الجمهورية الجزائرية الديمقراطية الشعبية
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قسم البيولوجيا الخلوية والجزيئية
DEPARTMENT OF CELLULAR AND MOLECULAR BIOLOGY

Serial

# Master's Thesis

In order to obtain a diploma of an Academic Master In biological sciences

Specialty: Applied Biochemistry

## Theme

**Preparation and characterization of some polyherbal formulations by using plants and evaluation of their biological activities in Oued souf / Righ**

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University year: 2022/ 2023

# Dedication

قال تعالى: (قل اعملوا فسيرى الله عملكم و رسوله والمؤمنون)

الاهي لا يطيب الليل إلا بشكرك ولا يطيب النهار إلا بطاعتك .

ولا تطيب اللحظات إلا بذكرك .. ولا تطيب الآخرة إلا بعفوك ..

ولا تطيب الجنة إلا برؤيتك الله جل جلاله

إلى من بلغ الرسالة وأدى الأمانة .. ونصح الأمة ..

إلى نبي الرحمة ونور العالمين سيدنا محمد صلى الله عليه وسلم

إلى العظيم أبي.. نور قلبي ...مشعل دربي ...عكازة ظهري

الى الذي بجوار ربي. أرجو من الله يرحمك ويتقبلك من الشهداء وستبقى كلماتك نجوم أهتدي بها اليوم وفي الغد وإلى الأبد والدي العزيز رحمه الله

إلى من كان دعائها سر نجاحي وحنانها بلسم جراحي إلى أغلى الحبايب أمي الحبيبة

إلى أفراد عائلتي الاولى و الثانية من كانوا سندا لي ودعما رغم إخفاقاتي و انكساراتي.. إلى من أحسنوا الظن بي ورأوا في الخير بأعينهم وقلوبهم. إلى اخوتي اخواتي الكرام اللذين كانوا السند والعضد

إلى الذين حملوا أقدس رسالة في الحياة إلى الذين مهدوا لنا طريق العلم والمعرفة .. أساتذتنا الأفاضل وعلى راسهم الاستاذ المشرف درويش سمير

إلى كل هؤلاء اهديهم هذا العمل المتواضع، راجية من المولى عز وجل ان يجعله صدقة جارية على روح الوالد الكريم

مديحة انفال

وآخر دعواهم ان الحمد الله رب العالمين

عظم المراد فهان الطريق

فجاءت لذة الوصول....لتمحي مشقة السنين

اهدي بحثي هذا

إلى من كلله الله بالهيبة والوقار ...إلى من احمل اسمه بكل افتخار....ابي العزيز الحاج أطال الله عمره

إلى معنى الحب والتفاني...إلى بسمة الحياة وسر وجودي...إلى من كان دعائها سر نجاحي...أمي صافية أطال الله عمرها

إلى رفاق الدعم في خطوتي الأولى...والخطوة الاخيرة...إلى من كانوا في السنوات العجاف سحابا ممطرا....إلى اخواتي أماني ، عائشة ، هاجرة ، توحة

إلى سندي في الحياة اخوتي الدكتور عبد الله ، أسامة ، محمد اليزيد

إلى رفيقة دربي حفظها الله ووفقها...وئام

إلى من أخذ بيدي نحو آفاق العلم والمعرفة...إلى من كان له الفضل بعد الله تعالى في إنجاز هذه الدراسة البروفيسور درويش سمير

إلى كل هؤلاء اهديهم هذا العمل المتواضع، سائلة الله العلي القدير أن ينفعنا به ويمدنا بالتوفيق

خديجة

نحمد الله عز وجل الذي وفقنا في اتمام هذا البحث العلمي والذي الهمنه الصحة و العافية و العزيمة، فالحمد لله حمدا كثيرا

... شكرا إلى من علمني النجاح والصبر إلى من افتقده في مواجهة الصعاب ولم تمهله الدنيا لأرتوي من حنانه.. أبي رحمة الله عليه

. وإلى من وهبتني الحياة ،ومن علمتني وعانت الصعاب لأصل إلى ما أنا فيه وعندما تكسوني الهموم أسبح في بحر حنانها ليخفف من آلامي .. أمي الأولى والثانية

إلى إخوتي من كان لهم بالغ الأثر في تجاوز كثير من العقبات والصعاب. إلى أستاذي الذي لم يبخل في مد يد العون لي وإشراف على بحثي . وجميع صديقاتي

أهدي إليكم بحثي.....

فاطمة الزهراء

الحمد لله الذي بنعمته تتم الصالحات

عظم المراد فهان الطريق فجاءة لذة الوصول لتهون مشقة الطريق .فالحمد لله الذي ما ختم جهدا وما تم سعيا إلا بفضله .

إلى أبي إبراهيم الذي وهبني عمره وقلبه أطال الله عمره .

إلى أمي زهرة مقنش قرة عيني وحبيبتي وطريقي إلى الجنة .

إلى أخي الغالي أكرم أدامك الله سندا لنا .

إلى أنيستي و حبيبتي وأبنتي ندى الريحان أختي الغالية .

إلى جدتي رحمها الله التي لم تيأس وظلت ترشدني وتنصحني. إلى عائلتي الكبيرة وكل من ذكرهم قلبي ونسيهم قلمي .

إلى جميع أساتذتي الكرام ممن لم يتوانوا في مد يد العون لي .و بالأخص أستاذي المشرف سمير درويش الذي سهر وكان بجانبنا في رحلة بحثنا و قام بتوجيهنا طيلة هذه الدراسة.

إلى صديقاتي ورفيقاتي دربي مديحة ،فاطمة ،خديجة وفقكم الله . وأخيراً ،أتقدم بجزيل شكري إلي كل من مدوا لي يد العون والمساعدة في إخراج هذه الدراسة على أكمل وجه.

**ربيعة**

# Acknowledge

At the end of writing this document, we are convinced
Memory is far away.
To be a unilateral act, in fact, we could not have achieved
This work is without
Support, generosity and coup d 'état of a large number of people
She gave the hand
Breath of life in doing this humble job.
Our respect is specifically directed at our Supervisor: Professor.
Derouiche Samir
The trust he placed in us in agreeing to lead this humble
For His Work
And all the hours he devoted to leading this
Work. We
highly sensitive to his human qualities in listening and
Understanding all over
Our course of study.
Our sincere thanks and deep gratitude to all
For my teachers
SNV College of the University Martyred Humma Green Valley without
Forget those who formed us
Throughout our course.

# Abstract

Polyherb is a selective mixture consisting of two or more herbs in order to treat a specific disease or many diseases. The aim of this work was to do a statistical study on plants used in several treatments such as cancer in the El Oued region and preparation of some polyherbal formulations by using some local plants and evaluation of their biological activities. For statistical study, 61 persons interrogated were chosen, interviews were carried according to a profile of the interviewed people, cancer- related characteristics and plants and their uses. For in-vitro study, new three polyherbal (F1, F2 and F3) formulated from the most popular plant in the El-Oued area. In –In-vitro analysis, Qualitative and quantitative phytochemical compounds for plants and herbal formulations were assessed by using standard protocol. Antioxidant activity was determined by DPPH and FRAP assays, Anti- inflammatory activity were assayed by hemolysis and protein denaturation tests and antidiabetic activity were tested on different herbal formulations. Results of statistical study show that *Ephedra alata,*, *Curcuma longa L.*, *Nigella Sativa L.*, *Portulaca oleracea L.* and *Atriplex halimus L.* . which are the most popular plant used in cancer treatment in the El-Oued region. Results of the phenols and flavonoids contents assay, plants and formulations were richness of the phytochemical components, as well as significant quantities of phenols, with preference for F2 by more than 102.29 mg/g. Also, through the results of study, we obtained that the formulations have an important antioxidant activities with a slight preference for formulation F1 (IC50=24.14mg/ml for FRAP test). In the other hand, As well as with regard to anti- inflammatory activity, our results confirm that the herbal formulations have significant activity, with F3 has maximum hemolysis activity (IC50=0.108 mg/ml) and the F2 has the maximum activity of Protein denaturation (IC50=0.059mg/ml). As for anti-diabetic activity, the results confirm that the herbal formulations have anti-diabetic activity, with preference for formulation F3 which was the best one. In conclusion, through this study, we conclude that herbal formulations produced from plants has a greater biological effectiveness than plants with great activity from F2 and F3, which encourages its use in diseases related to cancer, infections, and diabetes.

Keywords: plants anticancer, phenols, flavonoids, polyherbal formulations, antioxidant, anti-inflammatory

**ملخص**

التركيبة متعددة الأعشاب هي خليط يتكون من اثنين أو أكثر من الأعشاب يستعمل من أجل علاج امراض معينة. الهدف من هذا العمل هو إجراء دراسة إحصائية عن النباتات المستخدمة في علاج العديد من الأمراض كالسرطان في منطقة واد ريغ وواد سوف وايضا إعداد بعض التركيبات متعددة الأعشاب باستخدام بعض النباتات المحلية وتقييم أنشطتها البيولوجية. بالنسبة للدراسة الإحصائية، تم اختيار 61 شخصًا تمت مقابلتهم، وتمحورت الاسئلة حول تعريف الأشخاص المستجوبين من جهة وحول النباتات المتعلقة بالسرطان وخصائصها وطرق استخدامها. بالنسبة للدراسة في المختبر، تم تحضير ثلاثة تركيبات من المشروب المتعدد الاعشاب (ت1و ت2 وت3) وهذا انطلاقا من النباتات الأكثر شعبية واستخداما في المنطقة المدروسة. تم تقييم المركبات الكيميائية للنباتات من الناحية النوعية والكمية باستخدام الطرق المخبرية المعروفة وايضا تم تحديد النشاط المضاد للأكسدة من خلال اختبارات DPPH وFRAP كما تم تقييم النشاط المضاد للالتهابات عن طريق اختبار انحلال الدم واختبارات إزالة التشبع بالبروتين وايضا اختبار النشاط المضاد للسكري على مختلف التركيبات العشبية المحضرة. .النتائج المتحصل عليها من خلال الدراسة الإحصائية بينت أن نباتات الرجلة، الكركم ،حبة البركة، القطف والعلندة هي النباتات المستخدمة في علاج السرطان و الأكثر شيوعًا في منطقة وادي سوف وواد ريغ. من جهة اخرى نتائج فحص محتويات الفينول والفلافونويد تظهر ان النباتات والتركيبات الجديدة المتعددة الاعشاب غنية بالمكونات الكيميائية النباتية، بالإضافة إلى ان التركيبة 2 للمشروب هي الاكثر غنى من الفينول بقيمة اكثر من 102 مغ/غ. أيضًا، من خلال نتائج الدراسة المخبرية، وجدنا أن التركيبات العشبية الجديدة لها أنشطة مهمة كمضادات للأكسدة مع افضلية طفيفة للتركيبة ت1 بتركيز مثبط 24.14مغ/غ. من خلال اختبار(FRAP). من ناحية أخرى، وكذلك فيما يتعلق بالنشاط المضاد للالتهابات، تؤكد نتائجنا أن التركيبات العشبية لها نشاط كبير، حيث أن ت3 لها نشاط تحلل دموي كبير بقيمة تركيز تثبيطي ( 0.108 مليغرام/مل) و التركيبة ت2 لها أقصى نشاط من خلال اختبار تحلل البروتين بقيمة تركيز تثبيطي0.059 مليغرام/مل). أما بالنسبة للنشاط المضاد للسكري، فتؤكد النتائج أن التركيبات العشبية لها نشاط مهم مضاد للسكري، مع افضلية للتركيبة ت3. في الختام، من خلال هذه الدراسة، نستنتج أن التركيبات العشبية الجديدة لها فعالية بيولوجية أكبر من النباتات الاصلية وان النشاط البيولوجي الاكبر كان في التركيبة ت2 وت 3 وهو ما يشجع على امكانية استخدامها لعلاج الأمراض المتعلقة بالسرطان والالتهابات ومرض السكري.

**الكلمات الرئيسية**: نباتات مضادة للسرطان، الفينول، الفلافونويد، التركيبات العشبية، مضادات الأكسدة، مضادات للالتهابات

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# Acronyms

Abs: Absorption

ARNm: Acide Ribonucléique messger.

BSA: Bovine serum albumin

FeCl3: Iron(III) chloride

V: Volume

NK cells: Natural killer cells

1X PBS: Phosphate buffered saline

C: Concentration

COX2: Cylcooxygenase-2 -2tem CNS: Central nervous system

DPPH: 2.2-diphényl 1-pycrilhydrazyle

F: Formulation

FC: Folin-Ciocalteu

FRAP: Ferric Reducing Ability of Plasma

H 2 SO 4: Sulfuric acid

IC50: inhibition concentration at 50%

IP: inhibition percent.

Km: Kilometer

MCF-7: Michigan Cancer Foundation-7

Ml: Milliliter

Mm: millimole

Na 2 CO 3: Sodium carbonate

NADPH: Nicotinamide adénine dinucléotide phosphate

Nm: Nanomètre

PHF: Polyherbal Formulation

ROS: Reactive oxygen species

Rpm: revolution per minute

SOD: Superoxydedismutase

T1DM: Type 1 diabetesmellitus

T2DM: Type 2 diabetesmellitus

UV: Ultraviolet

# Introduction

A disease is a physical or mental disturbance involving symptoms, dysfunction or tissue damage, whileillness (or sickness) is a more subjective concept related to personal experience of a disease (Australian Institute of Health and Welfare, 2010).

There are many diseases that can afflict the human body, ranging from common colds to cancers. The2 main categories of disease that may lead to ill health are infectious and chronic diseases (Australian Institute of Health and Welfare, 2014).

Chronic and aggressive diseases are commonly incurable. Palliative and nursery care are widest practiced that aims to disease managements outside normal therapy.(Da-Yong et al., 2019).

Chronic diseasesare caused by multiple factors, including a person's genetic make-up lifestyle and environment. They are long-term conditions and cannot be directly spread from one person to another. Examples of chronic diseases include diabetes, asthma ,cancer and heart disease . (Australian Institute of Health and Welfare, 2014).

Oxidative stress is a state that occurs when there is an excess of free radicals in the body's cells. The body produces free radicals during normal metabolic processes.Oxidative stress can damage cells, proteins, and DNA, which can contribute to aging. It may also play a role in development of a range of health conditions, including diabetes, cancer, and neurodegenerative diseases such as Alzheimer's. (Stacy, 2019) Numerous studies for nearly five decades have contributed to understanding the role of reactive oxygen species (ROS) in oxidative stress, when the overwhelming production of ROS exceeds the ability of the cellular antioxidant system to neutralize reactive molecules.

The abundance of intracellular hydrogen peroxide (H2O2) appears to be the main cause of oxidative stress, which is frequently associated with the development of cancer, neurodegenerative disorders, and autoimmune diseases (Timothy et al., 2018).

The body naturally produces antioxidants to counteract these free radicals, oozing to their ability to inhibit the negative effects of ROS (Fraser et al., 2007). A person's diet is also an important source of antioxidants in particular flavonoids and related phenolic

compounds; vitamins C and E in fruits, vegetables, spices, and medicinal plants (Ullah et al., 2020; Fraser et al., 2007; Pourmorad et al., 2006).

Making certain lifestyle and dietary changes may help reduce oxidative stress. These may include maintaining a healthy body weight, regularly exercising, and eating a balanced, healthful diet rich in fruits and vegetables. Therefore, the search for therapeutic agents that could influence this pathological cascade by modulating the production of reactive species and/or antioxidant mechanisms seems to be a promising strategy. (Stacy, 2019).

The use of drugs and their side effects are of great concern, and most patients have perceived negative side effects, these circumstances, along with the failure of modern medicine in finding effective treatments for numerous diseases including cancer and diabetes, have promoted the resurgence of traditional medicine. Traditional medicinal practitioners use a variety of natural products for their health-care needs, the knowledge of such practices was collected and refined over hundreds or even thousands of years (Yman et al., 2016).

Phytotherapy or herbal medicine is one of the oldest forms of treatment on our planet. Worldwide, independent forms of plant healing have evolved over the centuries, such as Ayurveda in India, Campo Medicine in Japan, Sa-sang in Korea, and traditional Chinese medicine (Zimmermann et al., 2022). The availability of plants used in traditional medicine has long been considered a natural and renewable source of secondary metabolites that can be used to produce new drugs (Bahare et al., 2018). In the nineteenth century, during the dawn of modern pharmacies, plants, such as opium poppy, willow bark or coca leaves, contributed essential substances to important medicines (such as salicyls, morphia and cocaine). Modern plant pharmaceuticals contain specific amounts of to ensure consistency in the quantity of active substances (Weixlbaumer et al., 2020).

The aim of the study is to identify the most widely used anti-cancer plants in the region, through a statistical study, through a questionnaire distributed to plant users,

and also to find a polyherbal preparation from some well-known plants, and to study its properties and biological effects.

