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Theme

PREPARATION OF OINTMENT AND EVALUATION OF SYNERGETIC EFFECT OF POLYHERBAL EXTRACTS FOR ANTIDIABETIC ACTIVITY

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To the soul of the most precious person I lost. **My mother**, my God have mercy on her.

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Abstract

The aim of the present study was to evaluate synergetic effect of antioxidant and antidiabetic potential of methanolic and aqueous extracts of *Olea europaea* L., *Myrtus Communis* L., *Artemisia herba alba* L., *Commiphora myrrha* L. The extracts was analyzed by qualitative and quantitative tests, the antioxidant activity of extracts was determined using: TLC bioautographic test, DPPH scavenging method, reducing power and total antioxidant assay *as well as* alpha-amylase inhibitory activity of aqueous extract was carried out. *In vivo* antidiabetic activity of aqueous extract was orally administered to normoglycemic and alloxan-treated diabetic rats at 100, 300 and 500 mg/Kg to determine their hypoglycemic effect using metformin as control. The phytochemical screening carried out showed the presence of tannins, sterols and triterpenes, saponins, flavonoids and terpenoids. The qualitative test revealed yellow spots on the TLC plates sprayed with DPPH, and quantitative analysis showed significant levels of flavonoids with an amount of 439.93 mg EQ/g DW and 357.41 mg EQ/g DW in methanolic and aqueous extracts respectively. It was found that extracts exhibited the maximum inhibition of DPPH with 61.47% and 45.24% for the same order of extracts tested at 0.5 mg/mL, these extracts have the power to reduce iron and phosphomolybdate ion. Alpha-amylase inhibition activity of aqueous extract attaints 60% of inhibition in comparison to acarbose (80%), respectively. Aqueous extract exerted signicant decline in the fasting blood sugar in diabetic animals, however an increase in body weight was mentioned. It was also noted that this extract caused an improvement in biochemical profiles and improved the oxidative stress status in the tissues studied. Indeed, the decrease in MDA concentrations and the increase in GSH and GPx levels in the organs of treated rats were recorded. The histological study showed the beginning of regeneration of the pancreatic cells, and there was a significant improvement in the condition of the hepatocytes with a disappearance of vascular congestion in the liver. On the other hand, the histological study of the skin of the foot which has undergone massage of the ointment does not exert a toxic or irritating effect. Therefore, this polyherbal combination has the potential to be further developed as complex phytotherapeutic remedy for the treatment of diabetes.

Keywords: Polyherbal; polyphenols contents, antioxidant activity, α-amylase, oxidative stress and *in vivo* antidiabetic activity.

Résumé

L'objectif de cet étude était d'évaluer l'effet synergique du potentiel antioxydant et antidiabétique des extraits méthanoliques et aqueux d'*Olea europaea* L., *Myrtus Communis* L., *Artemisia herba alba* L., *Commiphora myrrha* L. Les extraits ont été analysés qualitativement et quantitativement, l'activité antioxydante des extraits a été déterminée par TLC bioautographique sur CCM, piégeage du radical de DPPH, un test de pouvoir réducteur et l'activité antioxydant total ainsi que l'activité inhibitrice de l'alpha-amylase de l'extrait aqueux ont été réalisés. L'activité antidiabétique *in vivo* de l'extrait aqueux (100, 300 et 500 mg/kg) a été administrée par voie orale à des rats diabétiques normoglycémiques et traités à l'alloxane pour déterminer leur effet hypoglycémique en utilisant la metformine comme contrôle. Le criblage phytochimique a montré la présence de tanins, de stérols et de triterpènes, des saponines, de flavonoïdes et des terpénoïdes. Le test qualitatif a révélé des taches jaunes sur les plaques CCM pulvérisées au DPPH, et l'analyse quantitative a montré des teneurs élevées en flavonoïdes avec une quantité de 439.93 mg EQ/g DW et 357.41 mg EQ/g DW et une inhibition maximale du DPPH avec 61.47% et 45.24% à 0.5 mg/mL respectivement de l'extrait méthanolique et aqueux. Ces extraits ont le pouvoir de réduire le fer et phosphomolybdate. L'inhibition de l'alpha-amylase de l'extrait aqueux atteint 60% d'inhibition par rapport à l'acarbose 80%. L'extrait aqueux a entraîné une diminution significative de la glycémie à jeun chez les animaux diabétiques, mais une augmentation du poids corporel a été mentionnée. Il a également été noté que cet extrait provoquait une amélioration des profils biochimiques et améliorait l'état de stress oxydatif dans les tissus étudiés. En effet, la diminution des concentrations de MDA et l'augmentation des taux de GSH et de GPx dans les organes des rats traités ont été enregistrées. L'étude histologique a montré un début de régénération des cellules pancréatiques, une amélioration significative de l'état des hépatocytes avec disparition de la congestion vasculaire au niveau du foie. En revanche, l'étude histologique de la peau des pieds ayant subi un massage par la pommade préparé n'exerce aucun effet toxique ou irritant. Par conséquent, cette combinaison de plantes médicinales a le potentiel d'être développée en tant que remède phyto-thérapeutique complexe pour le traitement du diabète.

Mots clés : Polyherbes ; Polyphénols, activité antioxydante, α-amylase, stress oxydatif et activité antidiabétique *in vivo*.

ملخص

تهدف هذه الدراسة إلى تقييم التأثير التآزري لمضادات الأكسدة و مرض السكري للمستخلصات الميثانولية والمائية لـ*Commiphora myrrha* L.، *Artemisia herba alb*a L.، *Myrtus Communis* L.، *Olea europaea* L.، فتم تحليل المستخلصات بطريقة نوعية وكمية كما تم تحديد نشاط مضادات الأكسدة للمستخلصات باستخدام: اختبار نوعي كروماتوغرافي TLC ، طريقة تثبيط الجذر الحر DPPH، طريقة القدرة الإرجاعية للحديد و النشاط المضاد للأكسدة الإجمالي. بالإضافة إلى النشاط المثبط للألفا أميلاز للمستخلص المائي. تم أيضا إعطاء المستخلص المائي عن طريق الفم إلى الجرذان المصابة بداء السكري بالألوكسان والمعالجة كذلك باستخدام التراكيز 100 و 300 و 500 ملغ/ كغ لتحديد تأثيرها الخافض لسكر الدم بالمقارنة مع الميتفورمين. أظهر الفحص الكيميائي النباتي الذي تم إجراؤه وجود التنينات والستيرول والصابونينات والفلافونويد والتيربينويدات. وكشفت طريقة TLC عن نتائج إيجابية من خلال ظهور بقع صفراء على لوحات التحليل الكروماتوغرافي التي رشت ب DPPH، كما أظهر التحليل الكمي مستويات كبيرة من مركبات الفلافونويد بكمية 439,934 ملغ مكافئ/غ وزن جاف و357,418 ملغ معادل/غ وزن جاف في المستخلصات الميثانولية والمائية على التوالي. لقد وجد أن المستخلصات أظهرت الحد الأقصى لتثبيط DPPH بنسبة 61,47% و45,24% لنفس ترتيب المستخلصات عند التركيز 0,5 ملغ/مل، وهذه المستخلصات لديها القدرة على تقليل أيون الحديد و النشاط المضاد للأكسدة الإجمالي. كما وصل نشاط تثبيط ألفا أميلاز في المستخلص المائي إلى 60% من التثبيط مقارنة بالأكاربوز (80%). أدى أيضا المستخلص المائي إلى انخفاض كبير في نسبة السكر في الدم للحيوانات المصابة بداء السكري، ولكن لوحظ زيادة في وزن الجسم. ولوحظ أيضًا أن هذا المستخلص ساهم في تحسين العوامل البيوكيميائية وتحسين حالة الإجهاد التأكسدي في الأنسجة التي تمت دراستها. وبالفعل تم تسجيل الانخفاض في تركيزات MDA والزيادة في مستويات GSH وGPx في أعضاء الفئران المعالجة. وأظهرت الدراسة النسيجية بداية تجديد خلايا البنكرياس، كما لوحظ تحسن كبير في خلايا الكبد مع اختفاء احتقان الأوعية الدموية. من ناحية أخرى، فإن الدراسة النسيجية لجلد القدم الذي خضع لتدليك بالمرهم المصنوع لم يسبب أي تأثيرات سامة أو مهيج للجلد. لذلك فإن هذا المزيج متعدد الأعشاب لديه القدرة على تطويره كعلاج نباتي بديل لعلاج مرض السكري.

كلمات مفتاحية: خليط نباتات؛ البوليفينولات، نشاط مضادات الأكسدة، ألفا أميليز، الإجهاد التأكسدي والنشاط المضاد لمرض السكري.

Abbreviations

MCH : Mean Corpuscular Hemoglobin

MCHC : Mean Corpuscular Hemoglobin Concentration

MCV : Mean Corpuscular Volume

CHOL : Cholesterol

PBS : Sodium Phosphate Buffer

PLT : Platelets

RBC : Red blood cell Count

RNS : Reactive Nitrogen Species

ROS : Reactive Oxygen Species

SPF : Sun Protection Factor

BHT : Butyl Hydroxy Toluene

BHA : Butyl Hydroxy Anizole

DPPH : 2,2-diphenyl-1-picrylhydrazyl

DTNB : 5,5'-dithiobis-2 nitro benzoic acid

GSH : Gluthathion reduced

MDA : Malondialdehyde

GPx : Glutathion peroxidase

IC_{50} : Inhibitory concentration at 50

TLC : Thin Layer Chromatographic

HE : Hematoxylin

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General Introduction

Introduction

Diabetes is known as one of the world's most common endocrine disorder and has been a major non-communicable disease over recent decades. The worldwide incidence of diabetes is 451 million which is likely to reach to 693 million in 2045 (**Cho et *al*., 2018)**. Diabetes mellitus as a group of metabolic diseases is mainly manifested by elevated plasma glucose level resulting from defects in insulin secretion and/or action or both **(Bhat et *al*., 2011)**. Exposure to chronic hyperglycemia can cause macro and micro vascular damages. Long-term hyperglycemia also increases ROS (Reactive Oxygen Species) production (King and Loeken, 2004). Elevation of oxidative stress and changes in antioxidant enzyme activity might play a main role in the development of micro vascular complications **(Moussa, 2008)**. Several types of anti-diabetic drugs have been produced and are in use for diabetic patients. Many of anti-diabetic synthetic drugs have undesirable side effects.

In the present era, there is a growing interest in herbal therapy owing to its safety, cost-effectiveness, and convenient availability. Several herbs are traditionally known as a remedy for diabetes whole plant or a part of it is used to treat serious disorders such as diabetes mellitus. Chronic hyperglycemia is often accompanied by oxidative stress which results in serious complications and disorders. Oxidative stress can be reversed by the use of antioxidants which reduce oxidative damage and, thus, prevent the long-term complications of Diabetes mellitus (**Ohnishi et *al*., 2004)**. Several plants and their individual constituents have been investigated for antidiabetic potential in animal and human studies. More than five hundred plants belonging to 140 different genera have exhibited antidiabetic activity when investigated on the basis of ethnomedicinal significance **(Salehi et *al*., 2019)**. Extracts prepared from *Olea europaea* leaves showed *in vitro* antidiabetic and antioxidant activities **(Chigurupati et *al*., 2021)**. Moreover, various phenolic acids, oleanolic acid, and oleuropein isolated from *Olea europaea* leaves have been attributed to the in vitro antidiabetic and antioxidant activities **(Sato *al*., 2003)**.

The *Artemisia herba alba* is the most popular medicinal and aromatic plants extensively used for treating inflammation, diabetes mellitus **(Rafiq et *al*., 2016; Réggami et *al*., 2021)** and for the treatment of human and livestock wounds **(Seddik et *al*., 2010)**.

The medicinal plant *Myrtus communis* L. (Myrtle) have been used have been reported to possess antihyperglycemic and antibacterial properties. Recent reports have described antioxidant activities of different extracts of myrtle and certain ingredients, implying its potential as medicine for the treatment of diseases related to oxidative stress, including diabetes mellitus **(Ferchichi et *al.*, 2011; Malekpour et *al.*, 2012)**.

In addition *Commiphora myrrha* L. was extensively used as anti-diabetic remedy due to their antioxidant, hypoglycemic, and hypolipidemic properties **(Shokoohi et *al.*, 2017)**. Therefore, the aim of this study was to formulate an ointment and investigate the synergetic effects of methanolic and aqueous extracts of a combination of *Myrtus communis* L., *Artemisia herba alba, Olea europaea* L. and *Commiphora myrrha* L. leaves on serum glucose and insulin levels, serum lipid profile, as well as lipid peroxidation, and antioxidant enzymes activities in the liver, pancreas and body weight changes in alloxan-induced diabetic rats, as well as evaluation of antioxidant activity with different methods and alpha amylase activity.

Chapter I

I. Definition of Diabetes

"A group of metabolic diseases characterized by chronic hyperglycemia resulting from a defect in insulin secretion or a defect in insulin action or both of these combined abnormalities**." (Buffet and Vatier, 2010)**.

Diabetes mellitus is a group of metabolic diseases characterized by chronic hyperglycemia resulting from a defect in insulin secretion or insulin action or both combined abnormalities. Chronic hyperglycemia is ultimately associated with specific organic complications particularly affecting the eyes, kidneys, nerves, heart and vessels **(Drouin et *al.*, 1999).**

I.1.Diabetes classification

A. Insulin-dependent diabetes (Type 1 diabetes)

This type of diabetes is a disease called "autoimmune". The person makes antibodies which have the characteristic of attacking its own pancreatic cells, in this case those that make insulin. The result is the destruction of the islets of Langerhans where manufactures insulin. When 90% of the islets are destroyed, diabetes appears **(WHO, 1999**).

B. Non-insulin dependent diabetes or Type 2 diabetes

Type 2 diabetes is a metabolic disease characterized by chronic hyperglycemia whose physiopathological elements include increased resistance of peripheral tissues (liver, muscles, adipose tissue) to the action of insulin, insufficient insulin secretion by β cells of the pancreas, inappropriate glucagon secretion, as well as a reduction in the effect of incretins, intestinal hormones stimulating postprandial insulin secretion (**Braillard et *al.*, 2017)**.

In this case the pancreas certainly still produces sufficient insulin at the beginning, but this is secreted too slowly and at the wrong time and does not act sufficiently to cause of excess weight (insulin resistance) **(WHO, 1999)**.

C. Gestational diabetes (GDM)

Gestational diabetes is observed in 3% of pregnant women, it can be considered as transient. This is a glucose intolerance leading to hyperglycemia of variable severity **(Rodier., 2001)**. It is characterized by hyperglycemia, that is to say an increase in blood sugar content, with values higher than normal, but lowers than those diagnosing diabetes, appearing during pregnancy. Women with gestational diabetes have an increased risk of complications during pregnancy and childbirth. Their risk, as well as that of their child, of having type 2 diabetes later in life also increases. It is very often diagnosed during prenatal screening and not following symptoms **(WHO, 2019)**.

D. MODY type diabetes

MODY diabetes (Maturity Onset Diabetes of the Youth) is “monogenic” diabetes due to pathogenic variants of a single gene, most often involved in the secretion of insulin and/or pancreatic development. They represent 2 to 3% of all diabetes. This type is characterized by an early onset (before the age of 35-40) and an autosomal dominant mode of inheritance. They are differentiated from type 1 diabetes (T1D) and type 2 diabetes (T2D) by the absence of autoimmunity and the absence of ketoacidosis at diagnosis and a level of corpulence comparable to that of the general population **(PNDS, 2022)**.

I.2.Pathophysiology

I.2.1.Physiopathology of type 2 diabetes

Type 2 Diabetes Mellitus (T2DM), one of the most common metabolic disorders, is caused by a combination of two primary factors: defective insulin secretion by pancreatic β-cells and the inability of insulin-sensitive tissues to respond appropriately to insulin. Because insulin release and activity are essential processes for glucose homeostasis, the molecular mechanisms involved in the synthesis and release of insulin, *as well as* in its detection are tightly regulated. Defects in any of the mechanisms involved in these processes can lead to a metabolic imbalance responsible for the development of the disease **(Unai Galicia-Garcia et *al.*, 2020)**.

I.2.2.Causes of diabetes

Type 2 diabetes, which represents up to 95% of diabetics, results mainly from factors linked to lifestyle (stress, sedentary lifestyle, etc.) as well as a combination of factors genetic and environmental. People with a genetic predisposition are thus the more likely to suffer from diabetes. The emergence of genetic research has made it possible to highlight correlations between certain genetic markers and the risk of diabetes (predisposition genes) **(ADA, 2008)**.

Obesity and particularly the accumulation of fat in the organs of the abdomen which lead to insulin resistance, also remain one of the main factors of onset of the disease. Indeed, to compensate for insulin resistance, the pancreas begins to produce more insulin until it is depleted and insulin secretion decreases. Therefore, a relative deficiency of insulin causes continuous hyperglycemia.

Type 2 diabetes is therefore considered to be the result of two phenomena: first insulin resistance, then exhaustion of the pancreas age also plays a role in the onset of diabetes. According to the Health Monitoring Institute, the maximum prevalence rate of treated diabetes is observed in people aged 70 to 79 years, with 17.7% among men and 11.5% among women. The average age from which the Diabetes risk of developing is thus 45 years.

I.2.3. Factors influencing type 2 diabetes

A. Genetic factors

Evidence of the involvement of genetic factors in the triggering of type diabetes 2 has been demonstrated by family studies: concordance is between 60 and 100% for monozygotic twins. It is around 40% for first-degree relatives **(Simonis-Bik et *al.*, 2010)**.

B. Environmental factor

Obesity, especially android obesity, sedentary lifestyle, smoking and age are increasing considerably the risk of developing type 2 diabetes **(Raverot, 2005)**. Also Insulin secretion disorders are ensured by the β cells of the pancreatic islets of Langerhansn, under physiological conditions, insulin secretion is characterized by kinetics called biphasic **(Figure 01)**. It is stimulated by an acute and transient increase in the extracellular concentration of glucose. The maximum level of secretion is reached after a few minutes (early peak). When the high concentration is prolonged, a second peak appears (late peak). This phase lasts as long as the stimulus remains applied **(Magnan and Ktorza., 2005).**

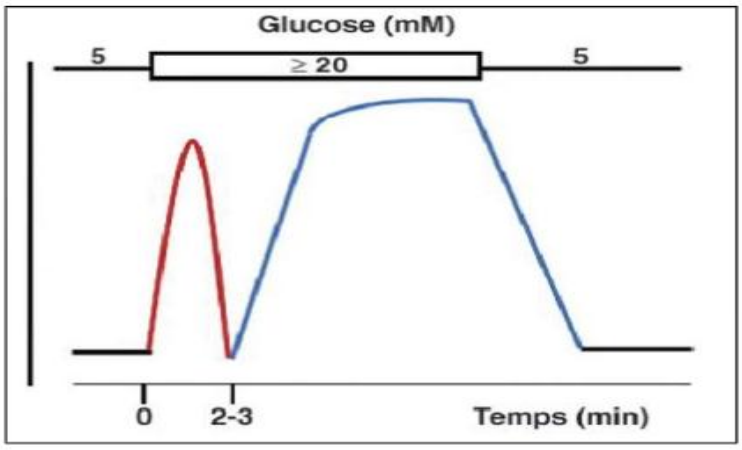

Figure 01: Biphasic secretion of insulin in response to a constant stimulus in glucose **(Magnan and Ktorza, 2005)**

C. Insulin sensitivity disorders

The reduction in the effects of insulin on insulin-sensitive tissues (muscle tissues, tissues adipose, liver) causes hyperproduction of glucose by the liver, explaining hyperglycemia in fasting and some of the interprandial hyperglycemia **(Henri, 2011)**. Insulin resistance is therefore characterizable at the level of peripheral tissues, in particular, the transport of glucose in muscle and adipose tissue and hepatic glucose production. This insulin resistance is aggravated by hyperglycemia and excess circulating free fatty acids or triglycerides stored in excess in the muscle. Excess hepatic glucose production is also increased by high levels of circulating fatty acids **(Halimi, 2006)**.

