinones are tried to develop through different strategies with a potential to treat leishmaniases, Chagas disease and cancer **(Da-Silva ,J and al. 2019)**. Quinones are very important phytochemicals for metabolic processes. Metabolic processes include the transfer of electrons in the Mitochondria through the respiratory chain in the process of respiration and photosynthesis **(Radhia and al.2018).**

3.2.2. Saponin :

Saponins are a group of secondary metabolites found widely distributed in the plant kingdom They form a stable foam in aqueous solutions such as soap, hence the name “saponin”. Chemically,

saponins asa group include compounds that are glycosylated steroids, triterpenoids, and steroid alkaloids. Two main types of steroid aglycones are known, spirostan and furostan derivatives . The main triterpene aglycone is a derivative of oleanane **(Bohlmann ,J and al. 1998)** . The carbohydrate part consists of one or more sugar moieties containing glucose, galactose, xylose, arabinose, rhamnose, or glucuronic acid glycosidically linked to a sapogenin (aglycone). Saponins that have one sugar molecule attached at the C-3 position are called monodesmoside saponins, and those that have a minimum of two sugars, one attached to the C-3 and one at C-22, are called bidesmoside saponins **(Lasztity, R.1998;Mamta, S and al .2013).**

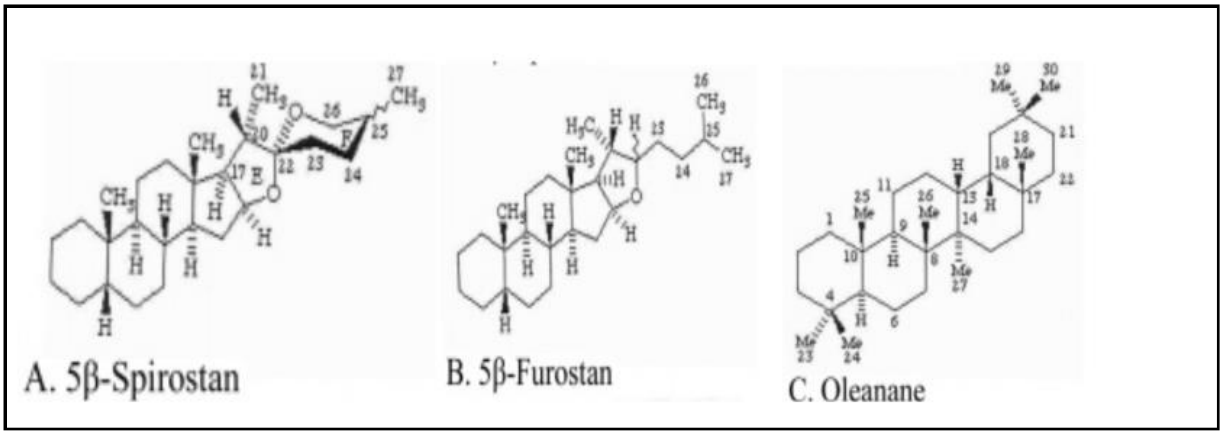

Figure n(08): Basic structure of steroid (A & B) and triterpenoid saponin (C) **(Mamta ,S and al .,2013).**

3.2.3. Terpenoids:

Perennial plants produce a major group of chemicals known as terpenoids. Thousands of terpenoids have been isolated so far **(Pichersky and Raguso. 2018).** Terpenoids are classified into monoterpenoids, hemiterpenoids and sesquiterpenoids and many others based on a number of their isoprene units. Terpenoids down regulate cytokine expression, reduce tissue loss and act as antioxidants. Infection is repressed by terpenoids by some mechanism **(Hossen and al.2022).**

3.2.4. Alkaloids:

The name of alkaloids derives from the “alkaline” and it was used to describe any nitrogen-containing base **(Mueller-Harvey, I and McAllan ,A.B . 1992)** Alkaloids are the very important constituent of plants with many therapeutic roles. The first alkaloid that was isolated was Morphine. It was isolated from poppy plant. It is used for the treatment of sleeping disorders and pain relieve **(Mamta S and al .2013).**

Alkaloids are natural product that contains heterocyclic nitrogen atoms, are basic in character. Alkaloids are naturally synthesis by a large numbers of organisms, including animals, plants, bacteria and fungi. Some of the fires natural products to be isolated from medicinal plants were alkaloids when they first obtained from the plants materials in the early years of 19th century, it was found that they were nitrogen containing bases which formed salts with acid **(Rao, R.V.K and al.1978).**

After that several other alkaloids were also identified, isolated and used. These include vincristine, vinblastine, atropine, brucine, colchicine etc. alkaloids are of great therapeutic importance, and they have proved their action in many researches. The high content of ammonia is present in the alkaloids which are the largest group of secondary chemical constituents. Amino acid building blocks synthesized the contents of nitrogen bases present in alkaloids. In a peptide ring, one or more hydrogen atoms are replaced with various radicals. Most of the peptide ring is occupied by an Oxygen atom. Nitrogenous compounds play role in guarding plants against herbivores and pathogens. **(Afzal I and al . 2023)** They are widely used as poisons, pharmaceuticals, narcotics and stimulants due to their strong biological activities. From a pharmacological view, alkaloids are used in the preparation of Central nervous system stimulants and anesthetics. For medicinal purposes, alkaloids are exploited very low although they are more than 12,000 residing in 20 % of plant species **(Mousavi and al.2018).**

3.3.Biological Activities of Phytochemicals :

The phytochemicals present in plants are responsible for preventing disease and promoting health have been studied extensively to establish their efficacy and to understand the underlying mechanism of their action. Such studies have included identification and isolation of the chemical components, establishment of their biological potency both by in vitro and in vivo studies in experimental animals and through epidemiological and clinical-case control studies in man. phytochemicals may reduce the risk of coronary heart disease by preventing the oxidation of lowdensity lipoprotein (LDL) cholesterol, reducing the synthesis or absorption of cholesterol, normalizing blood pressure and clotting, and improving arterial elasticity**(Mathai ,K.2000)** . Phytochemicals may detoxify substances that cause cancer. They appear to neutralize free radicals, inhibit enzymes that activate carcinogens, and activate enzymes that detoxify carcinogens. For example, according to data summarized by Meagher and Thomson, genistein prevents the formation of new capillaries that are needed for tumor growth and metastasis**(Meagher, E and Thomson, C.1999)**.

The physiologic properties of relatively few phytochemicals are well understood and more many research has focused on their possible role in preventing or treating cancer and heart disease**((Mamta, S and al .2013)**. Phytochemicals have also been promoted for the prevention and treatment of diabetes, high blood pressure, and macular degeneration**(American Cancer Society.2000)**.

While phytochemicals are classified by function, an individual compound may have more than one biological function serving as both an antioxidant and antibacterial agent **(Mamta Saxena and al .,2013).**

Table n(02): Bioactive Phytochemicals In Medicinal Plants**(Mamta, S and al .2013).**

Classification	Main groups of compounds	Biological function
NSA (Non-starch poly-saccharides.)	Cellulose, hemicellulose, gums, mucilages, pectins, lignins	Water holding capacity, delay in nutrient absorption, binding toxins and bile acids
Antibacterial & Antifungal	Terpenoids, alkaloids, phenolics	Inhibitors of micro-organisms, reduce the risk of fungal infection
Antioxidants	Polyphenolic compounds, flavonoids, carotenoids, tocopherols, ascorbic acid	Oxygen free radical quenching, inhibition of lipid peroxidation
Anticancer	Carotenoids, polyphenols, curcumine, Flavonoids	Inhibitors of tumor, inhibited development of lung cancer, anti-metastatic activity
Detoxifying Agents	Reductive acids, tocopherols, phenols, indoles, aromatic isothiocyanates, coumarins, flavones, carotenoids, retinoids, cyanates, phytosterols	Inhibitors of procarcinogen activation, inducers of drug binding of carcinogens, inhibitors of tumourogenesis
Other	Alkaloids, terpenoids, volatile flavor compounds, biogenic amines	Neuropharmacological agents, anti- oxidants, cancer chemoprevention

3.4. Importance of Medicinal Plants :

Therapeutic plants play an essential part in providing fitness to people mainly rustic people and eighty percent of the world's borderline population also uses them for health maintenance. The abundance of uses of aromatic plants in households also indicates their significance. Since the beginning of time, many traditional medicines were made through these therapeutic plants. The intricate chemical compounds are difficult to access for the innovation of new drugs, these therapeutic plants having many varieties provide purified components in addition to natural products for new drug formulation. Due to a lack of trace elements, uneven body growth takes place. Herbs play a major role by providing us with alimentary and trace elements. Chemicals

having medicinal value are also gained through therapeutic plants. Each one has an essential role when a deficiency occurs in the body **(Evanjelene and Velu. 2021).** The present review will help in enabling us to add new information to existing knowledge about the cost-effective treatment of different ailments with medicinal plants**(Afzal, I and al .2023).**

Medicinal plants have been playing an essential role in the development of human culture. As a source of medicine, Medicinal plants have always been at forefront virtually all cultures of civilizations. Medicinal plants are regarded as rich resources of traditional medicines and from these plants many of the modern medicines are produced. For thousands of years medicinal plants have been used to treat health disorders, to add flavor and conserve food and to prevent diseases epidemics. The secondary metabolites produced by the plants are usually responsible for the biological characteristics of plant species used throughout the world. The microbial growth in diverse situations is controlled by plant derived products. In this review we gave general overview of the medicinal plants**(Refaz ,A .D and al.2017).**

Chapter III

Phoenix Dactylifera L and Date Seed

I. *Phoenix dactylifera L*:

I. 1. Definition:

Phoenix dactylifera Linn are commonly known as date fruits and date palm belongs to the family of Palmaceae and it is grown in arid and semi-arid regions of the world **(Nasir and al.2015)** The tree is famous due to their advantageous health benefit and are considered as a high value commercial fruit crop in the international market **(Saryono and al. 2016).**

It is among primogenital cultivated plants as a source of income, and it has contributed to the Arabian cape's daily life for the past 7000 years. **(Sulaiman A and al .2022).**

Figure n (11): Date palm tree (Balaram Mahalder, CC-BY-SA-3.0) and fruits**(Al-Snafi1, A. E and Murshd -Thuwaini .M.2023)**

(Saryono and al. 2016)

I.2. Taxonomy :

(Mirshad ,M and Deepika ,S .2022)

Kingdom: Plantae

Division: Magnoliophyta

Class: Liliopsida

Order: Arecales

Family: Arecaceae

Genus: *Phoenix*

Species: *Phoenix dactylifera*

Binomial name: *Phoenix dactylifera Linn*

I.3. Botanical description:

The height of the tree is 36-40 m tall, Trunks are covered by persistent bases of petioles, the inferior part of the plant generally covered by a mass of offshoots.

- **Leaves:** The leaves are linear, keeled lesser pinnae adapted into spines, pinnate 20-40 cm long.
- **Flowers:** The flowers are small and gather in branched spadices. The colour of the flowers is yellow. The lower part of the flower is connected straight to the spikelets which after grow into fruits.
- **Fruits:** The fruits are called as dates and appear as oblong berry. The size fruit is 2.6-7.6 cm long and the diameter is about 2-3 cm. The colour of the fruit is red or yellow brown when it ripe and in unripe condition it looks like deep green in colour. The varieties of fruit depend upon the presence of sugar contained in fruit.
- **Seeds:** Seeds are oval-cylindrical in shaped. Every fruit has single seed with a longitudinal channel. Seeds appear bright red to bright yellow in colour when unripe **(Csir ,D.K.2003)**

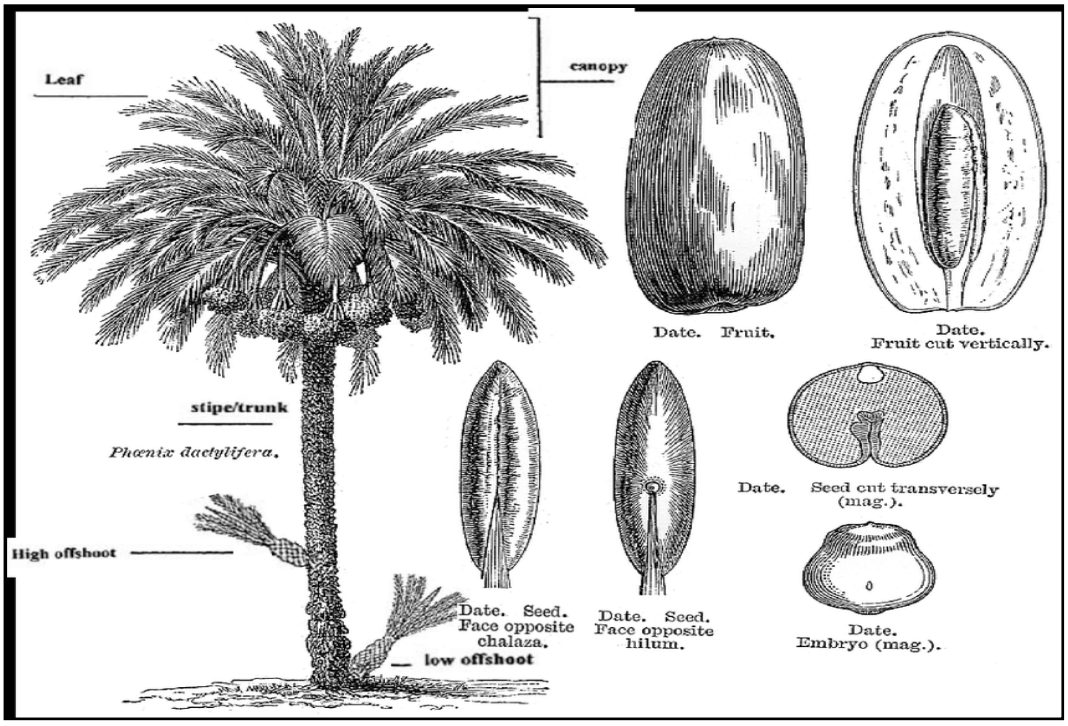

Figure n (12) : Botanical description of *Phoenix dactylifera L***(Mirshad ,M and Deepika ,S .2022)**

I.4. Ecology :

The plant date palm can resist high temperature for a long period of time but does not tolerate low temperature for very long time; below -8° is harmful for it. The appropriate conditions for growing

date palm are low humidity region and where rainfall is very low. The most preferable circumstances for flowering are dry, long and hot environment. And a mean temperature is required for actual ripening like 30°-35°. The date palm can grow in various types of soil and the alkalinity is between 5-8.2 pH ranges. Generally date palms are grown under providently controlled soaking **.(Kwaasi ,A.A.2003)**

I.5. Distribution:

Date was cultivated in the Middle East for more than 5,000 years **(Zohary, D;Hopf ,M. 2000)** According to the earliest record from Iraq (Mesopotamia), it was originated from the Mesopotamia **(Nixon ,R.W.1951).** It was depicted in ancient Babylonian and Assyrian tablets, including the Code of Hammurabi **(Chao, C.T and Krueger, R.R.2007)** Dates were one of the important fruits in the dry regions of the Arabian Peninsula, Middle East and North Africa. Over the past 3 centuries, it has been introduced in Pakistan, India, Australia, Mexico, South America, United States and Southern Africa. Dates were the staple food and essential income source in many countries **(Chao, C.T and Krueger, R.R.2007)** .Now, it was cultivated in wide areas. Long summers with high day and night temperatures, and mild, sunny, dry winters without prolonged frost are the ideal climatic conditions for this species **(Tengberg ,M.2011; Shabani ,F and al . 2011; Al-Snafi1 A. E and Murshd -Thuwaini M.2023)**

I.6. Chemical constituents and minerals of *Phoenix dactylifera L*:

Phytochemically, the whole plant (namely shoot tip, pollen grains, leaves, fruits, callus, embryogenesis and invitro leaf tissue) contains carbohydrates, alkaloids, steroids, flavonoids, vitamins and tannins. Four phenolic acids and nine bound phenolic acids were tentatively identified **(Ziouti and al.1996; Eong and al. 2006).** Dates contain at least six vitamins including small amount of vitamin C, vitamin B1 (thiamine), B2 (riboflavin), nicotinic acid (niacin) and vitamin A **(Al-Shahib and Marshall.1993).** Enzymes such as phytase, invertase and peroxidase have been isolated in dates **(Nadkarni.1976)**. Presence of estrone has been reported in the dried date seeds and pollens **(Heftmann and Bennet. 1965)**. 7 The date palm pollen grains showed the presence of alpha-amirin, triterpenoids, saponins and a crude gonadotrphic substance **(Mahran and al. 1976).**

The date palm (*Phornix dactylifera L.*) is considered the most important source of food for both human in arid and semiarid **(Besbes and al.,2004).** Dates contain a high percentage of sugars reaching 88% in some varieties **(Al Shahib and Marshall.2003)**. Dates are also rich in mineral salts and vitamins **(Booij and al.1992)**. For the date pit, the percentage of non-reducing sugars is 3.82% and in glucose and fructose is 1.68 and 1.53, respectively **(Fayadh and Al Showiman.1990).**

The pits of *Phoenix dactylifera* contains different chemical compounds such as saturated and

unsaturated fatty acids, Zinc (Zn), Cadmium (Cd), Calcium (Ca), and potassium (K). Saturated fatty acids include stearic and palmitic acid and unsaturated fatty Acids contain linoleic and oleic acids which could inhibit 5 -α reeducates enzyme **(Shariati and al. 2008).** Also, dates contain at least six vitamins including a small amount of vitamin C, and vitamins B1 (thiamine), B2 (riboflavin), nicotinic acid (niacin) and vitamin A **(Al-Shahib and Marshall .1993)**. Studies indicate that the aqueous extracts of dates have potent antioxidant activity **(Mansouri and al.2005)**. The antioxidant activity is attributed to the wide range of phenolic compounds in dates including p-coumaric, ferulic and sinapic acids, flavonoids and procyanidins **(Gu and al.2003 and Al-Farsi and al.2005).**

Table n (03): Phytoconstituents Reported In P. Dactylifera Linn.