# Bibliographic study

Chapter 1

# Pathophysiological factors of diseases

## 1.1 Inflammation

### 1.1.1 Defintion of Inflammation

Inflammation is a pervasive form of defense that is broadly defined as a nonspecific responseto tissue malfunction and is employed by both innate and adaptive immune systems to combatpathogenic intruders. A distinctive feature of inflammatory responses in relation to other facets ofantiparasite defenses is that damage to the self is unavoidable. Importantly, collateral damage frominflammation is not the same as immunopathology, which involves a specific immune-mediatedattack on target tissue that is no longer recognized by the immune system as self. Autoimmunepathology reflects dysregulation of adaptive immune components, such as antibody and cell- mediated functions, and has both genetic and environmental influences. Although inflammation-induced collateral damage can certainly contribute toimmunopathology (e.g., rheumatoid arthritis, multiple sclerosis, diabetes), the damage invoked byinflammation represents a basic biological trade-off between damage control and self-maintenanceand does not require the presence of self-antigens to become activated.(Noah et al., 2012)

### 1.1.2 causes of Inflammation

Microorganisms are one of the causes that may cause inflammation.Real world entities.chemicals.Unacceptable immune reactions.The demise of tissue.There are a number of factors that can lead to inflammation, including infectious agents such asviruses and bacteria. Through infiltration and destruction of cells in the body, cause virusesinflammation; Bacteria create internal toxins, which can cause inflammation. physical trauma,Burns, radiological damage and frostbite can cause tissue damage and inflammation. CorrosionChemicals such as acids, alkalis and oxidants can also cause inflammation. As in the pastimmune system disruption can lead to a harmful

inflammatory reactionBody. Another cause of inflammation is tissue death due to lack of oxygen or nutrients. (Johnkennedy, 2022)

### 1.1.3 Types of Inflammation

#### 1.1.3.1 Acute Inflammation

Tissue damage due to trauma, microbial invasion, or noxious compounds can induce acute inflammation. It starts rapidly, becomes severe in a short time and symptoms may last for a few days for example cellulitis or acute pneumonia. Subacute inflammation is the period between acute and chronic inflammation and may last 2 to 6 weeks. (Pahwa et al., 2023)

#### 1.1.3.2 Chronic Inflammation

Chronic inflammation is also referred to as slow, long-term inflammation lasting for prolonged periods of several months to years. Generally, the extentand effects of chronic inflammation vary with the cause of the injury and the ability of the body to repair and overcome the damage. This article reviews chronic inflammation (Pahwa et al., 2023)

### 1.1.4 Reponse

1. Initial tissue damage and activation of local inflammatory factors
2. Immune activation in the CNS and remodeling of the blood–brain barrier (BBB)
3. Recruitment of circulating leukocytes and subsequent secondary immunopathology
4. Engagement of anti-inflammatory responses that promote tissue repair and restoration of neurologic function.(Michael, 2016).

### 1.1.5 Cell types in inflammatory responses

The first cells attracted to a site of injury are neutrophils, followed by monocytes, lymphocytes (natural killer cells [NK cells], T cells, and B cells), and mast cells. (Chen et al., 2018)

### 1.1.6 Group of inflammatory mediators

Another group of inflammatory mediators, including histamine, cytokines, and lysosomal compounds, has a significant role on the capacity to exchange the permeability of vessels, even with its distinct characteristics. Histamines, for example, are the first line of agents released to increases vascular permeability, since they are easily accessed from mast cells and basophils. The activation of histamine H1 receptors promptly changes some pro-inflammatory pathways. Moreover, the histamine regulates the synthesis of cytokine in some inflammation process, such as allergies. In turn, cytokines released by different cells exhibit features that affect vessel dilatation and the ability to chemically transport white blood cells through the blood vessel into the interstitium of injured parenchyma (Ane ,2020)

### 1.1.7 Treatemnt of inflammation

Many drugs are available to ease joint pain, swelling, or inflammation and hopefully to keep your inflammatory disease from getting worse. These medications include:

Anti-inflammatory pain relievers (NSAIDs such as aspirin, ibuprofen, or naproxen)Corticosteroids (such as prednisone). Other medications including chemotherapy drugs, disease-modifying treatments, biologic therapy, and narcotic pain relievers. Some of these medications treat other conditions, such as cancer and inflammatory bowel disease, or they prevent organ rejection after transplants. But your doctor can prescribe them to help treat your symptoms. Your dose or side effects may be different. But these are strong medications, and your doctor will want to keep a close

eye on you while you take them.If you're taking any prescription drug, it's important to meet with your doctor regularly so they can check how well it's working and whether you have any side effects. (Ratini, 2022)

### 1.1.8 Diseases associated with inflammation

inflammation plays a role in many chronic diseases, some of which are described below.

- Rheumatoid arthritis and Psoriatic arthritis: These are autoimmune diseases in which a faulty immune system leads to a persistent local inflammatory response in the joints.
- Allergic asthma: This is a chronic inflammatory disorder in which an abnormal immune response leads to inflammation in the airways in the lungs.
- Chronic obstructive pulmonary disease (COPD): This is a chronic lung disease in which there is a long-lasting inflammatory response to inhaled irritants.
- Heart disease and Stroke: Studies show a strong association between chronic low-level inflammation and cardiovascular disease, including atherosclerosis (hardening of the arteries), heart attack, and stroke.
- Inflammatory Bowel Disease (IBD): This is a group of chronic inflammatory diseases that include ulcerative colitis and Crohn's disease, in which there is long-lasting inflammation in the lining of the digestive tract.
- Diabetes: This is a chronic inflammatory disease in which immune cells infiltrate the pancreas, the organ that makes insulin (the hormone responsible for controlling blood sugar levels).
- Chronic kidney disease (CKD): Low-level inflammation is commonly seen in patients with CKD (impaired kidney function).
- Cancer: Chronic low-grade inflammation is present in many types of cancers.

- Alzheimer's disease: Chronic low levels of inflammation in the central nervous system are linked to dementia and cognitive decline. (Bouchahm et al., 2013)

## 1.2 Oxidative stress

### 1.2.1 Définition

Oxidative stress, defined by an imbalance between theproduction and generation of reactive oxygen species ROS in cells and tissues and the capability of an organism to scavenge these molecules via antioxidant mechanisms. (Burgos Moron et al., 2019)

### 1.2.2 Free Radical

A free radical is any species that contains one or more unpaired electrons, that is, electrons singly occupying an atomic or molecular orbital. Because electrons are more stable when paired together in orbitals, free radicals are generally reactive with other species. Unpaired electrons have a strong tendency to form pair to become stable. Therefore, a radical might donate its unpaired electron to another molecule or it might steal an electron from another molecule in order to form a pair. However, if a radical gives one electron to another molecule or takes one from another molecule, that other molecule itself becomes a radical. Thus, an important feature of free radical mediated reactions is that they tend to proceed as chain reaction. (Subrata, 2016)

### 1.2.3 Reactive oxygen species

Superoxide radicals ($O2\bullet-$), hydrogen peroxide (H2O2), hydroxyl radicals ($\bullet OH$), and singlet oxygen (1O2) are commonly defined reactive oxygen species (ROS); they are generated as metabolic by-products by biological systems . Processes, like protein phosphorylation, activation of several transcriptional factors, apoptosis, immunity, and differentiation, are all dependent on a proper ROS production and presence inside cells

that need to be kept at a low level . When ROS production increases, they start showing harmful effects on important cellular structures like proteins, lipids, and nucleic acids. (Taibur et al., 2012)

#### 1.2.3.1 The generation of ROS and RNS by endogenous and exogenous sources:

The endogenous generation of these species by inflammation mechanisms and activation of immune cells, sever exercise, ischemia, mental activity stress, cancerous and infectious diseases, and aging (Bhattacharyya et al., 2014). Exogenous sources of ROS result from the pollution of water andair and water, alcohol drinking, smoking, some drugs, heavy metals, certain drugs (tacrolimus and cyclosporine), radiations, These compounds are decomposed into ROS after they penetrate the body. (Jîtca et al., 2022)

#### 1.2.3.2 Sources of reactive oxygen species

In the human body there are two main types of sources for reactive oxygen species (ROS), specifically the mitochondria and nicotinamide adenine dinucleotide phosphate oxidase (NADPH oxidase/NOX). (Jîtc et al., 2022)

### 1.2.4 Antioxidants

Antioxidants are compounds of endogenous or exogenous origin that serve to control thelevel of reactive species to neutralize them and minimize oxidative damage The antioxidant defence mechanisms can be divided into enzymatic and non-enzymatic defences. (Halliwell, 2007)

#### 1.2.4.1 Types of antioxidants

Antioxidants are grouped into two:

1. Primary or natural antioxidants
2. Secondary or synthetic antioxidants

Primary or natural antioxidants

They are the chain breaking antioxidants which react with lipid radicals and convert them into more stable products. They are mainly phenolic in structures and include the following:

1. Antioxidant mineral: These are cofactor of antioxidants enzymes. Their absence will definitely affect metabolism of many macromolecules such as carbohydrates. Examples include selenium, copper, iron, etc.
2. Antioxidant vitamins. They are needed for most body metabolic functions They include vitamin C, vitamin E. and vitamin B.
3. Phytochemicals: These are phenolic compounds that are neither vitamins nor minerals. These include. (Adwas et al., 2019)

Flavonoids:

These are phenolic compounds that give vegetables fruits, grains, seeds leaves, flowers, and bark their colors, Catechins are the most active antioxidants in green and black tea and sesamol. Carotenoids are fat solble color in fruits and vegetables. Zeaxanthin is high in spinach and other dark greens (Tarique et al., 2016)

Secondary or synthetic antioxidants

Secondary or synthetic antioxidants These are phenolic compounds that perform the function of capturing free radicals and stopping the chain reactions; the compound includes:

1. Butylated hydroxyanisole (BHA)
2. Butylated hydroxytoluene (BHT)
3. Propyl gallate (PG) and metal chelating agent (EDTA)
4. Tertiary butylhydroquinone (TBHQ)
5. Nordihydroguaiaretic acid (NDGA).

#### 1.2.4.2 Classification

Enzymatic antioxidants:

Cells deploy an antioxidant defensive system based mainly on enzymatic components, to protect themselves from ROS-induced cellular damage such as: (Taibur et al., 2012; Thomas, 2016)

- Superoxide dismutase (SOD)

These metalloproteins, which represent one of the first lines of defense against oxidizing stress, ensure the elimination of the superoxide anion O2 - by a dismutation reaction, converting it into hydrogen peroxide H2O2 and oxygen. (Adwas et al., 2019)

- Catalase (CAT).

The CAT maintains the physiological concentration of H2O2. It converts H2O2 catalytically into H2O and O2 and thus neutralizes it. The CAT exerts its peroxidase activity in vivo. It can also catalyze the reaction of oxidation, by H2O2, of numerous metabolites and toxins. Its basic function is to remove H2O2 and peroxide ROOH in molecular oxygen in order to prevent irreversible damage to themembranes . The CAT also binds NADPH as a reducing equivalent to prevent oxidative inactivation of the enzyme by H2O2 as it is reduced to water (Bhattacharyya et al., 2014).

- Glutathione peroxidase (GPx)

The role of GPx is achieved by the reduction of hydrogen peroxide, lipid hydroperoxides and other organic hydroperoxides

- Glutathione-S-transferases (GST)

representa major group of detoxifying enzymes, which form a family ofmultifunctional proteins involved in the cellular detoxification ofcytotoxic and genotoxic compounds and in the protection of tissuesagainst oxidative damage.

2.Secondary enzymes, for example, glutathione reductase, glucose-6-phosphate dehydrogenase (Adwas et al., 2019)

Non-enzymatic antioxidants

Non-enzymatic antioxidants include different chemical compounds such as tocopherol (vitamin E), ascorbic acid (vitamin C, Vit C), caretinoids, GSH (Carreon et al., 2023), phenolic compounds, ubiquinol (coenzyme Q10), phospholipids (proteoglycans and hyaluronic acid), lipoic acid,proteins binding free iron and copper (ceruloplasmin, transferrin, taurine, albumin), proteinhydrolysates, bilirubin, melatonin, uric acid, mucin,surfactant, amino acids, peptides, and phytates (Benmerzoug, 2022; Thomas, 2016).

## 1.3 Diabetes

### 1.3.1 Définition

Diabetes mellitus represents a set of autoimmune, metabolic and genetic disorders that share one major characteristic – hyperglycaemia. (Aoife et al., 2019) The recommended way of measuring plasma glucose and the threshold used to define what is normal or abnormal have gone through several iterations over the past few decades,betes mellitus is predominantly increasing due to sedentary lifestyles and the conse-quential upsurge in obesity. It has been estimated that about 171 million people world-wide suffer from diabetes mellitus . Oral hypoglycemic agents, insulin, and combina-torial approach are presently available pharmacotherapies for management of diabetesmellitus.(Saptadipa, 2021)

### 1.3.2 Type of diabetes

#### 1.3.2.1 diabetes Type 1

Definition: Type 1 diabetes is generally thought to be precipitated by an immune-associated, if not directly immune-mediated, destruction of insulin-producing pancreatic cells.1,2 Historically, type 1 diabetes was largely considered a disorder in children and adolescents. (Atkinson et al., 2015)

Symptomatic: Although the age of symptomatic onset is usually during childhood or adolescence, symptoms cansometimes develop much later. Although the aetiology of T1DM is not completely understood, thepathogenesis of the disease is thought to involve T cell-mediated destruction of -cells. Islet-targetingautoantibodies that target insulin, 65kDa glutamic acid decarboxylase, insulinoma-associatedprotein 2 and zinc transporter 8 — all of which are proteins associated with secretory granulesin -cells — are biomarkers of T1DM-associated autoimmunity that are found months to years before (Anastasia et al., 2017)

Treatment: To date, insulin therapy remains the only treatment provensafe and effective after clinically significant beta-cell de-struction takes place. Many different formulas of exogenousinsulin are currently available in the United States, includ-ing the following principal types with regard to onset andduration of action: ultra-short-acting insulin, short-actinginsulin, intermediate-acting insulin, and long-acting insulin.By using various combinations of these insulins, it is nowpossible to achieve acceptable control of blood glucose (Larissa et al., 2023)

#### 1.3.2.2 diabetes Type 2

Definition: Type 2 diabetes was previously known as non-insulin-dependent or adult-onset diabetes, and it results from the ineffective use of insulin by the body (Ota et al., 2017)

Symptomatic: The societal burden of T2DM has increased in the past decades due to population ageing and increasing prevalence of underlying risk factors, such as obesity,

increased sedentary activity and unhealthy diet. Even though T2DM can be treated and its consequences avoided or delayed with diet, physical activity, medication and regular screening and treat-ment of complications(Beulens et al., 2019)

Treatment: Type II diabetes has complicated basis and has various treatment options, each targeting different mechanism of action. One such option relies on digestive enzyme inhibition. Almost all of the currently used clinically digestive enzyme inhibitors are bacterial secondary metabolites. However in most cases understanding of their complete biosynthetic (Amruta et al,2010)

Chapter 2

# Phytotherapy and polyherb

## 2.1 Phytotherapy

Etymologically, from the Greek “phyton” which means plant and “therapein” which means cure.Phytotherapy is the use of plants for therapeutic purposes(Sophia (2015 Phytotherapy is part of alternative medicine, medicine based on plant extracts and natural active ingredients (Combe et al., 2019) Medicinal herbs have been used to treat diseases since ancient times. Some 3,000 to 4,000 years ago, works on medicinal plants were written in India, China, and ancient Egypt. In the East, especially in Central Asian folk medicine(Tokhirov et al., 2021)

### 2.1.1 Definition of medicinal plant

A medicinal plant is any plant which, in one or more of its organs, contains substances that can be used for therapeutic purposes or which are precursors for the synthesis of useful drugs. (Abayomi et al., 2013)

### 2.1.2 classifications of medicinal plants

There are two classifications of medicinal plants: 1) depending on the composition of the active substance-alkaloid, glycoside, essential oil, vitamin; 2) depending on the pharmacological parameters sedatives, analgesics, hypnotics, affecting the cardiovascular system, stimulating the central nervous system, lowering blood pressure. The active substances of medicinal plants are alkaloids, various glycosides (anthraglycosides, cardiac glycosides, saponins, etc.). flavonoids, coumarins, astringents and mucous substances, essential oils, vitamins, dyes, enzymes, phytoncides, polysaccharides, starch, proteins, starch, substances, fats and fatty acids and other compounds. (Tokhirov et al., 2021)

## 2.2 Polyherb

### 2.2.1 Ayurveda

Ayurveda is a traditional system of medicine and healthcarethat originated in the Indian subcontinent. More than 5,000 years ago.(Aslam et al., 2016) It means ”knowledge of life.” That is, it is a compound of twoThe words, ”ayu” (life) and ”veda” (knowledge)(Vasant et al., 2016) are used in multi-herbal herbal formulation therapy.(Karole et al., 2019)