I.3. Treatment of diabetes mellitus

I.3.1. Treatment of diabetes mellitus with insulin and oral antidiabetics

Treatment of diabetes mellitus depends on the type of diabetes and the degree of insulin deficiency. For the treatment of type 1 diabetes, insulin remains the most effective and most available means of obtaining normal, well-regulated blood sugar levels. The role of insulin administered to the patient in a strictly controlled manner consists of replacing the body's own insulin, the main expected effects on metabolic homeostasis are; stimulation of peripheral glucose use and activation of glycogenesis, glycolysis, lipogenesis and protein synthesis. In addition, insulin also attempts to inhibit gluconeogenesis and lipolysis **(Kelley et *al*., 1990; Bailey, 1999)**.

Type 2 diabetes can be controlled in the early stages of the disease through diet and physical activity, but if normal blood sugar levels are not achieved, a prescription for oral antidiabetics is mandatory, recourse to Insulin may also be necessary **(Koski, 2006)**.

Oral agents available for the treatment of type 2 diabetes can be classified into five pharmacological classes. Sulfonylureas, benzoic acid derivatives (hypoglycemic agents), biguanides, α-glucosidase inhibitors, and thiazolidinediones (antihyperglycemic agents) **(Harrigan et *al*., 2001)**. Also note the development of new insulin secretagogues, GLP-1 analogues and DDP-IV inhibitors **(Ahrén, 2007)**. The main points of difference between these pharmacological classes are: mechanism of action, toxicity and side effects.

I.3.2. Treatment of diabetes mellitus with medicinal plants

According to the WHO report, 80% of the world's population uses medicinal plants to treat various diseases. This remarkably high rate can be explained by the therapeutic effectiveness of these natural remedies proven within the population, and also by their availability and their low cost. This very old medical practice, which is based on the use of plant extracts and natural active ingredients, is known as phytotherapy **(Schlienger, 2014)**.

One of the remarkable applications of herbal medicine is the use of medicinal plants for the treatment of diabetes mellitus. Thus several plants are used by the population to maintain blood glucose levels within normal limits. This practice has fascinated researchers to undertake experiments in order to understand the mechanism of action of these natural remedies and to derive the active ingredients.

I.4. Oxidative stress

Oxidative stress has been defined as a disturbance in the antioxidants/oxidants balance in favor of oxidants (**Kirschvink et *al*., 2008; Abdal Dayem et *al*., 2010; Mühl et *al*., 2011**). This imbalance occurs by increasing the production of oxidants, decreasing the level of antioxidants or both **(Mühl et *al*., 2011; Kirschvink et *al*., 2008)**. Oxidative stress may cause cellular damages in macromolecules like proteins, lipids and nucleic acids, leading to cellular pathology and ultimately to cell death **(zadak et *al*., 2009; Abdal Dayem et *al*., 2010)**.

Oxidative stress plays an important role in the development and progression of many human pathologies including cardiovascular and neurodegenerative disorders (Alzheimer's and Parkinson's diseases) (**Pham-Huy et *al*., 2008**), atherosclerosis, cancer, diabetes, liver damages, rheumatoid arthritis, cataracts, inflammatory bowel disease, ulcers, pneumonia and human aging **(Makker et *al*., 2009)**.

I.4.1. Free radicals

Free radicals are defined as atoms, molecules or parts of molecules containing one or more unpaired electrons in their outer orbits. They are characterized by a very short half- life and a considerable degree of reactivity **(Valko et *al*., 2006)**.

Free radicals derivatives of oxygen like superoxide free radical anion ($O2^{\bullet -}$), hydroxyl free radical ($OH^{\bullet}$), nitric oxide radical ($NO^{\bullet}$), lipid alkoxyl ($LOO^{\bullet}$) and lipid peroxide (LOOH) as well as non-radical derivatives such as hydrogen peroxide (H_2O_2) and singlet oxygen ($1O_2$) are collectively known as reactive oxygen species (ROS). Whereas, reactive nitrogen species (RNS) include free radicals like nitric oxide ($NO^{\bullet}$) and nitrogen dioxide ($NO_2^{\bullet}$), as well as non-radicals such as peroxynitrite (ONOO-)**(Kalam et al., 2012)**.

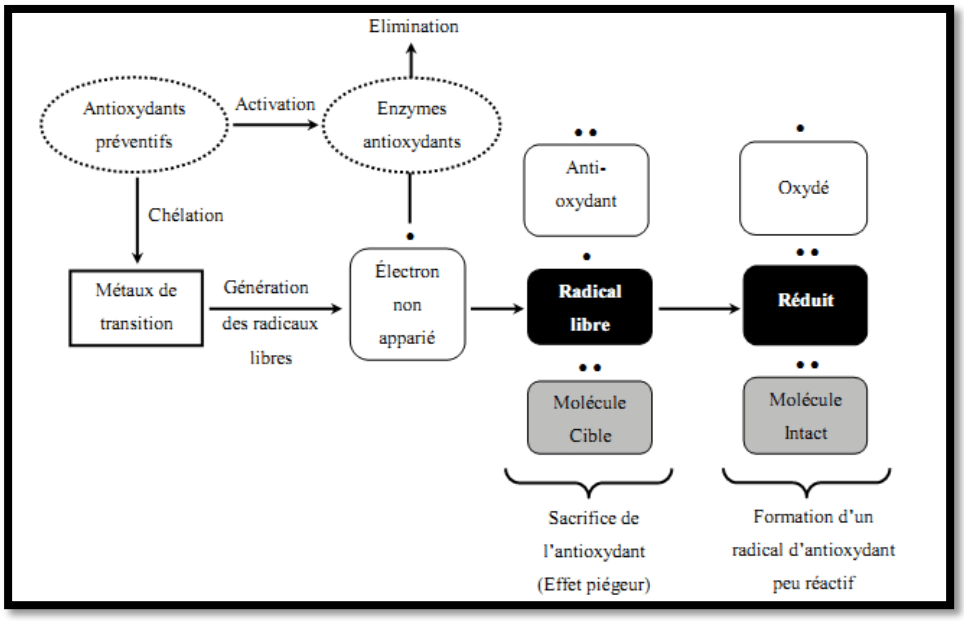

Figure 02: Mechanisms of action of antioxidants **(Kalam et *al.*, 2012)**

ROS are known to play an important role in biological systems, since they can be either harmful or beneficial to living systems. Beneficial effects of ROS involve physiological roles in defense against infections agents and the function of a number of cellular signaling systems. In contrast, at high concentrations, ROS can be important mediators of damage to cell structures, including lipids, proteins and nucleic acids. The harmful effects of ROS are balanced by the action of antioxidants (**Palmieri and Sblendorio, 2007**; **Salwa et *al.*, 2013**; **Rahman, 2007)**.

I.4.2. Antioxidant activity

Antioxidants are a loosely characterized group of compounds that are defined by their general ability to decrease or delay oxidation. Dietary antioxidants are recognized to have the ability to inhibit the formation of both reactive oxygen species (ROS) and reactive nitrogen species (RNS), which can adversely affect normal cellular processes and physiological functions **(Seifried and Pilch, 2013)**. Many herbal plants contain antioxidants. Most of the diseases such as tuberculosis are as a result of oxidative stress, which produces reactive oxygen species such as peroxyl ($^{\bullet}$OOH, ROO$^{\bullet}$), Hydroxyl (OH$^{\bullet}$) and superoxide ($O^{\bullet}_2$) radicals **(Ansari et *al.*, 2013)**. These reactive oxygen species are the ones that cause wide range of diseases.

Natural antioxidants have been shown to possess natural bioactive compounds that prevent these diseases. Natural antioxidants such as; ascorbic acid, carotenoids, and phenolic compounds are known to inhibit lipid peroxidation. Free radicals and active oxygen species reacts through a reaction cycle and chelate heavy metal ions **(Lee et *al.*, 2007)**. There has been growing attention on safety and toxic effects of some synthetic antioxidants such as butylatedhydroxyanisole users **(Balakrishnan and Agrawal, 2014)**.

Antioxidant activity is determined by different methods: β-Carotene or bleaching assay, DPPH (2,2-Diphenyl-1-picrylhydrazyl) assay, ORAC (oxygen radical absorbance capacity), ABTS ({2,2' - azinobis-(3- ethyl-benzothiazoline-6- sulphonic acid)}) assay, FRAP (ferric reducing antioxidant power assay, Copper reduction assay **(Preeti et *al.*, 2016)**.

I.4.3. Synergistic effect

The interaction of two or more drugs to give a combined efficacy which is greater than the sum of their individual effect of the drugs is called a synergistic effect. The pharmaceutical science classified the synergistic property of medicines in two categories namely pharmaco-dynamic and pharmacokinetic synergy. The pharmaco-dynamic synergistic illustrates the two or more drugs that interact on the same receptors and produce higher therapeutic effects through their positive interactions. Moreover the pharmacokinetic synergistic expressed the interaction of two or more drugs leads to improved absorption, distribution, metabolism and elimination of drug causing increase in the concentration of drug in the body and enhance their therapeutic properties. It has been reported that the complex synergistic effects produced by mixing medicinal plants in complex polyherbal formulation increases the bioavailability of active components, promote therapeutic effects, and reduce toxicity **(Williamson, 2001; van Vuuren and Viljoen, 2011; Zhou et *al.*, 2016)**. Hence the combinations of two or more than two herbs retaining antidiabetic properties exhibit the synergistic antidiabetic potential.

I.4.4. Combination of medicinal plants

The polyherbal therapy is defined as an herb-herb combination, used in Ayurveda, Unani and Chinese systems of medicine. It is therapeutically used since many past years, but till date, scientific confirmation of their therapeutic benefits is missing. The therapeutic property of herb-herb combination is higher compared to a single herb. The western medicines and availability of sophisticated instruments have well developed the herb-herb combination dosage form with better efficacy and safety. Presently, the herb-herb combination therapies into certain formulas offered potential interaction effects and presented new confidence to the diabetes patient for their healing **(Aslam et *al.*, 2016)**.

I.4.5. Polyherbal formulation

The polyherbal formulation is defined as a formulation incorporating two or more than two herbs for the treatment of various diseases. The researchers reported that the single medicinal plants contained ample antioxidant substances like phenolic and flavonoids which are responsible for the management of diabetes. The polyherbal formulation controlled diabetes more significantly instead of the individual herb due to synergistic effect and minimum side effects of polyherbal formulation. **Table 1** illustrated list of antidiabetic polyherbal formulation published in the research paper. The various studies described that the polyherbal formulation produces synergistic and improved antidiabetic effect compared to the individual herb.

Table 1: Synergetic antidiabetic polyherbal formulation for previous studies

Herbal ingredients	**Dosage form**	**Experimental protocol**	**Mechanism of action**	**Reference**
Bacopa monnieri*, *Hippophae rhamnoides*, and *Dioscorea bulbifera	Powdered	Streptozotocin induced diabetes rat	Polyherbal formulation significantly improved cognitive deficits, increased body weight and lowered plasma glucose levels, and suggested that the bacosides, quercetin, and diosgenin present in formulation imparts the activity	**(Upadhyay et *al*., 2015)**
Luffa acutangula* and *Madhuca longifolia	Tablets	Alloxan induced diabetes rat	Tablets exhibited antidiabetic activity due to their antioxidant	**(Singh et *al*., 2014)**

			property	
Catharanthus roseus* and *Leucas linifolia	Ethanol and Aqueous extract Alloxan	induced diabetic rats	Hypoglycemic and antioxidant activities,	**(Manthena et *al*., 2017)**
Curcuma caesia, Evolvulus alsinoides, Citrullus lanatus, Gymnema sylvestre, Tinospora cordifolia, Withania coagulans* and *Caesalpinia bonduc	Suspension of extract	Alloxan induced diabetic rats	Antioxidant	**(Mahajan et *al*., 2018)**
Phyllanthus emblica* and *Annona squamosa	Aqueous extract	Streptozotocininduced diabetes rat	Polyherbal formulation exhibited significant antidiabetic effect by controlling the blood glucose level, and it is due to the presence of a phenolic component in the formulation	**(Chaudhuri et *al*., 2016)**

I.5. *Olea europaea* L.

Since ancient times, Mediterranean countries have cultivated *Olea europaea* L. to produce both oil and medicinal compounds. In the last decades, the positive effects of olive biophenols on human health have been scientifically demonstrated on quite a few occasions **(Tuck and Hayball, 2002; De Leonardis et *al*., 2008)**. The Olive (*Olea europaea* L.) is a small tree belonging to the family *Oleaceae*. It is native to tropical and warm temperate regions of the world and it are one of the oldest known cultivated plants. The olive tree is commercially essential in the Mediterranean region as an important source for the production of olive oil **(Boskou, 1996)**. The tree is usually distributed in the coastal regions of the eastern Mediterranean basin, the neighboring coastal areas of southeastern Europe, western Asia, Arabian Peninsula, India and Asia and northern Africa as well as northern Iran at the south end of the Caspian Sea **(Somova et *al*., 2003; Parvaiz et *al*., 2013)**.

Algeria is considered one of the richest countries in medicinal plants with over 3164 species **(Zatout et *al*., 2021)**. It is considered as the main Mediterranean countries whose climate is more suitable for the cultivation of these species **(FAOSTAT, 2021).**

I.5.1. Taxonomy

Table 2. Classification of *Olea europaea* L. **(Selin and Bilgin, 2017)**

Class	**Magnoliopsida**
Sub class	Asteridae
Order	Scrophulariales
Family	Oleaceae
Sub family	Oleoideae
Tribe	Oleeae
Genus	*Olea*
Species	*europaea* L.

Figure 03: *Olea europaea* L. (Original photo)

I.5.2. Botanical description

The olive is an evergreen tree. Its vegetative phase continues throughout the year with a reduction in activity during winter. As a native to the dry subtropical Mediterranean area, it adapts very well to extreme environmental and agricultural conditions, often living for centuries **(Chiappetta et *al*., 2012)**. The root system is extensive and very superficial, consisting mainly of adventitious roots that spread laterally near the surface. The trunk has smooth greyish-green bark until about the tenth year of age, after which it becomes knotty, contorted and furrowed with bark of a darker color. Plants that have lived for centuries can become very tall and wide. The trunk gives rise to branches and fronds which carry the buds that produce annual growth **(Baldoni et *al*., 2012)**. The fruits of the olive tree are small oval drupes called olives. The olive tree is unique among the 600 species of *Oleaceae* as the only plant to have fruit that can be used directly for food (table olives) or after processing (olive oil). Fruiting takes place over a period of two years. The size of the ripe drupe varies with cultivar and growing conditions and does not exceed 2-3cm in diameter. Fruits have a thin exocarp, a fleshy mesocarp consisting of parenchyma cells rich in oil (the quantity of which varies with cultivar and season) and a central woody endocarp. Olives are produced every second year through a phenomenon known as induction.

Annual development and endogenous metabolic factors determine the transformation of undifferentiated tissue into vegetative and/or reproductive buds In general, a year of low production is accompanied by high vegetative activity and vice versa **(Baldoni et *al*., 2012)**. Leaves grow from spring to autumn and are shed after two years. They are arranged in

opposite distichous whorls and have entire margins. They are leathery, elliptic or lanceolate, variably dark green above, shiny due to waxes and opaque silvery-grey below. High sensitivity to light causes a large difference in photosynthesis between external and inner leaves, less exposed to light. Leaves are roughly flat and 30-80 mm long, but their dimension varies in a given cultivar in relation to age of plant, vigour of branch and phase of development in the span of a vegetative season: leaves that form shortly before Summer vegetative arrest tend to remain small **(Baldoni et *al*., 2012; Bartolini et *al*., 1998)**.

I.5.3. Polyphenolic compounds in olive leaves

There are differences in level and type of phenolic compounds in *Olea europea* L. leaves, fruits and seeds. The chemical composition of olive leaves varies depending on several conditions such as origin, proportion of branches on the tree, storage conditions, climatic conditions, moisture content and degree of contamination with soil and oils **(Molina and Yáñez-Ruiz, 2008); Goldsmith et *al*., 2014; Talhaouiet *al*., 2015)**. There are also significant changes in the phenolic composition of fruits and leaves during the maturation period **(Morales et *al*., 1996; Giannakopoulou et *al*., 2011)**.

Five groups of phenolic compounds are principally present in olive leaves: oleuropeosides (oleuropein and verbascoside), flavones (luteolin-7-glucoside, apigenin7-glucoside, diosmetin-7-glucoside, luteolin and diosmetin), flavonols (rutin), flavan-3-ols (catechin) and substituted phenols (tyrosol, hydroxytyrosol, vanillin, vanillic acid and caffeic acid). Additionally, the dialdehydic deacetoxy forms of the secoiridoid compounds oleuropein and ligstroside, oleocanthal and oleacein can be found (**Pereira et *al*., 2006; Ferreira et *al*., 2007)**. Phenolic compounds of olive leaves such as rutin, luteolin 7-O-glucoside, luteolin 7-O-rutinoside, luteolin 4-O-glucoside, apigenin 7-O-glucoside and apigenin 7-O-rutinoside are also reported in the literature **(Andrikopoulo et *al*., 2002; Makris et *al*., 2007)**. The most abundant compound in olive leaves is oleuropein, followed by hydroxytyrosol, the flavone-7-glucosides of luteolin and apigenin and verbascoside **(Salah et *al*., 2012)**.

I.5.4. Biological effects of olive leaves

Olive leaves are used orally for stomach and intestinal diseases, and chewed as a mouth cleanser. Decoctions of the dried fruit and leaves are taken orally for diarrhea and to treat urinary tract infections. Hot water extracts of the fresh leaves taken orally to treat high blood pressure (hypertension) and to induce urination (diuresis). Hot water extract of the dried plant is taken orally for bronchial asthma. Olive tree leaves are well known for their beneficial effects on metabolism when used as a traditional herbal drug. These properties are attributed to the phenolic compounds of olive leaves **(Hollman et *al*., 1995; Briante et *al*., 2002; Zarzuelo et *al*., 1991)**.