(Kamalika ,M and al .2022)

S. No	Type of chemical	Chemical constituents
1	Phenolic compounds	Coumaric acid, cinnamic acids and sinapic acid[11]
2	Flavonoid glycosides	Methyl luteolin, methyl quercetin, luteolin and quercetin[11]
3	Bound phenolic acids	Gallic acid, syringic acid, caffeic acid, p-hydroxybenzoic acid, vanillic acid, p-coumaric acid, o-coumaric acid, protocatechuic acid and ferulic acid[12]
4	Free phenolic acids	Vallic acid, ferulic acid, procatechuic acid and syringic acid
5	Flavones	Epicatechin and catechin[13]
6	Fatty acids	Palmitic acid, palmitoleic acid, linolenic acids, lauric acids, myristoleic acid, capric acids, and myristic acid[14]
7	Pigments	Anthocyanins[15]
8	Carotenoids	α-carotene and Lutein[16]
9	Steroids	Campesterol, cholesterol, stigmasterol, isofucosterol and ß- sitosterol[17]
10	Triterpenoid	α-amirin[18]
11	Enzymes	Peroxidase phytase and Invertase[19]
12	Vitamins	Vitamin A, B1, B2, B3, B5, B6, B9 and C[14]
13	Other constituents	Galactomannans α-D glucan and Heteroxylon[20,21]

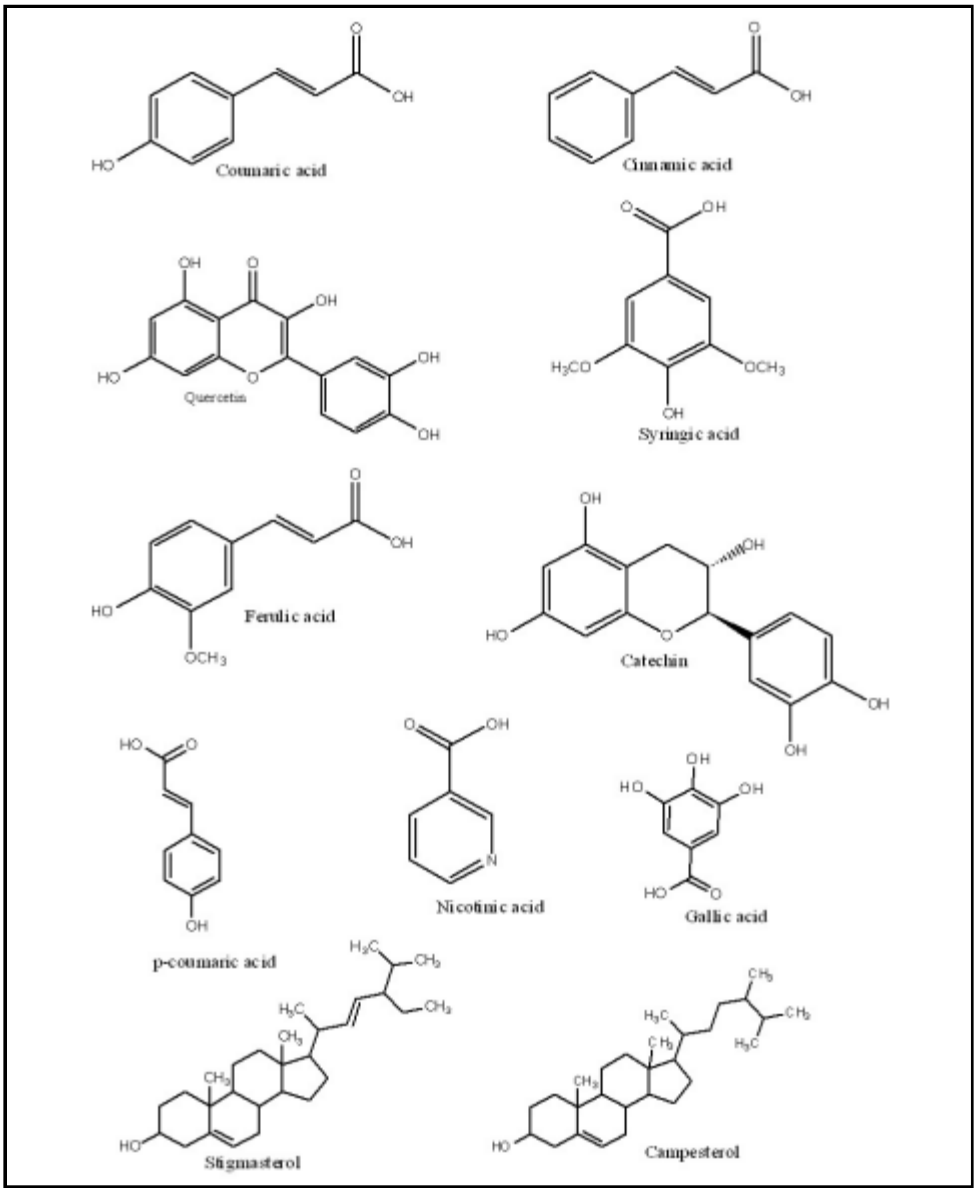

Figure n (13): Structures of chemical constituents of *P. dactylifera Linn*.

(Kamalika ,M and al .2022)

I.7. Traditional uses

The date fruit, was eaten fresh, in various processed forms and dried. Fruits were harvested when they were fully ripened (Tamar and Rutab stages), which were low in moisture, high in sugar and low in tannin, but in North Africa, the dates were eaten during the Khalal stage, which contained high tannin and very astringent **(Nixon ,R.W. 1951;Dowson ,V.H.W, and Aten, A.1962)**). Seeds roasted and ground into powder make a beverage like coffee called "date coffee" **(Nadkarni.1976).** All parts of the plant were used for some purpose. The trunk and wood were used as fuel. Fiber of the trunk and leaves were used to make baskets, bags, cords, food covers, crates, camel saddles, furniture, mats, fans, trays, ropes, paper, and twine. The dried leaves bundles were used to make shades, separating walls, roofs, and enclosures. Ribs of the leaves were used to make fishing traps and boats. The base of the leaves and fruit stalks were used as fuel. Palm heart was eaten as a salad.

Oil of date seeds was used for soap manufacturing. Date fruit also used medicinally **(Glasner, B and al . 2002;Nixon, R.W.1951;Barreveld, W.H.1993)**

I.8.Medicinal applications of *Phoenix dactylifera L*:

In traditional medicine, the use of date fruit is recommended for treatment of liver diseases and to be consumed by pregnant women before and after delivery **(Al-Mamary ,M and al . 2014).** Fruits were also used for fever, inflammation, loss of consciousness, memory disturbances, nervous disorders and paralysis **(Hassan HM. 2011).**

The water in which fresh dates are soaked for a while is a drink given to relieve alcohol intoxication. The milk in which clean and fresh dates are infused is a very nourishing and restorative drink to the children as well as adults especially during convalescence from fevers and smallpox. The sweet pulp of the date fruits is useful in dysentery. Date fruits are used as an ingredient in various aphrodisiac and tonic confections; they are also useful in asthma. Paste made from ground seeds is said to be applied for opacity of cornea and to the head to relieve headache. The smoke produced from burning of the date seeds in powder form is a useful fumigatory for piles. Some doctor's advice dates as consumptives as they promote expectoration, soothe the chest and prevent constipation **(WOI.1985).** A gum or juice obtained from the stem and named "laghi" is used as a demulcent, diuretic and refrigerant in genitourinary affections. The liquid distillate obtained from spathe of *Phoenix dactylifera L.* known as Maa Al-liqah or Maa Al-Tilal or water of the spathe, is believed to have certain medical uses as it relieves abdominal gases and pain especially after heavy meal and claimed to have anti-spasmodic activity . The flower of the plant is used as a purgative **(Zoghet and Alsheikh. 2000)**. The pollen grains of date palm have been used in Egyptian local practice to improve fertility in women **(Amin and al. 1969;Elberry ,A.A and al.2011).** It was used as nutrient, heart tonic, aphrodisiac, laxative, demulcent and expectorant. It was traditionally used in fatigue, diabetes, hypertension, chest complaints, cough, asthma and gastroenteritis **(Tahraoui ,A and al . 2007; Khare ,C.P.2007).**

II. Date seed:

II.1. Definition :

Common names Date seeds, dates pits, date kernels, date stones, date pips, **(Heuzé ,V and al .2015)**. Date seeds are the by-product of date stoning, either for the production of pitted dates or for the manufacture of date paste **(Barreveld. 1993)**

Figure n (14): The date seed **(Heuzé, V and al . 2015).**

II.2.Description :

The date seed is a hard coated seed, usually oblong, ventrally grooved, with a small embryo. Date pits weigh 0.5 g to 4 g and represent 6 to 20% of the fruit weight depending on maturity, variety and grade **(Daghir. 2008; Zaid and al. 2002).**

II.3.Distribution :

Date by-products are available in countries of production. Date seeds are available near to where dates are packed or processed. The date industry is the main source of date pits, but they are also produced at farm level in date paste production units **(Heuzé, V and al .,2015)**.

II.4. Chemical composition:

The date seed contain different chemical compounds such as saturated fatty acids as stearic and palmitic acid, unsaturated fatty acids such as linoleic and oleic acids which could inhibit the 5-α reducates enzyme, **(Shariati. M.2008).**The date seed is mainly composed , protein, carbohydrates, phenols **(Mrabet, A and al . 2015)** and minerals (potassium, magnesium, calcium, phosphorus, sodium, Zinc (Zn), Cadmium (Cd), Calcium (Ca) and potassium (K) and iron) **(Shariati ,M.2008).** 3.1–7.1% moisture, 2.3–6.4% protein, 5.0–13.2 fat, 0.9–1.8% ash and 22.5–80.2% dietary fiber **(Sahar, H.O. 2014).** Also, seeds contain high levels of phenolics (3102– 4430 mg gallic acid equivalents/ 100 g), antioxidants **(Al-Farsi ,M.2007).**

Such substances perform several functions from a biological point of view, such as antioxidant, antibacterial, and antiviral activities **(Mrabet ,A and al. 2020; Ekpa, O.D and al .1996)** . Date seeds are also a good source of oil (5 to 13%), which is rich in phenolic compounds, tocopherols, and phytosterols (**Nehdi, I and al.2010).**

II.5.Uses and benefits of date seed:

utilization of such waste is very important to date cultivation and to increase the income to this sector. In addition, date seeds could be considered as a valuable source of functional ingredients and could therefore be used for different food products' formulation and for the development of different functional and medicinal supplements.

used in making non-caffeinated coffee **(Habib and Ibrahim.2009)**. They are also listed in folk remedies for the management of diabetes, liver diseases and gastrointestinal disorders in traditional Egyptian medicine **(Kchaou and al. 2016).** It has been reported that the extracts of date seeds ameliorate gastric ulceration in rats **(Al-Najada and al. 2014**;**Al-Sayyed and al. 2014**) and possess an anti-inflammatory activity in the rat adjuvant arthritis model **(Doha and Al-Okbi. 2004 ; Rahmani and al. 2014; Salah and Al-Maiman .2005),** have reported that feeding the defatted date seed flour to rats reduced the plasma triglycerides, total cholesterol and low-density lipoprotein **(Hamza, A .2018)**.

Date seeds are traditionally used for animal feed. They can also be used as a source of oil (which has antioxidant properties valuable in cosmetics), as a raw material for activated carbon or as an adsorbent for dye-containing waters **(Lecheb. 2010; Banat and al.2003)**

Chemical composition of date seeds showed high amount of fiber (75–80%), fat (10–13%), proteins (5– 6%), and ash **(Besbes and al. 2004; Al-Farsi and al. 2007 ;Al-Farsi and Lee.2008)**. Thus, utilization of such waste is very important to date cultivation and to increase the income to this sector. In addition, date seeds could be considered as a valuable source of functional ingredients and could therefore be used for different food products' formulation and for the development of different functional and medicinal supplements.

Date seeds can be discarded, used in animal feeding or used in making non-caffeinated coffee **(Habib and Ibrahim. 2009).** They are also listed in folk remedies for the management of diabetes, liver diseases and gastrointestinal disorders in traditional Egyptian medicine **(Kchaou and al. 2016)**. It has been reported that the extracts of date seeds ameliorate gastric ulceration in rats **(Al-Najada and al. 2014; Al-Sayyed and al.2014)** and possess an anti-inflammatory activity in the rat adjuvant arthritis model **(Rahmani and al. 2014; Salah and Al-Maiman.,2005)** have reported that feeding the defatted date seed flour to rats reduced the plasma triglycerides, total cholesterol and low-density lipoprotein**. (Hamza, A .2018)**

Date seeds are traditionally used for animal feed. They can also be used as a source of oil (which has antioxidant properties valuable in cosmetics), as a raw material for activated carbon or as an adsorbent for dye-containing waters **(Lecheb. 2010; Banat and al. 2003)** Date by-products are

usually fed to animals during winter, though they can be used at any time of the year **(Genin and al. 2004).**

Few researches have been carried out on incorporation of date seed powder into wheat flour for bread production **(Golshan Tafti and al. 2017**), pan bread containing 15% date seed had the highest score in overall acceptability when compared to control and other levels of seeds. They also concluded that date seed powder had improving effect on the nutritive value of pan bread and also it had a hypoglycemic effect and could decrease the risk of diabetic diseases. It is reported that Saudi Mafrood flat breads containing 10% coarse seed powder had similar sensory properties to flat breads containing wheat bran. Flat breads containing fine seed powder had lower sensory scores (color, flavor, odor, overall acceptability) than wheat bran controls **(Habibi ,N. 2011;Hejri ,Z and al.2013)** milled date seeds into germ and residue and incorporated them in formulation of Iranian Barbari flat bread at the levels ranged in 0.5-3 g/100 wheat flour. The results of texture analysis during 5 days of bread storage showed that both fractions of germinated date seeds reduced the bread staling. The higher levels of flavonoids and antioxidant capacity were observed in Arabic bread containing date seed powder **(Platat and al. 2013).**

It is thought that date fruits and seeds may have an effect on human health due to the vitamins, amino acids, fat, protein, bioactive components, phenolic components and other phytochemicals in their composition . For this reason, it is thought that different parts of consumable herbal materials should be made ready for use as food supplements. Because of these nutrients and phytochemicals in the palm kernel, the use of palm seed flour can be encouraged to fortify food industry products **(Mehmet, M. O.2022) .**

Second part

Experimental part

Chapter I

Materials and methods

1. Material

1.1. Vegetal Material

The plant used in this work is *phoenix dactylifera, L* collected in El Oued Province in July 2023. To prepare the extract, the stones were separated from the dates then washed to remove traces of pulp and all kinds of impurities that stick to it, then left to dry away from sunlight and at room temperature, The kernels are ground by a blender to obtain a fine powder.