### 2.2.2 Polyhebral formulation

It is a selective mixture consisting of two or more herbs in order to treat a specific disease or many diseases, and it is based on two Ayurvedic principles (one drug and the use of more than one drug) in its formulation, so that the herbal ingredients are combined in precise proportions to give an improved therapeutic effect and reduce toxicity. Because the herbs become more powerful And effectiveness when combined with each other, and this is due to the synergistic effect due to the revitalizing or stimulating action on each other, for therapeutic activity. (Karole et al., 2019)

### 2.2.3 Distention geographic

The culture of polyherbal formulation is prevalent in many Countries of the worlde: India, Nigeria, South, Korea ,Pakistan, Bangladesh ,China ,Ghana South Africa, Iran, Thailand ,Indonesia ,The United State of America, Malaysia ,Spain, Canada ,Cameroon ,Sri Lanka and Tanzania. (Aslam et al., 2016)

### 2.2.4 Limitations of polyherbal formulation

When combinations of plants with these constituents are combined together it may show better activity when compared to the individual extract. But at the same time presence

of many constituents may lead to chemical incompatibility which may result in instability (Karole et al., 2019)

Toxicology studies and clinical trials on herbal formulations are not required, per acceptable clinical standards, for the filing of patents and granting of manufacturing licences to the maker of ayurvedic herbal formulations, which may be sometime crucial for patient compliance. (Sonalika et al., 2022)

### 2.2.5 Advantages of polyherbal formulation

polyherbal formulation gives the anticipated activity in combination. The existence of many active substances that when combined can have a potentiating impact that may not be possible with a single substance. Plant-based pharmaceuticals that have a variety of related active principles that can operate in synergistic, potentiative, agonistic, and antagonistic ways. Because of synergism, polyherbalism has many advantages over single-herb formulations. A multi-constituent formulation can achieve a superior therapeutic impact. In order to obtain the desired pharmacological action for this, a lesser dose of the herbal preparation would be required, lowering the possibility of harmful side effects. By removing the need to take multiple herbal formulations at once, patients can enjoy greater. convenience, which in turn improves compliance and has a positive therapeutic impact. Polyherbal formulations that contain a variety of compounds that fight illness complications in different ways to offer a whole course of treatment for a disease state. (Karole et al., 2019; Sonalika et al., 2022)

### 2.2.6 Exemple of polyherbal formulation along with the different pharmacological activities

- Diabrid:(Gymnemasy lvestre, Momordica charantia,EugeniaJambolana,Trigonella Graeceium). It is Anti-diabetic

- Hepax-A: (Plumbago zeylanica, Picrorrhiza kurroa, Piper nigrum, Zingiber oficinale, Sodii carbonas impura, Phyllanthus emblica, Terminalia chebula, Calcii oxidum Potassii carbonas impure). It is Hepatoprotective
- Praneem : (Azadirachta indica (Neem) along with puriied Saponins from Sapindus mukerosi and Mentha citrata oil). It is treatment of Vaginal microbicides
- Zylamend : (Ocimum sanctum, Curcuma longa, Zingiber of icinale, Camellia sinensis, Rosmarinus of icinalis, Polygonum cuspidatum, Berberis vulgaris, Origanum vulgar, Scutellariabaicalensis and Coptis chinensis). It is treatment of Prostate cancer. (Aslam et al., 2016)

Chapter

# 3 Medicinal plants

## 3.1 Atriplex halimus L.

### 3.1.1 Geographical description and Taxonomy

*Atriplex halimus L.* is native of Mediterranean frequently encountered on marginal soils and degraded lands in southern Europe and North Africa. (Tapia et al, 2013)

- Kingdom: Plantae
- Phylum: Spermaphytes (phanerogams)
- Subphylum: Angiosperm
- Class: Dicotyledons
- Subclass: Apetalous
- Order: Centrospermales
- Under order: Chenopodawings
- Family: Amarantaceae (Chenopodiaceae)
- Genre: Atriplex
- Species: *Atriplex halimus L.* (Quezel et Santa ,1962)

### 3.1.2 Botanical description

Shrub branched from the base, 1 to 2.5 m from above. Stem woody with whitish-grey bark; alternate leaves, evergreen, slightly leathery, silver-grey on both faces, variable in shape: oval-rhomboidal to lanceolate, long1-3cm by 0.5-2 cm broad, attenuated into a short petiole at thebase. Flowering from July to October. Generally described as monoecious, at least some individuals of A. halimus would be poly-games with, in addition to unisexual male and unisexual female flowers, some bisexual flowers, which makes them

Figure 1. *Atriplex halimus L.*
(Guillaume, 2015).

trimonoecious individuals. Male flowers yellowish, small, with 5tepals and 5 stamens; greenish female flowers, without perianth, with two opposite brac-teoles. Inflorescences of 20-50 cm in compound racemes, bare or slightly leafy at the base; valves surrounding the whitish fruits, entire, rounded kidney-shaped, wider (4-5 mm) than high (3-4 mm), free (fused just at their base), smooth (or with weak protuberances), without ribs; red seeds 1.5 to 2 mm. (Guillaume, 2015)

### 3.1.3 Chemical Constituents

Atriplex revealed the presence of phytoecdysteroids, flavonoids, triterpenoid, saponins , coumarins , saikosaponins and alkaloids .(Basharat et al., 2021) (Abdelhamid et al., 2022)

This plant material is very rich in protein, fiber, in vitamin A, C and D and saponins, alkaloids and flavonoids . (Ouldkadour., 2019)

### 3.1.4 Pharmacological effect

The aqueous extract is rich in tannins and flavonoids, components to which the authors ascribe a possible action on the pancreas by the increase of beta cell insulin secretion. (Hamza et al., 2019) .Decoction of leaves of A. halimus is used as blood purifier and

also to treat fever, jaundice and liver diseases. (Basharat et al., 2021) .A. halimus is classifiedAmong the plants most used by the population Steppe for the treatment of hyperglycemia . (Nedjimi et ol,2013 ) /Native Arabic Herbal practitioners use the leaves to treat heart conditions and Diabetes mellitus (boiled) and rheumatism (extract prepared with Boiled water is added to the bath water) . (Walker et al., 2014) , A.halimus (Mediterranean saltbush) is a good source for vitamins A, Cand D. (Shaheen et al, 2021)

## 3.2 Curcuma longa L.

### 3.2.1 Geographic distribution and taxonomy:

*Curcuma longa L.*. in a plant distributed from India to South China, Southeast Asia, Papua New Guinea, and Northern Australia.(Ezra et al., 2018),(Saba et al., 2020; Saensouk et al., 2021)

- Kingdom: Plantae
- Subkingdom: Tracheobionta
- Super division: Spermatophyta
- Division: Magnoliophyta
- Class: Magnoliopsida
- Sub class: Zingiberidae
- Order: Zingiberales
- Family: Zingiberaceae
- Genus: Curcuma L.
- Species:*Curcuma longa L.* (Saba et al., 2020)

Figure 2. *Curcuma longa L.* .
(Ezra et al., 2018)

### 3.2.2 Botanical Description:

*Curcuma longa L.* is a perennial herb, grows to a height of 60-90cm. Its leaves are very large, in tuft up to 1.2 m or longer including the petiole which resembles blade, oblong lanceolate, tapering to the base . Turmeric is main lycultivated at 20° to 30° C in tropical regions in Southeast Asia especially in India (Punjab, Bihar, Tamil Nadu) and China. It is an important medicinal and aromatic plant considered as one of the golden resource with massive exports prospective as medicine, cosmetic, cooking spice dye. Flowers are yellow, 10-15 cm in length and grouped together in dense spikes, which appears from the end of spring till mid of autumn. This plant is devoid of fruits. Rhizome is used which is ovate or pear shaped and resembles the bulb known as round turmeric measuring 2.5-7.0 cm in length and 2.5 cm in diameter with finger like projection branching off. It is yellowish brown with a dull orange from interior section that looks bright yellow or when powdered. (Saba et al., 2020)

### 3.2.3 Chemical composition

Curcumin (diferuloylmethane) Chemical constituents of turmeric have been extensivelyinvestigated by many researchers. At the moment, more than 235 compoundis the active principal curcuminoid present in Curcuma longa(Prafulla et al.,2013). The other two curcuminoids are desmethoxycurcumin and bisdesmethoxycurcuminand various

volatile oils, including tumerone, atlantone, and zingiberone. Other constituents include sugars, proteins, and resins. The curcuminoids are polyphenols and are responsible for the yellow colour of turmeric. Derivatives of curcumin are demethoxycurcumi bis-demthoxycurcumin 5 -methoxycurcumin dihydrocurcumin cyclocurcumin (Prafulla et al., 2013; Saba et al., 2020)

### 3.2.4 Pharmacological effect

Turmeric is good source of macro and micronutrients such as protein, energy, vitamin and minerals, and it is known for various medicinal properties with antioxidant activities and is useful in conditions such as inflammation, ulcer and cancer. It has also antifungal, antimicrobial, renal and hepatoprotective activities. Turmeric is being used since past era to modern era; the major application of turmeric is due to antifungal and anti-bacterial property in skin and hair. The recently published papers in international cite as PubMed/ Medline, Science Citation Index and Google Scholar about turmeric were searched. Therefore, turmeric is widely used in treatment of various diseases such as diabetes, osteoarthritis etc. The nutrient content present in the curumin longa is in significant amount, so it can be helpful in combating the nutrients deficiency. Turmeric is also used for Ayurvedic and various cosmetic purposes like blood purification and different types of skin products(Prafulla et al., 2013; Sabaet al., 2020; Sunidhi and Bharti, 2020)

## 3.3 Ephedra.alata:

### 3.3.1 Geographical distribution and Taxonomy:

The genus is indigenous to the temperate and subtropical latitudes of Europe, Asia, and America, and grows especially in northern and western China, northern India, and Spain. In the United States, ephedra plants grow along the Rocky Mountains. is distributed in Africa: Algeria; Egypt, Libyan, Morocco, Tunisia, Mauritania, Chad, Mali , Saudi Arabia, Iraq, Iran, Palestine, Lebanon, Jordan and Syria. (Al Snafi, 2017). E. alata grows wildly

Figure 3. *Ephedra alata* subsp.alenda (STAPF).
(Hadjadj et al., 2020).

on the gravely rocky, sandy and clay soil in arid environments often near shifting sand dunes (Jaradat, 2015)

- Kingdom: Plantae
- Phylum: Tracheophyta
- Division: Gnetophyta
- Class: Gnetopsida
- Order: Ephedrales
- Family: Ephedraceae
- Genus: Ephedra
- Species: *Ephedra alata* (Al Snafi, 2017).

### 3.3.2 Botanical description

Ephedra is a medicinal plant belonging to the Ephedraceae family (Leila et al., 2020). There are many Ephedra species present worldwide among these are *Ephedra alata* , (Hadjadj et al., 2020; Al Rimawi et al., 2017). *Ephedra alata* is short, evergreen and almost leafless shrubs that grow about 60 to 90 cm high.The stems are green in color,

slender, erect or reclining, small ribbed and channeled, about 1.5 mm in diameter and usually terminating in a sharp point. Nodes are 4 to 6 cm apart, and small triangular leaves appear at the stem nodes. The nodes are characteristically reddish brown. The stems usually branch form the base. They bear minute, yellow-green flowers and fruits, and emit a strong pine-like odor and have an astringent taste.(AL-snafi, 2017).

### 3.3.3 Chemical Constituents:

E. alata has been a natural source of alkaloids such as ephedrine, pseudoephedrine, nor ephedrine, nor pseudoephedrine, methyl ephedrine and methyl pseudoephedrine (AL-Snafi, 2017; Daphne et al., 2020). In addition to alkaloids, Ephedra isa source of phenolic compounds and therefore possesses high antioxidant capacity. Ephedra has been reported to contain various phenolic compounds, such as trans-cinnamic acid, catechin, syringin, epicatechin, symplocoside, kaempferol 3-O-rhamnoside 7-O-glucoside, isovitexin 2-O-rhamnoside, which contribute significantly to the antioxidant activity of the plant(Al Rimawi et al., 2017).

### 3.3.4 Pharmacological effect:

E. alata is used in traditional medicine to treat allergies, bronchial asthma, chills, colds, coughs, nasal congestion,edema, fever, flu, headaches (Gonzalez-Juarez et al,2020). It is used in the treatment of joints, lack of sweating, breathing, and low blood pressure Heartbeat (Schaneberg et al., 2003). This plant also shows antimicrobialand anticanceractivities (Al Rimawi et al., 2017). The plant stems are usually chewed to treat bacterial and fungal infections .Plant with vascular and hypotensive effects (Al-Snafi, 2017)

Figure 4. *Nigella Sativa L.*
(Sabira, 2015)

## 3.4 Nigella sativa L.

### 3.4.1 Geographical distribution and taxonomy:

*N. Sativa L.* . is native to Southern Europe, North Africa and Southwest Asia and it is cultivated in many countries in the world. (Sharma, 2009)

- Kingdom: Planate
- Division: Magnoliophyta
- Order: Ranunculales
- Family: Ranunculaceae
- Genus: Nigella
- Species: *Sativa L.* . (Sharma, 2009).

### 3.4.2 Botanical description

The plant grows to 20-90 cm tall, with finely divided leaves, the leaf segments narrowly linear to threadlike. The flowers are white, yellow, pink, pale blue or pale purple, with

5-10 petals. The fruit is a capsule composed of several united follicles, each containing numerous seeds (Aftab et al., 2013; Sharma, 2009)

### 3.4.3 Chemical Constituents

Their secondary metabolites are; Linoleic acid, alkaloids (nigellicin...), essential oils (thymoquinone, limonene...) (Aftab et al., 2013). Moreover, N. sativa seeds also contain alpha-hederin, a water soluble pentacyclictriterpene and saponin, a potential anticancer agent (Al-Jassir,1992; Atta-Ur-Rahman, 1994; Sharma, 2009).

### 3.4.4 Pharmacological effect:

Plant of N. sativa L. has been widely used as antihypertensive, liver tonics, diuretics, digestive, anti-diarrheal, appetite stimulant, analgesics, anti-bacterial and in skin disorders.The plant has anti-cancer effects thanks to some of its active compounds, such as thymoquinone and alpha-hederin (Randhawa and Alghamdi, 2011). She proven effective in vitro in inactivating MCF-7 breast cancer liver tonics, immunomodulator, analgesic, antimicrobial, anti-inflammatory, spasmolytic, bronchodilator, renal protective, gastro-protective, antioxidant properties, anthelmintic etc .stimulant, analgesics, anti-bacterial (Abel-Salam, 2012; Goreja, 2003; Khandi, 2009).

## 3.5 Portulaca oleracea L.

### 3.5.1 Geographical distribution and taxonomy:

*Portulaca oleracea L.* a plant distributed widely in the tropical and subtropical areas of the world including many parts of the United States and is eaten extensively as a potherb and is added to soups and salads around the Mediterranean and tropical Asian countries (Palaniswamy, 2002). It has a cosmopolitan distribution in Africa.

- Kingdom: Plantae


Figure 5. *Portulaca oleracea L.*.
(Habibian, 2020).

- Subkingdom: Viridiplantae
- Infrakingdom: Streptophyta
- Superdivision: Embryophyta
- Division: Tracheophyta
- Subdivision: Spermatophytina
- Class: Magnoliopsida
- Superorder: Caryophyllanae
- Order: Caryophyllales
- Family: Portulacaceae
- Genus: Portulaca L
- Species: *Portulaca oleracea L.* (Okafor et al,2014)

### 3.5.2 Botanical description

It is a warm climate, annual green herb, with branched and succulent stems which are decumbent near the base and ascending near the top to a height of 15–30 cm. The plant is fleshy, stout, succulent (water content of over 90%), with obovate to spathulate, obtuse

opposite leaves tapering towards the base. The flowers are small, yellow, and sessile in clusters of three to five on the forks and tips of the branches, opening in the morning only. The fruit is oblong and transversely dehiscent. The seeds are orbicular and 0.5 mm in diameter (Rashed, 2003). , China, India, Australia, Middle East, Europe, and United States (Rashed, 2003).