Traditionally, olive tree leaves have been used as a folk remedy for combating fevers and other diseases, such as malaria (**Benavente-Garcia et al., 2000; Soler et *al*., 2000)**, relieve arrhythmia and prevent intestinal muscle spasms **(Benavente-Garcia et *al*., 2000)**. Olive leaf extracts have been recently marketed as dietary product (**Briante et *al*., 2002)**. Commercial products in the form of herbal teas or food supplements are available all over the world, as complete dried leaves, powder, extracts or tablets (**Tsimidou, 2010)**. It has been shown that encapsulation of olive leaf extracts with the aid of β-cyclodextrin increases the aqueous solubility of the polyphenolic residue from olive leaves (**Mourtzinos et *al*., 2007)**.

In recent years, there has been great interest in the health effects of various herbal teas. Olive leaf tea is one of the most common, traditional herbal teas used among Mediterranean people to cure certain diseases. For this reason, interest in the potential health benefits of olive leaves has increased among scientists in various fields. Several studies have shown that olive leaf extract exhibits a large spectrum of *in vitro* and *in vivo* properties, including antioxidant activity (**Castillo et *al*., 2010; Cabarkapa et *al*., 2014)** , radio-protective effects (**Castillo et *al*., 2010)**, anti-proliferative effect on leukemia cells by inducing apoptosis (**Salah et al., 2010; Abaza et *al*., 2007; Fareset *al.*, 2011; Samet et *al*., 2014)**, cytotoxic activity against human breast cancer cells (**Shaoping Fu et *al*., 2010; Taamalli et *al*., 2012)**, anti-HIV virus effect **(Lee-Huang et *al.*, 2003)**, antifungal activity **(Korukluoglu et *al*., 2006)**, gastroprotective activities (**Dekanski et *al*., 2009)**, attenuation of diabetic neuropathic pain (**Kaeidi et *al*., 20011)** and amelioration of gentamicin nephrotoxicity **(Tavafi et *al*., 2012)**. Several biological properties such as antioxidant, anti-atherosclerotic, hypoglycemic and cardio-protective effects of olive leaves have been reviewed in the literature **(Sedef and Karakaya, 2009; Tsimidou and Papoti, 2010**).

Olive leaf extracts help skin with the presence of flavonoids and oleanolic acid, which stimulate the components of the connective tissue and regularize the tissue thereby boosting the health of the skin protecting it from aging (**Sabry, 2014)**. Olive leaves, particularly *Olea europaea* L., are rich in phenolic compounds including flavones, flavonols, catechin and substituted phenols. The determination of polyphenols in plants is of great interest because of the natural antioxidant activity of these compounds **(Abaza et *al*., 2015)**.

I.6. Myrtle (*Myrtus Communis* L.)

Myrtle (*Myrtus Communis*), coming from the *Myrtle* family, is an evergreen, long-lived plant that can be described as a shrub or small tree, with a height of around 1.8-2.4 meters, small leaves and deep lines on its bark. The Latin words "*Myrtus*" and "*communis*", meaning myrtle, mean common plants that grow in groups. The fruits are pea-sized, spherical or oval, have hard kidney-shaped seeds and can be blue, black or white in color (**Özcan, 2017)**.

Myrtus communis is primarily found in the Mediterranean region and southwest Europe, thriving at elevations of up to 1000 m above sea level. In contrast, M. nivellei BATT. and TRAB. is an indigenous species of the central Sahara .Wild *M. communis* is abundant in coastal areas of numerous countries bordering the Mediterranean Sea, along with the Mediterranean islands. This plant serves as a rich source of essential oils obtained from its leaves, berries, and occasionally its flowers. These extracts are widely utilized in perfumery, cosmetics, culinary practices, pharmaceuticals, aromatherapy, and various industrial applications.

In Algeria, *M. communis* can be found in the North region and along the coasts of Algiers and Constantine. On the other hand, *M. nivellei* is restricted to the Sahara region, predominantly distributed in central areas such as Tefedest, Mouydir, and Tassili n'Ajjer, with fewer populations in the northern zone. Typically growing in scattered populations within rocky and sandy areas near underground water sources, *M. communis*, known as "rihan" or "raihan" in Arabic, or "Mersin" in Berber in Algeria, is highly esteemed for its widespread application in traditional medicine (**Bouzabata et *al*., 2016)**.

I.6.1. Taxonomy

Table 03. Classification of *Myrtus Communis* L. **(Quezel and Santa, 1963)**

Kingdom	**Plantae**
Phylum	Streptophyta
Class	Equisetopsida
Subclass	Magnoliidae
Order	Myrtales
Family	Myrtaceae
Genus	*Myrtus*
Species	*communis* L.

Figure 04 : *Myrtus Communis* L. (Original photo)

I.6.2. Botanical description

Myrtus L. is a minor genus in the *Myrtaceae* family, which includes around 100 genera and 3000 species found in temperate, tropical, and subtropical climates. *M. communis* is the sole *Myrtaceae* species native to Europe. It is found throughout the Mediterranean and Middle East, where it grows wild and is farmed. It is the only member of the genus found in the Northern Hemisphere. It is an evergreen shrub that grows to a height of 1–5 meters.

The oppositely arranged leaves are ovate-lanceolate, 2-5 cm long, coriaceous, glabrous, punctate-glandular, and whole. When crushed, they emit a lovely aromatic odor. From June to September, white, star-shaped flowers develop, each with five petals, five sepals, and a mass of tufted stamens. After the summer, subglobose to ellipsoid berries appear around November, turning bluish-black (or infrequently yellowish-white) when ripe.

The flowers are pollinated by insects, and they have a specific seed dissemination technique that involves birds, animals, and autumn and winter rains during the critical early stages of growth. These characteristics can enhance reproductive performance, including seedling establishment and survival in Mediterranean environments (**Özkan, 2009)**.

I.6.3. Phytochemical components

Fruits and leaves are the most studied parts of the myrtle in terms of phytochemical composition. Polyphenols are the key compounds, crucial for the plant's functions and human health due to their various biological activities. Total polyphenols, flavonoids, and anthocyanins in myrtle berries vary depending on ecotypes, geographic location, and extraction methods, ranging from 14.68 to 138.21 mg GAE/g, 52.03 to 158.94 mg/g, and 5.355 to 60.25 mg/g, respectively. Anthocyanins, particularly delphinidin 3-O-glucoside, petunidin 3-O-glucoside, malvidin 3-O-glucoside, and peonidin 3-O-glucoside, dominate the phytochemical composition of myrtle berries, contributing to their purple-dark color and health benefits like antioxidant and anti-inflammatory properties. Other compounds present in myrtle berries include cyanidin 3-O-glucoside, delphinidin–pentose, and petunidin–pentose.

In addition to polyphenols, myrtle berry extracts contain phenolic acids (such as gallic acid, caffeic acid, and syringic acid), flavanols (like (–)-epigallocatechin), flavanones (e.g., naringin), and flavonols (including myricetin, myricetin 3-O-galactoside, myricetin 3-O-rhamnoside, quercetin 3-glucoside, and quercetin 3-rhamnoside).

Myrtle leaf extracts are rich in tannins (such as eugeniflorin D2, tellimagrandin I and II, and oenothein B), flavonoids (including (+)-catechin, quercetin 3-O-rhamnoside, quercetin-galactoside-gallate, (–)-epigallocatechin, (–)-epigallocatechin 3-O-gallate, myricetin galactoside, myricetin 3-O-rhamnoside, myricetin arabinoside, and kaempferol-O-galloyl-hexoside), phenolic acids (like ellagic acid, gallic acid, syringic acid, t-ferulic acid, and caffeic acid), and gallomyrtucommulones (such as gallomyrtucommulone A and C)**(Giampieri et *al*., 2020)**.

I.6.4. Biological effects of *Myrtus communis* L.

All the aerials' parts, fruits, leaves, and essential oil are medicinal. The applications of myrtle are in food, spices, and traditional medicine. The decoction of myrtle aerial part was used as hypotensive, hypoglycemic, anti-inflammatory, and anti-diarrhea in the treatment of bleeding, conjunctivitis, epistaxis, peptic ulcers, palpitation, urethritis, hemorrhoids, headache, leukorrhea, excessive perspiration, skin diseases such as pulmonary treating cough, gastrointestinal disorders (i.e., peptic ulcers, diarrhea, and hemorrhoids), urinary diseases (i.e., urethritis), and skin ailments (i.e., reddened skin), as well as for inactivating microorganisms and wound healing **(Sumbul et *al*., 2010; Boudjelal et *al*., 2013; Aleksic and Knezevic 2013, Aleksic et *al*., 2014).** Fruits have a wide range of applications: as astringent, analgesic, antiseptic, carminative, demulcent, anti-inflammatory, diuretic, antidiabetic antiemetic, nephroprotective, homeostatic, and for stomach disorders. Leaves were applied as flavoring agent, astringent, antiseptic, blood purifier hypoglycemic, laxative, analgesic,

homeostatic, stimulant, and in the treatment of constipation and respiratory diseases **(Sumbul et *al*., 2012; Dellaoui et *al*., 2018)**.

Myrtle extracts have antibacterial properties by affecting cell wall permeability and membrane, disrupting essential membrane functions and inhibiting the growth of various bacteria strains. Myrtle essential oils inhibit the growth of different bacterial strains and fungi through the activity of monoterpenes and polyphenols. Two studies evaluated myrtle effects against Protozoa: one showed essential oils inhibited *Leishmania tropica* growth with IC_{50} of 8.4 and 11.6. Myrtle's antimicrobial potential warrants further investigation due to issues with synthetic drugs **(Giampieri et *al*., 2020)**.

I.7. Chih (*Artemisia herba alba* L.)

The *Artemisia* genus comprises over 500 species, commonly known as "Chih" in Algeria, thriving in arid and semi-arid climates with precipitation ranging from 0 to 50 cm. *Artemisia herba-alba*, a wild shrub found in steppes and arid Mediterranean regions (Morocco, Algeria, Egypt, etc.), is relatively abundant in the Iberian Peninsula, particularly in the east, southeast, and south of Spain. This extensive genus of medicinal plants houses more than 300 species recognized for their healing properties in arid and semi-arid regions.

A. herba-alba, a compact evergreen shrub, possesses physiological and root adaptations enabling it to thrive in arid regions, optimizing soil moisture retention. Its small leaves minimize surface area for evaporation. Typically reaching heights of up to 40 cm, this shrub features small, upright, rigid stems, with blooming commencing toward the end of summer.

A. herba-alba, a medicinal and aromatic dwarf shrub, grows wild in arid Mediterranean areas, extending to the northwestern Himalayas. Most prevalent in the Iberian Peninsula, it thrives predominantly in central Spain, spreading across the eastern, southeastern, and southern regions. This plant prospers in nitrogen and gypsum-rich substrates **(Mohamed et *al*., 2010)**.

I.7.1. Taxonomy

Table 04. Classification of *Artemisia herba alba* L. **(Gacem et *al*., 2020)**

Kingdom	**Plantae**
Division	Magnoliophyta
Class	Magnoltiopsida
Subclass	Magnoliidae
Order	Asterales
Family	Asteraceae
Genus	*Artemisia*
Species	*herba-alba* L.

Figure 05: *Artemisia herba alba* L. (Original photo)

I.7.2. Botanical description

Artemisia herba-alba is a herbaceous plant with woody and branched stems, 30-50 cm, very leafy with a thick stump. The leaves are small, sessile, pubescent and silver looking. The flowers are grouped in clusters, with very small (3/1.5 mm) and ovoid heads. The involucre is interlocking bracts, the external orbicular and pubescent. Thefloral receptacle is bare with 2 to 5 yellowish flowers per flower head all hermaphrodite. It is a low and always green woody plant. Its morphological and physiological characteristics make it a species well adapted to arid climatic conditions. The seasonal dimorphism of its foliage allows it to reduce the sweat surface and thus avoid water loss **(Mouchem, 2015).**

I.7.3. Phytochemical components

Biochemical studies have identified a total of 839 compounds from the various parts of fourteen *Artemisia* species. These species primarily consist of terpenoids, flavonoids, coumarins, caffeoylquinic acids, sterols, and acetylenes. The most abundant compounds in the genus Artemisia are hydrocarbon and oxygenated terpenes. These predominantly include acyclic monoterpenes (such as citronellol, myrcenol, linalool, artemisia ketone, Artemisia alcohol, etc.), monocyclic monoterpenes like p-menthanes (including menthol, α-terpinene, p-cymene, terpinen-4-ol, 1,8-Cineole piperitone, etc.), bicyclic monoterpenes such as camphanes (borneol, camphor, etc.), pinanes (α-pinene, myrtenol, myrtenal, 3-pinanol, etc.), thujanes (α-thujene, sabinene, sabina ketone, etc.), acyclic sesquiterpenes like farnesanes (farnesal, farnesol, etc.), monocyclic sesquiterpenes including bisabolanes (α-bisabolol, cis-lanceol, etc.), germacranes (germacrene A, germacrene B, germacrene C, germacrene D, etc.), elemanes (α-elemene, β- elemene, γ-elemene, δ-elemene, etc.), humulanes (α-Humulene, Humulene epoxideI, etc.), caryophyllanes (β-caryophyllene, γ-caryophyllene, etc.), bicyclic sesquiterpenes like eudesmanes (α-selinene, β-eudesmol, kongol, artemisin, etc.), cadinane (artemisinol, δ-Cadinene, γ-Cadinene, etc.), muurolanes (γ-muurolene, δ-Muurolene, etc.),

amorphanes (4,7(11)-Amorphadien-12-al, 4-Amorphen,3,11-diol, Arteannuin A, Arteannuin B, Arteannuin C, Arteannuin D, etc.), guaianes (α-guaiene, β-guaiene, γ-gurjunene, etc.), aromadendranes (α-Aromadendrene, globulol, etc.), tricyclic sesquiterpenes like cedranes (cedrol, cedryl acetate, etc.).

These species also contain higher terpenoids such as diterpenes (phytol, isophytol, etc.) and triterpenes (β-amyrin, α-amyrin, friedelin, etc.). The various classes of compounds mentioned here exhibit numerous pharmacological properties. For example, Limonene, being a monoterpene, has various medical and pharmaceutical uses, such as its anti-carcinogenic effects in liver tumor models and its application as a topical medication for dermal and sub-dermal injuries. Another monoterpene, p-Cymene, has demonstrated notable antioxidant and antimicrobial activity.

The next significant group of compounds comprises flavonoids (like apigenin, luteolin, chrysoeriol, cirsiliol, kaempferol, rhamnocitrin, quercetin, tamarixetin, mikanin, casticin, cirsilineol, eupatin, mearnsetin, chrysosplenol E, etc.) and flavonoid glycosides (such as kaempferol-3-O-glucoside, isorhamnetin 3-glucoside, etc.).

Artemisia also yields many other compounds such as cyclic and acyclic hydrocarbons, alkynes, lignans, cyaromatic acids, saturated and unsaturated fatty acids, alcohols, ketones, esters, among others. **(Koul, 2017).**

I.7.4. Biological effect of *Artemisia herba alba*

In the folk medicine many plants are used to control diabetes **(Eddouks et al., 2002; Tahraoui et *al.*, 2007; Almasad et *al.*, 2007; Ziyyat et *al.*, 1997)**. In Morocco, Artemisia herba alba locally called "chih" is used as flavoring for tea and is considered as an important medicinal plant used for the treatment of some diseases such as diabetes **(Twaij and Al-Badr, 1988; Abu-Irmaileh and Afifi, 2003; Jouad et *al.*, 2001; Hudaib et *al.*, 2008; Alzweiri et *al.*, 2011; Azaizeh et *al.*, 2003)**. A previous study has demonstrated that the aqueous extract of the aerial parts of *A. herba alba* caused a significant fall in plasma glucose levels in both normoglycemic and alloxanized rabbits **(Twaij and Al-Badr, 1988)**. In addition, another study has demonstrated that the *A. herba alba* extract had a clear preventive effect on the appearance and development of insulin resistance without affecting body weight **(Hamza et *al.*, 2010)**. This plant in the last study seems to act as antidiabetic by restoring insulin sensitivity. Furthermore, aerial parts of *A. herba alba* have been shown to act as an anti-diabetic agent and exerted a significant hypoglycaemic action **(Wang and Ng. 1999).**

Plants often contain wide variety of antioxidant molecules, such as phenolic acids, flavonoids and other natural antioxidants. Generation of free radicals may be, at least partially, the basis of many human diseases and conditions. Therefore, the antioxidant action of *A. herba alba* may explain its claimed usefulness in folk medicine. The yields and the aromatic essential oil which is rich in monoterpenes and sesquiterpene lactones widely distributed in this plant and possess anti-inflammatory and anticarcinogenic activities.

It has also shown that essential oils were found to have some antioxidant abilities for preventing the linoleic oxidation and to reduce DPPH and ABTS radicals. This effect seems to be due to the rich phenoic compounds **(Al-Mustafa and Al-Thumbat, 2008; Kadri et *al.*, 2011; Tawaha et *al.*, 2007)**.

I.8. Myrrha (*Commiphora myrrha* L.)

The species *Commiphora myrrha* found in the southern part of Arabia to the north-eastern part of Africa and the north-eastern part of Kenya is responsible for the production of the true myrrh. Other types of *Commiphora* species that produce resins are found in Sudan, south of Arabia, Eritrea, Kenya, Ethiopia, and Somalia **(Hanus et *al.*, 2005)**.

Myrrh, which has its origin from Arabia, is an extract that is produced by secretory tissues found on the bark of *Commiphora* species. *Commiphora* is from the family of *Burseraceae* which has more than 150 species of plant spread across subtropical and tropical regions, particularly north-east Africa, southern Arabia, and India **(Demissew, 1993)**. Species of the genus *Commiphora* are defined as small trees or shrubs having spinescent branches and bark that has pale-gray discharge or a reddish-brown resin. Resins produced by *Commiphora* have applications in fragrances, bouquet and as ointment in embalmment process. They are reported as having applications in traditional system of medicine for the management of all manner of ailments like wounds, ache, joint inflammation, fractures, parasitic infection, obesity, and gastrointestinal diseases **(Abdul-Ghani et al., 2009)**.

I.8.1. Taxonomy

Table 5. Classification of *Commiphora myrrha* L. **(Nees and Engler, 1883)**

Kingdom	**Plantae**
Phylum	Streptophyta
Class	Magnoliopsida
Order	Sapindales
Family	Burseraceae
Genus	*Commiphora*
Species	*myrrha* L.

Figure 06: *Commiphora myrrha* L. **(**Original photo**)**

I.8.2. Botanical description

Commiphora myrrha is a small, thorny tree up to 3 meters tall, with a broad trunk and gnarled, irregular branches ending in sharp thorns. The leaves are trifoliate, the petioles short. Its fruit is elongated and pyriform. This plant is present in Ethiopia, Somalia, Sudan and northeastern Arabia, with approximately 150 to 200 species listed in the pharmacopoeia. It belongs to the *Burseraceae* family.