Figure n (15): The kernels

Figure n (16): kernel powder

1.2. Laboratory equipment

- Rotary evaporator type Büchi Rotavapor R- 200
- CHRISA BETA1 type the lyophilisation
- SHIMADZU type HPLC
- INOLAB type PH meter
- KIKA-WERK type mechanical stirrer
- RETSCH type grinder
- SIGMA type horizontal centrifuge
- UV-VIS-1240 molecular transmission spectrophotometry
- MEMMERT type oven
- MEMMERT type water bath
- Analytical balance type KERN ABJ/ABS
- Electric scale type KERN EMB 2200-O
- Hotplatestirrer
- Caliper
- Refrigerator.
- Blender

1.3. Glassware and plastic equipment

Micro pipette ;Test tubes with cas ; Bottles ; Erlenmeyer ; Test tube holder ; plastic collection tube Spatula ; Pipette tips ; Filter paper ; Graduated test tubes ; Beakers ;Vial ; Aluminium foil ; Glass funnel

1.4. Chemical products

Ethanol ; Hydrochloric acid (HCl) ; Distilled water ; Sodium hydroxide (NaOH) ; Gallic acid ; Sodium carbonate (Na2CO3) ; Ascorbic acid ; Aluminum chloride (AlCl) ; ether/chloroform ; Trichloroacetic acid (TCA) ; Tanic Acid ; Phosphoric acid (H3PO4) ; Catechim ; Quercetin ; Copper sulfat (CuSO4); ferric chloride(FeCl3); Sulfuric acid (H2SO4) ; Vanillin ; Phenol ; sodium phosphate buffer; Magnesium filings ; FCR reagent (Folin-Ciocalteu); citric acid sodium citrate ; potassium ferricyanide [K3Fe(CN)6]; dextrose ; sodium chloride ; Sodium-potassium nitrat (O64H2.kNaC4H4) ; 2,2-diphenyl-1-picrylhydrazyl (DPPH) ; Burchard's reagent ; Dragendorff reagent ; Mayer's reagent ; Sulphate reagent ; Ammonium Hydroxide (NH4OH)

1.5. The blood

The blood taken to carry out an anti-inflammatory test is collected from volunteers who have not taken anti-inflammatory drugs for 21 days.

2. Methods

2.1. morphological analysis

To carry out this analysis, 10 date stones of the "Ghars" variety were sorted. This operation consists of measuring the dimensions (length and width), weight and determination of the color of the nucleus and assessed visually.

2.2. Preparation of the Hydroalcoholic extract

Was carried out according to the method described by. **(BAHRIZ & BENYOUCEF, 2023)** with some modifications. Briefly We put 100 g of kernel powder dissolved in 500 ml of (70% ethanol and 30% distilled water) to soak for 48 hours at room temperature with stirring. After filtering on filter paper, this process is repeated on the sediment 3 times, The liquid resulting from repeated filtering operations is collected, then we put it in the rotary evaporator device at a temperature of 37 °C for 24 hours. Then we take the remaining solution and put it in the freezer for 24 hours, then it is placed in the lyophilisation device. This process takes 48 hours for the sample to dry completely,The extract was weighed and stored for upcoming analysis at 4 °C.

2.3. Yield calculation

The yield of the plant in extracts is the ratio between the weight of the extract and the weight of the plant to be treated, it is expressed as a percentage (**CHENNI & HACHELA, 2019)** and calculated by the following formula :

$$R\ \% = (PE / PA)*100$$

R: extract yield in percentage.

PE: extract weight in grams.

PA: dry plant weight in grams.

2.4. PHYTOCHEMICAL STUDY

2.4.1. Qualitative phytochemical analyzes (Phytochemical screening)

Phytochemical tests (Screening) are qualitative tests which make it possible to characterize the different chemical groups contained in a plant organ. These reactions are based on precipitation or coloring phenomena using reagents specific to each family of compounds. **(Sabeur & Saidi,2019)**

2.4.1.1. Flavonoids

Pieces of magnesium ribbon and 1ml concentrated HCl were mixed with 5 ml ethanolic extract after a few minutes The presence of flavones is confirmed by the appearance of a color changing to purple red (flavonals), purplish red (flavonones and flavonols). (**KADRI and al,2021)**

2.4.1.2. Tannins

A quantity of plant matter is mixed in 2 ml of water distilled, a few drops of concentrated HCl are added, everything is heated in a boiling water bath. The formation of a red precipitate indicates a positive test **(BÉKRO and al,2007)**

2.4.1.3. anthocyanins

The anthocyanins were characterized by a mixture of 2ml, 10% H2SO4 and 10% NH4OH. The appearance of a blue color in a basic medium indicates the presence of anthocyanins.

(Sabeur & Saidi,2019)

2.4.1.4. coumarins

covered with paper soaked in diluted NaOH, then brought to the boil. Any yellow fluorescence indicates the presence of coumarins after UV examination**. (Boubekeur,2019)**

2.4.1.5. Alkaloid

2.4.1.5.1. MAYER test

The test consists of adding to 1.5 ml of each extract 0.5 ml of 1% Hcl and 3 drops of Mayer's reagent, the appearance of a creamy white precipitate reveals the presence of alkaloids.

(HADDAD & HADROUG, 2021)

2.4.1.5.2. Dragendorff test

The test consists of adding 5 ml of powdered plant material and 2 ml of 2% HCl to it. The solution obtained is introduced into a test tube then 5 drops of Dragendorff reagent are added.
The appearance of a precipitate confirms the presence of alkaloids. **(Belouadah & Attoui, 2020)**

2.4.1.5.3. Bouchardat test

0,1 g de résidu est repris dans 6 ml d'éthanol à 60%, puis reparti dans tube à essai, sont ajoutées 2 gouttes de réactif de Burchard. L'apparition d'un précipité brun indique un test positif. **(BÉKRO and al,2007)**

2.4.2. Quantitative phytochemical analysis

2.4.2.1. Estimation of total phenolics

Total phenolic content was calculated using the Folin–Ciocalteu method (0.2 mL) of the sample of the extract and 1mL of Folin – Ciocalteu reagent were added diluted of 10% and added 800 µL of saturated sodium carbonate (7,5%) after 4 min. The absorbance was measured at 765 nm after 30 minutes of cuddling in the oven at 30 degrees. The total phenolic content of the extract was measured in milligrams of gallic acid equivalent per gram of extract. **(CHOUIKH and al, 2020)**

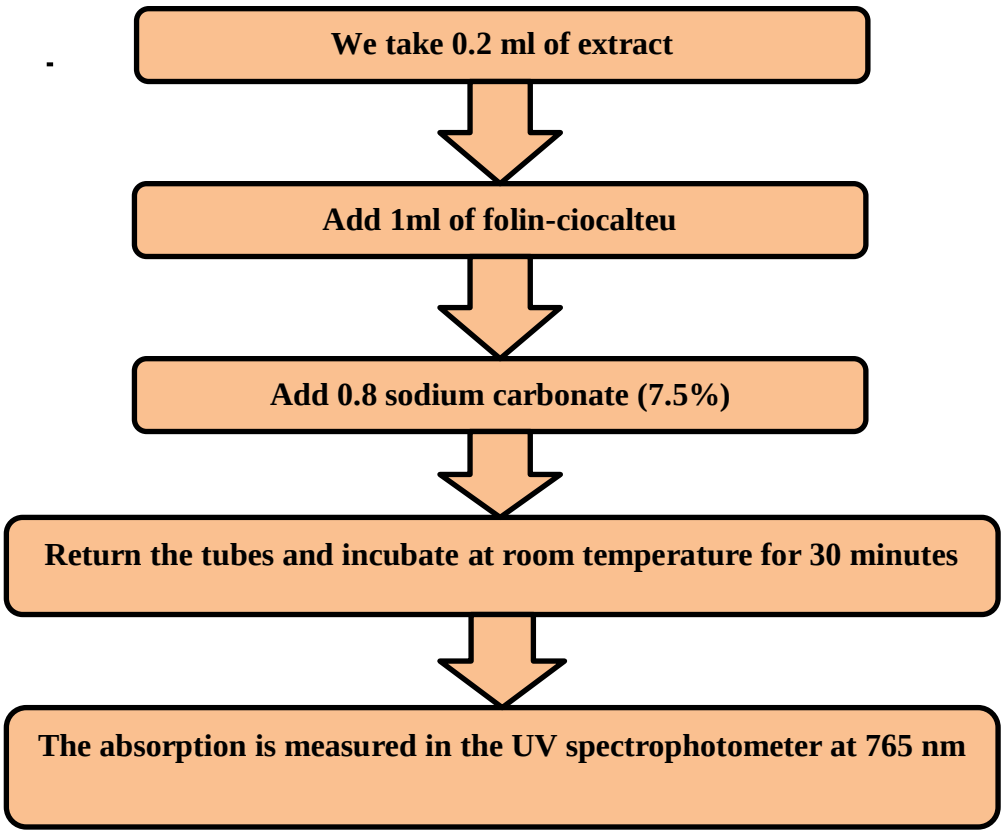

Figure n (17): Scheme for estimation of polyphenols in extract

2.4.2.2. Estimation of total flavonoids

We blended 1 ml of the extract with 1 ml of AlCl3 solution (2%). After stirring the test tubes, we let them rest for 60 min at room temperature, based on the measured absorbance 430 nm,

The results were expressed in milligrams of Quercetine per gram of extract. **(CHOUIKH and al, 2020)**

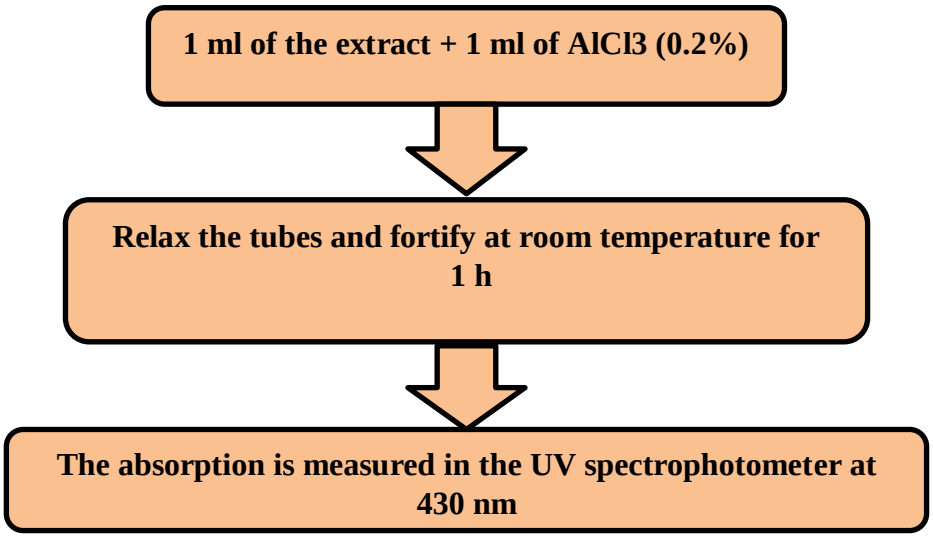

Figure n (18): Scheme for estimation of Total Flavonoids in extract.

2.4.2.3. Estimation of total tannins

2.4.2.3.1. Estimation of total hydrolysable tannins

The Folin-Ciocalteu colorimetric method was used to calculate the total hydrolysable tannin concentration. A 10 mL test tube containing 8.4 mL distilled water, 0.5 mL Folin-Ciocalteu reagent, and 0.1 mL 7 percent Na2CO3 solution was filled with an aliquot of 1 mL tannic acid in distilled water of each concentration. Absorbance was measured at 700 nm against a blank after incubation for 30 minutes. All of the tests were repeated three times. Tannic acid equivalents (TAE) per gram of dry extract (mg/g) were used to assess the total tannin content of the extract **(Laib & Boutlelis, 2022)**

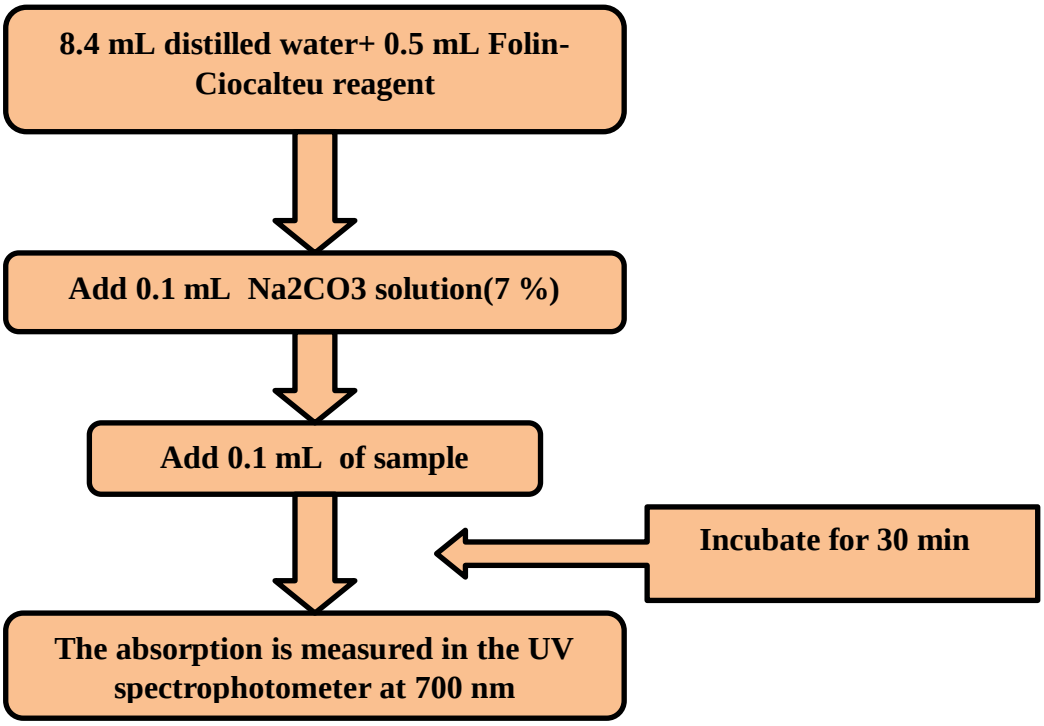

Figure n (19): Scheme for estimation of Total hydrolysable tannins in extract

2.4.2.3.2. Estimation of condensed tannins

Added 3 mL of freshly made vanillin reagent (4%vanillin in methanol) and 1.5 mL of strong hydrochloric acid were pipetted into an aluminum Foil wrapped tube with 0.5 ml the sample. The mixture was thoroughly mixed before the hydrochloric acid was added. The reaction's absorbance against water was evaluated at 500 nm after 15 minutes at 20 to 2°C. Catechin was utilized to create the calibration curve. **(Laib & Boutlelis, 2022)**