### 3.5.3 Chemical Constituents

Diverse compounds have been isolated from *Portulaca oleracea L.* ., such as flavonoids, alkaloids, polysaccharides, fatty acids, terpenoids, sterols, proteins vitamins and minerals (Okafor et al, 2014). Purslane is also reported as an excellent source of the antioxidant vitamins -tocopherol, ascorbic acid, and -carotene as well as glutathione, andthe amino acids isoleucine, leucine, lysine, methionine , cystine, phenylalanine, tyrosine, threonine, and valine (Panel et al, 2022) *Portulaca oleracea L.* also an excellent source of omega-3 fatty acids, which is usually present in oil and fat of fishes but not normally found in plants. (Zhou et al., 2015)

### 3.5.4 Pharmacological effect:

*Portulaca oleracea L.* has been used as a folk medicine in many countries, acting as a febrifuge, antiseptic, vermifuge, and so forth (Lee et al., 2015). It exhibits a wide range of pharmacological effects, including antibacterial (Okafor et al., 2014) antiulcerogenic, anti-inflammatory, antioxidant, and wound-healing (Rashed, 2003). properties.neuroprotective, antimicrobial, ant diabetic, antiulcerogenic, and anticancer activities. (Panel et al., 2022) .Omega-3 fatty acids play an important role in the enhancement of immune function and prevention and treatment of hypertension, coronary artery disease, cancer, and other inflammatory and autoimmune disorders (Zhou et al., 2015)

# Experimental part

Chapter

# 4 Materials and methods

## 4.1 Presentation of the study area

This study was carried out in the regions of Oued Souf and Oued Rig (Figure 6). Information was obtained by conduction several field courses by visiting alternative medicine centers .Elderly people with experience in the field of herbal medicine ;the general public ;and herbalists

## 4.2 Methodology

The study was conducted from Septemberuntil November 2022 in the studied area where 61 people from different categories (Vender ; the general public ; herbalists ) where interviewed. All registered people more 20 and eligible for the study.

The data were collected through semi-structured interviews, and subsequently codified and categorized for proper statistical analysis. Interviews were carried according to a three-part questionnaire. The first part includes the profile of the interviewed people (sex, age, professional activity and residential area). The second part contains cancer-related characteristics (type of cancer, medical background), while the third part concerns plants and their uses (vernacular names,The growing area ;the season of harvesting the plant; part used, the method of preserving it ,reasons for using medicinal plants, mode of preparation,specified dosage ,the duration of administration, use phase and side effects).

All participants were made aware that this study is for research purposes and their participation was voluntary.

At first, a list of vernacular names of medicinal plants used by this population was created. These plants were identified according to common names in the region. Scientific names of plant species were determined according to the Plant List (http://www.theplantlist.org).


Figure 6. Geographical location of the study area (DHW, Oued Souf).
(Hana, 2009)

### 4.2.1 Plant material

### 4.2.2 Collect

Atriplex halimus and Portulaca oleracea were earned in11december in Oued Righ region is one of the oldest cultivated regions and one of the best known in the Sahara northern Algeria. It is constituted of about fifty oases which count totally about 16000 ha cultivated and more than one and a half million palm trees date palms producing dates of excellent (This area is characterized by The depression is elongated from south to nort ( Fekraoui et Djabri, 2013) *Ephedra alata* was earned in Late August to mid September in the oases of Souf located in the heart of the Grand Erg Oriental which is located 600 km as the crow flies south-east of Algiers. Covering an area of 120,000 km2, the Grand Erg Oriental is an immense sea of sand. It is between the large dunes that the Soufi built their oases(Remini and Miloudi, 2021). But about the other plant (Curcuma longa and Nigella sative) were obtained from herbalists

#### 4.2.2.1 Drying and Grinding

Drying depends on spreading the plant in a thin layer, and that is in the shade, in order to preserve the percentage of oils,The volatile is in the plant, and the plant is stirred from time to time to ensure good drying and to prevent fermentation of the plant in case high humidity. After drying, the plant sample was ground well in a grinding machine to ensure good extraction. Methode

## 4.3 Preparation of extract

For preparation of plant we take a weight of 10 grams of dry and ground plant. add 100ml of distilled water inside Arlen Meyer.you heat up two hours at 50C°,cover the mix for 24 hours and leave in the dark,nominate several times by (refinery .medical gauze .cotton),we pass the machine on the centrifuge for 5 minutes 2000 cycles/minute. steam water at 60 degre,Calculate the yield .

## 4.4 Yield

Yield were calculated using the following formula :

$$Y(\%) = \frac{FP}{DM} \times 100$$

- Y: Yield
- FP: Final product
- DM: Dry Matter .

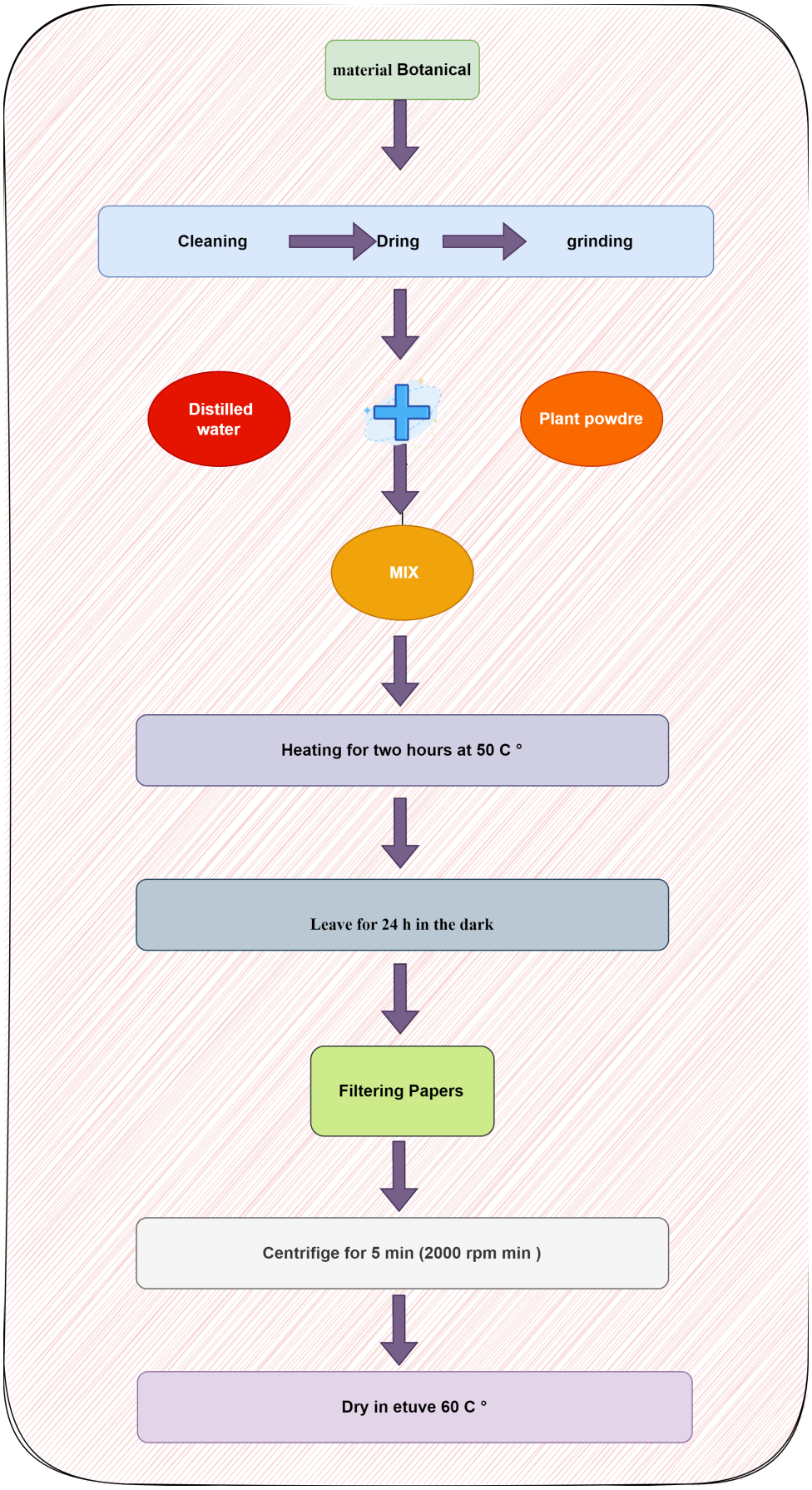


Figure 7. Scheme represents Preparation of extract

## 4.5 Phytochemical analysis

### 4.5.1 Qualitative analysis

The phytochemical analysis were carried out on the aqueous extracts prepared from the plant by qualitative characterization method according to (Evans., 2009; Harborne., 1998; Wadood et al., 2013 Harborne., 1973)

#### 4.5.1.1 Phenols

Introduce 5 ml of extract In a test tube and drops few of natural 5% ferric chloride solution. A dark green color indicates the presence of phenolic compounds.

#### 4.5.1.2 Flavonoids

In a test tube, introduce 5ml of extract, 5ml of diluted ammoniac and 1ml of H2SO4. The appearance of a yellow color indicates the presence of flavonoids.

#### 4.5.1.3 Alkaloids

1 ml of aqueous extract were treated with a few drops of hydrochloric acid then 1–3 drops of Wagner reagent were added. The appearance of brown precipitate reveals the presence of alkaloids in the sample.

#### 4.5.1.4 Tannins

In a test tube, introduce 5 ml of extract and add 1 ml of a 2% aqueous solution of ferric chloride (FeCl3). The presence of tannins was indicated by a greenish or bluish-blackish coloration.

#### 4.5.1.5 Terpenoids

The formation of a reddish brown color indicates the presence of terpenoids, through the addition of chloroform (2ml) and concentrated sulfuric acid (3 ml) to 5 ml of plant extract.

#### 4.5.1.6 Reducing compound

Add Fehling's liquor (1ml of reagent A and 1ml of reagent B) to the extract and incubate the whole in a boiling water bath, The appearance of a brick-red precipitate indicates the presence of reducing sugars.

#### 4.5.1.7 Saponins

In a test tube, introduce 5ml of extract, mixed with 5ml of distilled and with vigorous manual agitation. The formation of a steady foam indicates the presence of saponins.

#### 4.5.1.8 Steroids

For 1ml of plant extract, add 0.5ml of acetic acid solution, followed by 0.5ml of concentrated H2SO4. If the solution does not give any green color, it proves the presence of unsaturated steroids. In a second tube, the same volume of H2SO4 was added. The presence of the red color indicates the presence of steroid derivatives.

### 4.5.2 Quantitative analysis

#### 4.5.2.1 Total phenols compounds

Determination of the total polyphenols was carried out according to the Folin-Ciocalteu(FC) method (BoizotCharpentier., 2006): 100 ul of artichoke extract are

mixed with 500 ul of the FC reagent and 400 ul of Na2CO3 at 7.5% (w / v). The mixture is stirred and incubated in the dark and at room temperature for ten minutes and the absorbance is measured at 760 nm by a UV spectrophotometer. The results are expressed in mg gallic acid equivalent/ g of dry vegetable material with reference to the calibration curve of gallic acid. Calibration curve is carried out by gallic acid at different concentrations (20 - 40 - 60 - 80 - 100 - 120 g/ml) under the same conditions and the same steps of the assay. The results are thus expressed in milligrams of gallic acid per gram of dry extract (mg of EAG / g). All measurements are repeated 3 times.

#### 4.5.2.2 Total flavonoids compounds

The determination of total flavonoids was carried out according to the method described by (Dehpour A et al., 2009): 500 ul of each extract, 100 ul AlCl3, 100 ul of 1 M sodium acetate and 2.8 ml of distilled water. The mixture is stirred and then incubated in the dark and at room temperature for 30 minutes. The blank is made by replacing the extract with 95% methanol and the absorbance is measured at 415 nm using a UV spectrophotometer. The results are expressed in mg equivalent quercetin / g of dry vegetable material with reference to the quercetin calibration curve. The quercetin calibration curve is performed by quercetin at different concentrations (20 - 40 - 60 - 80 - 100 – 120 g/ml) under the same conditions and the same steps of the assay.

## 4.6 Preparation of polyhernal Formulation

The three formulation are differente we relied on three criteria for their composition The first one is based on phenol values in plants ,the second is based on flavonoidsvalues in plants and finally the third is based on equality in the values

Table 1. Rate of plants for polyherbalformulation.

| Plants | *Ephedra alata* | Nigell sative L. | Partulaca olercea L. | *Curcuma Longa L.* | *Atriplex halimus L.* |
|---|---|---|---|---|---|
| F1 | 30% | 30% | 20% | 10% | 10% |
| F2 | 30% | 20% | 20% | 20% | 10% |
| F3 | 20% | 20% | 20% | 20% | 20% |

## 4.7 Biological activity

### 4.7.1 Antioxidant activity

#### 4.7.1.1 FRAP assay

Principle

The Antioxidants are determined by colorimetry. The ferric-tripyridyltriazine complex is reduced to the ferrous-tripyridyltriazine in presence of the antioxidants; the complex loses its yellow color to a dark blue. This coloration measured at 595 nm is proportional to the concentration of antioxidants present in the samples. The method is standardized by Trolox (Oyaizu., 1986).

Procedure

1. Take 500µl of sample.
2. Ferrocyanide potassium (1%).
3. Add 1.25ml of the buffer solution (0.2 M, PH = 6.6).
4. Incubation during 20 min in a water bath at 50 ° C.
5. Add 1.25ml of the aqueous TCA solution (10%) to stop the reaction.
6. Centrifugation at 3000 rpm for 5 minutes.
7. 1.25 ml of supernatant are then mixed with 1.25 ml distilled water and 250 µl FeCl3 (0.1%).
8. Reading at 700 nm against a blank.

The results expired by IC50, after calculating of the ferric reducing antioxidant power values according to (Yazdani., et al 2019) as follows:

$$FRAP(\%) = 100 - \frac{OD\_controle}{OD\_sample} \times 100$$

#### 4.7.1.2 DPPHassay

A DPPH (1,1-diphenyl-2-picryhydrazyl) radical scavenging assay was employed to determine, by a spectroscopic method, relative plant antioxidant ability. Anti-radical activities of plant extracts were estimated, according to the method of Nwidu et al. Stock solutions of extracts (5 mg/ml) were prepared and diluted to final concentrations of 200, 100, 50, 25, 12.5 and 6.25 µg/ml in ethanol. One hundred and 60 µL of 0.1 mM DPPH in ethanol solution was added to 20 µL of the extracts or standard, and then mixed with 20 µL of H2O. vit c(as a control solution) over the concentration range of 1100,75,50,25,10 and 5 mg/ml was assayed under similar conditions. The mixtures were incubated at 37 °C for 40 min in the dark. Sample absorbance was read at 517 nm, as described in(Nwidu et al.,2019)

### 4.7.2 Anti-inflammatory activity

#### 4.7.2.1 Protein denaturation assay

Principal

The anti-inflammatory activity is measured of protein denaturation inhibition in presence of the anti-inflammatory compound, which is studied through in vitro assay. The measured turbidity at 660 nm is proportional to the concentration of anti-inflammatory compound present in the sample (Vennila., et al 2018).

Procedure

1. Add different concentrations (10–100mg ml-1) of the sample to bovine serum albumin (BSA) solution (1

2. Incubation during 30 min at room temperature.
3. The pH of the solution was adjusted to 2 using dropwise addition of concentrated HCl.
4. After incubation, the mixture is heated at 72 °C for 30 min.
5. The all tubes are cooled for 10 min.
6. The turbidity is measured at a wavelength of 660 nm. Diclofenac is used as standard.

The results expired by IC50, after calculating of inhibition percentage (IP) as follows:

$$IP(\%) = A0 - \frac{A1}{A0} \times 100$$

#### 4.7.2.2 Hemolysis assay

Principal

The Hemolysis assay is done as described by (Vinjamuri., et al 2015) that determined the protective effect of the antioxidant compound presented in the sample against the membrane erythrocyte lysis which induced by 1X PBS. The detection of membrane RBCs lysis by measuring the concentration of hemoglobin in blood plasma at 540 nm by spectrophotometer.

Procedure

1. 5mL of blood was collected from healthy volunteers in the tubes containing 5.4 mg of EDTA to prevent coagulation.
2. The blood centrifuged at 1000 rpm for 10 min at 40C.
3. Plasma is removed carefully and the white buffy layer was completely removed by aspiration with a pipette with utmost care.
4. The erythrocytes were then washed for additional three times with 1X PBS, pH 7.4 for 5 min.
5. The Washed erythrocytes were stored at 4oC and used within 6 h for the hemolysis assay.

6. Add 50 µL of 10 dilutions (100 µL Erythrocytes suspension and 900 µL 1XPBS) of erythrocytes suspension was mixed with 100 µL of test samples (10-100mg/mL), 100 µL of 1XPBS was used as a control.
7. Reaction mixture is incubated at 370C water bath for 60 min.
8. The volume of reaction mixture is made up to 1 mL by adding 850 µL of 1XPB.
9. The reaction mixture is centrifuged at 300rpm for 3min
10. The resulting hemoglobin in supernatant is measured at 540 nm by spectrophotometer to determine the concentration of hemoglobin.

The percentage hemolysis is calculated as follows:

$$Hemolysisinhibition(\%) = 100 - \frac{OD\_sample}{OD\_control} \times 100$$

## 4.8 Anti-diabetic activity

### 4.8.1 Glucose uptake in yeastcells

Glucose uptake eassay by yeast cells was performed (Cirillo et al., 1963). The yeast, Saccharomyces cerevisiae suspended in distilled water was subjected to repeated centrifugation (3000 × g, 5 min) until clear supernatant fluids were obtained and 10% (v/v) of the suspension was prepared in distilled water. Various concentrations of plant extracts (50 to 250 g/ml) were added to 1 ml of glucose solution (5 mM) and incubated together for 10 min at 37 °C. Reaction was started by adding100 l of yeast suspension followed by vortexing and further incubation at 37 °C for 60 min. After60 min, the tubes were centrifuged (2500 × g, 5 min) and amount of glucose was estimated in the supernatant by using a spectrophotometer (UV 5100B) at 520 nm. Metronidazole was used as standard drug.