Towards the end of August, white and green flowers appear, forming knots on the trunk of the tree. Myrrh is a resinous gum naturally secreted by the tree, but it requires an incision to be harvested. This oily and fragrant resin solidifies into white-yellow tears which turn red or brown as they dry, thus constituting the precious myrrh. The quality of myrrh is distinguished by a rough, dusty surface, semi-transparent, with white traces at the break. Good quality is characterized by a slightly sticky texture when broken, indicating a high essential oil content, with a characteristic, aromatic odor and flavor **(Batiha et *al*., 2023)**.

I.8.3. Phytochemical Components

Myrrh can be defined as an oleo-gum resin produced by different *Commiphora* species. It is constituted by 3–4% impurities, 7–17% volatile oils, 25–40% alcohol soluble resins, and 57–61% water soluble gum **(Massoud et *al*., 2001)**. Myrrh resin constituents soluble in alcohol are commiphorinic acids, commiphoric acids, commiferin, heerabomyrrhols, and heeraboresene **(Rao et *al*., 2001)**. Moreover, these resins have been reported to contain commiphorinic acid, α-, β-, and γ-commiphoric acids, commiferin, α- and β-herrab-omyyhols, cholestrerol, heerboresene, compesterol, kerto steroids, β-sitosterol, 3-epi-α-amyrin, and α-amyrone **(Rao et *al*., 2001)**.

The aromatic and transuding resins produced by the stem-bark of *Commiphora* species (Burseraceae family) are characterized as yellowish to reddish brown powder having a nicely bitter taste with a slight irritable balsamic smell (**Hanus et *al.*, 2005)**.

The major composition of terpenes in myrrh is furano-sesequiterpenoids with elemane, eudes-mane, guaiane, or runcates, structures **(Bisset and Wichtl, 1994)**. It has a peculiar odor resulting from furano-sesquiterpenes **(Bruneton, 1995)**, which is likely to be a significant constituent of pharmaceutical myrrh **(Wichtl, 1994)**.

I.8.4. Biological effect of *Myrrha*

Myrrh is recognized for its therapeutic properties since the ancient times and has been used extensively in the treatment of wounds, management of aches, inflammation of the joints, parasitic infections, obesity, and gastrointestinal diseases in the ancient Egypt **(Abdul-Ghani et al. 2009**).

The most studied and most frequently used species of *Commiphora* are *C. molmol, C. myrrha, C. mukul, and Commiphora opobalsamum*. These species resins have shown an array of pharmacological activities in treating wound, mouth ulcer, aches, fracture, stomach disorders, microbial infection, and inflammatory disease. In Unani medicine, gums are used as antiseptic, astringent, anthelmintic, carminative, emmenagogue, expectorant, and stomachic. They are also applied in combination with other drugs in preventive medicine against epidemic diseases and used ectopically in gouty and painful joints, and for the treatment of wounds. Sesquiterpenoids having an array of pharmacological properties were resin extracts menthofuran and furanoeudesma 1,3-dieneare known as major components in myrrh oil (**Sileshi et *al.*, 2023)**.

Myrrh extracts have been found to have antibacterial and antiviral effects on various virus strains, as well as inhibiting the growth of bacteria and virus strains. They have also shown potential in inhibiting the growth of bacteria and virus strains. Additionally, myrrh extracts have demonstrated antimicrobial potential on various bacteria and fungi, *as well as* neuroprotective activity on induced neuronal cell death. Acetylcholinesterase inhibitors, whether natural or synthetic, are commonly used as insecticides or nootropic drugs to improve memory in amnesia patients.

Commiphora myrrha has been found to inhibit AchE and has been used in traditional medicine for diabetes management. Myrrh's analgesic properties derive from bioactive compounds that act as pain relievers. Sesquiterpenoids in myrrh act on opioid receptors, providing anesthetic effects. These compounds alleviate nerve pain and improve hyperalgesia. Myrrh extracts can be used as a substitute for nerve pain management **(Batiha et *al.*, 2023)**.

Chapter II

II. Plant materials

The aerial part of the *Myrtus Communis* L. and *Olea europaea* L., *Artemisia herba alba* L. in addition to myrrha *Commiphora myrrha* L., were purchased on March 16, 2024 from the popular market in El-oued. Algeria, the plant components were cleaned well from impurities and ground into a fine powder using an electric grinding machine. The powder of all plants was stored at room temperature in a glass jar individually and tightly closed until the beginning of the experiment.

II.1. Qualitative phytochemical Screening

A systematic and complete study of crude drugs should provide a thorough picture of both primary and secondary metabolites derived as a result of plant metabolism **(Sofawora 1982).** Different qualitative and quantitative phytochemical tests are to be performed for establishing profile of a given extract for its nature of chemical composition **(Tona et *al.*, 1998; Brinda et *al.*, 1981).** The following tests were carried out on the extracts to detect various phytoconstituents present in them.

II.1.1. Preparation of methanol, diethylether and aqueous extracts

About 20 g each of the powdered of the plant was macerated three times with 60 mL of diethyl ether for 15 min. Theextracts were filtered using Whatman filter paper and concentrated to 25 mL. The filtrates were labelled appropriately asdiethyl ether extract. The marc of each part was then macerated in methanol two times using the same above protocol. The obtainedextracts were labelled as methanol extract.Another 5 g of each plant material was extracted by infusion in 50 mL of distilled water. After shaking for 15 min, the extracts were filtered through Whatman filter paper and labelled as water extract. The three extracts were used for thephytochemical screening **(Vaghasiya and Chanda, 2007)**.

Alkaloids

10 mL of the etheric extract was evaporated then dissolve the residue obtained in 1.5 mL of 2% HCl. Then, 1 to 3 drops of Mayer's reagent were added to the alkaline aqueous solution. The formation of a yellowish-white precipitate indicates the presence of basic alkaloids **(Dohou et *al.*, 2003)**.

Emodols

Evaporate 3 mL of the etheric extract, and then add 1 mL of NH_4OH. The appearance of a bright color varying from orange-red to purple-purple indicates the presence of emodols **(Bruneton, 2009)**.

Coumarins

Evaporate 3 mL of the etheric extract; then treat the residue with 1 mL of hot water. Divide the volume in half, 0.5 mL is treated with 10% NH_4OH and the remainder serves as a control. The appearance of intense fluorescence at UV 265 nm or UV 365 nm indicates the presence of coumarins **(Bouhadjera, 2005)**.

Sterols or triterpenes

Evaporate 5 mL of the etheric extract, and then dissolve the residue in a mixture of 1 mL of acetic anhydride and 1 mL of chloroform. The mixture is divided into two test tubes. One serving as a control and in the second, using a pipette, 1 to 2 mL of concentrated H_2SO_4 are added. At the contact zone of the two liquids, a brownish red or purple ring is formed, while the supernatant becomes green or purple; this reveals the presence of sterols or triterpenes **(Bruneton, 2009)**.

Terpenoids

Mix 5 mL of the etheric extract with 2 mL of chloroform and 3 mL of concentrated sulfuric acid. The appearance of a red-brown coloring at the interface layer indicates the presence of heterosidic triterpenes **(Khan et *al.*, 2011).**

Free quinones

A few drops of 0.1N NaOH were added to 10 mL of the extract studied. The presence of free quinones is confirmed if the aqueous phase turns yellow, red or purple **(Dohou, 2004)**.

Fatty acids

Evaporate 15 mL of the etheric solution, and then dissolve the residue obtained in ethanol. The ethanolic solution obtained was then concentrated to dryness and the residue was saponified by adding 10 mL of 2N KOH. Then extract the mixture with diethyl ether. After a second evaporation to dryness, obtaining a fatty residue indicates the presence of fatty acids **(Bougandoura, 2011)**.

II.1.2. Phytochemical screening of the methanolic extract

Alkaloids salts

Evaporate 20 mL of the methanolic extract, then add 5 mL of 10% HCl to the residue and heat the mixture in a water bath for 15 minutes. Filter the mixture and alkalize it with a few drops of a 10% NH_4OH solution to pH 9. Extract the solution with ethyl ether and then concentrate the mixture to dryness. Dissolve the residue in 2% HCl. The volume was divided into three test tubes: the first tube will serve as a control. On the other hand, treat the other two tubes with Mayer and Wagner reagents respectively. By adding Mayer's reagent, we obtain turbidity or a yellowish-white precipitate and for Wagner's, we obtain a brown turbidity or precipitate **(Memelink et *al.*, 2001)**.

Tannins

To 1 mL of the solution to be tested, add 2 mL of water and 2 to 3 drops of 2% $FeCl_3$ solution. Leave to rest for a few minutes. A positive test is revealed by the appearance of a dark blue color (presence of gallic tannins) or a green coloring (presence of cathechy tannins) **(Bruneton, 2009)**.

Reducing compounds

To 1 mL of the aqueous solution, 2 mL of distilled water and 20 drops of Fehling liqueur were added; then the solution was heated. The appearance of a brick red precipitate indicates the presence of carbohydrates **(Togola, 2002)**.

Flavonoids

Flavonoids are extractable with alcohol or hot water; they are poorly soluble in cold water. To do this, treat 5 mL of the methanolic extract with a few drops of concentrated HCl and 0.5 g of magnesium turnings then leave to act for a few minutes. The presence of flavonoids is demonstrated if a pink, red or purple color develops within 3 min **(Soulama et *al.*, 2014)**.

Sterolic and triterpene glycosides

Evaporate 10 mL of the methanolic extract then treat the residue obtained with 0.5 mL of chloroform and 0.5 mL of acetic anhydride. Then divide the mixture into two test tubes: one will serve as a control and in the bottom of the second and using a Pasteur pipette, add 1 to 2 mL of concentrated H_2SO_4. At the contact zone of the two liquids, a brownish red or purple ring is formed and the supernatant becomes green (presence of sterolic glycosides) or purple (presence of triterpene glycosides) **(Bruneton, 2009)**.

II.1.3. Phytochemical screening of the aqueous extract

Saponosides

In a 250 mL volumetric flask, put 2 g of leaf powder and 100 mL of distilled water, and then make the mixture under decoctionprocess for 30 min. After filtration and cooling, the volume of filtrate was adjusted to 100 mL of distilled water. From this stock solution, 10 tubes were prepared at a rate of 1, 2, 3,… and 10 mL per tube respectively. The final volume of each tube was readjusted to 10 mL with distilled water. Each of these tubes is vigorously shaken in a horizontal position for 15 seconds. After resting for 15 minutes in a vertical position, the height of the persistent foam was measured in cm **(N'Guessan et *al.*, 2009).**

Starch

5 mL of the aqueous extract was placed in a test tube and then treated with the starch reagent. The appearance of a purplish blue color indicates the presence of starch **(Benmehdi, 2000)**.

II.1.4. Preparation of the methanolic extract

About 20 g of powdered root material was macerated with 200 mL of methanol for 48 h. The methanol extract was filtered using whatman filter paper and then concentrated under vacuum at 40 °C using a rotary evaporator. Finally, crude extract was obtained. Later this was stored at 4 °C prior to antioxidant test **(Motamed and Naghibi, 2010)**.

II.1.5. Preparation of the aqueous extract

A quantity of 20 g of the plant material is decocted in 200 mL of distilled water for half an hour at 60°C. After filtration, the recovered solution is dried at 40°C in an oven. The dry residue obtained is stored at 4°C until used **(Boubacar, 2005)**.

II.1.6. Quantitative phytochemical analysis

II.1.6.1. Determination of total phenol content

The amount of total phenol content of methanolic and aqueous extracts was determined by Folin-Ciocalteu's reagent method as described by **Singleton and Rossi (1965)**. 0.2 mL of extract mixed with1 mL of Folin-Ciocalteu's reagent (diluted 10 times), then we add 0.8 mL sodium carbonate (7.5%). The mixture was incubated at room temperature for 30 min. The absorbance was measured at 765 nm using a spectrophotometer UV visible, against a blank sample. Total phenolic content is expressed in terms of gallic acid equivalent (mg/g of extracted compounds).

II.1.6.2. Determination of flavonoid content

The amount of flavonoid contents of extracts tested was determined by Aluminium chloride colorimetric method (**Zhishen et *al.*, 1999).** The reaction mixture consisted of 500 µL sample, 1.5 mL distillated water, 150 µL nitrite sodium (5%), after five minutes 150 µL aluminium chloride (10%) was added. The solution mixture was incubated at room temperature for 6 min then we add 500 uL of sodium hydroxide (NaOH, 1M). The absorbance was measured at 510 nm using a spectrophotometer UV visible, against a blank sample. The flavonoid content is expressed in terms of quercetin equivalent (mg/g of extracted compounds).

II.1.6.3. Determination of condensed tannin contents

The tannin content was measured using a colorimetric assay **(Julkunen-Titto, 1985)**. An aliquot (500 µL) of plant extract or standard solution of catechin was mixed with 1.5 mL of 4% vanilline methanol solution and 750 µL of concentrated hydrochloric acid, and the mixture was allowed to stand for 20 minutes. Absorbance was read at 550 nm against the blank. Tannin content was calculated as mg CAT eq/g DW, using a catechin calibration curve.

II.1.6.4. Determination of flavonols contents

Total flavonols contents of different extracts were determined by method described by (**Almaraz-Abarca et *al*., 2007)**. The reaction mixture consisting of 0.5 mL of the sample, 0.5 of aluminium chloride prepared in (2%) and 1.5 mL of sodium acetate solution (50 g/L) was allowed to incubate for 2 h at 20 °C. Absorbance at 440 nm was measured. Total flavonol content was calculated as mg eq/g of quercetin equivalent from the calibration curve.

II.2. *In vitro* antioxidant assays

II.2.1. DPPH assay on TLC

Five microliter of standards, methanolic and aqueous extracts was applied to the TLC plate. The plate was sprayed with a 0.2% DPPH reagent in methanol and left at room temperature for 30 min. As explained above, yellow spots formed from bleaching of the purple color of DPPH reagent, were evaluated as positive antioxidant activity (**Sharififar et *al.*, 2007**).

II.2.2. DPPH radical scavenging activity

The radical scavenging activity using free-radical DPPH assay was carried out using the spectrophotometric method. 100 µL aliquot of each extract was added to 1.9 mL of a DPPH methanolic solution (0.04%). The mixture was vigorously shaken and left to stand in the dark for 30 min at room temperature. The antioxidant activity was then measured by the decrease in absorption at 517 nm using UV-Visible spectrophotometer and corresponds to the extract ability to reduce the radical DPPH to the yellow-colored Diphenylyldrazine. The Anti-radical activity was expressed as IC_{50} (µg/ml). The Anti-radical percentage inhibition calculated by the following equation:

$$DPPH\ Inhibition\ (\%) = \left[\frac{\mathrm{Ao} - \mathrm{A1}}{\mathrm{Ao}}\right] \times 100$$

Where Ao is the absorbance of control test after 30 min. A1 is the absorbance of the sample extract after 30 min **(Samarth et *al.*, 2008)**.

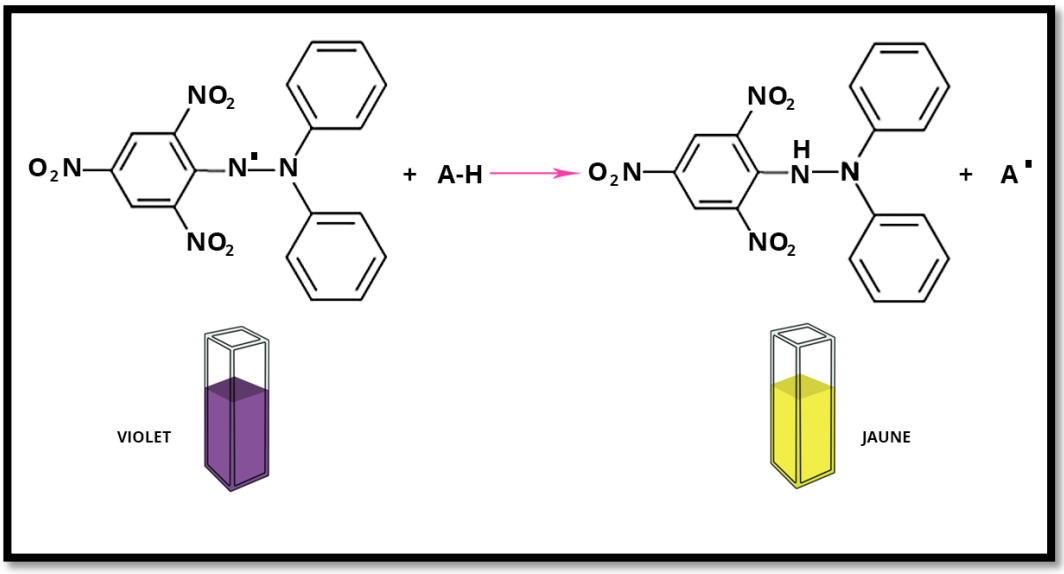

Figure 7: Reaction mechanism of 2,2-diphenyl-1-picrylhydrazyl (DPPH) with antioxidant **(Boligon et *al.*, 2014)**

II.2.3. Reducing power assay

The reducing capacity of fractions was determined using the method described by (**Karagôzler et *al.*, 2008)**. 1 mL of different concentrations diluted by distilled water was mixed with 2.5 ml phosphate buffer (0.2 M, pH 6.6) and 2.5 mL $K_3Fe(CN)_6$ 1%. The mixture was then incubated at 50 °C for 30 min. Thereafter, 2.5 mL of TCA 10% was added to the reaction mixture and then centrifuged for 10 min at 3,000 rpm. The upper layer of solution (2.5 mL) was mixed with 2.5 mL distilled water and 0.5 mL $FeCl_3$ (0.1%), and the absorbance was measured at 700 nm using spectrophotometer, against a blank sample. Ascorbic acid was used as positive control**.**

II.2.4. Phosphomolybdenum assay

The assay is based on the reduction of Mo (VI) - Mo (V) by the extract and subsequent formation of a green phosphate/Mo (V) complex at acidic pH. Different concentration of (0.3 ml) extracts was combined with 3 ml of reagent solution (0.6 M sulfuric acid, 28 mM sodium phosphate and 4 mM ammonium molybdate). The tubes containing the reaction solution were incubated at 95°C for 90 min. After the mixture had cooled to room temperature, the absorbance of each solution was measured at 695 nm against a blank. The antioxidant capacity was expressed as equivalent AA /g DW (**Prieto et *al.*, 1999)**.