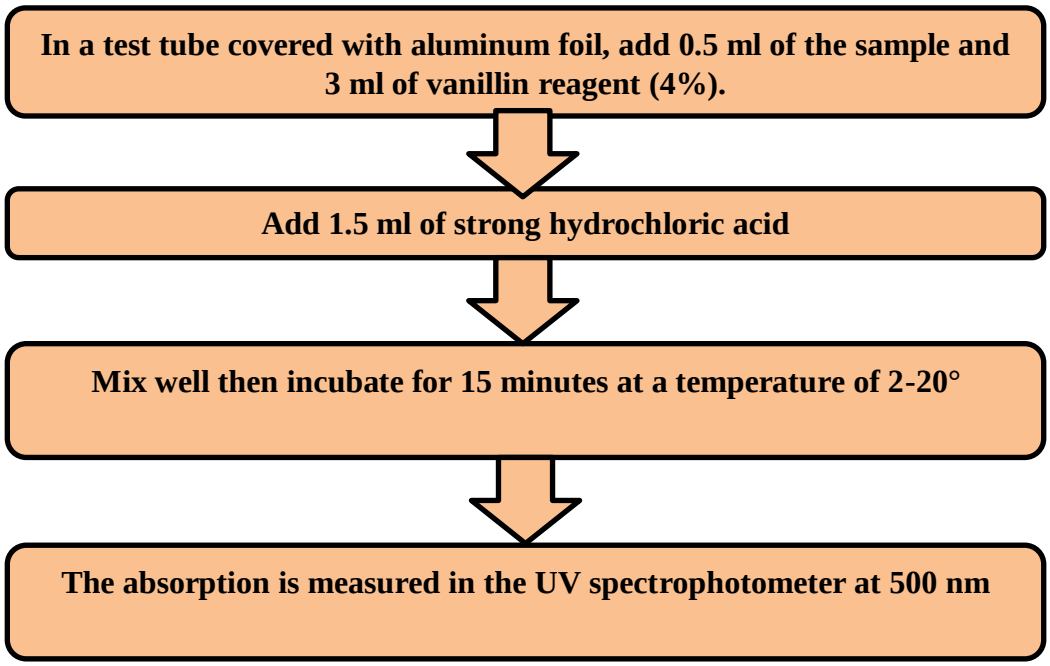

Figure n (20): Scheme for estimation of Total condensed tannin in extract

2.5. Antioxidant Activity

2.5.1. Reducing power assay FRAP

Known as the FRAP test is a method based on the change of coloring during the reduction of iron, from the ferric ion (Fe3+) to the ferrous ion (Fe2+) by electron transfer. This reduction is due to the presence of an antioxidant. (**Chenni & Hachela, 2019)**

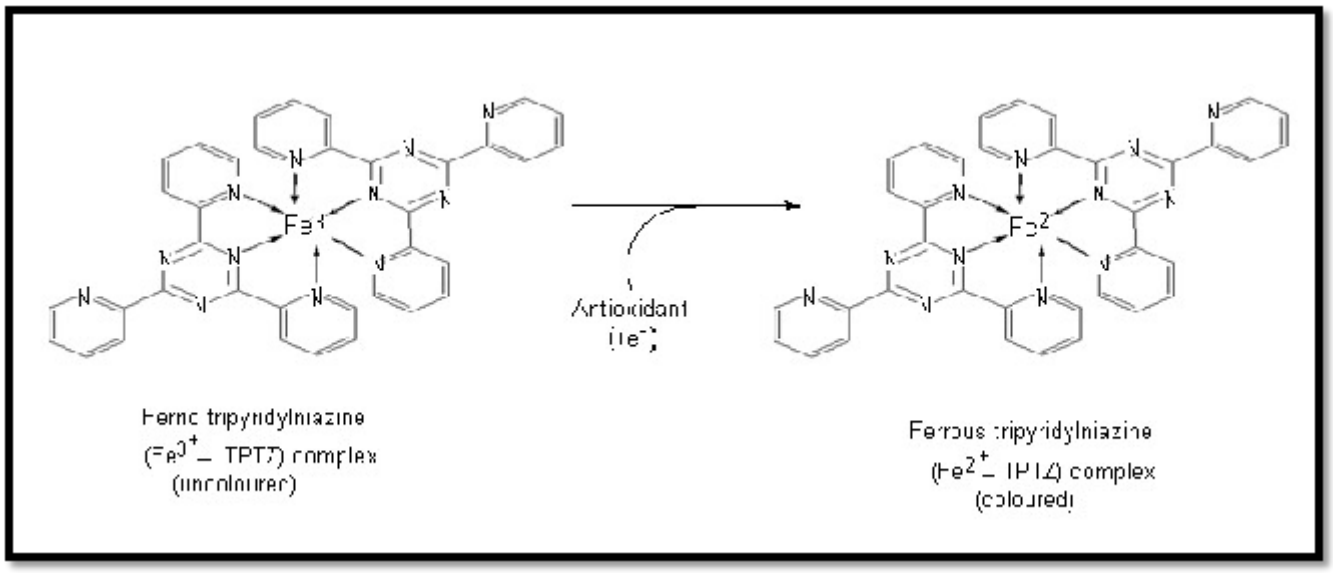

Figure n (21): FRAP test reaction **(Haimoud,A. 2017)**

- Different concentrations of the ethanolic extract of phoenix dactylifera.l (10-100 µg/mL) was added to 2.5 mL of 0.2 M sodium phosphate buffer (pH 6.6) and 2.5 mL of 1% potassium ferricyanide [K3Fe(CN)6] solution.
- The reaction mixture was vortexed well and then incubated at 50°C for 20 min.
- At the end of the incubation, 2.5 mL of 10% trichloroacetic acid was added to the mixture and centrifuged at 3000 rpm for 10 min.
- The supernatant (2.5 mL) was mixed with 2.5 mL of deionised water and 0.5 mL of 0.1% ferric chloride.
- The colored solution was read at 700 nm against the blank with reference to standard using UVSpectrophotometer. Here, ascorbic acid was used as a reference standard, the reducing power of the samples were comparable with the reference standard. **(Maruthamuthu,V and al, 2016)**

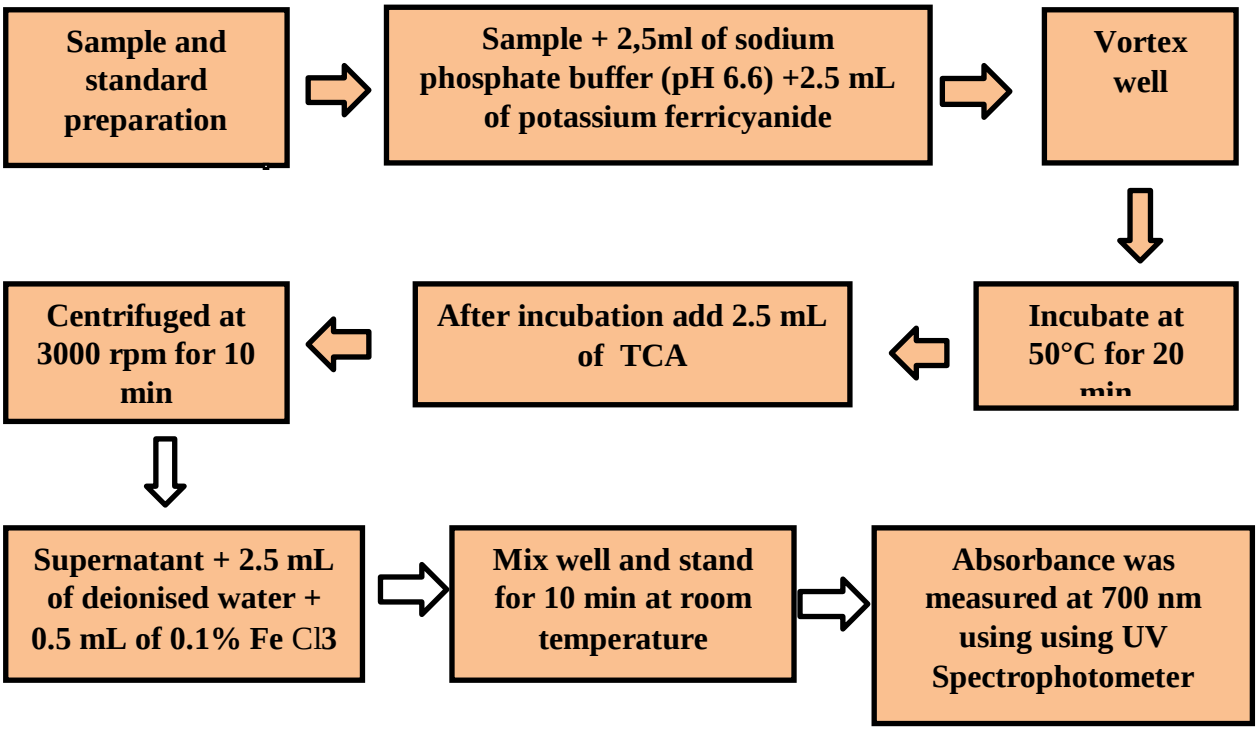

Figure n (22): Diagram representing the iron reduction protocol (FRAP test)

2.4.2. DPPH free-radical scavenging activity

The DPPH (1,1-Diphenyl-2-Picryl-Hydrazyl) test is a widely used method in the analysis of antioxidant activity. Indeed, DPPH is characterized by its ability to produce stable free radicals. This stability is due to the delocalization of free electrons within the molecule. The presence of these DPPH• radicals gives rise to a dark purple color of the solution. Reduction of DPPH• radicals by an antioxidant agent results in discoloration of the solution. The color change can be monitored spectrophotometrically at 517nm and in this way the antioxidant potential of a substance or plant extract can be determined. (**Degaa & Latreche, 2019)**

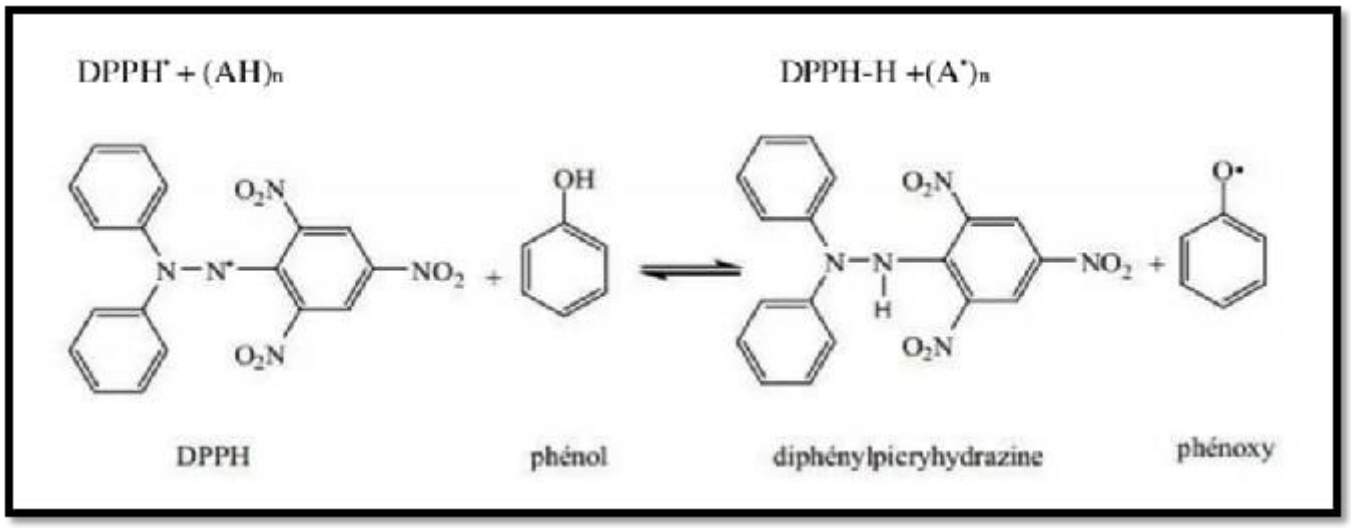

Figure n (23): DPPH radical modification during electronic transfer **(Boubekeur,2019)**

The effect of each extract on DPPH is measured by the procedure. A volume of 1mL of the freshly prepared DPPH˙ solution (0.1mM) is mixed with 500 µL of extract solution at different concentrations or ascorbic acid. After 30 min of incubation in the dark and at room temperature, the absorbance is read at 517 nm against a control where the extract is replaced by ethanol **(BAHRIZ & BENYOUCEF, 2023).**

The percentage inhibition (I%) is calculated by the following relationship:

I%= [(Control Abs - Test Abs)/ Control Abs] x 100

I %: Percentage of antiradical activity (AAR%).

Abs test : Sample absorbance

Abs Contrôle négatif : Absorbance of negative control

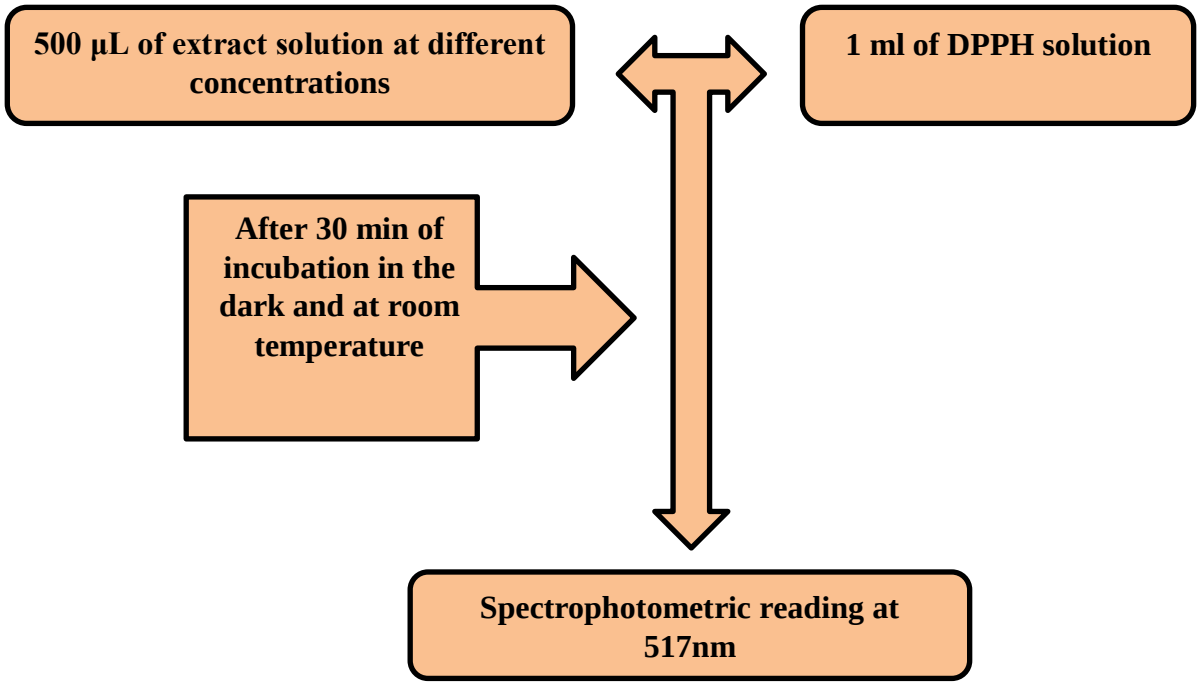

Figure n (24): Experimental protocol representing the DPPH test

2.6. Anti-inflammatory activity

Anti-inflammatory activity is determined in vitro by membrane stabilization method **(Shinde and al., 1999)**

Anti-inflammatory activity was studied in vitro using human red blood cells (HRBC) because the globular membrane is analogous to the lysosomal membrane and can be used for the evaluation of membrane stabilizing power by plant extracts or essential oils .