% increase in glucose uptake :

(Abs cont – Abs sampl)/abs cont ×100

## 4.9 Statistique analysis

### 4.9.1 Data analysis

The data reported on the questionnaire were entered and listed on a Microsoft Excel, Mintab (MTB13FR) data base and analysed to determine the proportions of different variables.

Chapter 5

# Results

## 5.1 Yield

The following table represents the yield of various plants as the largest yield of *Atriplex halimus L.* and lowest yield of *Curcuma Longa L.*

Table 2. Represents the yield of various plants

| Plants | *Nigella Sativa L.* | *Artiplex halimus L.* | *Curcuma Longa L.* | *Portulaca oleracea L.* | *Ephedra alata* |
|---|---|---|---|---|---|
| Yield(%) | 13.6 | 24.2 | 7.4 | 10.536 | 10.68 |

## 5.2 Phytochemical analysis plants

### 5.2.1 Qualitative Analysis

Phytochemical tests allow the detection of the main chemical compounds found in parts of many plants, including *Nigella Sativa L.* , *Atriplex halimus L.* , *Curcuma Longa L.* *Portulaca oleracea L.* and *Ephedra alata* .The results of the tests carried out on the aqueous extract,are mentioned in the Table 3.

Table 3. Representephytochemical analysis of plants

| Plants | Phenols | Flavonoids | Alkaloids | Tannins | Terpenoids | Reducing compound | Saponins | Steroids1 | Steroids2 |
|---|---|---|---|---|---|---|---|---|---|
| *Nigella Sativa L.* | + | - | ++ | ++ | - | ++ | - | + | - |
| *Atriplex halimus L.* | +++ | +++ | +++ | +++ | - | + | + | - | - |
| *Curcuma Longa L.* | ++ | +++ | +++ | + | ++ | +++ | ++ | ++ | + |
| *Portulaca oleracea L.* | +++ | ++ | +++ | +++ | +++ | +++ | + | ++ | + |
| *Ephedra alata* | +++ | +++ | ++ | +++ | +++ | +++ | +++ | - | + |

NB: On The Table 3: (+) means present and (-) means absence

## 5.2.2 Quantitative analysis

### 5.2.2.1 Totale phenolicsand flavonoids contentofplants

Totale phenolics content was estimated by galic acid curve (Annexe) and expressed as mg galic acide equivalent (GAE)gof extract .Samples were analyzed in triplicate.Table04 represents the analytical data for phenolics content of the aqueous extract of *Nigella Sativa L.* , *Atriplex halimus L.* ,*Curcuma Longa L.* .,*Portulaca oleracea L.* and *Ephedra alata*. Results were obtained from the compensation equation Y=0.007x 0.067- R2 = 0.908.

Table 4 represents a phenol and flavonoid longitude of plants *Nigella Sativa L.* ,*Atriplex halimus L.* *Curcuma Longa L.* ., *Portulaca oleracea L.* and *Ephedra alata* where we note; that the *Ephedra alata* , contains the highest concentration of phenol 119.29±4.18mg/g , followed by *Nigella Sativa L.* . 102.62 ±5.59mg/g , *Portulaca oleracea L.* and Curcuma

Longa L. at succession at 70.5 ±12.5mg/g and 41.19± 2.24mg/g , and the last *Artiplex halimus L.*. 39.14± 1.89mg/g.

Total flavonids content of aqueous extract of *Nigella Sativa L.* , *Artiplex halimus L.* , *Curcuma Longa L.* , Portulaca olerace L and *Ephedra alata* was expressed as mg Qeuercetin equivqlents/g of (Annexe). Samples were analyzed in triplicate. Table 04 represents the analytical data forflavonoid content of the aqueous extract of *Nigella Sativa L.*, *Artiplex halimus L.* , *Curcuma Longa L.* , Portulaca L. and *Ephedra alata* Results were obtained from the compensation equation od standard curve Y=0.004x 0.013- R2 = 0.947.

Table 4 represents flavonoids and flavonoids for plants where we note that Karkima has the highest flavonoid concentration of 44.17 ±1.69mg/g , followed by *Nigella Sativa L.*of 31.667 ±0.363mg/g, *Ephedra alata* and Portulaca olerace L.of 26.1±0.01mg/g and 25.50 ±0.289mg/g , followed by *Artiplex halimus L.* of 13.667 ±0.22mg/g

Table 4. Total phenol and flavonoid content of the aqueous extract of plants

| Plant | Total phenol(mg/g) | Total flavonoid (mg/g) |
|---|---|---|
| *Ephedra alata* | 119.29±4.18 | 26.1±0.01 |
| *Nigella Sativa L.* | 102.62 ±5.59 | 31.667 ±0.363 |
| *Artiplex halimus L.* | 39.14± 1.89 | 13.667 ±0.22 |
| *Curcuma Longa L.* | 41.19± 2.24 | 44.17 ±1.69 |
| *Portulaca oleracea L.* | 70.5 ±12.5 | 25.50 ±0.289 |

## 5.3 Phytochemical analysis of herbal formulations

### 5.3.1 Qualitative Analysis

Table 5 represents the results of phytochemical analysis of different herbal formulation where we note that: F1 contains Phenols, Tannins and steroid 2 in large quantities, followed by alkaloids and terpenes in medium quantities and after flavonoids and steroid 1 in small quantities and total absence of saponin. Between F2 contains large quantities of

Phenols, flavonoids and terpenes followed by all alkaloids, Tannins, Reducing compound, steroid 2 in medium quantities. Steroid 1in small quantities while there are no soaps. The last f3 is characterized by large quantities of Phenols ,alkaloids, flavonoids, , Tannins, terpenes and Reducing compound in medium quantities. It also contains 1.2 steroids in small quantities and total absence of saponin.

Table 5. Represent the resulte of phytochemical analysis of formulation.

| Formulation | Phenols | Flavonoids | Alkaloids | Tannins | Terpenoids | Commound Reducing | Saponins | Steroids1 | Steroids2 |
|---|---|---|---|---|---|---|---|---|---|
| F1 | +++ | + | ++ | +++ | ++ | + | - | + | +++ |
| F2 | +++ | +++ | ++ | ++ | +++ | ++ | - | + | ++ |
| F3 | +++ | ++ | +++ | ++ | ++ | ++ | - | + | + |

NB: On The Table 5: (+) means present and (-) means absence

### 5.3.2 Quantitative analysis

Table 6 represents the results of the length of phenol and flavonoid of the three herbal formulations as we note that the highest phenol ratio at f2 is 102.29 ±5.07mg/g, followed by f1 with a value of 88.95 ±1.11mg/g and last f3 with a value of 73.857 ±0.675mg/g . While we note that the highest flavonoid ratio at F3 is 70.42 ±2.59mg/g , followed by F2 and F1 on sequence 59.750 ± 0.433 mg/g and 41.667± 0.464mg/g

Table 6. Total phenol and flavonoid content of the Formulationof F1.F2 and F3

| | Total phenol(mg/g) | Total flavonoid(mg/g) |
|---|---|---|
| F1 | 88.95 ± 1.11 | 41.667 ± 0.464 |
| F2 | 102.29 ±5.07 | 59.750 ± 0.433 |
| F3 | 73.857 ±0.675 | 70.42 ±2.59 |

## 5.4 Biological activity

### 5.4.1 Antioxidant activity

Table 7 represents the results of IP,ascorbic acide and three Formulations with different concentration from 5 mg/ml to 125mg/ml in the FRAP and DPPH assay .

Table 7. Represent the Antioxidant activityby FRAP and DPPH methods

| C mg/ml | IP% | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | FRAP | | | | DPPH | | | |
| | vitC | F1 | F2 | F3 | VitC | F1 | F2 | F3 |
| 5 | 10,82 | 55,46 | 33,68 | 11,06 | 18,17 | 1,78 | 5,73 | 0,95 |
| 10 | 14,43 | 56,85 | 35,48 | 13,66 | 20,15 | 2,21 | 6,02 | 1,21 |
| 25 | 25,26 | 61 | 40,86 | 21,46 | 26,09 | 3,52 | 6,89 | 1,99 |
| 50 | 43,31 | 67,93 | 49,84 | 34,46 | 35,99 | 5,69 | 8,34 | 3,29 |
| 75 | 61,36 | 74,8 | 58,81 | 47,46 | 45,89 | 7,87 | 9,79 | 4,59 |
| 100 | 79.41 | 81,78 | 67,79 | 60,46 | 55,79 | 10,04 | 11,24 | 5,89 |
| 125 | 97,46 | 88,70 | 76,76 | 73,45 | 65,79 | 12,22 | 12,69 | 7,19 |

Figure 8: represent IC50 for ascorbic acid and three different formulations are in test FRAP where observed that IC5O for F3 (80mg/ml)is more then IC50 for ascorbic acid 60,21mg/ml

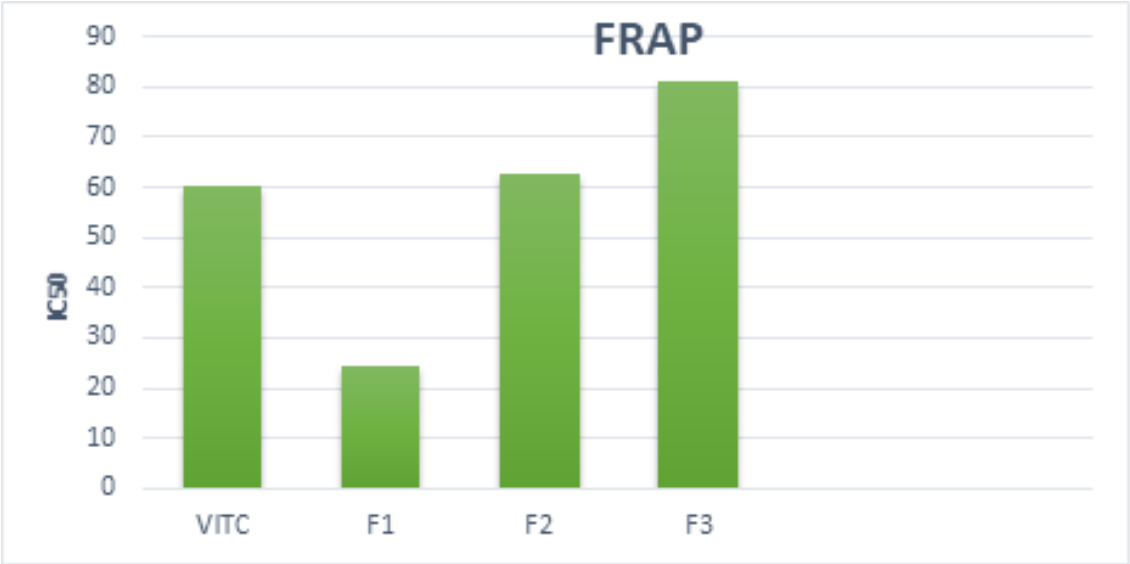


Figure 8. IC 50 value of Antioxidant activity by FRAP

Figure 9 representsthe DPPH radical-scavenging capacity in the studies was reported after 30 min reaction time. The parameter used to measure the radical scavenging activity of extract evaluated is IC50 value, defined as the concentration of antioxidant required for 50% scavenging of DPPH radicals in this specified time period. The IC50 value for aqueous extract F 1 was 550.11mg/ ml, which was comparatively lower than the IC50 (88.29mg/ ml) of ascorbic acid

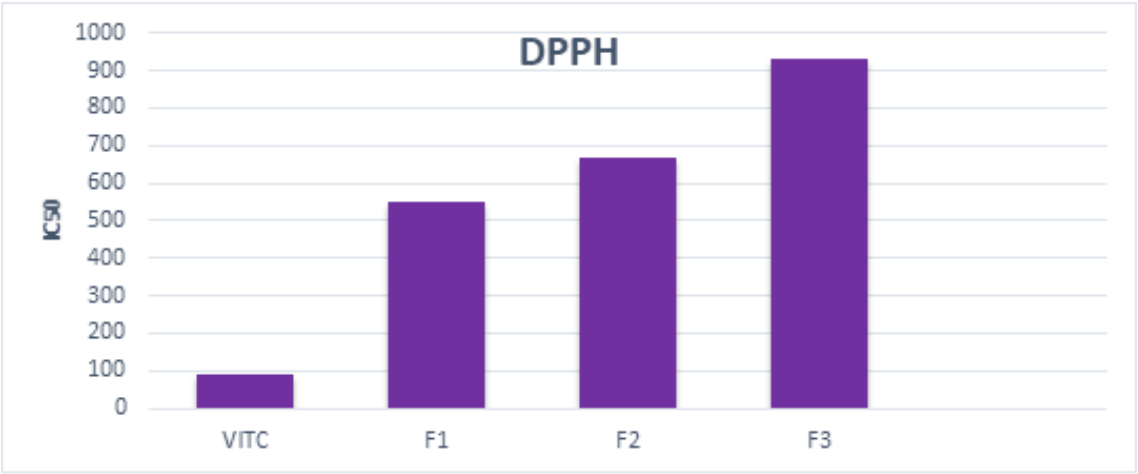


Figure 9. IC 50 value of Antioxidant activity by DPPH

### 5.4.2 Anti-inflammatory activity

Table 8 represents the results of IP, Diclofenac and three Formulations with different concentration from 10 ug/ml to 100 ug/ml in Antiinflmmatory assays.

Table 8. Inhibitory percentage of Anti-inflammatory activity

| C ug/ml | IP% | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | Hemolysis | | | | Protein denaturation | | | |
| | Diclofeanc | F1 | F2 | F3 | Diclofenac | F1 | F2 | F3 |
| 10 | 16,05 | 9,59 | 5,09 | 9,4 | 25,72 | 2,33 | 7,81 | 4.47 |
| 20 | 22,41 | 12,86 | 6,32 | 16,4 | 31,68 | 9,58 | 16,31 | 9,48 |
| 30 | 28,77 | 18,24 | 10,9 | 17,66 | 37,64 | 16,83 | 24,08 | 19,06 |
| 40 | 39,52 | 19,4 | 13,79 | 21,97 | 43,6 | 24,08 | 33,31 | 29 |
| 50 | 39,75 | 22,67 | 16,93 | 24,52 | 49,56 | 31,33 | 41,81 | 38,76 |
| 60 | 42,22 | 25,94 | 19,89 | 29,63 | 55,52 | 38,58 | 50,31 | 48,52 |
| 70 | 54,21 | 29,21 | 24,83 | 32,09 | 61,48 | 45,83 | 58,81 | 58,28 |
| 80 | 60,57 | 30,99 | 25,81 | 38,31 | 67,44 | 53,08 | 67,31 | 68,04 |
| 90 | 66,93 | 35,75 | 28,77 | 42,44 | 73,4 | 60,33 | 75,81 | 77,8 |
| 100 | 73,29 | 39,02 | 31,57 | 46,57 | 79,36 | 67,58 | 84,31 | 87,56 |

Figure 10 represents Percentage of protction 50 for Diclofenac and three different formulations are in test Hemolysis where observed that Percentage of protction 5O for F3 (108,25ug/ml)is more then Percentage of protction50 for Diclofenac 64,49ug/ml.

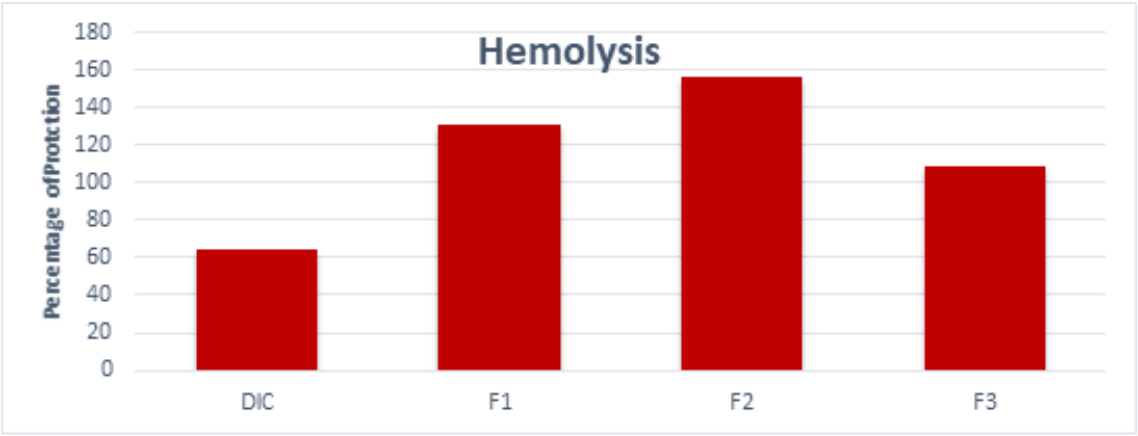


Figure 10. Protective percentage of Hemolysis activity for herbal formulation

Figure 11 represents Percentage of protction50 for Diclofenac and three different herbalformulations forprotein denaturation assay where observed that Percentage of protction50 for F1 (75,99 ug/ml)is more then Percentage of protction50 for Diclofenac 53,32 ug/ml.