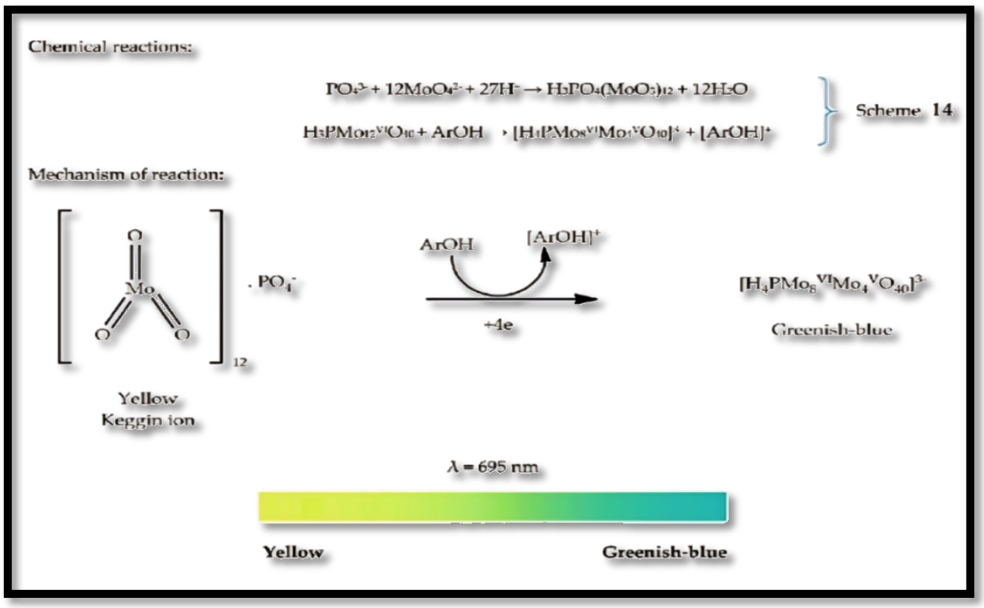

Figure 8: Reaction mechanism of Phosphomolybdenum assay

(Bibi Sadeer et *al.*, 2020)

II.2.5. Sun Factor Protection (SPF) test

The *in vitro* method measures the reduction of the irradiation by measuring transmittance after passing through a film of product. As in the operative conditions of the transmission measurement are correct, this to be a very precise and single value, always reproducible for the same product and expressed as a single UV curve, in the absorbance scale.

The crude methanolic, aqueous and extract ointment formulation were analyzed for the in vitro SPF. The samples were dissolved in methanol and ointment in ethanol. Scans of the samples in solution were run from 290 to 320 nm using 1 cm glass cuvettes in a UV-Visible spectrophotometer. The commercial sun-screens Venus, were used for the calculation of the correction factor. The SPF model used in this study was based on the following equation proposed by Mansur et al. **(Osterwalder and Herzog, 2009)**:

$$\mathbf{SPF} = \sum_{k=290}^{320} \mathrm{EE}\,\lambda \times \mathrm{I}(\lambda) \times \mathrm{abs}\,(\lambda)$$

where CF is correction factor (constant), EE (l) the erythemal efficiency spectrum; I (l) the solar simulator spectrum as measured with a calibrated spectrophotometer; where, 290-320 nm in 5 nm increments; abs (l) is the spectrophotometer measure of sunscreen product absorbance.

II.2.6. Preparation of ointment

The method followed of **Riyazullah et *al.*, (2021)** with modification. The four plants tested were crushed by an electric grinder until a fine powder was obtained. An ointment formulation of 8% was then prepared by adding 20 g of the leaves' powder to 250 mL of Olive oil heated until being melted 40°C in water bath for 2h mixed gently and then let it for 6 days in dark conditions. After filtration we add about 10 g of beeswax under heating till homogenization of the mixture. The prepared herbal ointments were put in ointment jars, labeled and were stored at room temperature.

II.2.7. Evaluation of ointment: The evaluations were carried out on the ointment by using the following parameters

- **Colour and odour:** Colour and odour of ointment, examine by visual examination.
- **Loss on drying**: 1 g of ointment was placed in the Petri dish and heated in the water bath at 105 °C every 30 min until it get constant weight.
- **pH:** The pH of ointment was determined by digital pH meter. 1 g of ointment was dissolved in 50 ml of distilled waterand the pH was measured.
- **Diffusion study**: The diffusion study was carried out by preparing agar nutrient medium of any concentration. It was poured into Petridis. A hole bored at the center and ointment was placed in it. The time taken for the ointment to get diffused was noted.
- **Stability study**: The stability studies are carried out for the prepared ointment at temperature of $37C^{o}$ for 2 months (**Bharskar and Siddheshwar, 2022)**.
- **Consistency:** Smooth and no greediness are observed **(Sakthivel et *al.*, 2023)**.
- **Non irritancy test**: The test is performed by applying the small amount sample to the hand and observed for 24 hours to check the effect like redness, erythema, inflammation etc. Hence, no such effect was observed, it is non irritant to the skin **(Mulla et *al.*, 2022)**.

Figure 9: Steps for preparing ointment

II.2.8. α-Amylase inhibition activity

The aqueous extract of mixture was tested for their antidiabetic activity by using α-amylase, inhibitory activity **(Marmouzi et *al.*, 2016)**. The α-amylase inhibitory was determined by reacting different concentrations of the aqueous extract (25 µL) with α-amylase enzyme in phosphate buffer (50 µL) and starch solution (50 µL). The reaction was stopped after 10 min by adding 25 µL of HCl (1 M) and 100 µL of iodine-potassium iodide solution at 37°C. Then, the absorbance was measured at 630 nm. Acarbose was used as a positive reference.

The inhibitory activity was estimated as follows:

$$Inhibition\ (\%) = 1 - \left(\frac{Absorbance\ of\ sample}{Absorbance\ of\ control}\right) \times 100$$

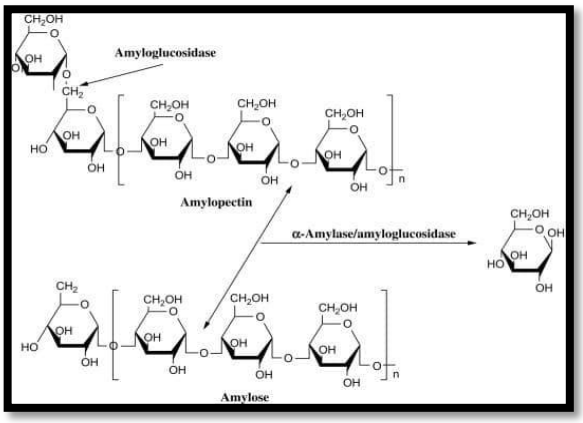

Figure 10: Reaction mechanism of α-Amylase **(Colin et *al.*, 2010)**

II.2.9. α-glucosidase inhibition activity

The aqueous extract was tested for their antidiabetic activity by using α-glucosidase inhibitory activity **(Marmouzi et *al.*, 2016).** The α-glucosidase inhibitory capacity of the extract was calculated using p-nitrophenyl-α-D-glucopyranoside (pNPG), as substrate. The mixture contained 50 µL of sample, 50 µL of glutathione and 50 µL of α-glucosidase solution in phosphate buffer (pH 6.8) and PNPG (50 µL) in a 96-well micro plate. The reaction was stopped after 15 min by adding 50 µL of sodium carbonate (0.2 M). The absorbance was read at 400 nm. Acarbose was used as a positive reference. The inhibitory activity was estimated as follows:

$$Inhibition\ (\%) = 1 - (\frac{Absorbance\ of\ sample}{Absorbance\ of\ control}) \times 100$$

II.3. *In vivo* study of anti-diabetic activity

II.3.1. Experimental Animals

A total of 48 male albino rats, weighing (153-228 g) acquired from Hamma Lakhdar University, the Faculty of Natural Sciences and Life, Algeria. Animals were housed in plastic cages at 25±02°C with a 12 hour day and night cycle with free access to standard pellet diet and water. The study was approved and conducted according to the guidelines of the Animal Ethics committee.

II.3.2. Acute toxicity study

Phase 1: This phase requires nine animals. The nine animals are divided into three groups of three animals each. Each group of animals are administered different doses (10, 100 and 1000 mg/kg) of test substance. The animals are placed under observation for 24 hours to monitor their behavior *as well as* if mortality will occur. The following **Figure 11** showed the nine animals after sacrifice.

Figure 11: Rats after sacrifice phase 1

Phase 2: This phase involves the use of three animals, which are distributed into three groups of one animal each. The animals are administered higher doses (1600, 2900 and 5000 mg/kg) of test substance and then observed for 24 hours for behavior *as well as* mortality and then sacrificed **(Figure 12).** Then the LD_{50} is calculated by the formula:

$$LD50 = \sqrt{\mathrm{D0} \times \mathrm{D100}}$$

D_0= Highest dose that gave no mortality, D_{100} = Lowest dose that produced mortality (**Lorken, 1983)**.

Figure 12: Rats after sacrifice phase 2

II.3.2. Diabetes induction

After period of adaptation for 15 days, the animals were fasted overnight (12 h) with free access to water, the induction of diabetes was achieved by administering alloxan monohydrate (98%) (Sigma-Aldrich, USA) *via* intraperitoneal injection at a dose of 150 mg/kg. Following the injection, the water bottles were replaced with bottles containing a 10% glucose solution for 24 hours. This was done to counteract the hypoglycemic effects caused by alloxan, which leads to the subsequent excessive insulin secretion. It is important to note that this severe drop in blood glucose levels can be fatal for the experimental subjects. Diabetes was confirmed after a period of 72 hours, and fasting blood glucose levels were measured using a blood glucose meter.

Figure 13: Intraperitoneal injection of rat by alloxan

II.3.3.Treatment animals

Once diabetes was induced, all the rats, whether diabetic or not, were divided into 6 groups of 5 rats each and kept in identical conditions. The treatment with the aqueous extract began 72 hours after confirming diabetes and lasted for 21 days. The chosen dosage was based on previous toxicity studies.

After stabilization of diabetes:

- **Group I (Negative control):** were serving as a control and received normal water throughout the experiment.
- **Group II (Positive control):** Were treated orally 50 mg/kg Body Weight/day of Metformin daily for 21 day.
- **Group III (diabetic group untreated):** were serving as a control and received normal water throughout the experiment.
- **Group IV (Treated group):** were treated orally 100 mg/kg of BW/day of aqueous extract daily, in addition to applying the ointment in their feet for 21 day.
- **Group 5 (Treated group):** were treated orally 300 mg/kg of BW/day of aqueous extract daily, in addition to applying the ointment in their feet for 21 day.
- **Group 6 (Treated group):** were treated orally 500 mg/kg of BW/day of aqueous extract daily, in addition to applying the ointment in their feet for 21 day.

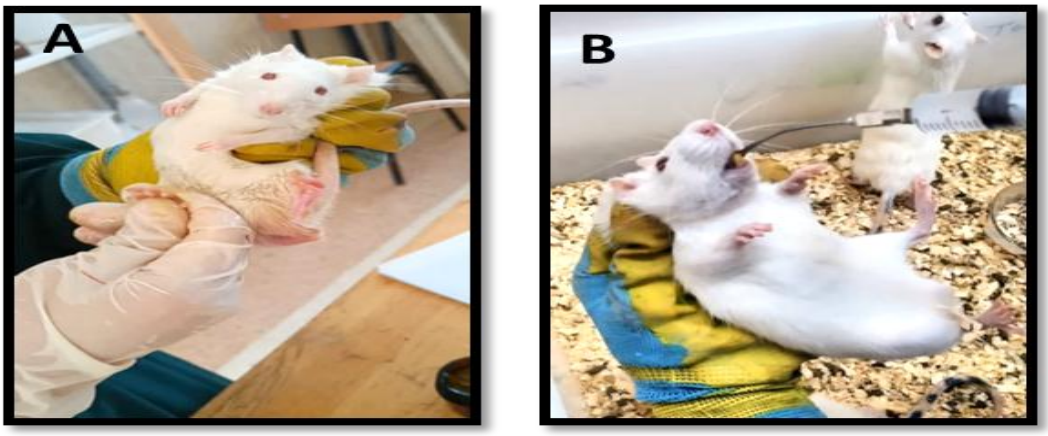

Figure 14: Massage of feet's rats (A) and Gavage of rats (B)

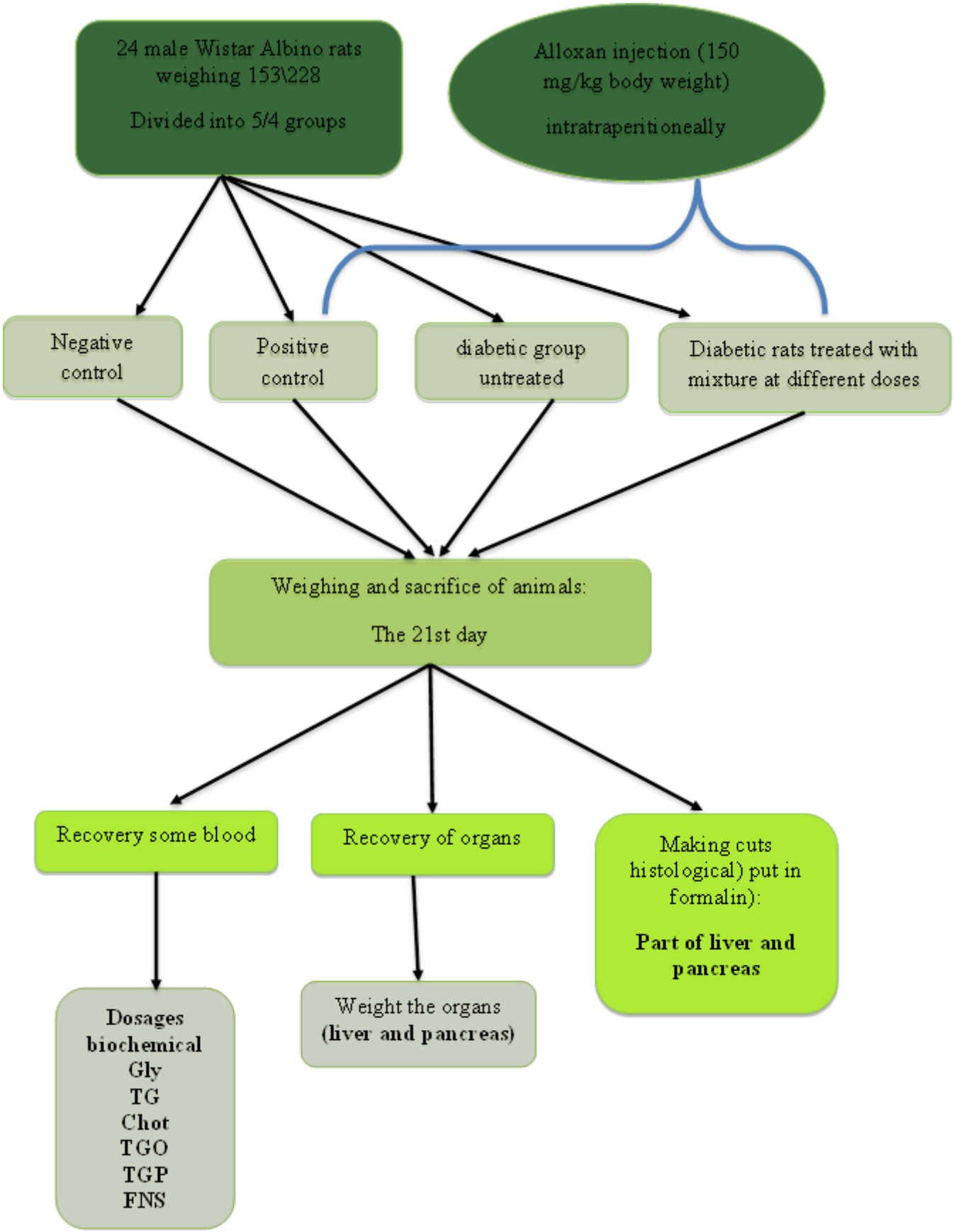

Figure 15: Experimental protocol of the study

II.4. Monitoring of animals during treatment

II.4.1. Body weight and glycemia measures

To monitor the animals during the treatment, we conducted regular measurements. We used an SF-400 scale to weigh the rats every 7 day.

We measured the rats' blood glucose levels every7 day. Blood samples were collected from the rats' tails after administering the extracts. We used aVital Check glucometer to determine the blood glucose levels, which were expressed in milligrams per deciliter (mg/dl).

II.4.2. Sacrifice, collection of blood sample and isolation of organs

At the end of the treatment, animals were fasted overnight, but allowed access to water ad libitum. They were subsequently anesthetized with chloroform and blood samples were obtained and collected in two tubes. The first one containing EDTA and it was processed immediately for hematological parameters analysis. The second containing heparin and it was centrifuged at 4000 g at 4°C for 15 min to obtain serum stored at −20°C until analysis of biochemical parameters. The organs (liver, Pancreas) were weighed and fixed in 10 % formalin for histopathological examination. The pathological samples were analyzed following the standard operating procedures for analyze measurement at 19 March Hospital, Eloued, Algeria.

Figure 16: Collect of blood after sacrifice

II.4.3. Determination of biochemical parameters

The different biochemical parameters are measured by the spectrophotometric method for the determination of parameters: cholesterol, Serum enzyme activities of aspartate amino-transaminase (ASAT), alanine amino-transaminase (ALT), according to the principles and the protocols assay kits.

II.4.4. Determination of hematological parameters

The blood count formula (FNS) was performed by an automatic hematology analyzer. The parameters determined are: red blood cell (RBC), white blood cell (WBC), hemoglobin (Hb), hematocrit (HT), lymphocytes (LY), mean corpuscular volume of red blood cells (MCV) and mean corpuscular concentration in hemoglobin (CCMH).

II.5. Assay of oxidative stress parameters

II.5.1. Preparation of organ homogenates

One gram of tissue (liver, Pancreas) from each organ of each group was used. After grinding and homogenization of the tissues in TBS (tris 50 Mm, Nacl 150 mM, pH 7.4) and by centrifuge of the tissue suspension (3900 rpm, 20 min), then the supernatant obtained is stored at -20°C while waiting for carry out the dosages of oxidative stress parameters.

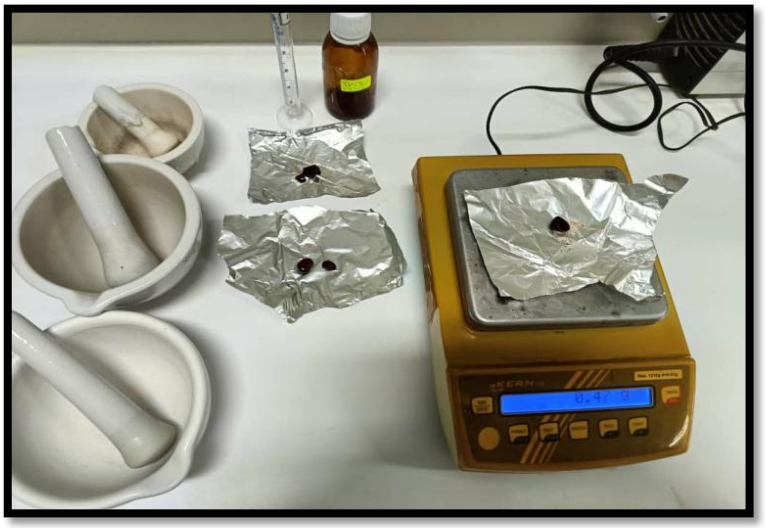

Figure 17: Weighting of organ's rat

II.5.2. Tissue protein assay

The tissue proteins were determined using a colorimetric method using Comassie blue as a reagent which is reacted with the amine group (NH_2) of the proteins to form a blue colored complex. (The appearance of the blue color reflects the degree of ionization of the acidic environment and the intensity corresponds to the protein concentration). Absorption is measured at 595 nm **(Bradford, 1976)**.