- Alsever solution is prepared by dissolving 2% dextrose, 0.8% sodium citrate, 0.05% citric acid and 0.42% sodium chloride in distilled water and the solution is sterilized.
- Human blood is mixed with an equal volume of sterilized Alsever solution and centrifuged at 3000 rpm for 10 min.
- The pellet is washed three times with isosaline solution (0.9%, pH = 7.2) and a suspension of 10% (v/v) isosaline is prepared.
- A reaction mixture (4.5 mL) is prepared by mixing 1 mL phosphate buffer (pH = 7.4), 2 mL hyposaline solution (0.45%), 1 mL sample/standard (1 mg/mL) and 0.5 ml of red blood cell suspension.
- The reaction mixture without plant sample is used as control and phosphate buffer as blank.
- The mixtures are incubated at 37 °C for 30 minutes and centrifuged again.
- The hemoglobin content in the supernatant solution is estimated spectrophotometrically at 560 nm.

percentage inhibition is calculated by the following relationship:

I%= [(Control Abs - Test Abs)/ Control Abs] x 100

I %: Percentage of anti inflammatory activity.

Abs test : Sample absorbance.

Abs Contrôle négatif : Absorbance of negative control.

2.7. High performance liquid chromatography (HPLC)

HPLC is a very flexible and simple way to isolate and identify different compounds in a mixture.

HPLC can be quite broadly described by common theories. A fluid called mobile phase passes through a column which contains a solid phase (silicas, fused silicas, grafted silicas).

At the initial instant, the mixture to be separated is injected into the inlet of the column where it is carried along by the mobile phase. If the stationary phase has been chosen well, the constituents of the mixture having different retention times are separated by elution. A detector placed at the outlet of the column coupled to a recorder makes it possible to obtain a trace called a chromatogram**.(Laouini, 2014)**

2.7.1. Equipment

The different parts constituting HPLC are described below:

Pump: A pump is powered from several bottles containing solvents of different polarities, allowing programming of the solvents to ensure a gradient system.

- In isocratic mode: 100% of a mixture of the same eluent throughout the analysis,

- In gradient mode, the concentration of the constituents of the eluent mixture varies over time.

Injector: It is an injection valve which carries a sampling loop.

Column: It contains the stationary phase which defines the type of chromatography, either normal phase or reversed phase.

- The normal phase : In the case of normal phase chromatography, the silica is grafted by polar groups and the eluent used is non-polar.

- The reverse phase :In the case of the reversed phase, the silica is grafted by linear carbon chains (C8, C18 and the eluent used is polar (ACN (acetonitrile), MeOH, H2O).

Detector : A UV-visible detector with variable wavelength (190-400 nm) which makes it possible to detect the different compounds contained in the sample to be analyzed. Note that it is important that the products to be detected carry a chromophore which absorbs in this wavelength range.

Solvents: Adsorption equilibrium is a competition phenomenon. Mobile phase molecules compete with solute molecules for polar adsorption sites (liquid-solid separation). The greater the interaction between the mobile phase and the phase stationary is strong, the less the adsorption of the product in solution will be significant. Solvents are therefore classified according to their strength which goes in the same direction as their polarity. **(Laouini, 2014)**

Table n (04): Properties of some solvents useful in liquid phase chromatography

Solvents	Polarity (P)	Boiling point (°C)	Viscosity (cP)
Isooctane	0.1	99	0.50
Hexane	0.1	69	0.31
Toluene	2.4	111	0.59
Methyl-t-butyl ether	2.5	55	0.27
Dichloromethane	3.1	40	0.44
Propanol-1	4.0	97	2.30
Tetrahydrofuran	4.0	66	0.55
Chloroform	4.1	61	0.57
Ethanol	4.3	78	1.08
Ethyl acetate	4.4	77	0.45
1,4-Dioxane	4.8	101	1.37
Acetone	5.1	56	0.36
Methanol	5.1	65	0.55
Acetonitrile	5.8	82	0.38
Acetic acid	6.0	118	1.10
Water	10.2	100	1.00

2.7.2. HPLC operating conditions

The analysis of phenolic compounds in the extracts studied was carried out using SHUMADZU HPLC brand high-performance liquid chromatography coupled with a UV-visible multi-wavelength detector. The analysis is carried out according to the operating conditions:

Steel column length: 250 x 4.6 mm

Stationary phase : C18.

Elution solvent: aqueous, hydroalcoholic (30\70)

Wavelength: 254 nm.

Injection loop: 10 µl.

Detector: UV-Visible.

Peaks were identified by retention times of congruents relative to standards.

3. Statistical analysis

Data from in vitro studies were analyzed using (Excel), version 2013. Experimental data were presented as mean. The results were analyzed by Two-Samples Assuming Equal Variances (T-TEST) and a significant difference was considered at $P < 0.05$.

Chapter II

Results and discussion

1. Results

1.1. Morphological characterization of Ghars seeds

The table below displays the results of the morphological analysis conducted on the external characteristics of palm seeds of the “Ghars” cultivar.

Table n (05): Results of morphological analyzes

Characterization morphological	**Results obtained**	
Structure	Shape	Ovoid
	Size	Average
	Color	Brown
	Surface	Smooth wrinkled
	Germinal pore	Central
Measures	Weight (g)	0,70±0,150
	Length (cm)	2,56±0,700
	Width (cm)	0,58±0,100

1.2. Yield

After extraction of the compounds *phoenix dactylifera L* seed variety "Ghars" following the protocol of **(Bahriz and Benyoucef, 2023)**, the obtained ethanolic extract was characterized by dark brown color and powder appearance. The yield calculated and the results obtained are presented in the table.

Table n (06): The appearance, color and yield of ethanolic extract of *phoenix dactylifera L* seed.

The extract	**Appearance**	**Color**	**Yield (%)**
Ethanolic	**Powder**	**Dark brown**	**11.425 %**

From the results shown in the table, we note that the hydroethanolic extract of seeds of " *Phoenix dactylifera L*" variety "Ghars" recorded a yield estimated at 11.425%. This value is higher than the value obtained by **(Maamoun and al ,2021)**, which amounted to 8.2% from the hydro methanolic extract of date pits, variety "Ghars", and higher than the value of the hydro methanolic extract from date pits, variety "Deglet Nour", which amounted to 5.06%. It is Also the higher than the value obtained by **(CHOUAT and AZZI, 2020),** which amounted to 4.60% from the aqueous extract of date pits of the "Deglet Nour" variety, and higher than the value obtained by **(BAFFI and DJEDID, 2020)** in the aqueous extracts, which amounted to 8.68% of the "Ghars" date pits and the rate of 8.80% for date pits of the "Deglet Nour" variety.

the percentage obtained is lower than the percentage recorded by **(Warsinah and al, 2022)** for the ethanolic extract of date pit seeds "phoenix dactylifera L" which amounted to 19.60%.

Differences in yield values from our results and other studies can be explained by the experimental conditions and methods applied as well as the solvents used for extraction, which is probably the main factor in this difference because the solubility of the active ingredients is not the same in all solvents.

This difference in production rates between our results and the results of previous studies can be attributed to the method of collection and drying and the duration of preservation of the samples, which may play a role in this difference, as plant compounds are affected by the external factors surrounding them, such as lighting, heat, and humidity. The geographical location, the nature of the climate prevailing in the growing environment, the presence of the plant, and the age stage of the plant at the time of the study can also have an impact on the results **. (Shrada and Al-Awadi, 2019)**.

1.3. PHYTOCHEMICAL STUDY

1.3.1. Qualitative phytochemical analyzes (Phytochemical screening)

The qualitative analysis of the *Phoenix dactylifera L* seed extract makes it possible to highlight the presence of certain types of secondary metabolite, through qualitative characterization reactions which are based on precipitation or coloring phenomena using specific reagents for each family of compounds or examination under UV light. The results of the phytochemical tests of the prepared extract are represented in the following table.

Table n (07): Different phytochemical groups highlighted in the extract.

Chemical groups	Testing	Ethanolic extract
Alkaloids	Mayer	-
	bouchardat	-
	Dragendorff	-
Anthocyanins		+
Coumarins		-
Flavonoids		+
Tannins		+

(-): Negative test.

(+): positive test.

The table above shows the results of the preliminary phytochemical screening test for the extract, where the phytochemical analysis showed the presence of flavinoids, tannins, and anthocyanins, and the absence of both alkaloids and coumarins.

These results were consistent with what was recorded by **(Dekdak and al,2021)** in terms of the presence of flavinoids and tannins and the complete absence of alkaloids in the ethanolic extract of the same variety "Ghars".

1.3.2. Quantitative phytochemical analysis

1.3.2.1. Estimation of total phenolics

The content of polyphenolic compounds obtained from Ghars date seed extract was estimated using a calibration curve, which was performed using the reference extract, which is gallic acid at different concentrations. A calibration curve **(Figure 25)** was generated with a correlation coefficient of $R^2 = 0.99$

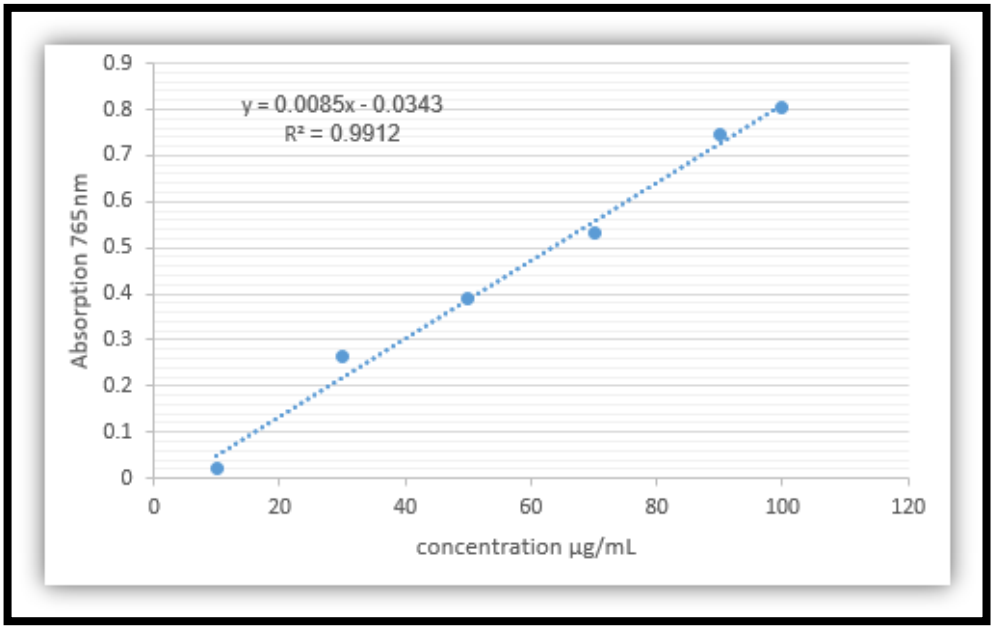

Figure n (25): Gallic acid calibration curve for the determination of total polyphenols.

1.3.2.2.Estimation of total flavonoids

The content of flavonoids obtained from Ghars date seed extract was estimated using a calibration curve, which was performed using the reference extract, quercetin acid, at different concentrations. A calibration curve **(Figure 26)** was generated with a correlation coefficient of $R^2 = 0.96$.

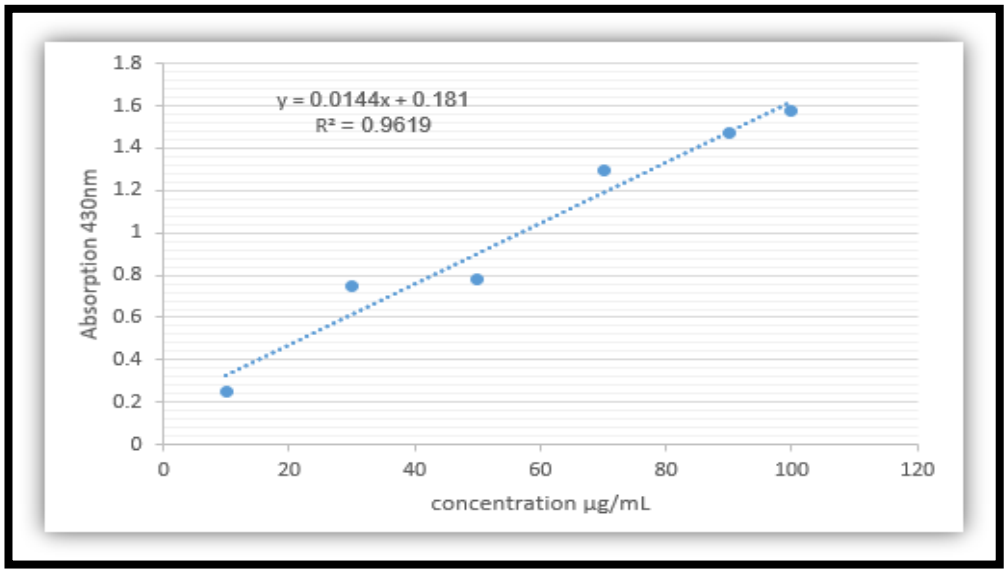

Figure n (26): Quercetin acid calibration curve for the determination of total flavonoids

1.3.2.3. Estimation of total hydrolysable tannins

The content of hydrolysable tannin compounds obtained from Ghars date seed extract was estimated using a calibration curve, which was performed using the reference extract, tannic acid at different concentrations. A calibration curve **(Figure27)** was generated with a correlation coefficient of $R^2 = 0.92$

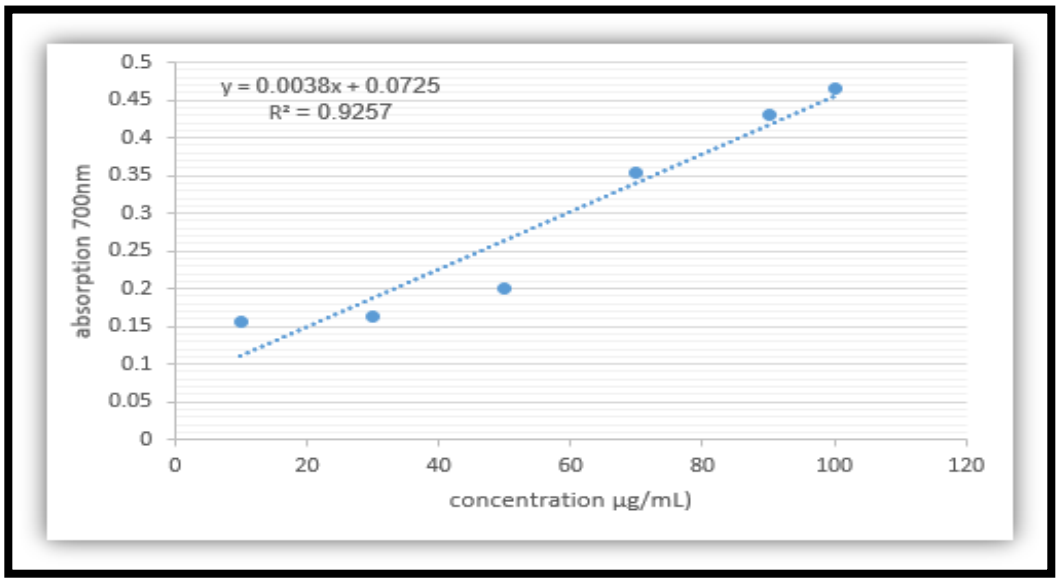

Figure n (27): Tannic acid calibration curve for the determination of total hydrolysable tannins

1.3.2.4. Estimation of condensed tannins

The content of Condensed tannins compounds obtained from Ghars date seed extract was estimated using a calibration curve, which was performed using the reference extract, Catechin acid at different concentrations. A calibration curve **(Figure28)** was generated with a correlation coefficient of $R^2 = 0.90$.