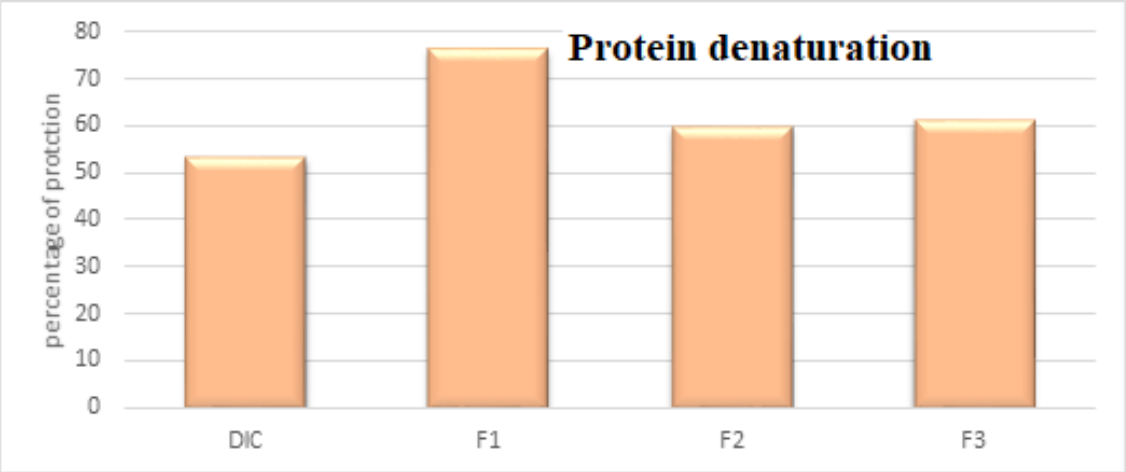


Figure 11. Protective percentage of protein denaturation assay for herbal formulation

## 5.5 Anti-diabetic activity

### 5.5.1 Glucose uptake in yeast cells

Figure 12 represents a multi-curve of five different concentrations from 50mg/g to 250 mg/ml of glucose ratio in terms of changing the concentration of water extract Herbal Formula 1 where we observe the inverse relationship of glucose ratio and the concentration of water extract Herbal Composition 1: from which we note at the concentration of 150mg/ml of water extract there is a lower value of glucose ratio.

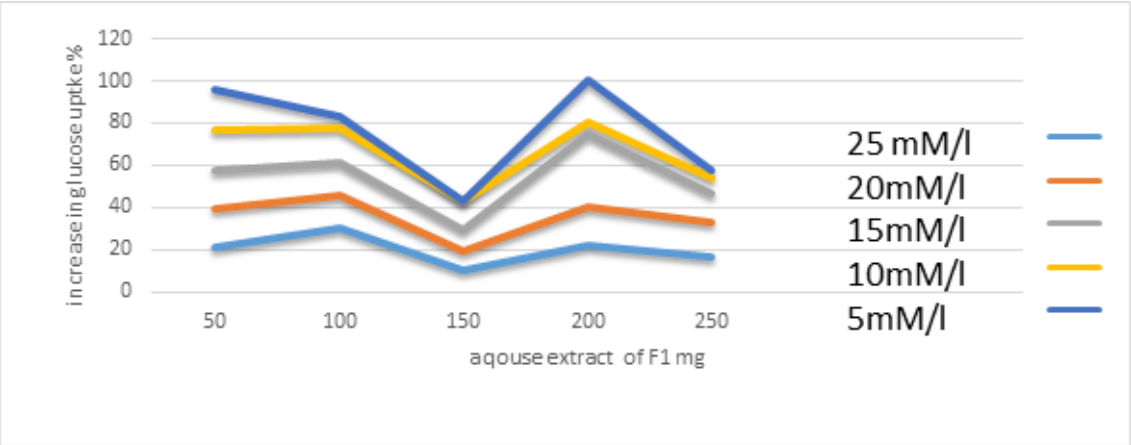


Figure 12. Effect of F1 on glucose uptake by yeast cells

Figure 13 represents a multi-curve of tuo different concentrations from 100mg/ml and 250mg/ml of glucose ratio in terms of changing the concentration of water extract Herbal Formula 2 where we observe the inverse relationship of glucose ratio and the concentration of water extract Herbal Composition 1: from which we note at the concentration of 100mg/ml of water extract there is a lower value of glucose ratio.

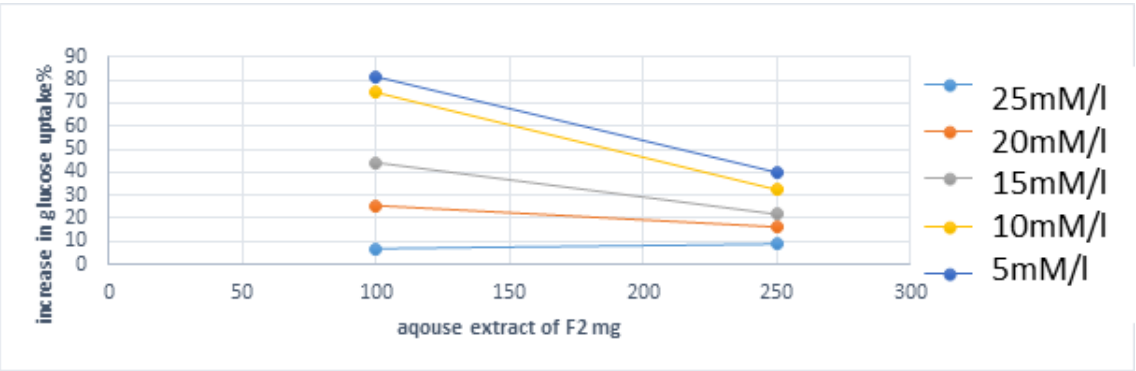


Figure 13. Effect of F2 on glucose uptake by yeast cells

Figure 14 represents a multi-curve of five different concentrations from 50mg/g to 250 mg/ml of glucose ratio in terms of changing the concentration of water extract Herbal Formula 1 where we observe the inverse relationship of glucose ratio and the concentration of water extract Herbal Composition 1: from which we note at the concentration of 100mg/ml of water extract there is a lower value of glucose ratio.

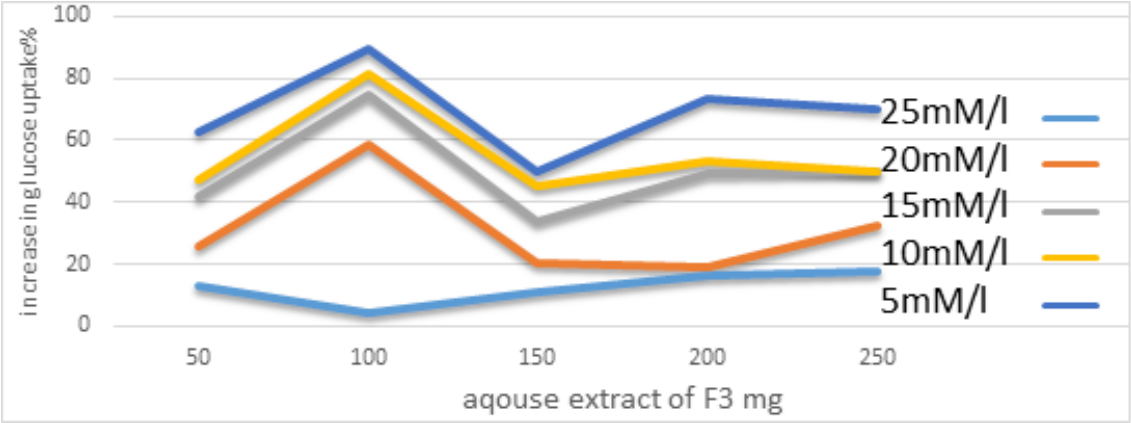


Figure 14. Effect of F3 on glucose uptake by yeast cells

Chapter

# 6 Discussion

## 6.1 Discussion

### 6.1.1 Yield

According to the results the aqueous extraction of plants was done, then the yield of these extracts was calculated based on 100 grams of the dry weight of the plant. It showed the highest yield with one plant *Artiplex halimus L.* followed by the yield of the plant *Nigella Sativa L.* while the yield of the plant *Ephedra alata* was the lowest. These results are in line with the results of *Portulaca oleracea L.* .and they are also consistent with the results of *Curcuma Longa L.* add comparison with other study.

### 6.1.2 Phytochemical analysis of plants

we note that the major compounds are present significant quantities in powder *Nigella Sativa L.* is: Alkaloids and Tannins, there are Reducing compound moderately t while there are the rest compounds such as Phenols and Steroids1 in small amounts .In addition ,our plant is characterized by the absence of Flavonoids, Terpenoids, Saponins and Steroids2. The results of our study matched a study by Mohammed et al. In 2021 where phenol, tannins, steroid, redius componds and alkaloids were found, and a difference was recorded in some compounds: alkaloids, phenols, tannins and steroids were established. protective percentage of Hemolysis activity for herbal formulationration of difference in the presence of saponins. As N. sativa seeds possess these important phytochemical constituents to which their pharmacological activities are ascribed , this signifies their potential use as medicine against microbial infections. (Festus et al., 2022)

In the other hand, we note that the major compounds are present significant quantities in powder Atriplex halimus is: Phenols ,Alkaloids, Flavonoids and Tannins, there are Reducing compound and Saponins moderately. In addition ,our plant is characterized by the complete absence of Terpenoids, Steroids1 and Steroids2 .Our results are in agreement witha study conducted by (Basharat et al., 2021) in the presence of saponins, phenols,

flavonoids, Reducing compound and tannins and the lack of terpenes. As well as the study presented in (ounaissia et al., 2020). It matched the results of our study.

According the results Curcuma Longa we note that the major phytochemical compounds are Alkaloids and Reducing compound .there are Phenols,Flavonoids, Steroids1 , Saponins and Terpenoids moderately t while there are the rest compounds such as Tannins and Steroids2 in small amounts .The results of the current study showed that Curcuma Longa roots are rich in active substances, and it is consistent with other studies, such as a study by Dudu et al.,2018 confirming the presence of flavonoids, alkaloids, tannins, and saponins, as well as with a study in Pakistan by Saba Irshad et al,2018 confirming the presence of most of the compounds. Curcumin (diferuloylmethane) is the active principalcurcuminoid present in Curcuma longa. There are polyphenols and are responsible forthe yellow colour of turmeric. (Saba et al., 2020)

Phytochemicals results of *Portulaca oleracea L.* showed the presence of Phenols ,Alkaloids, Terpenoids ,Reducing compound and Tannins .there are Flavonoids and Steroids1 moderately, while there are the rest compounds such as Saponins and Steroids2 in small amounts. The results of our study are in accordance with the other studies by (Okafor et al., 2014) but the latter showed a difference in the absence of steroid, alkaloids and saponins. Diverse compounds have been isolated from *Portulaca oleracea L.*, such as flavonoids, alkaloids, polysaccharides, fatty acids, terpenoids, sterols, proteins vitamins and minerals. (Saffaryazdi et al., 2020)

Concerning the results of *Ephedra alata* , we note that the major compounds are Phenols, Flavonoids Reducing compound, Terpenoids, Saponins and Tannins .there is Alkaloids moderately t while there are the rest compounds such as Steroids2 in small amounts. In addition,our plant is characterized by the complete absence of, Steroids1. The results of our study showed agreement with other studies, including two studies here in Algeria, where the first was conducted by (Hibi et al., 2022), as well as the second study, which was conducted by (Elyacout et al.,, 2023), that the *Ephedra alata* plant contains most of the chemical compounds, so that the first study did not mention anything about the type of steroids shown in table above. It has been a natural source of alkaloids such as

ephedrine, pseudoephedrine, nor ephedrine, nor pseudoephedrine, methyl ephedrine and methyl pseudoephedrine (Al-snafi, 2017; Daphne et al., 2020).

However, the results of our current study are very little different from those mentioned earlier in terms of the presence and absence of certain compounds. This difference is due to the change in climatic conditions, the life of the plant and the period between harvest and drying. Bioactive constituents such as alkaloids, flavonoids, phenols, tannins and terpenoids are known to elicit broad antimicrobial responses against bacteria, fungi, viruses and parasites. A number of studies documented that these phytochemicals also possess many pharmacological properties, including but not limited to anticancer, antioxidant, anti-inflammatory, cytotoxicity, anti-diarrheal, anti-hemostatic, anti-hemorrhoidal, anti-apoptosis, anti-aging, and growth regulation. (Festus et al., 2022).

#### 6.1.2.1 Quantitative phytochemicals compounds

According to we note that E.A. aqueous extract contains a high percentage of polyphenols 119.29 ± 4.18 mg/g so E.A. represents a good choice for forming a herbal formula rich in polyphenols. A study by the writer showed that E. A is rich in phenolic compounds. Comparing the water extracts of other plants, we note that Nigell Sativa's extract contains a greater proportion of polyphenols estimated at 102.62 ±5.59 mg/g followed by Portulaca oleracea at 70.5 ±12.5 mg/g and Curcuma Longa and Atriblex halimus at 41.19 ± 2.24 mg/g and 39.14 ± 1.89 mg/g respectively. In the current study, alopecia was selected in the rich herbal formulation Boulevinol. This was in line with a study by (AL Rimawi et al., 2017) that proved that Alainda is a source of phenolic compounds and thus possesses a high antioxidant potential that reduces the risk of heart disease and cancer.

According to the results of flavonoids analysis, we note that the aqueous extract of Curcumalonga contains a high percentage of flavonoids 44.17 ±1.69 mg/g which qualifies it to participate in the herbal formula. In comparison with other plants' water extracts: note that the Nigell Sativa extract has the highest ratio of 0 read by *Ephedra alata* and Portulaca oleracea at 26 ± 0.01 mg/g and 25.50 ± 0.289 mg/g respectively and finally

Atriplex halimus 13.667 ± 0.22 mg/g .In the current study Curcuma Longa was selected within the herbal formula rich in flavonoids. This was in line with a study by (Septi et al., 2022) that identified antioxidant activity, phenol and flavonoid medical examination. The result showed that compounds of these herbs are expected to contain functional biological compounds, including Curcuma longa.

### 6.1.3 Phytochemical analysis of formulation

Results show that the major compounds are present significant quantities in powder F1 is: Phenols , Steroids2 and Tannins .there are Alkaloids and Terpenoids moderately t while there are the rest compounds such as Flavonoids ,Reducing compound and Steroids1 in small amounts In addition ,our plant is characterized by the complete absence of Saponins. Also results indicated that the major compounds are present significant quantities in powder F2 are: Phenols , Flavonoids and Terpenoids.there are Alkaloids,Steroids2,Reducing compound and Tannins moderately t while there are the rest compounds such as Steroids1 in small amounts. In addition ,our plant is characterized by the complete absence of Saponins.

In the other side results of quantitative analysis show that the major compounds are present significant quantities in powder F3 are: Phenols , and Alkaloids.there are, Tannins Flavonoids Terpenoids and Reducing compound moderately t while there are the rest compounds such as Steroids1 and Steroids2 in small amounts. In addition ,our plant is characterized by the complete absence of Saponins.

Through the results of individual plants and the three herbal formulations in the phytochemical analysis were itoshemek test, some compounds such as alkaloids appeared in Formula 1 and the disappearance of soaponin in Formula 3. This is explained by Pizzino is synergistic reaction (2019), where he defined it as an interaction to produce new and different effects from individual ingredients. As Parasuraman et al stated (2014), plant chemical compounds of a single plant are activated in the presence of components of another plant and have been proven by the use of alitane, the kinetics of the drug, and

the dynamics of the drug. As a result, Puspitarini (2022) proves that when plants are used together, the effects can be complicated by their diversity. And interactions between multiple individual elements can get into the mix.