Take 1 mL of the homogenate, and then add 4 mL of Comassie blue Shake and after 5 minutes read the optical densities at 595 nm. The protein concentration is determined by comparison with a standard range of bovine serum albumin (20-100 µg) previously carried out under the same conditions.

II.5.3. Assay of tissue malondialdehyde (MDA)

The principle of this dosage is based on the condensation of MDA in an acidic medium and hot with thiobarbituric acid. The reaction results in the formation of a pink-colored complex between two molecules of thiobarbituric acid which can therefore be measured by absorption spectrophotometry at 532 nm **(Yagi, 1976)**.

Take 100 µL of homogenate and 400 µL of TBA reagent into the glass and screw test tubes, and close tightly. Heat the mixture in water bath at 100°C for 15 minutes. Then cool in a cold water bath for 30 minutes, leaving the tubes open to allow the gases formed during the reaction to escape. After centrifuge at 3000 rpm for 5 minutes and read the absorbance of the supernatant at 532 nm using a spectrophotometer.

The quantity of MDA in the sample is expressed in nM/gram of tissue.

$$\text{MDA (nmoles MDA/mg protein)} = \frac{\text{DO} \times 10^6}{\text{E} \times \text{L} \times \text{X} \times \text{Fd}}$$

DO: Optical density read at 530 nm;

E: Molar extinction coefficient of MDA (E = 1.56×105 M^{-1} cm^{-1});

X: Protein concentration (mg/mL);

L: Optical path length L: 1cm;

Fd : Dilution factor.

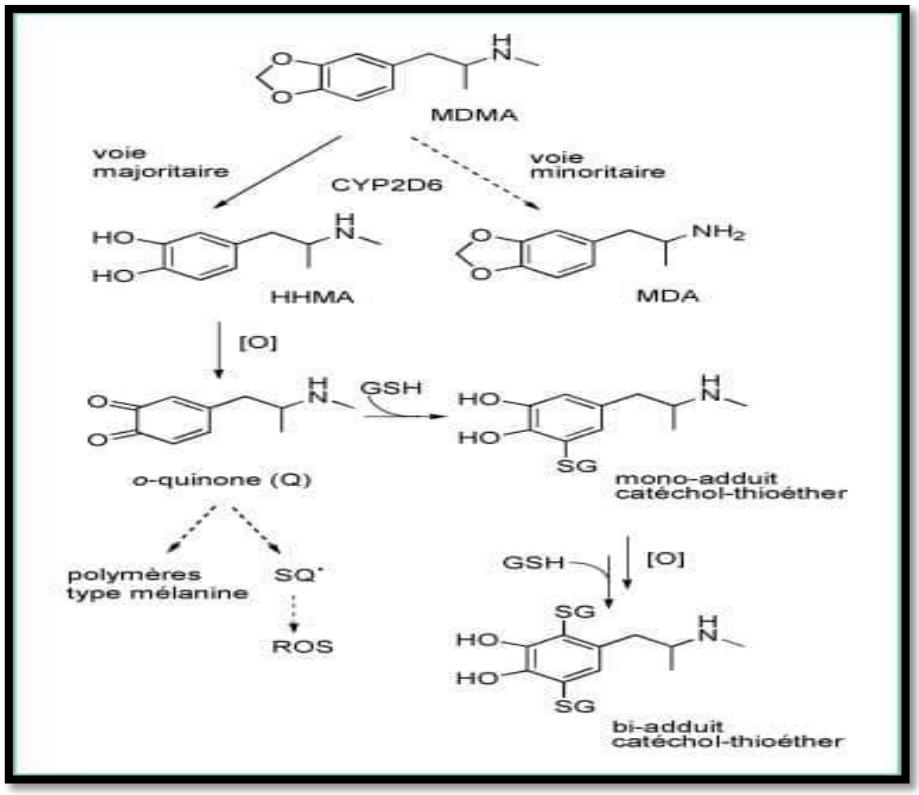

Figure 18: Reaction mechanism of malondialdehyde **(Fleury et *al.*, 2009)**

II.5.4. Determination of reduced glutathione (GSH)

Reduced glutathione was determined by the method of **Wekbeker et Cory, (1988)**.The assay is based on the oxidation of GSH by 5,5'-dithiobis(2-nitrobenzoic acid) (DTNB), DTNB and glutathione (GSH) react to generate 2-nitro-5-thiobenzoic acid (TNB) which has yellow color. Therefore, GSH concentration can be determined by measuring absorbance at 412 nm. According to the following reaction **(Figure 19)**:

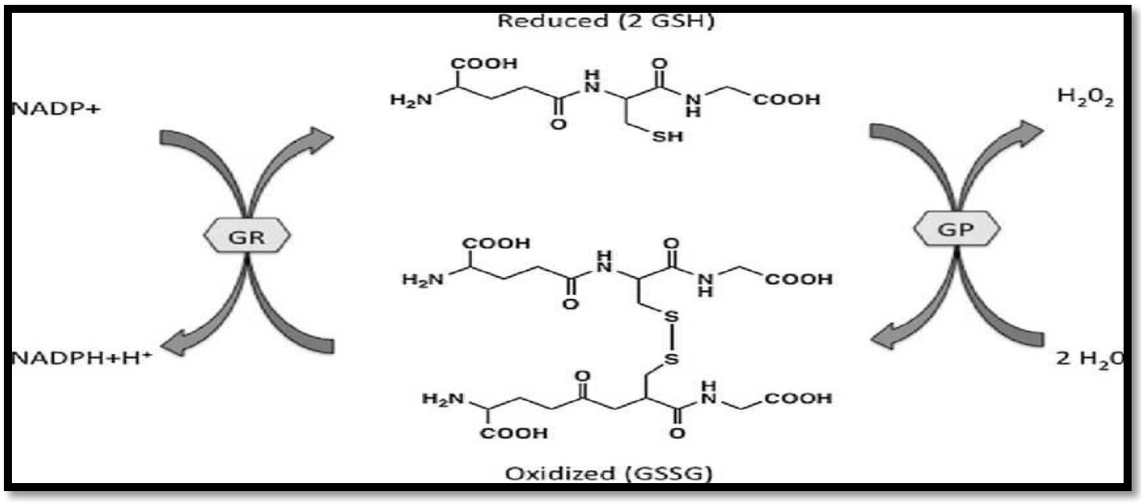

Figure 19: Reaction mechanism of reduced glutathione **(Ying et *al*., 2011)**

For this assay, 50 µL of the tissue homogenate was diluted in 10 mL of phosphate buffer (0.1 M, pH 8). To 3 mL of the mixture of dilution, 20 µL of DTNB (0.01 M) was added. Absorbance is read at 412 nm against a blank prepared under the same conditions. The concentrations of GSH are expressed in µmol/g of the tissue were obtained from calibration curve of GSH realized in the same condition.

II.5.5. Glutathione peroxidase (GPX) enzymatic activity

GSH-Px activity is measured using the technique of Floche and Gunzler (1984). This method is based on the reduction of hydrogen peroxide (H_2O_2) in the presence of reduced glutathione (GSH). The latter is transformed into (GSSG) under the influence of GPx according to the following reaction: H_2O_2+2 GSH ‹ GSSG + 2H_2O

Take 0.2 mL of the homogenate and add 0.4 mL of GSH (0.1 mM); 0.2 mL of TBS buffer solution (50 mM Tris, 150 mM NaCl, pH 7.4). Incubate in a water bath at 25°C for 5 min then add 0.2 mL of H_2O_2 (1.3 mM) to initiate the reaction, leave to act for 1 minute; Add 1 mL of TCA (1%) to stop the reaction, put the mixture in ice for 30 minutes; after centrifugation for 10 minutes at 3000 rpm take 0.48 mL of the supernatant mixed with 2.2 mL of TBS buffer solution then add 0.32 mL of DTNB (1.0 mM), Mix the solution and after 5 minutes read the optical densities at 412 nm.

The enzymatic activity of GPx is expressed in nano moles of GSH oxidized per milligram of proteins (nmol GSH/mg protein) according to the equation:

$$\text{GPx (µmol/mg prot.)} = \frac{(\text{DO sample} - \text{DO etalon}) \times 0{,}04}{\text{DO etalon}} + \frac{5}{\text{mg protein}}$$

- DO: Optical density read at 412 nm.
- 0.04: Substrate concentration (GSH).

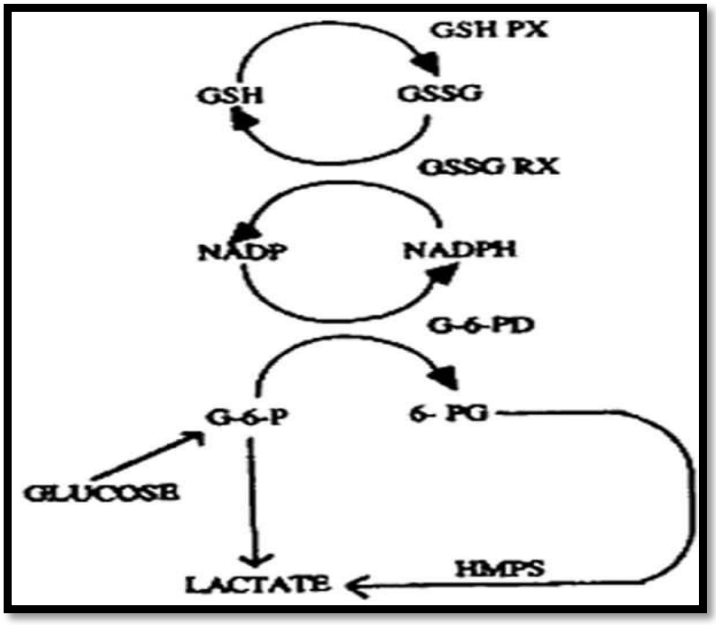

Figure 20: Reaction mechanism of reduced glutathione **(Gallo and Martino, 2009)**

II.5. Histological study

The histological studywas carried out at pedagogy laboratory of the biology department of Hamma Lakhdar University Eloued, Algeria. This study makes it possible to validate the possible antidiabetic activity of the aqueous extract tested or not, through the microscopic visualization of the livers and pancreas of the different groups and detect for each one the presence or absence of lesions. The part of each organ recovered in formalin intended for histological study was cut longitudinally to obtain segments.

II.5.1. Fixation

The organs tested were placed in cassettes, then fixed in formalin (10%) for 24 h. Place the cassettes in the basket and pass it through the cycle through the automate dehydration device which has 12 baths, this cycle is made up of three phases; dehydration phase, clarification phase and the inclusion phase.

II.5.2. Dehydration

Passage of the samples in eight ethanol baths of increasing concentration (from 70% to 100%) for 45 min for each bath. Passing the samples through the two baths of an intermediate liquid for 30 minutes for each bath, in order to eliminate traces of absolute ethanol. In this impregnation step xylene is used as an intermediate solvent favorable to membrane exchanges between ethanol-xylene on the one hand and xylene-paraffin on the other hand.

Figure 21: Dehydration by a dehydration machine

II.5.3. Inclusion and creation of blocks

Then samples were remained for 1h 30min in each latbaths, which allows the penetration of the paraffin inside the tissues (cells) due to the solubility of the paraffin in xylene, and this aims to give certain rigidity to the tissues in order to allow the production of thin sections with a thickness of 2 to 4 µm.

The inclusion was carried out in molds allowing the making of cassettes which are then insert on the microtome. The different segments were entirely immersed in hot paraffin (68°C – 70°C) then frozen at a temperature varying between 5.8°C and 6.1°C.

Figure 22: The stages of coating (blocking)

The sections were then made with a thickness of 3 to 5 µm on the microtome and transferred to a water bath at 50°C. Sections were spread on slides and left on a hot plate at 52°C for approximately 10 min. Thus, the slides were placed in slide holders and heated in the oven to a temperature of 78°C for 2 to 3 h.

Figure 23: Cut of slides

The slides are placed in an oven set at a temperature of 74°C for 24 hours, in order to eliminate peripheral paraffin. They are then immersed in a xylene bath for 30 min, which allows the elimination of intracellular paraffin.

Figure 24: Placement of the slides in the oven at 60°C

II.5.4. Coloration

The slides are embedded in ethanol for 10 min and then in water, latter penetrates inside the cells which encourage the dye itself to penetrate the cell membranes. After rinsing, the rehydrated sections were placed in a hematoxylin bath for 2 min to stain the nuclei. Excess dye is removed by rinsing with water. They were then put in an eosin bath for 30 seconds to color the cytoplasm, then rinsed with water and put in an alcohol bath (3 min) to remove excess dye.

The sections were then incubated in a xylene bath (15 min) to remove the alcohol and clean the slides. After staining, the slides were covered with coverslips using a Eukit (assembly step), then left in the open air for drying. After drying, they were cleaned with xylene to remove excess Eukit. Thus the slides are ready for microscopic observation using an optical microscope (OPTIKA) at different magnifications G (×4; ×10; ×40) with integrated camera.

Figure 25: The steps of hematoxylene eosin staining

Statistical analysis

The results are expressed as the mean 3 values ± standard deviation. One-way analysis of variance (ANOVA) followed by the Tukey test was performed to assess differences between groups. Statistical analyses were performed with the software Excel.

Chapter III

III. *In vitro* study

III.1. Extraction yield

Secondary metabolites were found in good proportion in methanolic extract when compared to aqueous extract. The maceration of the mixture powder with methanol was given a yield of 35.87%. Moreover, the decoction in distilled water of the mixture was giving a yield 34.8%. Yield of each extract are shown in **Figure 26**.

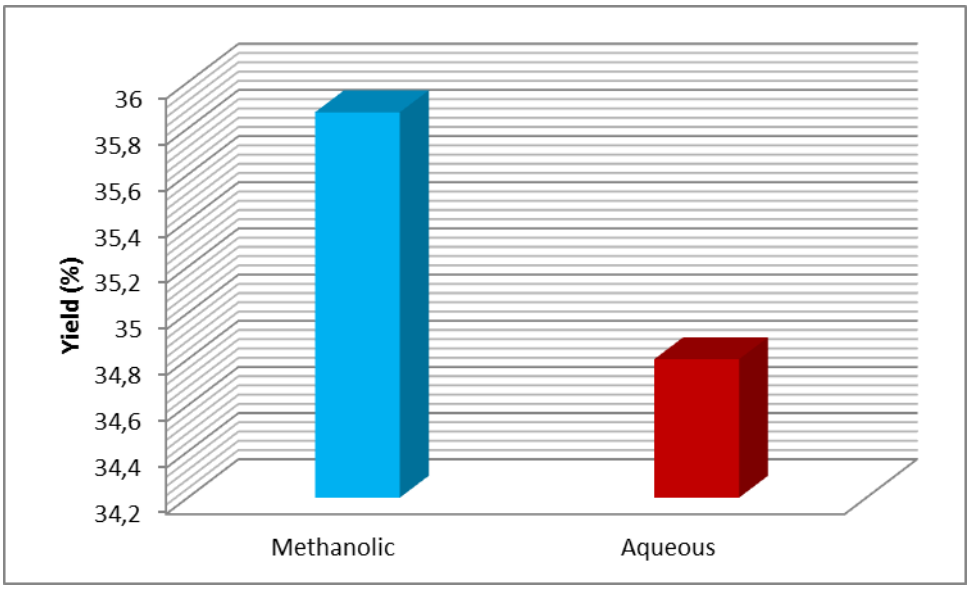

Figure 26: Yield of methanolic and aqueous extracts

The results of **Benmeziane et *al.*, (2023)** showed the methanolic and distilled water extracts of *Artemisia Herba Alba* from from Jordan, exhibited the same yield value with (24.4%). Whereas, 80% aqueous ethanolic extract exhibited higher extraction yield (30.6%) they explain the difference in extraction yield to the different organic solvents used.

The extract yield is affected by the solvent extraction and its polarity, pH, temperature, time, and composition of the sample. The combined use of water and organic solvent may enhance the extraction yield. Compounds such as proteins and carbohydrates other than phenolic compounds may be more solubilized in water/alcohol than in pure solvents **(Do et *al.*, 2014)**.

Also the previous study of **Belmimoun et *al.*, (2016)** show that the yield of decoction for aqueous extract of *M. communis* was 24.65%.

III.1.1 Phytochemical screening of extracts

III.1.2. Qualitative Analysis

This test aims to detect the different chemical compounds present in a mixture of plant leaves. It involves using specific color reactions as qualitative tests. These reactions rely on phenomena such as precipitation or staining using specific reagents, allowing for the identification of the presence or absence of certain secondary metabolites in the plant. The results of these chemical tests are presented in the table below.

Table 6: Phytochemical compounds of different extract of plants mixture

Phytocompounds	Etheric extract	Methanolic extract	Aqueous extract
Emodol	+		
coumarin	+		
Triterpenes	+		
Free quinones	+		
Fatty acids	+		
Terpenoids	+		
Alcaloïds salts		-	
Tannins		+	
Reducing compounds		+	
Flavonoids		+	
Sterol and Triterpene		+	
Saponins			+
Starch			-
Anthraquinones free			+

+(Present), - (Absent)

Phytochemical screening of different extracts demonstrated the presence of several bioactive constituents. Amongst them, reducing compounds and saponins were found to be most abundant, terpenoids, tannins, flavonoids and anthraquinones free, while starch was absent.

These results are in agreement with many phytochemical studies which revealed that, the aerial part of *Artemisia herba-alba* is rich in secondary metabolites such as polyphenols, alkaloids, flavonoids, lactones, tannins. This medicinal herb has a broad spectrum of phytochemicals or secondary metabolites **(Nigam et *al.*, 2019)**, like polyphenols, tannins, flavonoids, flavonols, terpenoids, coumarins, glycosides, sterols, polyacetylens **(Choi et *al.*, 2013)**, anthracenosids **(Laouini et *al.*, 2018; Boukhennoufa et *al.*, 2020).**

According to the results obtained by **Mezouar et *al.*, (2022)** they noticed the presence of flavonoids, tannins, sterols and triterpens, terpenoids and saponins in hydromethanolic extract of *Olea europaea* leaves, whereas coumarins, reducing compounds, anthraquinones and alkaloids were negative.

III.2. Quantitative analysis

III.2.1 Total phenolics, flavonoids, tannins and flavonols contents

Phenolics or polyphenols are secondary plant metabolites that are ubiquitously present in plants and plant products. Many phenolic compounds including flavonoids, tannins and phenolic acid, exhibit a strong antioxidant activity **(Rohman et *al.*, 2010)**. Therefore, the amounts of total phenolics, flavonoids, tannins and flavonols in the mixture extracts tested were measured in this study.