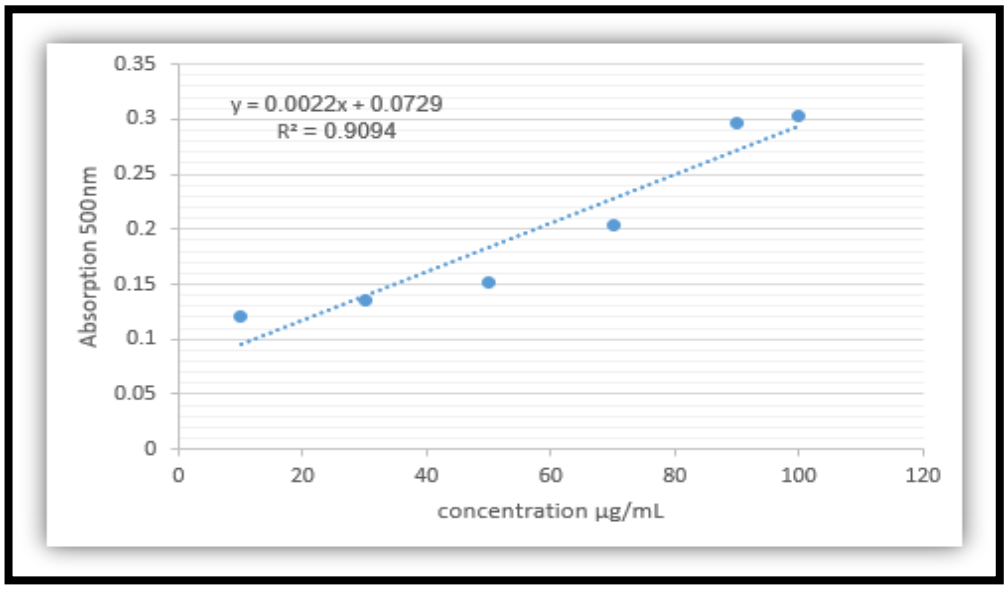

Figure n (28): Catechin acid calibration curve for the determination of total condenses tannins

The polyphenol content in ethanolic date pit extract is about 290.5 mg/ml. This value is higher than the values obtained by **(SAITI and al., 2020)**, which amounted to (118.3 mg/ml) from the aqueous extract of natural date pits and (130 mg/ml) from the aqueous extract of roasted dates. Date pits of the "Ghars" variety and more than the value obtained by **(Baraa,2020)** from date kernel extract from the "Degles Beida" variety. These results indicate that the ethanolic extract of date seeds is more effective in extracting polyphenols than the aqueous extract.

However, the results of the determination of phenolic compounds do not indicate exact values of polyphenol contents, because despite its high sensitivity, the Folin-Ciocalteu method can cause interference problems, in fact the Folin-Ciocalteu reagent can react with amino acids (tyrosine and tryptophan) and sugars. reductases such as glucose and fructose **(BEKHECHI and BENOSMAN, 2021).**

The flavonoid content in the ethanolic date pit extract is about 12.2 mg/ml. This value obtained is much lower than the value obtained by **(Baraa, 2020)** which was 201.12 mg/ml, and **(ABED, 2023)** its values are close to the value we obtained, as the flavonoid contents were 13.3 mg/ml of the extract in the MECH DEGLA extract, followed by those found in the DEGLET-NOUR extracts. and HMIRA with 15.7 and 20.8 mg/ml. This difference in contents can be explained by external factors (such as geographical and climatic factors), harvest period, as well as genetic factors and experimental conditions.

The hydrolysable tannin content in the ethanolic date pit extract is 68.81 mg/ml, and the condensed tannin content is 118.6 mg/ml.

Due to the absence of work regarding condensed and degradable tannin contents in the species studied in addition to planting, we compared our results with those of **(Bentrad and Gaceb-Terrak,2020)** From the Bint Qabbala variety, originating in Ghardaia, Algeria, with a content of 31.3 mg/ml, which was lower than the results we obtained.

The phytochemistry of dates is very rich in various compounds, such as polyphenols, flavonoids, tannins, and others. The concentration and percentage of these phytochemicals depend on the stage of date maturity, the type of dates, the condition of the soil at the growing site, the nature of the solvent, and dosing methods. The phenolic composition of plant extracts is affected by various factors such as variety, climate, storage, processing, etc.

1.4. Antioxidant Activity

1.4.1. Reducing power assay

Using this method, we measured the reducing power of iron in the kernel extract and in ascorbic acid as a synthetic antioxidant. The results obtained made it possible to draw the following curves:

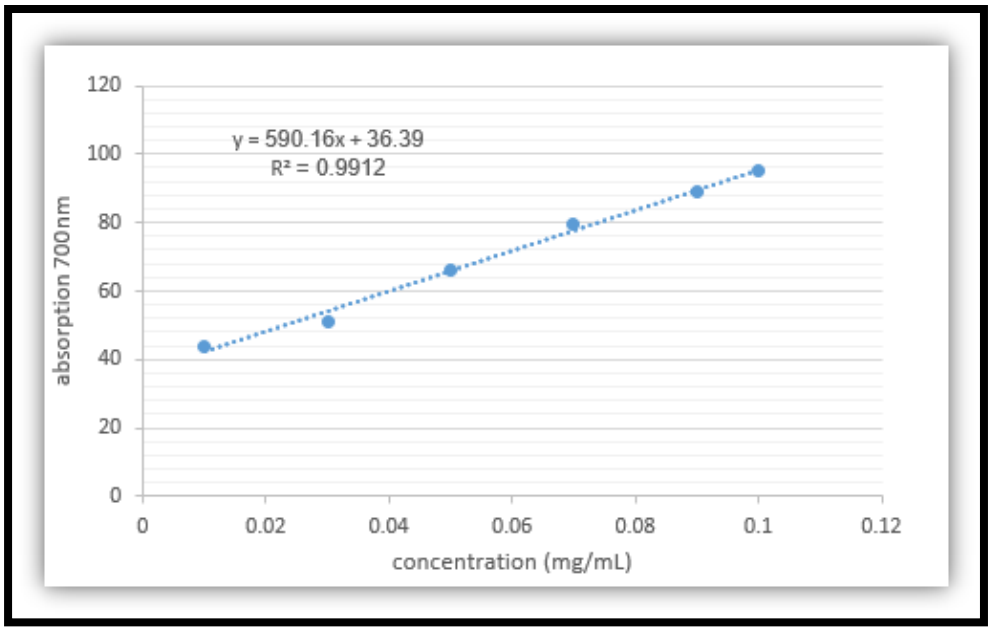

Figure n (29): Reducing power of iron as a function of extract concentrations

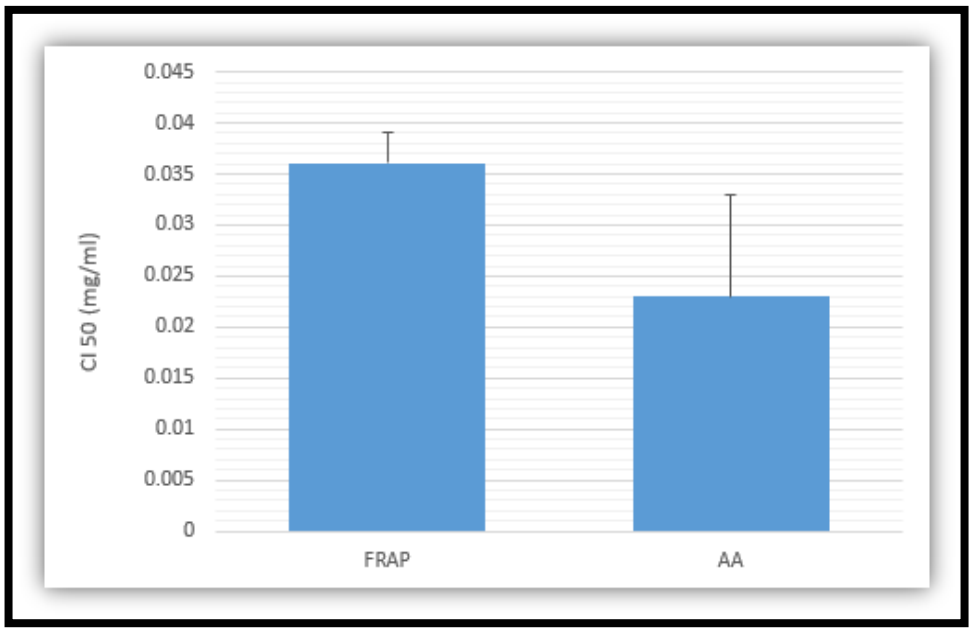

Figure n (30): IC50 for extract and ascorbic acid (FRAP)

The reduction potential refers to the ability of the extract to donate an electron and convert ferric iron to ferrous iron. When added to a potassium ferricyanide solution, the yellow color changes to a blue-green hue, with intensity reflecting the extract's reducing power.

Our study revealed significant reducing power for the examined extract, with notable IC50 values of (0.0361mg/ml), compared to (0.023mg/ml) for ascorbic acid. Consequently, this extract demonstrates substantial antioxidant activity.

(P=0.35>0.05) This is evidence that there is no difference between date pit extract and ascorbic acid

In contrast, the evaluation of "Ajwa" kernel extract's iron-reducing capacity by **(Belbachir and Belaid. 2021)** yielded an effective concentration with an IC50 value of (0.416mg/ml). Our findings significantly diverge from theirs, indicating a substantial difference. Our results strongly support the efficacy of date seed extracts as antioxid**a**nts. This variance likely stems from differences in extraction methods or solvents used.

1.4.2. DPPH free-radical scavenging activity

The antioxidant activity of our date stones (natural and roasted) of the Ghars variety was evaluated by DPPH test; In this test we use ascorbic acid as a standard, the results obtained (percentage of inhibitions I%) are represented in the calibration curve

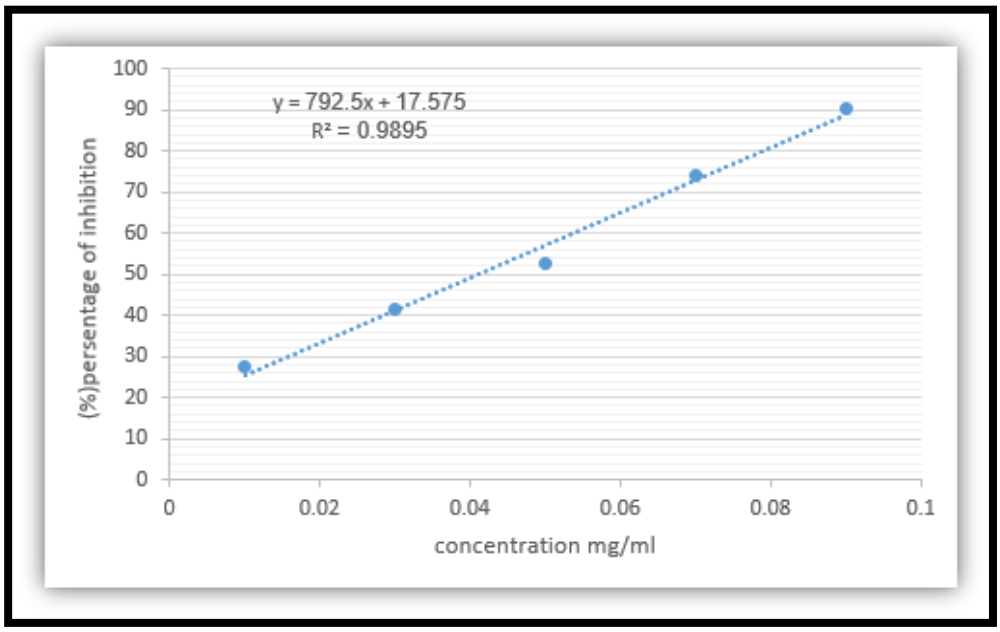

Figure n (31): Percentage of inhibition of the free radical DPPH depending on the different concentrations of date stone extracts

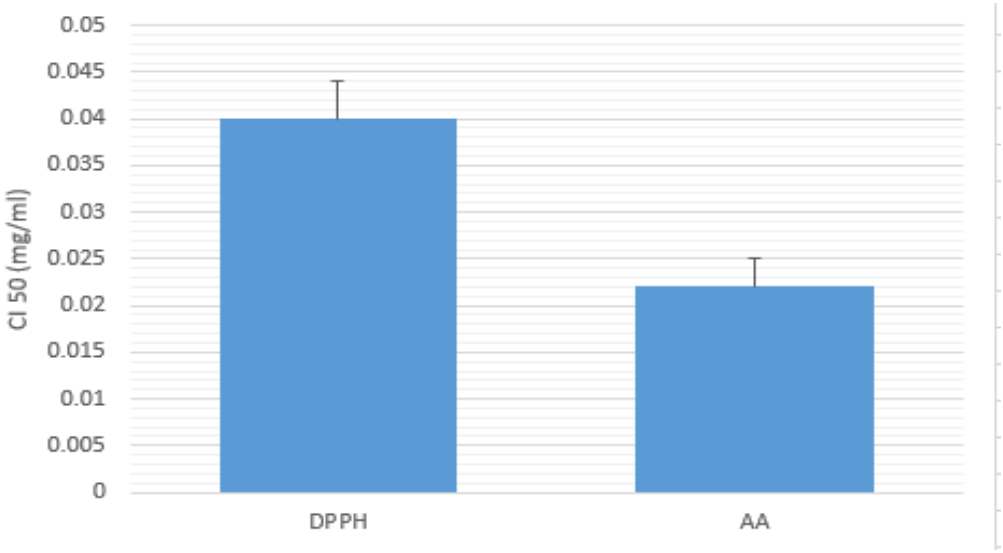

Figure n (32): IC50 for extract and ascorbic acid (DPPH)

In this study, the DPPH (1,1-Diphenyl-2-Picryl-Hydrazyl) method was utilized to assess the antioxidant activity of date kernels. This method enables a more accurate estimation of the concentration of inhibitory radicals by active molecules **(Saiti and Terbagou 2020)**.

At a concentration of (90 µg/mL), the synthetic antioxidant showed DPPH radical scavenging capacity of 94.1%, while the dry extract of Ajwa kernel powder showed 90.5% inhibition at the same concentration. The IC50 value of the extract 0.04mg/ml indicates a lower activity compared to the value of ascorbic acid (0.022mg/ml) , which indicates that the kernel powder possesses strong DPPH radical trapping capabilities relative to the activity of the stronger ascorbic acid, for which there is no significant difference between them.

(P=0.31>0.05) This is evidence that there is no difference between date pit extract and ascorbic acid.

Conversely, natural date kernels demonstrate a robust ability to combat free radicals. When compared to the results of **(Saiti and Terbagou. 2020)** regarding roasted date seeds, their IC50 value was recorded as (0.041mg/ml), suggesting a decrease in antioxidant activity due to the roasting process.

However, according to the findings of **(Belbachir and Belaid. 2021)**, the IC50 value of the Ajwa kernel extract was determined to be IC50 = 0.011. Consequently, the neutralization potential of the DPPH radical by the "Ajwa" kernel extract in this study surpassed that of the "Ajwa" kernel powder. This disparity could possibly be attributed to the solvents employed in the extraction process.