#### 6.1.3.1 Quantitative analysis of herbal formulation

Theaqueous extract of herbal F2 appeared at the highest polyphenol ratio of 102.29 ±5.07 mg/g, followed by F1 at 88.95 mg/g±1.11 and in the latter F3 at 73.857±0.675 mg/g. It was expected that the F1 formula selected on the basis of the highest value of polyphenol was the highest, but the F2 formula selected on the basis of flavenoid was higher. Furthmore, we also note that the aqueous extract of the herbal F3 recorded the highest ratio by 70.42±2.59 mg/g, followed by F2 by 59.750± 0.433 mg/g, followed by F1 by 41.667 ± 0.464 mg/g. It was expected that the selected F2 on the highest ratio in flavenoid recorded this value, but the selected F3 on the basis of equal quantities recorded the highest value. From it, we note that the amount of flavonoids in most of the herbal formulations was higher than the amount of flavonoids from individual plants, where the value was greater in turmeric and on the one hand than in the herbal formulations. This may be due to the synergistic interaction between the components of secondary metabolism, which was known above. A study revealed Because of the synergy, polyherbalism confers some benefits not available in a single herbal formulation. Obviously, a better treatment effect can be achieved with a single multi-component formulation. (Parasuraman et al., 2014)

### 6.1.4 Antioxidant activity

According to the results of the table 7 All formulations showed concentration-dependent increases in the inhibition capacity IP, where the higher the concentration of the plant, the greater the scavenging ability

According to the DPPH results, various herbal formulations showed low free anti-radical activity as this activity is achieved by% as we note at the highest extract

concentration 125mg/ml that the witness vitamin C has the highest free anti-rolling activity by 65.79 and thereafter the three herbal formulations observed that the herbal formula 1 is the highest free anti-radical activity by 12.69 mg/ml and the herbal composition 3 recorded the lowest anti-activity by 7.19 mg/ml. The greatestDPPH racdical scavenging potency of with a minimum IC50 value for F1 was 550.11mg/ml, followed by F2 with 665.04 mg/ml, followed by F3 with 933.32 mg/ml. All data were compared with the IC50 value of standard ascorbic acid 88.29mg/ml. DPPH is a stable organic free radical, which loses its absorption spectrum band at 515–528 nm when it accepts an electron or a free radical species. The DPPH assay is a simple, acceptable and most widely used technique to evaluate the radical scavenging potency of plant extracts. The antioxidants are the components of the plants which are capable of enacting the visually noticeable quenching of the stable purple-coloured DPPH radical to the yellow-coloured DPPH (Sushant et al., 2019)

Concerning to the FRAP assay results, herbal formulations have shown high activity against free radicals FRAP expresses this activity percent. The results indicated that the herbal formulations at the highest 125 concentration recorded a relatively low FRAP free anti-radicals activity with the vitamin C witness that we estimated to see 97.46% and then followed by F1 where the 88.70% activity ratio was then F2 and F3 by 76,76% and 73,455% respectively. It follows that F1 is an activity on FRAP free anti-oxidant activity. The greatest FRAP radical scavenging potency with a minimum IC50 value was recored for F1 24.41 mg/ml ,followed by F2 with 62.75 mg/ml, followed by F3 with 81.29 mg/ml. All data were compared with the IC50 value of standard ascorbic acid 60.21mg/ml, The transformation ability of compounds from Fe3+/ferricyanide complex to Fe2+/ferrous form acts as a potential indicator for antioxidant activity (Sushant et al., 2019). In the FRAP assay, the yellow colour test solution changes to green and blue depending on the reduction capacity of extracts or compounds. The presence of reductants in the test solution reduces Fe3+ to Fe2+, which can be monitored by measurement of Perl's Prussian blue colour at 700 nm . The FRAP assay of antioxidants is convenient, reproducible and linearly concentration-dependent. Through the study, I observed herbal

formulations 1 and 2 have a large antioxidant activity (FRAP and DPPH) and this is due to their containment of the amount of polyphenols and flavonoids. This is consistent with the study of (Pourmorad et al., 2007) where it proved that the high content of polyphenols gives good antioxidant activity (FRAP and DPPH).

### 6.1.5 Anti-inflammatory activity

Aqueous extracts of herb formulations showed a large degradation inhibition of the direction of red blood pellets and the inhibitory activity of dissolution is expressed in%. The results indicated that the water extract of the F3 herb formula has the highest inhibitory activity and is estimated at 46. 57% where it has the lowest inhibitory activity by 31. 57% Compared to witness Diclofenac, the three herbal formulations have a lower activity than 73. 29%.

We note that the percentage of protection 50 comparison of herbal formulations that F3 possesses the lowest concentration of 108. 25 ug/ml Where F2 has the highest concentration 155.92 ug/ml, by the way, with the witness, we note that he has the lowest concentration, and from which we conclude that F3 is the best formula in antifolitic activity. It can be due to the good flavenoid content that protects biofilms from oxidative damage caused by free radicals and this is proven by the study presented in (Asgary et al., 2005) study has proven that flavenoid keeps the red blood cell membrane safe.

Concerning the protein denaturation assay, aqueous extracts of herbal formulations showed anti-inflammatory activity from medium to large and this activity is expressed%. The results indicated that F1 has the lowest anti-inflammatory activity and is estimated to 67.58 and exceed F2 and F3 respectively by 84 .31% and 87.56% compared to herbal formulations with witness Dicofinac which is estimated 79.36% note that F1 has the lowest anti-inflammatory activity.

According to our results we note that Porcentag of Pretction for Formula 1 has the highest concentration 100ug/ml and F2 has the lowest concentration compared to Diclofenac with the lowest concentration, and from it we conclude that the best herbal

formula of anti-inflammatory activity is F2. This can be seen from the fact that the herbal composition is rich in polyphenols that affect enzymatic systems and signals that are involved in inflammatory processes and this is consistent with the study (Tarique et al., 2016)

### 6.1.6 Anti-diabetic activity

#### 6.1.6.1 Glucose uptake in yeast cells

The mechanism of transporting glucose through the yeast cell membrane was Reduce attention as an important means of laboratory examination of hypoglycemia for various herbal compounds/plants. It was noted that the three formulations promote the transfer of glucose through the yeast cell membrane. The glucose absorption rate in yeast cells was linear in all the five glucose concentrations mentioned in formula 1 and 3. Concentrations in formula 3 mentioned in the study. Our results are consistent with previous reports listed below;

Previous study suggest that *Ephedra alata* subsp. alenda leaves aqueous extract exerts a pronounced effect against type 2 diabetes and obesity by reducing body weight, hyperlipidemia and hyperglycemia and by the prtotection liver, kidney and testes functions in obese rats. (Saber et al., 2022)

Several phenolic compounds and flavonoids possess marked anti-diabetic activity. Possibly the insulin-like activity of these bioactive compounds inherent in aqueous extract A. halimus is responsible for the antihyperglycemic effects. (Chikhi et al., 2014)

As revealed by another study (Lekshmi et al., 2014) turmeric root extracts have a high ability to inhibit glucosidase activities and glucose interactions. It has proven to be a source of preventive and therapeutic factors for the management of diabetes and related disorders.

*Nigella Sativa L.* its active constituents have preserved pancreas b- cell mass by inhibiting COX-2 mRNA expression under oxidative stress condi-tions. (Nadeem et al., 2022).

Okafor et al had reported the anti diabetic activity in aqueous extract of *Portulaca oleracea L.* in rosiglitazone induced diabetics. Polemice treatment markedly lowered blood
*Portulaca oleracea L.* ighceride. (Okafor et al., 2014)

Treatment group and other positive effects of PHF (Formulated with extracts of *Nigella Sativa L.* Trigonella foenum, Linum usitatissimum ,Cuminum Cyminum) in this research. The effectiveness of PHF on the endogenous antioxidant system as one of the strategies to reduce diabetes-induced oxida-tive kidney damage was also investigated. Like previous research. (Anwar et al., 2022).

# Conclusion

- The results of the survey show that the *Ephedra alata* , Curcuma long, Nigella sativa, Portulaca oleracea and Atriplex halimus are widely used and emphasizes the importance of its effectiveness in treating many cancer diseases in the regions study
- The phytochemical screening showed that the *Ephedra alata* , Curcuma long, Nigella sativa, Portulaca oleracea and Atriplex halimus plants extracts contain a mixture of phytochemicals as phenol, saponins, flavonoids, steroids, tannins, terpenoids, reducing compond and alkaloids with important quantitative value of total phenols and flavonoids which may contribute to the plant being a source of treatment for many diseases.
- Herbal Formulation prepared by plants have an important source of phytochemical compounds with high amount of polyphenol for new formulation F2
- The in vitro study of polyherbal formulations appeared an essential antioxidant activity which may suggest that F3 products to be an effective treatment for many diseases associated with oxidative stress factors.
- In-vitro inflammatory study of polyherbal formulations appeared an important anti-inflammatory activity of F2 and F3 which may suggest these products to be an effective treatment of many diseases associated with inflammatory factors.
- In-vitro antidiabetic study of polyherbal formulations looked an important anti-diabetic activities of F3 which may suggest this product to be an effective treatment of diabetes and its complications.

## Perspective

For future studies, we hope that studies will focus more on:

- Detect all compounds of F1, F2 and F3 ,
- Testing them in anti-diabetic and anticancer action by cell culture and in-vivo methods,

- Try to make extraction and the separation these compound
- Try to make a specific drug form these formulations

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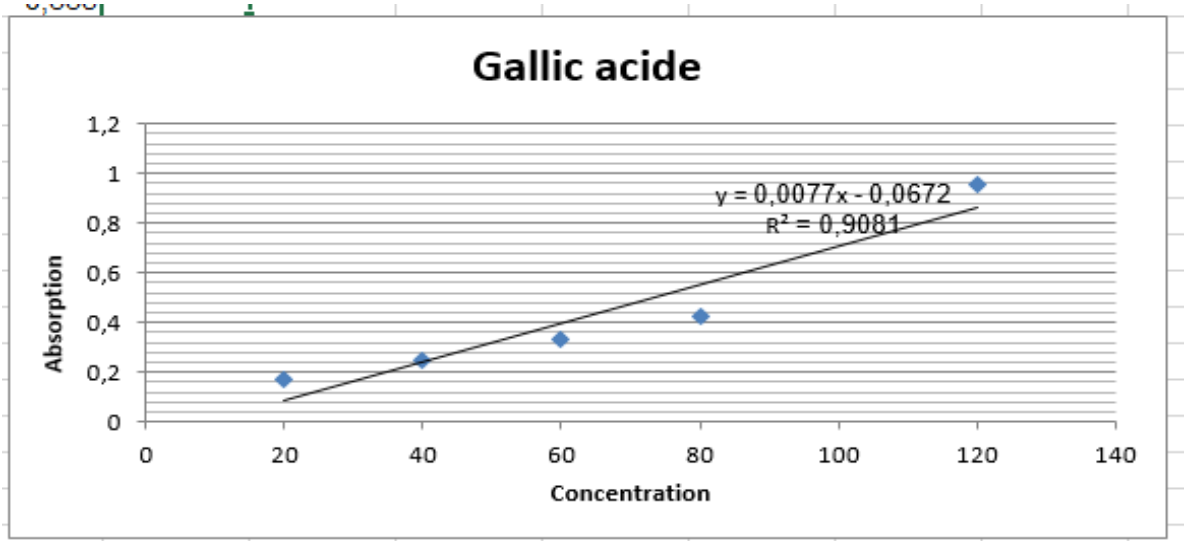
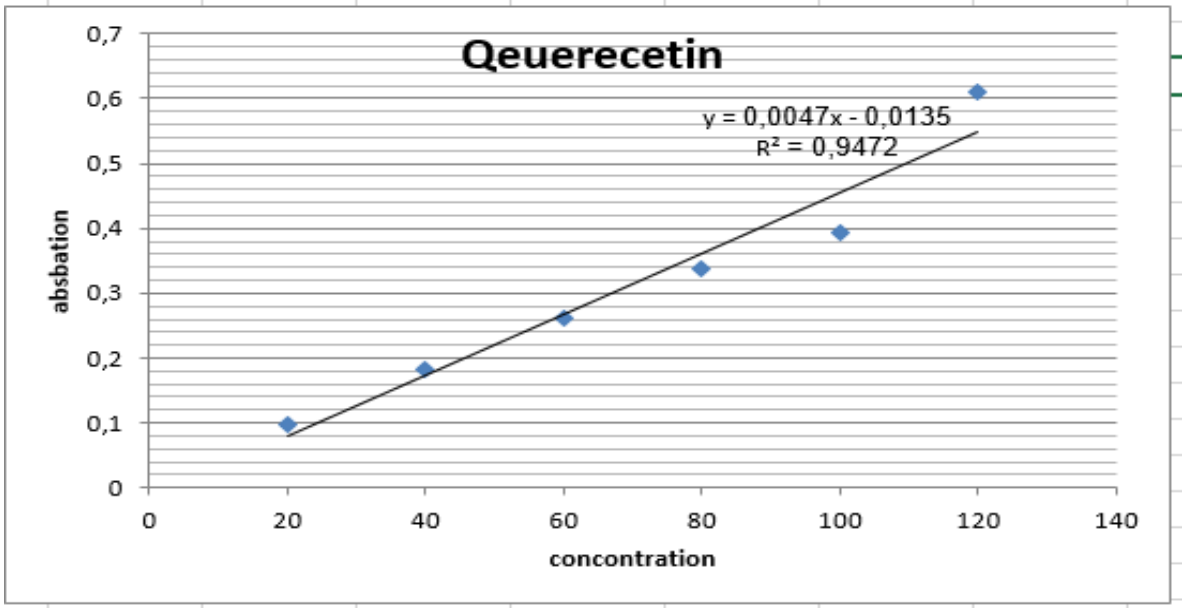
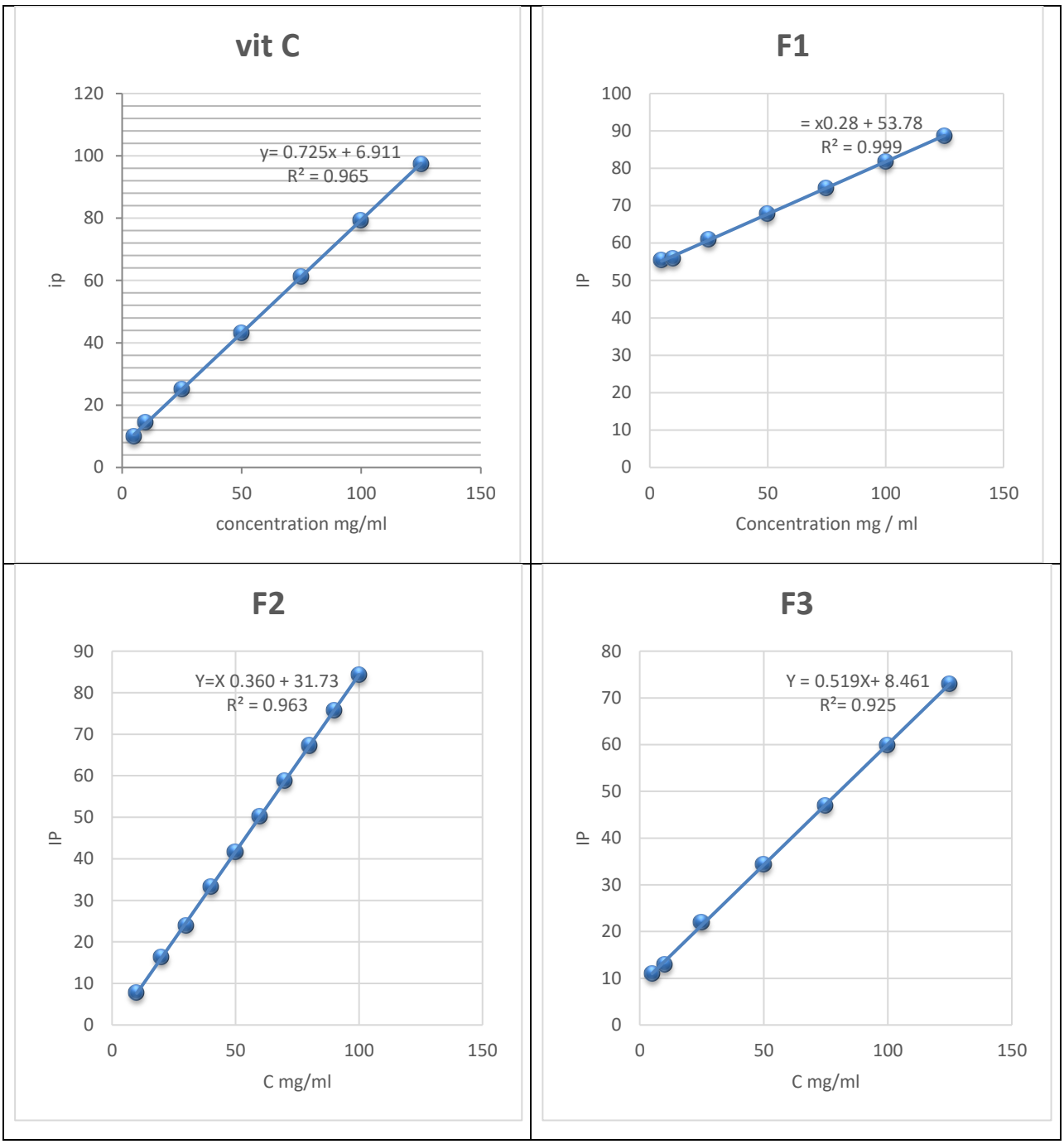
# Appendix