The total phenolics in extracts was determined according to the Folin-Ciocalteau method using gallic acid as a standard and expressed as milligrams of gallic acid equivalents per gram of extract (mg GAE/mg DW). A linear calibration curve of Gallic acid was obtained **(Figure 27)**.

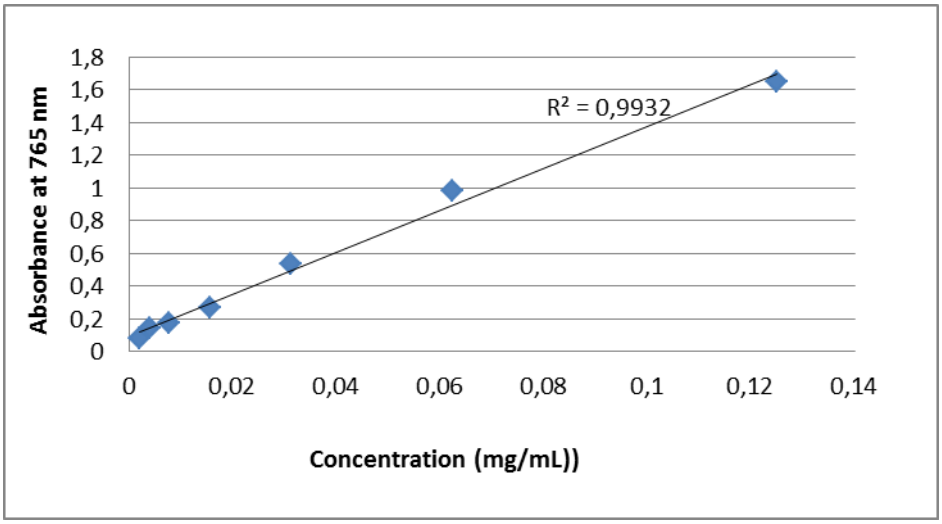

Figure 27: Curve of gallic acid for determination of total polyphenols of extracts tested.

Whereas, the flavonoids content was estimated by the $AlCl_3$ method **(Bahorun et *al*, 1996)** and expressed as miligram of quercetin equivalents per gram of extracts. The calibration curve of quercetin was shown in (**Figure 28)**.

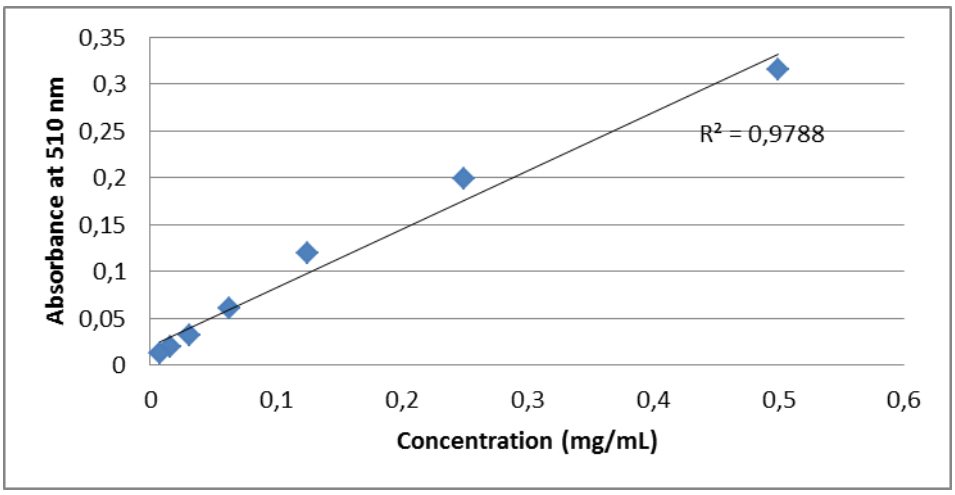

Figure 28: Curve of Quercetin for determination of total flavonoids of the extracts tested.

The determination of tannins was carried out according to the method of vanillin-HCL. The amount in tannins is expressed in milligram of catechin equivalent per gram of dry extract (mg EC/g DW) (**Figure 29)**. While flavonols were carried out according to the aluminium trichloride ($AlCl_3$) method and the content is expressed in milligrams of quercetin equivalent per milligram of dry plant matter (µg EQ/mg DW).

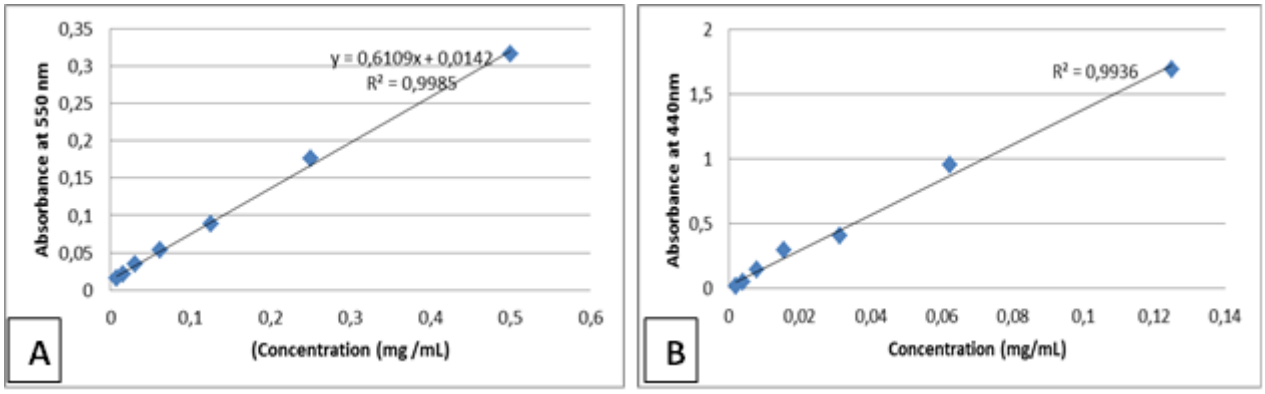

Figure 29: Curve for determination of tannins (A) and flavonols (B) of the extracts tested.

Total phenolic content and flavonoids tannins and flavonols were higher in methanolic extract than aqueous extract with an amounts varied between (13.39 – 439.93 mg E/g DW) and 11.56 – 357.41 mg E/g DW for aqueous extract. While methanolic and aqueous extracts showed higher flavonoids content in the mixture of plants than the other contents with an amount of 439.93 and 357.41 mg E/g DW mg E QER/g DW respectively.

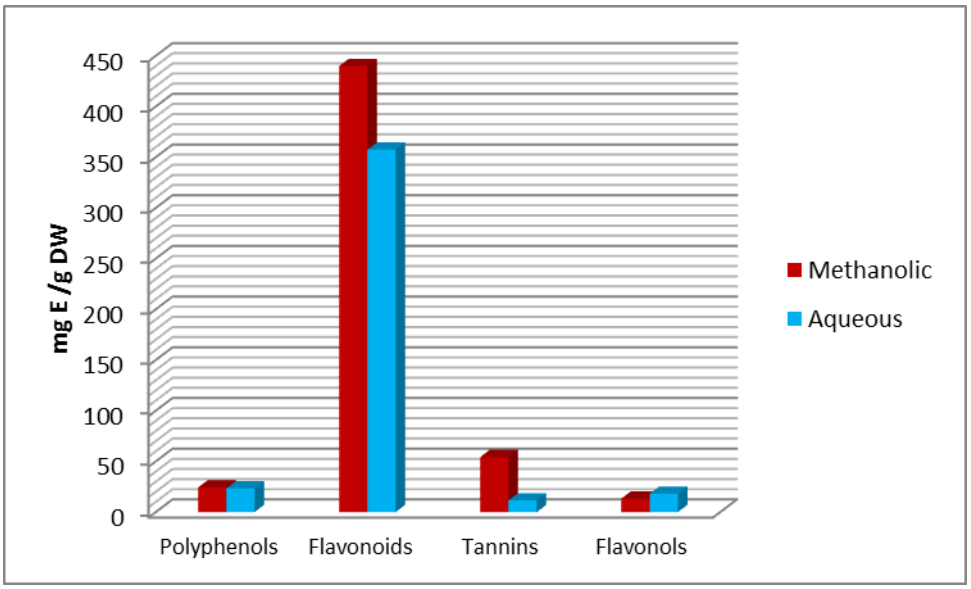

Figure 30: Total amount of polyphenols, flavonoids, tannins and flavonols of methanolic and aqueous extracts

Our results is compared to the study of **Younsi et *al.*, (2016**) working on *A. herba-alba* which they obtained contents of total phenols, flavonoids and flavonols respectively 27.65 ± 0.08 mg GAE/g DW, 13.96 ± 0.07 mg RE/DW and 0.085 ± 0.001 mg QE/g DW.

The contents and the composition of total phenolic and flavonoid of *A. herba-alba* extracts varied according to plant origin, the solvent of extraction and the analytical method used **(Seddik et *al.*, 2010)**.

While the study reported by **Benchikh et *al.*, (2018)** show that methanolic extract had the highest total phenolic content (149.25±3.11 mg GAE/g of dry extract), the aqueous extract had the highest tannins content (83.35±0.36 mg TAE/ g of dry extract), whereas, ethyl acetate extract had the highest total flavonoid content (38.4±0.9 mg QE/ g of dry extract).

Regarding myrtle leaves, the concentration of total polyphenols and flavonoids ranges from 31.25 to 298.63 mg GAE/g and 129.96 to 376.82 mg/g, respectively, depending on the myrtle variety and extraction methods used **(Amensour et *al.*, 2010; Özcan et *al.*, 2020)**.

III.2.2. Physicochemical parameter of ointment

The present study was done to prepare and evaluate the herbal ointment. For this the herbal extracts were prepared by using simple maceration process to obtain a good yield of extract and there was no any harm chemical constituents used. The physicochemical properties were studied which shows satisfactory results for diffusion study, pH, Loss on drying and others **(Table 7)**.

Table7: Physicochemical parameters of herbal ointment formulation

Physicochemical parameters	Observation
Colour	Green
Odour and taste	Characteristic
Consistency	Smooth and no greediness
Loss of drying	0,01 %
pH	6,22
Diffusion study	8 mm in 1 min
Non irritancy	Non irritant
Stability (20°C)	Stable at pH 6,22

III.3. Biological activity

III.3.1. *In vitro* antioxidant activity

The antioxidant power of the extracts tested of our mixture was carried out by four different chemical methods: TLC bioautographic (qualitqtive test), free radical scavenging activity of DPPH, the reducing power of Iron and phosphomolybdem assay.

III.3.2. DPPH assay on TLC bioautographic

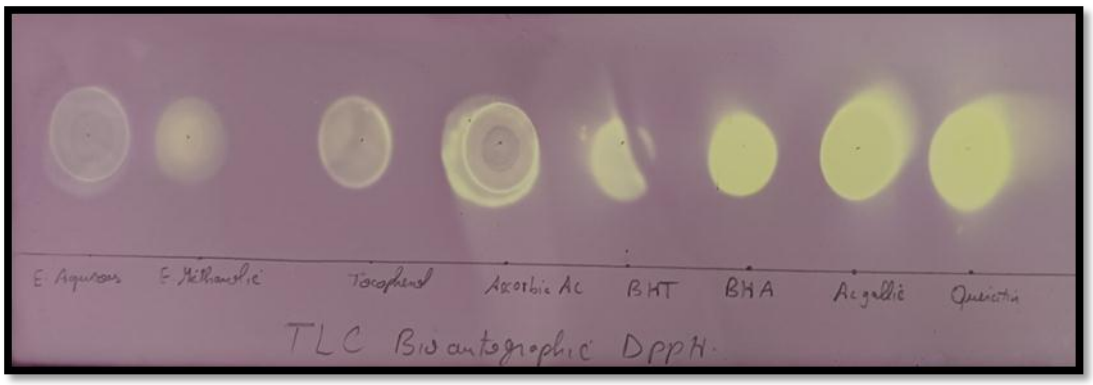

Figure 31: TLC bioautography for antioxidant activity in methanolic and aqueous extracts

Antioxidants bands were observed in the TLC plate exposed to DPPH solution. TLC confirmed the presence of polyphenolic compounds extracted by methanol and distillated water, the polarity of these solvents might have favoured this isolation **(Figure 31)**.

Purple colour of DPPH reagent was bleached by yellow spots was the indication of positive antioxidant activity of our extract tested in comparison to standards used tocopherol, Ascorbic acid, BHT, BHA, Gallic acid and Quercetin. There is a strong link between phenolic content and free radical scavenging activity **(Oki, et *al.*, 2002; Reza et *al.*, 2010)**.

III.3.3. DPPH-scavenging assay

The DPPH radical is often used as an indicator to test the ability of the extract to donate a hydrogen atom or an electron and therefore the antioxidant activity **(Tepe et *al.*, 2005)**.

The inhibition of radical DPPH was evaluated for the aqueous and methanolic extract *as well as* for the positive control ascorbic acid. The values obtained made it possible to draw a curve of the percentage of anti-radical activity for the extracts. According to the results presented in **Figure 32**, we note that the anti-radical activity is dose-dependent because it is proportional to the increase in the concentration of the extract. *In vitro* free radical scavenging activity of extracts tested showed that the methanolic extract exhibited the maximum percentage inhibition (61,47%) at 500 mg/mL concentration followed by aqueous (45,24%) at the same concentration. the results were compered to standard of ascorbic acid (92,05%). The decreasing order of DPPH scavenging activity was ascorbic acid > methanolic > aqueous.

Our results is compared to that of **Belmimoun et *al.*, (2016)** where the highest antioxidant capacity was exhibited by the aqueous extract of *Myrtus communis* with IC_{50}= 29.08 mg/mL.

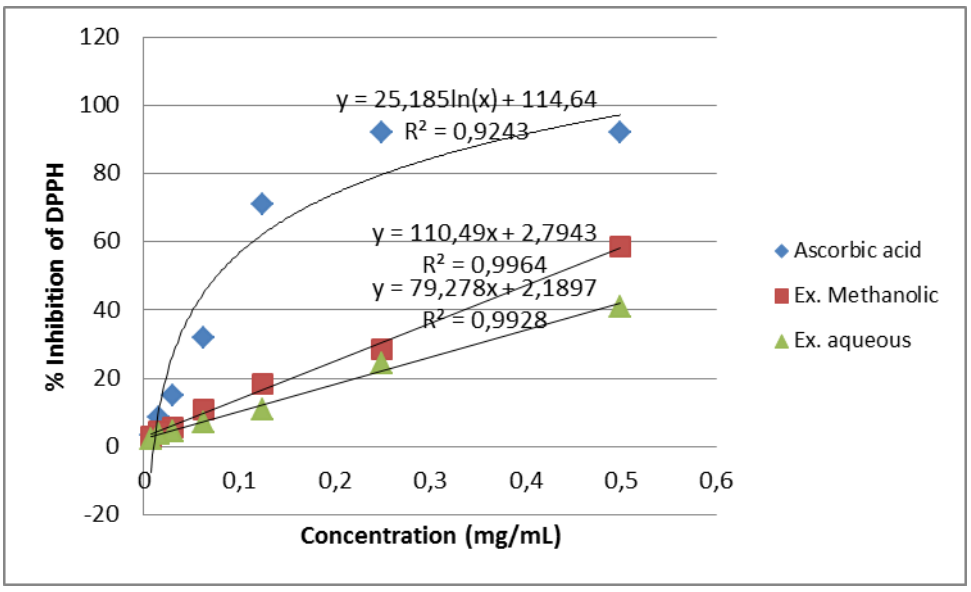

Figure 32: Percentage of anti-radical activity of methanolic and aqueous extracts and ascorbic acid

Crude extracts and phenolic fractions of *M. communis* were found to have considerable antioxidant activity and inhibition capacity against enzymes involved in several human diseases such as 5-lipoxygenase, cyclooxygenase-1, and acetylcholinesterase **(Amessis-Ouchemoukh et *al.*, 2014; Moein et *al.*, 2015; Babou et *al.*, 2016; Ozbeyli et *al.*, 2020)**.

III.3.4. Reducing power assay

In this test, the presence of a reducing agent in the extract to be tested will reduce the Fe^{3+}/ferricyanide complex [$FeCl_3$/$K_3Fe(CN)6$] to the ferrous form (Fe^{2+}) which is accompanied by the passage from yellow color to green or blue color which will absorb at 700 nm, thus, an intense color means a powerful reducing effect **(Oliveira et *al.*, 2008)**.

The **Figure 33** reveals that the reduction capacity is proportional to the increase in the concentration of the extracts which has a dose-dependent reducing potential (0.0078 mg/mL up to 0.5 mg/mL).

The reducing power of myrrha resin extracts and its essential oil increased with the increase of concentrations. The essential oil was superior to methanolic extract and ethyl acetate extracts. The reducing powers of essential oil were 0.348 **(Mohamed et *al.*, 2014)**.

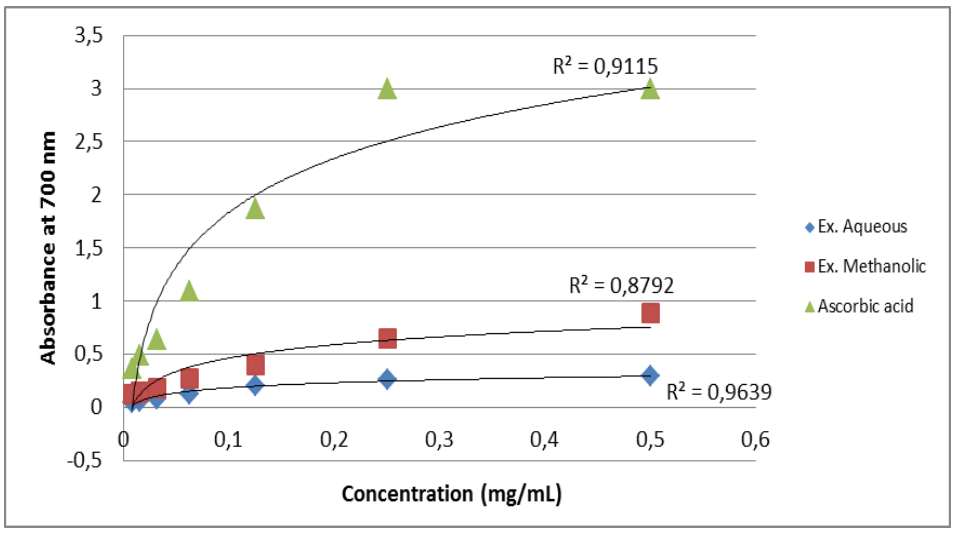

Figure 33: Reducing power of methanolic and aqueous extract and ascorbic acid

This richness in polyphenols gives the extract antioxidant activity, because the antioxidant power of an extract is related to its contents of phenolic compounds and particularly flavonoids **(Oyedeji et *al.*, 2011)**. Therefore, we proceeded to evaluate the antioxidant activity *via* differents methods (DPPH method, reducing power and the total antioxidant assay).