1.5. Anti-inflammatory activity

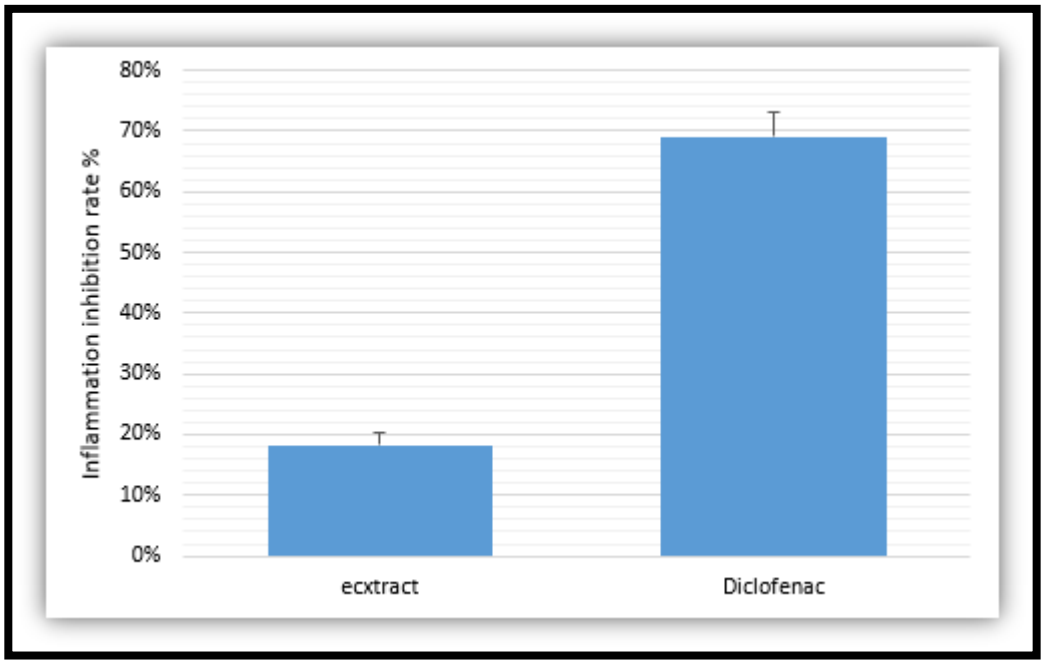

Figure n (33): Inflammation inhibition rate of the extract and diclofenac

As for the ethanolic extract of the "Ghars" date kernel, the inhibition rate reached 18.29%, while for diclofenac, it reached (69%). Diclofenac, an anti-inflammatory drug used as a standard, showed an inhibition rate at the same concentration, indicating that the date pit extract has an anti-inflammatory effect.

In a study conducted by (Begum et al. 2024) on type I Ajwa date kernels, after 3 hours at a dose of (300 mg/kg), the methanol fraction showed a significantly high inhibition rate of (71%) against carrageenan-induced edema, followed by chloroform (64.6%) and hexane (62.5%). The group treated with diclofenac sodium showed an inhibition rate of (79.4%). The inhibition ratio of the standard drug is similar to that of methanol, hexane, and chloroform.

In the case of type II Ajwa date kernel (Begum et al., 2024), the methanol fraction showed a significant inhibition of 71% against carrageenan-induced edema after 3 h at a dose of 300 mg/kg, followed by n-hexane (59.2%) and chloroform (35%). The rate of inhibition in the group treated with diclofenac sodium was determined to be 78.7%. The inhibition percentage of the standard drug is similar to the inhibition percentage of the fractions (methanol, n-hexane, and chloroform).

Excessive generation of free radicals by inflammatory leukocytes is extremely harmful, especially in cases of chronic inflammation, leading to exacerbation of conditions such as arthritis and diabetes. Therefore, controlling it is crucial to improving health and vitality. Date fruit pulp extracts showed anti-inflammatory activities in a rat adjuvant arthritis model, increasing blood antioxidant levels and promoting body weight gain and Fe, suggesting their beneficial effects. Several studies have indicated that the pulp extract is safe and may possess anti-inflammatory properties contributing to its therapeutic benefits **(Begum et al., 2024)**.

In a study conducted by **(Bouguedba and Dekik 2020),** the rate of inhibition of denaturation of the Prunus ameniaca L. kernel extract was significant, reaching (74.15%). This is similar to diclofenac, an anti-inflammatory drug used as a standard, which shows a similar rate of inhibition at the same concentration. Protein denaturation is among the causes of inflammation **(Barros et al., 2008)**.

1.6. HPLC analysis of phenolic compounds

The different extracts of seed *Phoenix dactylifera L* variety "Ghars" were analyzed in order to compare their chromatographic profiles and to obtain information on the composition of these extracts in polyphenols and their comparisons with the different standards of the standards used

(Ascorbic acid, Pyrocatechol, Pyrogallol, Salicin, Esculin, Chlorogenic acid, Catechin, Caffeine, Vanillic acid, Rutin, Vanillin, Caffeic acid, Salicylic acid, Quercetin, Cinnamic acid).

The quantity of these compounds was reported in microgram per milligram of the equivalent plant material in standard solution.

The chromatographic curves of the studied extracts listed in **(Figure 34, 35)** showed that they contain some known polyphenolic compounds and flavinoids.

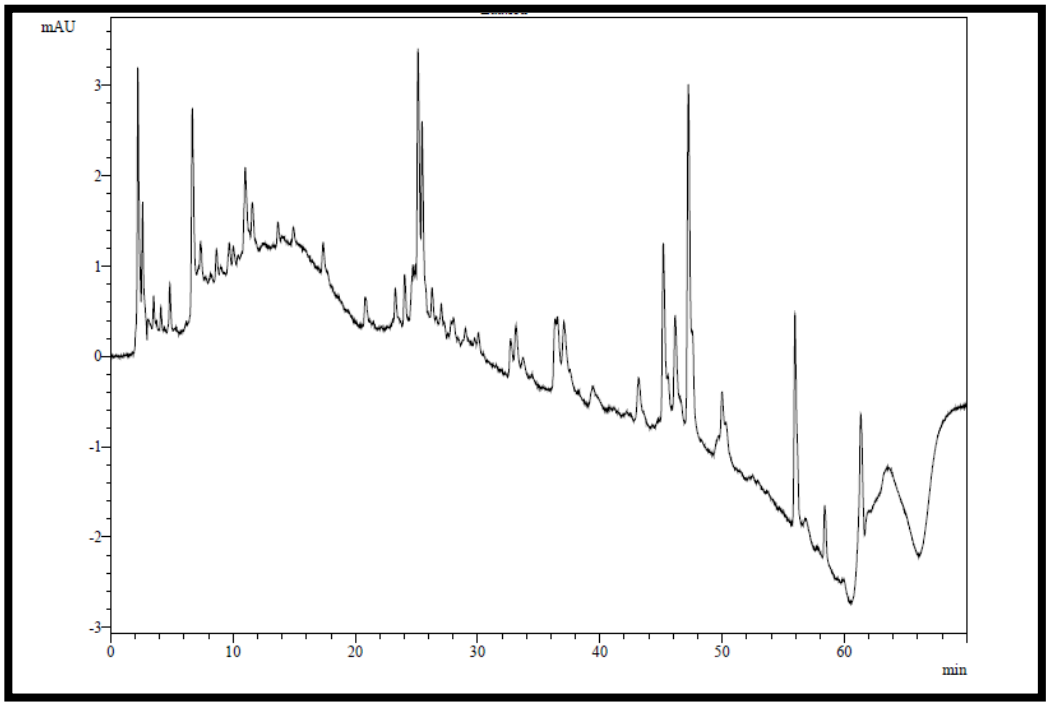

Figure n (34): HPLC chromatographic profile of the aqueous extract of "Ghars" date seeds.**EA**

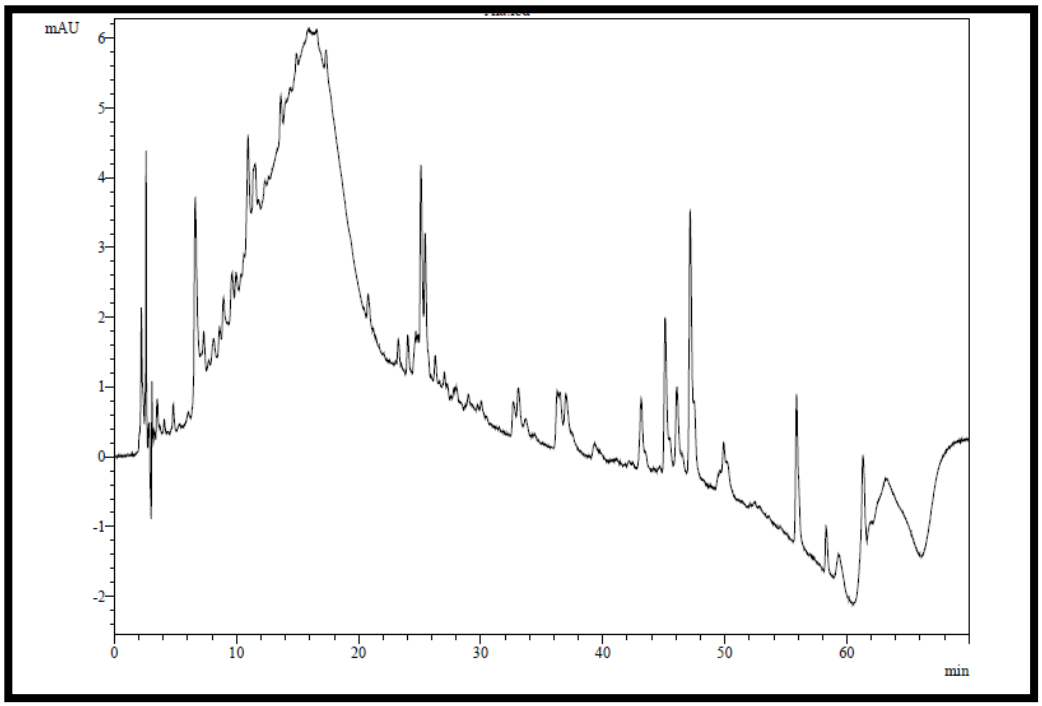

Figure n (35): HPLC chromatographic profile of the hydraulic extract of "Ghars" date seeds. **EAL**

Through the results obtained based on the chromatographic analysis of extracts from date seeds of the "Ghars" variety, and based on the tables (see the appendix), we notice a variation in the number of phenolic compounds for each extract, as the highest number was recorded in the hydroalcoholic extract with (65 compounds), followed by the aqueous extract with (49 compounds).

It was also noted that there was a difference in the number of occurrences of known standard compounds from one extract to another, as each appeared: Ascorbic acid, Pyrocatechol, Esculin, Caffeine, Vanillic acid and Caffeic acid in both extracts.

While the presence of some phenolic compounds was limited to one extract but not the other, as catechin was present in the hydroalcoholic extract of date seeds and its absence was recorded in the aqueous extract. While Chlorogenic acid, Cinnamic acid and Rutin were present only in the aqueous extract.

Table n (08): Concentration of known phenolic compounds in the various studied extracts

Compounds	Concentration (µg/ mg)	
	EA	EAL
Ascorbic acid	0,0035	0,0031
Pyrocatechol	0,0016	0,0043

Esculin	**0,0029**	**0,0033**
Caffeine	**0,0014**	**0,0027**
Vanillic acid	**0,0014**	**0,0021**
Caffeic acid	**0,0306**	**0,0039**
Chlorogenic acid	**0,0019**	**–**
Catechin	**–**	**0,0272**
Rutin	**0,0009**	**–**
Cinnamic acid	**0,0004**	**–**

The data in the table above revealed significant differences in concentration levels of known compounds. Catechin had the highest concentration of (0.0272 µg/mg) in the hydroalcoholic extract containing the largest number of compounds. In contrast, no catechin was detected in the aqueous extract.

The aqueous extract had the highest caffeic acid concentration of (0.0306 µg/mg), while the hydroalcoholic extract had the lowest concentration of (0.0039 µg/mg)

The results also showed that chlorogenic acid is only present in the aqueous extract at a concentration of (0.0019 µg/mg). In addition, rutin and cinnamic acid compounds were found exclusively in the aqueous extract, but at very low concentrations of (0.0009 µg/mg) and (0.0004 µg/mg), respectively.

Returning to the common compounds between the two extracts, we notice a variation in the concentrations of the compounds pyrocatechol, esculin, caffeine and vanillic acid. The highest concentrations were recorded in the hydroalcoholic extract and were estimated at (0.0043µg/mg), (0.0033µg/mg), (0.0027µg/mg), (0.0021µg/mg) respectively. In the aqueous extract, their concentrations were low, estimated at (0.0016 µg/mg), (0.0029 µg/mg), (0.0014 µg/mg) and (0.0014 µg/mg), respectively.

The results also showed that the ascorbic acid that appeared in both extracts was characterized by similar concentrations, as it was estimated in the aqueous extract at (0.0035 µg/mg), while in the hydroalcoholic extract it was estimated at (0.0031 µg/mg).

Note that during our analyzes not all compounds were identified due to lack of adequate standards.

Several studies have focused on the identification of phenolic compounds in date stone extracts by HPLC analysis. Indeed, the study by**(Hmidani and al. 2020)** showed that the maximum contents of caffeic acid, chlorogenic acid, ferulic acid,gallic acid, p-coumaric acid, syringic acid, vanillic acid, luteolin, quercetin and rutin from the methanolic extracts of four varieties of date stones grown in Morocco, are 88.64, 10.34, 12.16, 17.62, 143.6, 1.43, 10.81, 12.68, 36.39 and 80.26 mg/100 g DM respectively.

(Bijami and al. 2020) quantified the phenolic compounds in the methanolic extract of date stones of the Mozafati variety at different stages of maturity, the quantities of chlorogenic acid, gallic acid, p-coumaric acid, sinapic acid, catechin and vanillin recorded are 0.53, 11.02, 0.68 to 11.72, 26.84, 14.43 to 32.56 and 2.95 to 8.3 mg/100 g DM respectively.

Analysis of phenolic compounds from Saudi Arabian date pit variety by HPLC revealed the presence of benzoic acid, caffeic acid, cinnamic acid, ferulic acid, p-coumaric acid, protocatechuic acid, salicylic acid, syringic acid , coumarin, kaempferol, resorcinol and vanillin whose contents are 0.128, 0.512, 3.513, 7.676, 1.004, 6.672, 7.835, 20.478, 0.663, 14.878, 0.919 and 0.068 mg/100 g respectively **(El-Rahman and Al -Mulhem. , 2017).**

(El-Mergawi and al, 2016) noted that chromatographic analyzes of date stones from ten varieties from Saudi Arabia revealed the dominance of phenolic acids such as protocatechuic acid, p-hydroxybenzoic acid, caffeic acid and coumaric acid with contents up to 395.1, 171.2, 112 and 478.4 mg/100g DM compared to 18.73, 34.73, 5.11, 9.89 and 2.23 mg/100g DM for luteolin, quercetin, naringinin, kaempferol and isorhamnetin respectively.

Moreover, **(Messaoudi and al, 2013)** studied phenolic acids in pits of seven Algerian date fruit varieties and found that catechin or epi catechin derivatives, sinapic, cinnamic, and coumaric acid derivatives are the main compounds of all varieties.

These differences between date seed varieties in terms of seed phenolic profiles are due to many factors such as genetic diversity among varieties, growing conditions, water availability, diseases, maturity, harvest dates, storage conditions, geographical origin, light, and temperature. Fertilizers, soil type, as well as the extraction system and analysis method **(Bouhlali and al., 2018).** Our findings indicate that date seeds are an important source of phenols and flavonoids known as natural antioxidant compounds that can be used in nutritional formulations.

Conclusion

Conclusion

The work we have done is dedicated to studying the effect of *phoenix dactylifera L* on inflammation and oxidative stress. The aim of this work is to study whether treatment with the ethanolic extract of the roots of *phoenix dactylifera L* has an effect on inflammatory disease and its complications. In light of the results obtained, we can conclude the following:

- The plant "*Phœnix dactylifera.L*" contains a diverse richness in primary and secondary metabolites, including carbohydrates, lipids, proteins, polyphenols, flavonoids, condensed tannins and hydrolyzable tannins, so our plant has anti-radical activity, which can It represents a new potential source of bioactive molecules that have an important therapeutic effect against many diseases. .
- And the full antioxidant capacity, and we test it in comparison with ascorbic acid. We have achieved it through the group that our local data "planting" provides better ability to resist DPPH oxidation (0.04 mg/ml), and provides significant antioxidant activity of FRAP with IC50 of (0.036 mg/ml).
- A test was conducted to determine the rate of inhibition of inflammation and compared it with the drug diclofenac. A rate of 18.29% was obtained for the ethanolic extract and 69% for the drug diclofenac. This indicates that the ethanolic extract contains anti-inflammatory activity.

We can conclude that the plant *Phoenix Dactylifera L*. has great therapeutic benefit against inflammation and its complications.