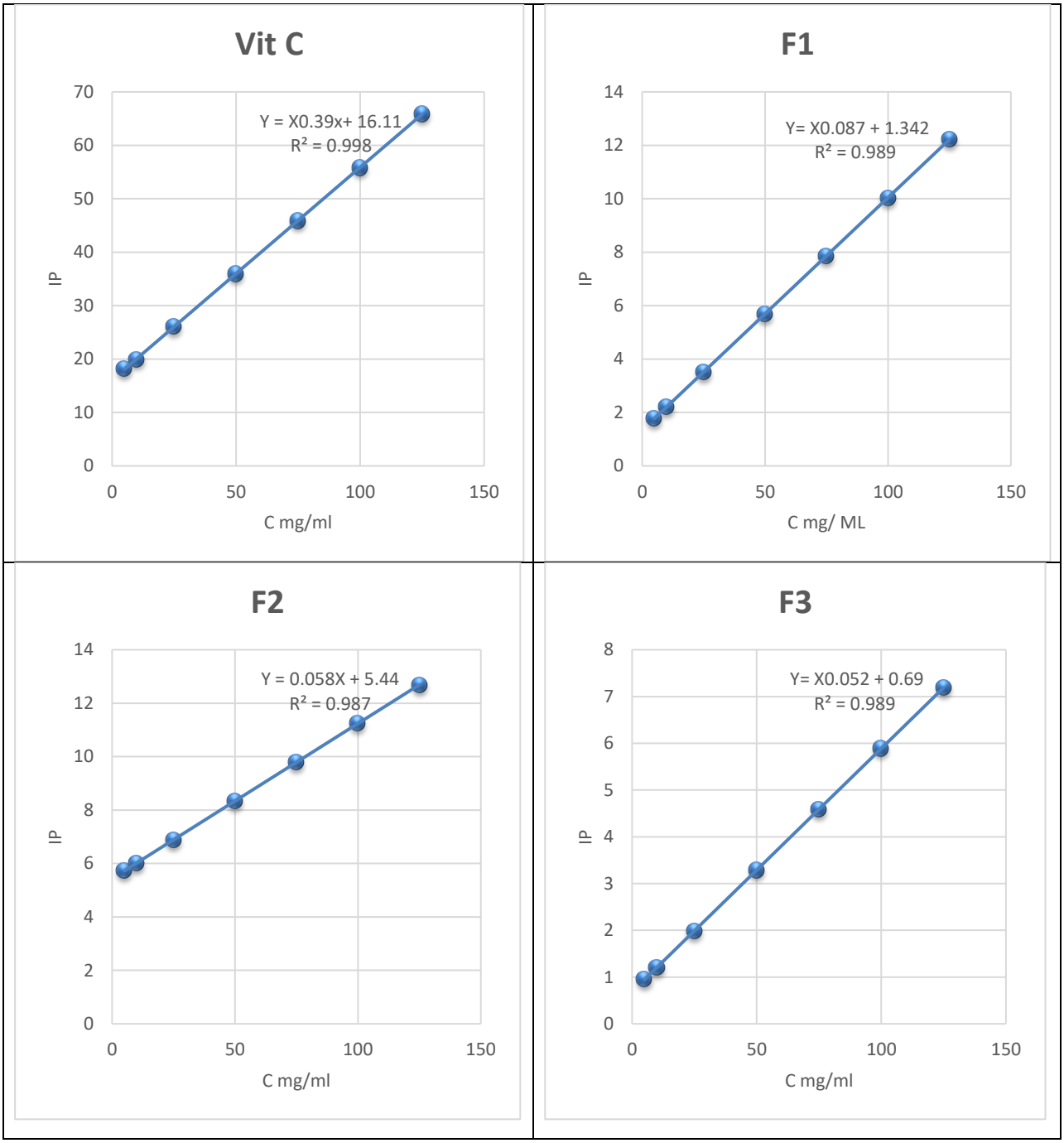

Appendix 01: Calibration curve of Gallic acid .Each point represents the mean of three analyze.



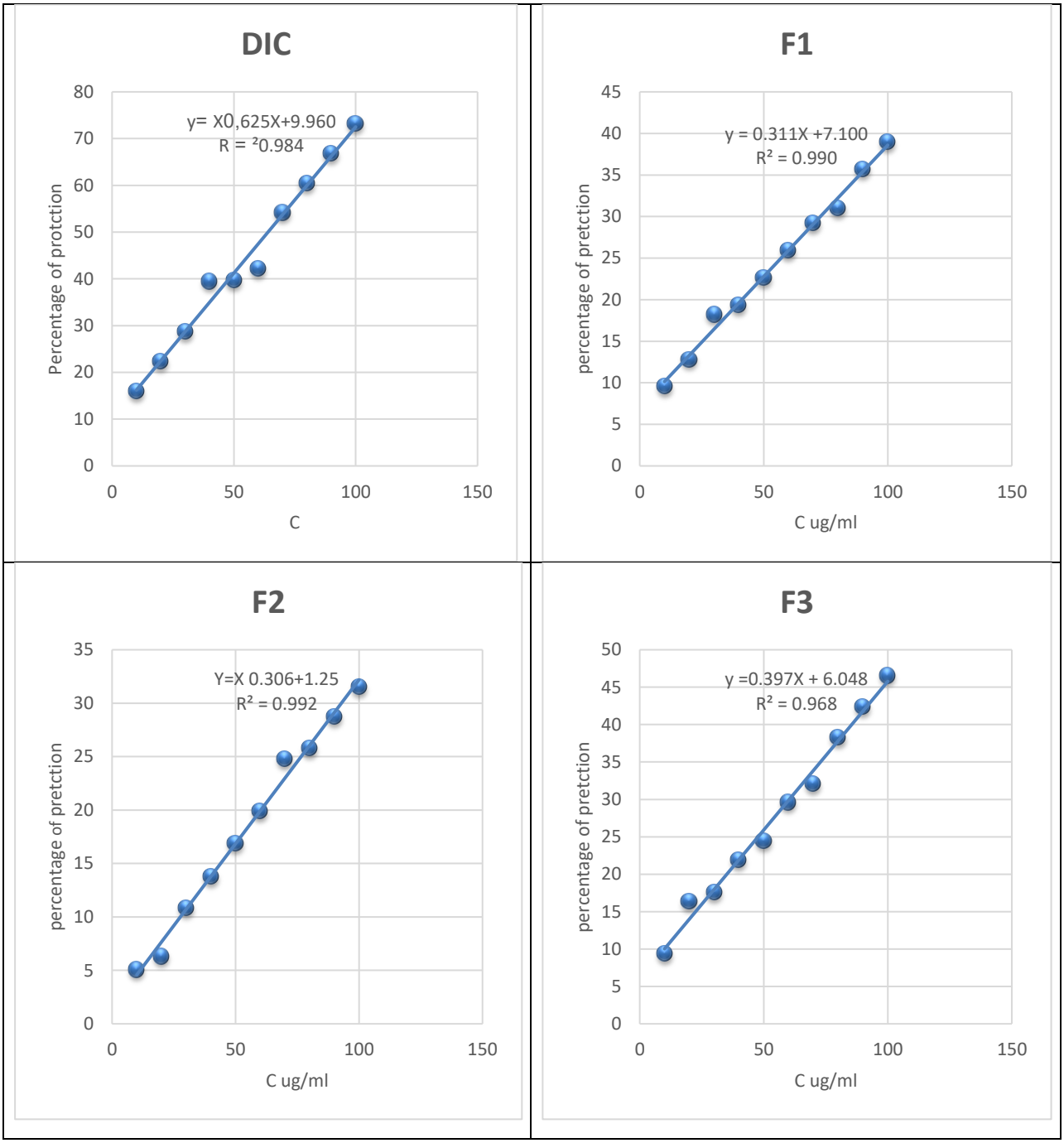

Appendix 02: Calibration curve of Querecetin. Each point represents the mean of threeanalyze.



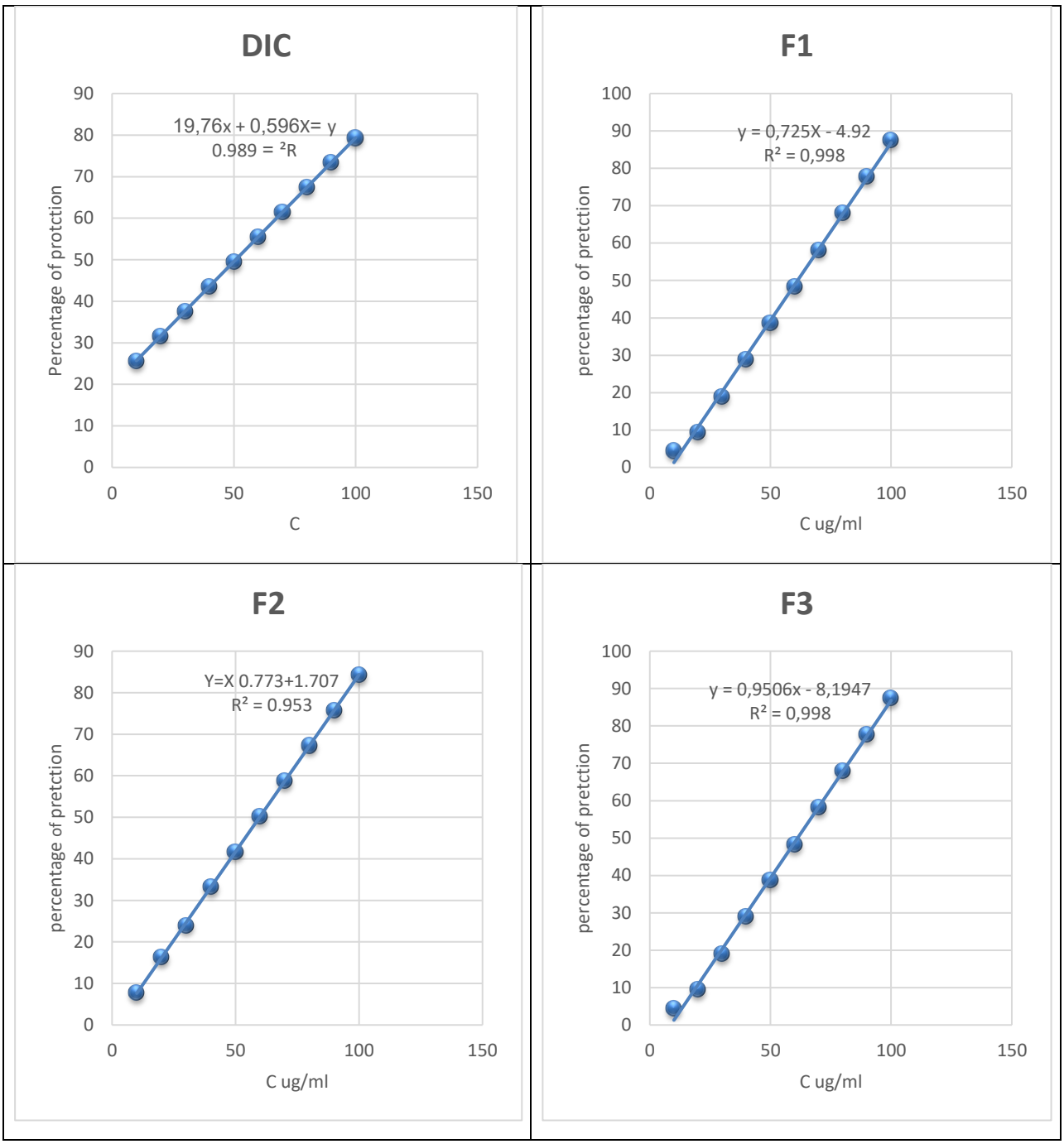

Appendix 03: FRAP Assay test.




Appendix 04: DPPH Assay test.




Appendix 05: Hemolysis.




Appendix 06: Protein denaturation.

**الجمهورية الجزائرية الشعبية الديمقراطية**
**وزارةالتعليم العالي والبحث العلمي**
**كليةعلوم الطبيعة والحياة**
**قسم بيولوجيا الخلوية والجزيئية**

# استبيا ن حول الأعشاب الطبية الشائعة في علاج السرطان

**ضع علامة Xأماماالاجابة المناسبة:**

**الجزء الاول:** معلومات الشخصية

**1** الجنس **:** - ذكر **( )** أنثى**( )**

**2**العمر **:** - اقل من **20( ) 60 – 35 ( ) 35 – 20 ( )** أكثرمن**60 ( )**

**3**التخصص**:** - بائع**( )** طب بديل **( )** مريض **( )** عامةالناس**( )**

**4**المنطقة **:** - وادي ريغ **( )** وادي سوف**( )**

**الجزء الثاني** معلومات تتعلق بالسرطان

**1**-نوع السرطان **:** - سرطان الثدي **( )** سرطان الرحم **( )** سرطان الرئة**( )**

سرطان الدم **( )** سرطان الفم **( )** سرطان الكبد**( )**

سرطان الأمعاء **( )** سرطان الغدد **( )** سرطان البروستاتا**( )**

السرطان عامة **( )** نوع أخر**..... ( )**

**2**-الحالةالصحية للمريض **:** - مريض سرطان فقط **( )** يعاني من أ مراض أخرى**) ( )**

**الجزءالثالث** حول النباتات واستخدامتها **:**

اسم النبتة.....

**1** -منطقة النمو **:** - المنطقةالتلية **( )** المنطقةالصحراوية**( )**

**2**-موسم القطف **:** - الشتاء **( )** الربيع **( )** الصيف **( )** الخريف**( )**

**3**-الجزء المستعمل **:** - الأوراق**( )**البذور **( )** الثمار**( )**

الساق الأزهار**( )** الجذور**( )** الأغصان**( )**

**4**-طريقة الحفظ **:** - تجفيف في الظل **( )** تجفيف في الشمس **( )** تجفيف يالفرن **( )** تمليح**( )**

غيردلك**.....**

**5**-كيفية الاستعمال **1 :** - مسحوق بودرة **( ) :** - محلى بالعسل **( )** مع الماء**( )**

**2** -تغلى في الماء **3 ( )** مستحلب **4 ( )** - مخلوط مع دهن**( )**

**6**-الجرعة المحددة **:** - قليلة **( )** متوسطة **( )** كبيرة**( )**

**7**-مدةالعلاج **:** - مدة قصيرة **( )** طول فترةالمرض**( )**

**8**-مرحلة الاستخدام **:** - قبل العلاج الكيماوي **( )** مع العلاج الكيماوي **( )** بعد العلاج الكيماوي**( )**

**9**-الأعراض الجانبية **:** - غثيان **( )** صداع **( )** ارق**( )** غيردلك.....

**Democratic And Popular Algerian Republic**

**Ministry Of Higher Education And Scientific Research**

**Echahid Hamma Lakhdar University Of El-Oued**

**Faculty Of Natural Sciences And Life**

**Department Of Cellular And Molecular Biology**

# Questionnaire On Medicinal Herbs Common In Cancer Treatment

Put an (**X** ) in front of the appropriate answer:

***The first part*** : The profile of the interviewed people

**-Sex** : male ( ) female ( )

**-Age** : Less than 20 ( ) 35-20 ( ) 60-35 ( ) more than 60 ( )

Professional activity : Harbalists ( ) ; alternative medicine ( ) ؛patient ( ) ; the general public ()

**-residential area** : Oued Souf ( ) ; Oued Rig ( )

***1.The second part*** :Cancer-related characteristics

1. Type of Cancer: Breast Cancer ( )؛ Uterine cancer ( )؛ lung cancer ( ) Blood cancer ( ) Oral cancer ( ); liver cancer ( ) ؛Cancer of the intestine ( ) Prostate cancer( ) ;Cancer ingeneral( ); Other type ().....

**2**. **Medical background** : Cancer patient only ( ) He suffers from other diseases ( )

***The third part :***

*Plants and their uses*

Vernacular names:.....

- The growing area: Tell Area ( ) , Desert Area( )
- The season of harvesting the plant:Winter () Spring () Summer () Autumn ()
- Part used: Seed Leaf () Fruits ( ) Leg ( ); flowers ( ); Roots ( ) ;Twigs ( )
- The method of preserving it :drying in the shade ( ) , drying in the sun ( ) , drying in the oven () , salting ( )
- Reasons for using medicinal plants: Only cures cancer ( ) ,other diseases ( )
- Mode of preparation :

1-Powder: () Sweetened with honey () with water ()

2 -Boiled in water ( ), 3- Emulsion ( ) , 4- Mixed with fat( )

- Specified dosage: in small quantities ( )؛medium ( )؛large ( )
- The duration of administration: Short duration ( ) ؛Length of sickness ( )
- Use phase: Pre-chemo( )؛with chemo( )؛after chemo( )
- Side effects : nausea ( ); headache ( ); insomnia ( )

other effects : .....