Perspectives

Given the importance of these results, this work requires further in-depth studies to understand the molecules responsible for the pharmacological effect and the mechanisms and mode of their action on different biological systems during anti-inflammatory and antioxidant action.

The use of medicinal plants may lead to the depletion of species, and stresses the necessity of cultivating and propagating them to preserve them. Medicinal plants have a role in finding treatments for diseases, and one must be careful of the risk of losing biodiversity and exploiting medicinal plants irresponsibly, and the necessity of protecting these.

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Annexes

ANNEXE

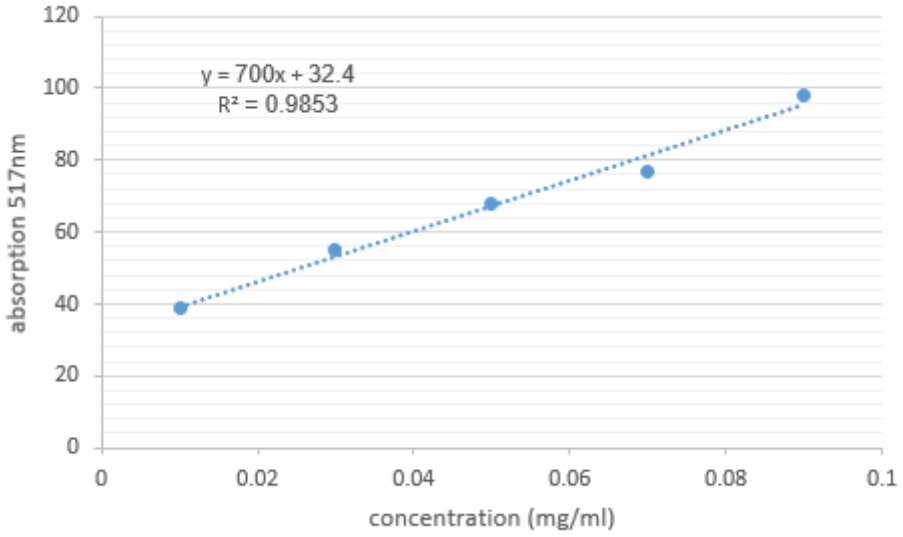


Figure (01): Reduction of iron strength as a function of ascorbic acid concentrations

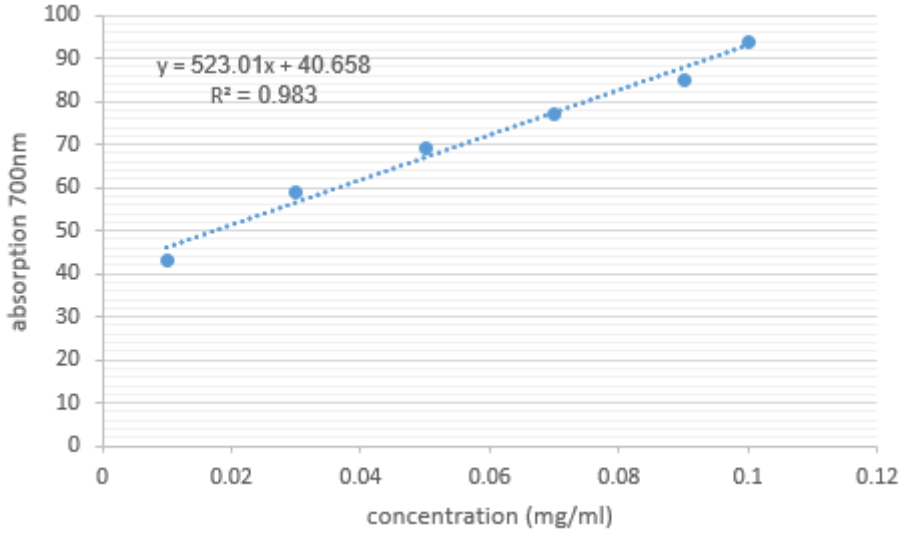
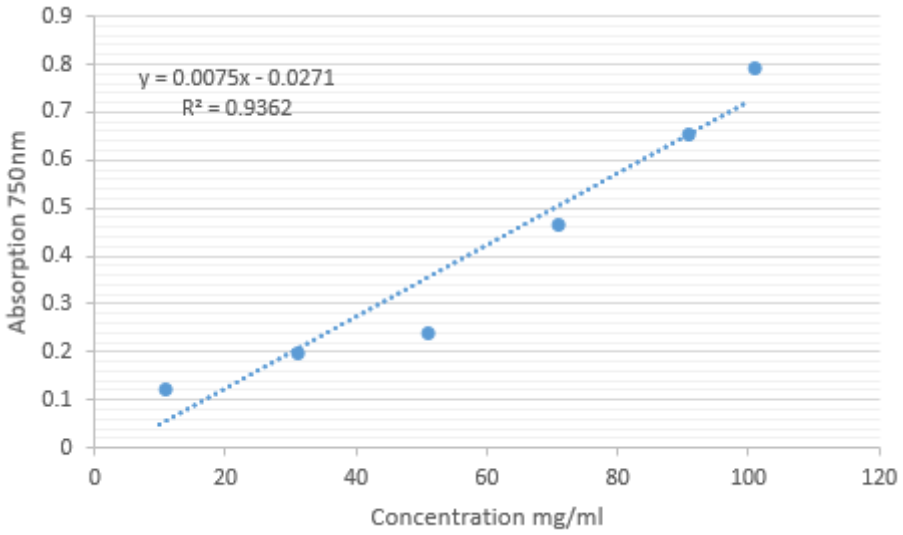
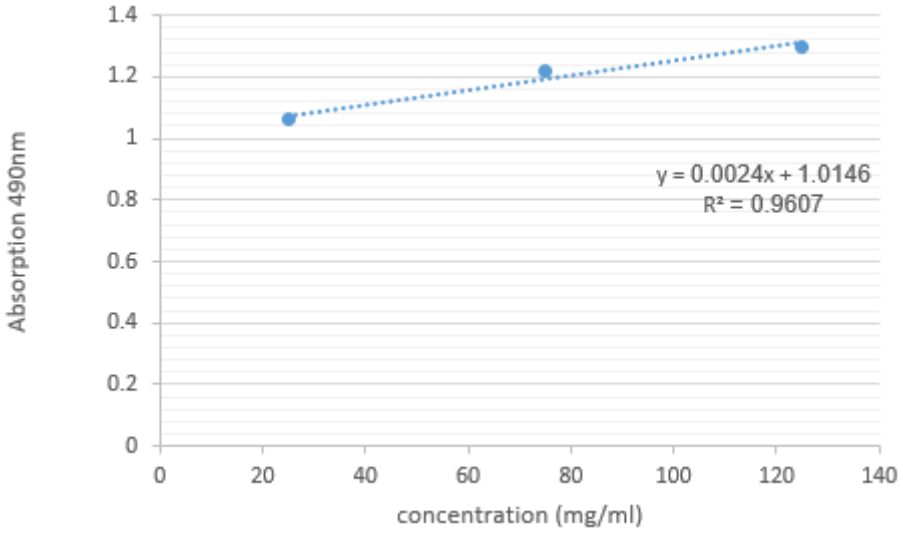


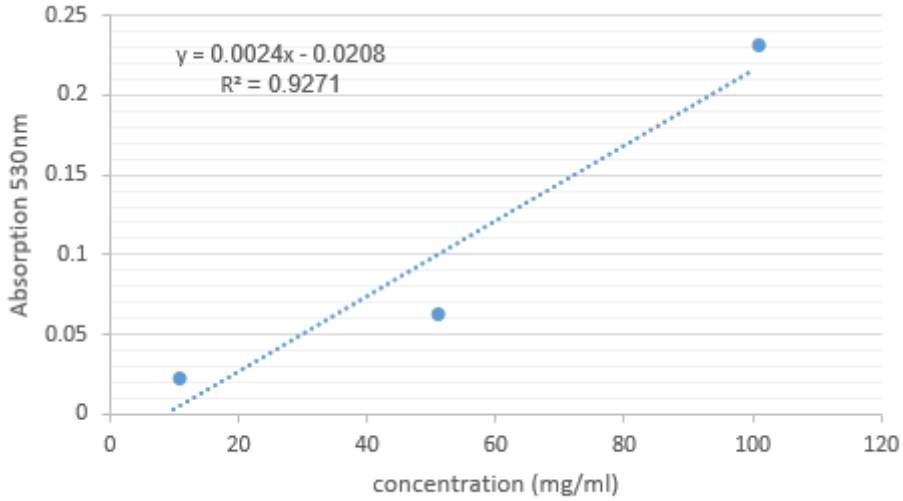
Figure
(02): Percentage of inhibition of DPPH free radicals depending on different concentrations of ascorbic acid



Figure(04) : Protein standard curve.



Figure(05) : Glucose standard curve.



Figure(06) : Fat standard curve.

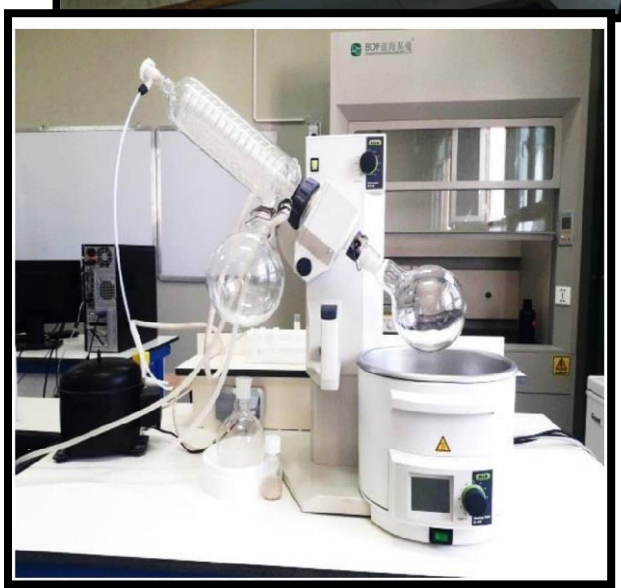


Figure : The lyophilisation

ANNEXE

Figure : Rotary evaporator

Figure : High performance liquid chromatography device HPLC

Table : Different standards of phenolic compounds

ANNEXE

PDA Ch1 254nm

Peak#	Name	Ret. Time	Area	Height	Area%
1	Acide ascorbique	2.469	10009958	1715563	21.885
2	Pyrocatéchol	3.790	1776794	199065	3.885
3	Pyrogallol	5.406	620584	48758	1.357
4	Salicine	6.764	98148	5321	0.215
5	Esculine	7.302	1197043	81136	2.617
6	Acide chlorogénique	8.810	1745586	143373	3.816
7	Catéchine	9.134	243648	18966	0.533
8	Caféine	9.679	2936592	248416	6.420
9	Acide vanillique	11.239	10955414	643954	23.953
10	Rutine	13.471	2493551	261394	5.452
11	Vanilline	14.592	2539286	157906	5.552
12	Acide caféique	17.314	178485	12121	0.390
13	Acide salicylique	19.581	285981	15252	0.625
14	Quercétine	21.425	2305955	156193	5.042
15	Acide cinnamique	23.545	8350959	508561	18.258
Total			45737984	4215978	100.000

Table: Some phenolic compounds of the hydroalcoholic extract

PDA Ch1 254nm

Peak#	Ret. Time	Area	Height	Area%
1	2.209	31610	2328	4.234
2	2.591	28737	4914	3.849
3	2.834	12659	1218	1.696
4	3.075	12614	1903	1.690
5	3.261	10642	1030	1.426
6	3.504	15287	1223	2.048
7	3.723	7657	637	1.026
8	4.084	6183	366	0.828
9	4.822	3250	367	0.435
10	6.050	1118	102	0.150
11	6.621	39415	2737	5.280
12	7.317	4030	418	0.540
13	8.121	5037	329	0.675
14	8.619	3208	312	0.430
15	8.937	6630	536	0.888
16	9.642	8190	581	1.097
17	9.970	4473	386	0.599
18	10.592	2836	256	0.380
19	10.940	27391	1759	3.669
20	11.523	23253	980	3.115
21	11.806	3386	297	0.454
22	12.309	4126	253	0.553
23	12.619	1191	122	0.159
24	13.625	6814	603	0.913
25	14.027	4420	235	0.592
26	14.389	2567	176	0.344
27	14.914	3607	282	0.483
28	17.340	9102	424	1.219

Peak#	Ret. Time	Area	Height	Area%
29	20.781	5018	389	0.672
30	23.247	4319	405	0.579
31	24.025	6558	560	0.878
32	24.687	9125	636	1.222
33	24.879	5907	597	0.791
34	25.107	38395	3030	5.143
35	25.448	27487	2057	3.682
36	26.275	3879	377	0.520
37	27.039	1736	207	0.233
38	27.815	1431	161	0.192
39	27.994	2282	200	0.306
40	29.009	1632	165	0.219
41	29.696	1058	77	0.142
42	30.046	2442	194	0.327
43	32.672	8306	496	1.113
44	33.091	13494	688	1.808
45	33.698	5121	251	0.686
46	36.275	10668	770	1.429
47	36.496	11162	718	1.495
48	36.973	12037	614	1.612
49	43.149	16930	1001	2.268
50	43.541	2548	206	0.341
51	45.114	33844	2223	4.533
52	45.515	7346	495	0.984
53	46.064	23911	1249	3.203
54	46.592	4096	249	0.549
55	47.159	58522	3795	7.839
56	47.488	13729	1101	1.839
57	49.579	6355	285	0.851
58	49.901	11346	722	1.520
59	50.240	9336	445	1.251
60	55.873	33398	2140	4.474
61	58.291	8263	658	1.107
62	59.308	12103	391	1.621
63	61.307	33813	1658	4.529
64	61.899	6999	251	0.938
65	62.763	2533	33	0.339
Total		746562	54264	100.000

R % = (PE /PA)*10 0

ANNEXE

Table: Some phenolic compounds of the aqueous extract

PDA Ch1 254nm

Peak#	Ret. Time	Area	Height	Area%
1	2.218	35340	3118	6.377
2	2.608	19233	1578	3.471
3	3.509	2929	366	0.529
4	4.101	1877	257	0.339
5	4.834	5414	536	0.977
6	6.673	25363	2119	4.577
7	7.350	3533	353	0.638
8	8.648	3361	328	0.607
9	9.686	4167	319	0.752
10	10.020	2759	236	0.498
11	11.003	15424	946	2.783
12	11.349	1676	229	0.302
13	11.574	7488	542	1.351
14	13.669	2378	245	0.429
15	14.925	1960	172	0.354
16	17.374	5479	364	0.989
17	20.803	4364	306	0.787
18	23.269	3644	363	0.658
19	24.039	6398	559	1.155
20	24.706	8836	618	1.595
21	24.904	5845	602	1.055
22	25.125	38812	2982	7.004
23	25.462	28261	2150	5.100
24	26.275	3438	335	0.620
25	27.040	1683	199	0.304
24	26.275	3438	335	0.620
25	27.040	1683	199	0.304
26	28.034	4620	228	0.834
27	29.015	1236	128	0.223
28	29.728	1163	86	0.210
29	30.069	2448	205	0.442
30	32.706	6458	420	1.165
31	33.133	11033	572	1.991
32	33.699	4316	208	0.779
33	36.358	10788	781	1.947
34	36.537	13134	811	2.370
35	37.066	18852	750	3.402
36	37.643	2992	176	0.540
37	43.172	7470	432	1.348
38	45.192	30261	1972	5.461
39	45.568	7575	510	1.367
40	46.162	22875	1183	4.128
41	46.635	3163	247	0.571
42	47.234	58632	3793	10.581
43	47.563	13120	1119	2.368
44	49.653	3903	188	0.704
45	49.986	11640	701	2.101
46	50.336	5244	354	0.946
47	55.957	35025	2332	6.321
48	58.387	7647	613	1.380
49	61.335	30891	1576	5.575
Total		554146	39206	100.000