III.3.5. Total antioxidant assay

The determination of the total antioxidant capacity TAC of the different extracts is estimated by the method of **Prieto et *al.*, (1999)**. The total antioxidant capacity was estimated using a calibration curve, carried out with a reference substance, Ascorbic acid at different concentrations. The results are expressed in mg Ascorbic acid equivalent per g of extract (mg EAA/ g of dry extract) (**Figure 34)**.

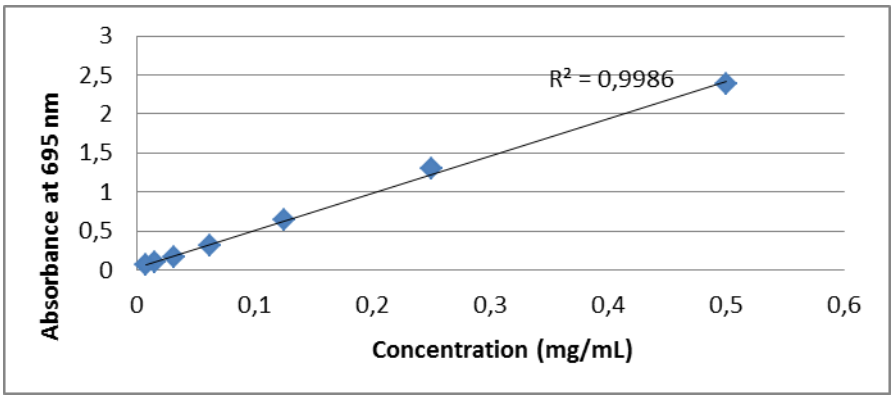

Figure 34: Calibration line of ascorbic acid for measuring antioxidant capacity

The methanolic extract show a total antioxidant better than aqueous extract but near to standard used BHA, the BHT exhibited the best activity with the tocopherol. The previous study of **Belmimoun et *al.*, (2016)** show that the total amount of antioxidant assay for aqueous extract of *M. communis* was 68.05±7.55 (mg /g DW).

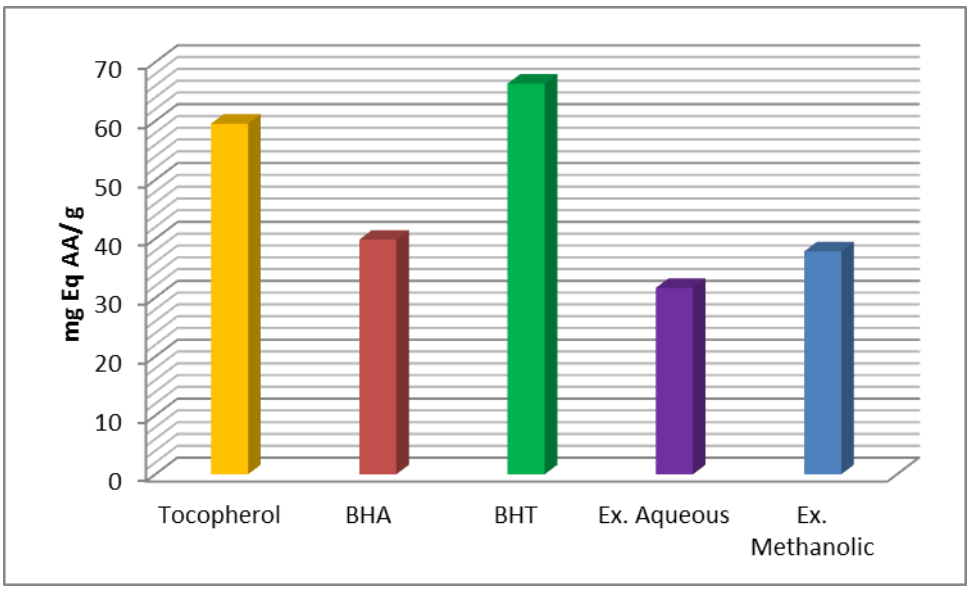

Figure 35: Comparative histogram of the total antioxidant capacity of the extracts and standards tested.

III.3.5. Sun Factor Protection test

The SPF was evaluated by the methodology developed by Mansur method. The analysis was carried out on an ultraviolet spectrophotometer, where the values of the obtained absorbances were placed in the table below. The photoprotective results demonstrated that our ointment gave the best value with Sun Factor Protection equal to 19.25% classified in medium category.

Table 8: SFP value of all samples

Sample	SPF value	Category	Potection
Equeous Extract	18,373	medium	Moderate protection
Methanolic extract	17,737	medium	Moderate protection
metabolic	19,258	medium	Moderate protection

SPF value classification according to the European Commission (EC) Recommendation in **Osterwalder and Herzog (2009)** are as follows: SPF value 6-10, provide low protection. SPF value of 15-25, provides moderate protection. SPF value of 30-50, giving high protection. 50+ SPF value, provide very high protection. The higher SPF value desired, required amount of sunscreen active ingredient higher **(Schulze 1956).**

Current study of Myrrh formulations have been categorized as mild sensitizers in local lymph node assay (LLNA) evaluations **(Lv et *al.*, 2009; Zhou et *al.*, 2019)**. Caryophyllene (C15H24) present in myrrh oil contributes to its antibacterial, antitumor, and anti-inflammatory properties **(Kamil et *al.*, 2018)**. *Commiphora myrrha* is used to treat wounds, infections, acne, boils **(Grbić et *al.*, 2018**; **Zhou et *al.*, 2019)**, hair, and the scalp **(El Ashry et**

***al.*, 2003; Shameem et *al.*, 2018).** It has been used to manufacture perfumes and cosmetics and used in aromatherapy **(Gadir et *al.*, 2014)**. The extract affected chapped and irritated skin types **(Ashry et *al.*, 2010)** and improved skin tone, firmness, and elasticity **(Happy et *al.*, 2021)**, and had a UV protection effect **(Chakravarty et *al.*, 2018)**.

III.3.6. Alpha-Amylase inhibitory activity

In this study, the α-amylase inhibitory activity of aqueous extract was investigated. The percentage inhibition of α-amylase by the extract was studied in a concentration range of 50–300 µg/mL. The result was compared to the acarbose which recorded an inhibition greater than extract tested attaint 80% of inhibition **(Figure 36)**. The results showed that the crude extracts contained bioactive molecules that can inhibit the enzyme activity.

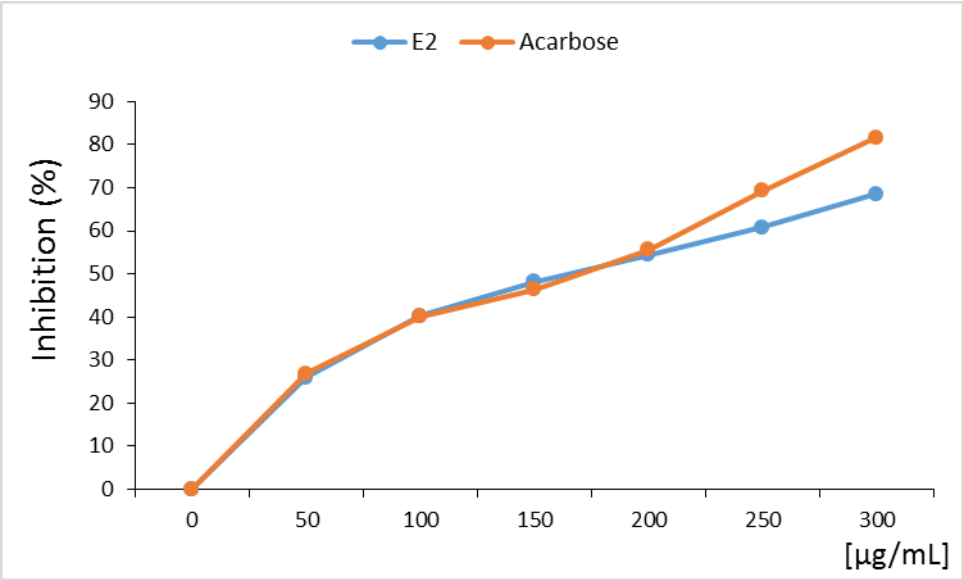

Figure 36: Inhibitory effect of aqueous extracts on α-amylase activity

In vitro studies have reported the inhibitory effect of α-amylase activity via a 70% ethanolic extract of Artemisia herba-alba. This resulted in an 11% decrease in α-amylase activity, which may be a mechanism by which this extract can lower blood glucose **(Awad et *al.*, 2012)**.

The results demonstrated that the plant showed α-amylase inhibition activity in a concentration dependent-manner. Furthermore, the previous studies have been displayed the strongest inhibitory activity on α-amylase of *Commiphora myrrha* methanolic extract ranged from 15.32% ± 0.58% to 71.32% ± 1.2%. And the maximum inhibition was recorded for Acarbose which was found to be 37.81% ± 1.2% to 86.32% ± 0.63% (**Abdel-Hady et *al.*, 2019).**

III.3.7. Alpha-glucosidase inhibitory activity

The inhibitory activity of aqueous extract of α-glucosidase enzymes was shown in below **(Figure 37)**. The results demonstrated that the extract showed increase in the percentage of inhibition with augmentation of concentration with higher inhibition of 65% compared to the acarbose which had the maximum inhibition 76%.

Our results are compared to that of **Abdel-Hady et *al.*, 2019** which they recorded an inhibition of *Commiphora myrrha* methanolic extract and acarbose respectively with 51.44±0.58% and 71.34 ± 1.5% at the same concentration (250 µg/mL).

AlShaal et *al.*, 2020, reported that the olive leaf extracts showed an 81.34% inhibition of α glucosidase at a concentration of 3.85 mg/mL with IC_{50}=0.34±0.12 mg/mL, while **Mansour et *al.*, (2023)** reported that aqueous extract of *Olea europaea* had inhibition on α-glucosidase with IC_{50}=14.14±0.41 µg/ml.

The evaluation of α-glucosidase inhibitory effect of extract in the present study may contribute to the understanding of their mechanism of action in reducing blood sugar and developing alternative natural and safe antidiabetic food supplements.

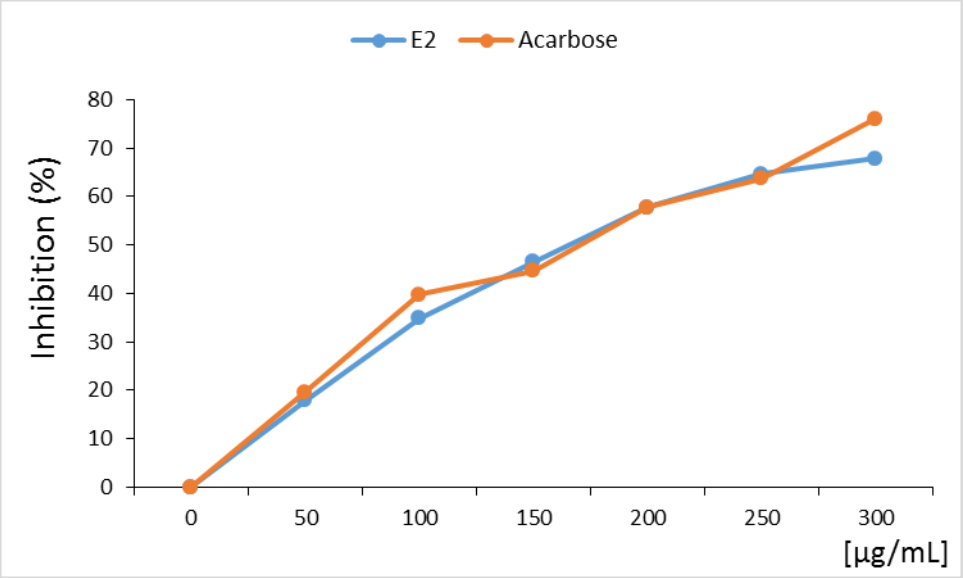

Figure 37: Inhibitory effect of aqueous extracts on α-glucosidase activity

III.4. Study *in vivo*

III.4.1. Acute toxicity

No mortality was observed during the first phase of the observation period after administration of aqueous extract at the doses of 10, 100 and 1000 g/kg of body weight, and in the second phase at doses 1600, 2900 and 5000 mg/kg. According to Hodge and Sterner **(Frank, 1992)**, substances with LD_{50} higher than 5 g/kg by oral route are regarded as being safe or practically nontoxic. The behavioral signs of toxicity such as diarrhea, vomiting, convulsion and difficulty in movement were not observed.

III.4.2. Assessment of body weight and glycemia during period of treatment

At the end of the study period (21 days), the body weight of diabetic animals and groups treated with herbal combination (doses 100, 300 and 500 mg/kg) showed slight increase compared to control group, significant higher increases in body weight of metformin rats was found. The aqueous extract exerted signicant decline in the fasting blood sugar in diabetic animals especially at dose of 300 mg/kg.

In previous study, the body weight of alloxan-induced diabetic rats increased significantly after treatment with *C. myrrha* extract. They argued that this may be induced by stimulation of carbohydrate metabolism **(Helal et *al.*, 2005)**. Alcoholic extract of *C. mukul* gum resin could prevent weight loss in diabetic rats. The authors attributed this effect to the enhancement of insulin secretion, which is the main glycogenolysis regulator in the liver and muscle tissues **(Bellamkonda et *al.*, 2011)**.

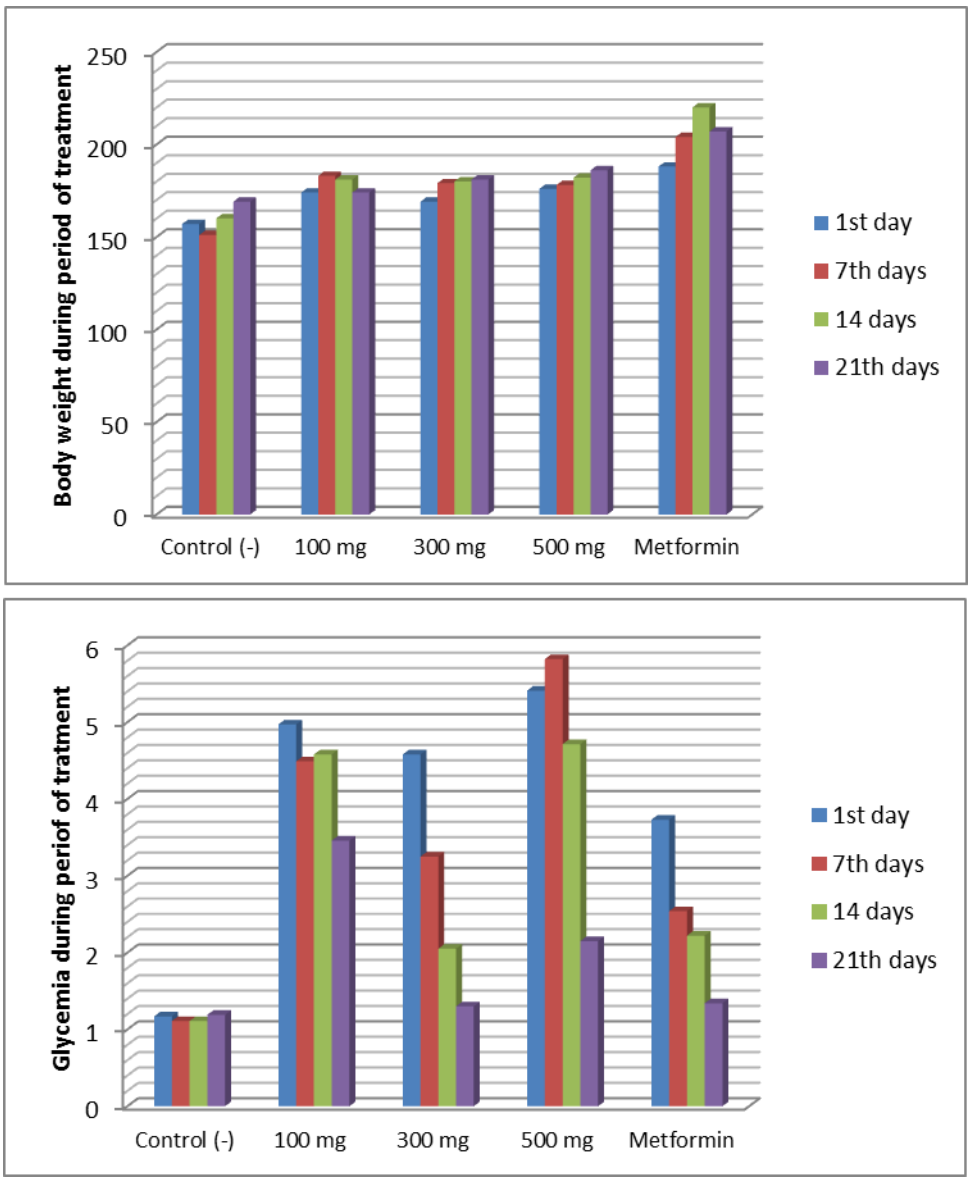

Figure 38: The change of body weight and glycemia during period of treatment

A study reported by **Boudjelal et *al.*, (2015)** confirm that the oral administration of 300 mg/kg b. w. of the aqueous infusions of *Artemisia herba-alba* and *Ajuga iva* exhibited a significant reduction in blood glucose content, showing a much more efficient antidiabetic activity compared to glibenclamide as a positive control in this study.

Other plants of the *Artemisia* genus, such as *Artemisia campestris* and *Artemisia herba-alba Asso*, are used in traditional medicine to treat diabetes in countries such as Algeria, Morocco, Pakistan and Mexico. The blood glucose-lowering effect of extracts of *Artemisia absinthium* by inhibiting α-glucosidase has been confirmed in other studies **(Cruz and Andrade-Cetto, 2015)**.

Additionally, studies have shown promising results in the use of myrtle extracts to regulate glucose and lipid levels in animal models, suggesting suggests that the extracts may stimulate the β-cells of pancreas to release insulin **(Panjeshahin et *al.*, 2016)**.

III.4.3. Assessment organ weight

The results obtained presented in rats treated with the aqueous extract are presented in the following table, we observe a slight increase in relative weight of the liver and feet, while there is a slight decrease in relative weight of kidney in comparison to the control.

Table 9: Changes in organs weight's rat after treatment with aqueous extract at different doses and control

	Liver	Pancreas	Feet
Control (-)	2.35 ±0.36	0.34 ±0.10	1.29 ±0.11
100 mg	2.86 ±0.58	0.25 ±0.10	1.42 ±0.17
300 mg	2.63 ±0.35	0.358 ±0.10	1.44 ±0.11
500 mg	3.89 ±1.57	0.21 ±0.08	1.57 ±0.22
Metformin	2.48 ±0.09	0.14 ±0.02	-

The increase in the weights of liver can be explained by the tissue hypertrophy of these organ caused by alloxan. According to studies carried out on the diabetogenic effect of alloxan, a loading of the liver marked by the increase in the hepatic concentration of free TG and AG which is due to their mobilization of adipose tissues towards the liver and the reduction of their degradation **(Mihaela et *al.*, 2006)**.

III.4.4. Hematological parameters of tested rats

The hematological parameters of the treated and control groups are presented in table below. The results show that there is an increase in the number of red blood cells (RBC) and hemoglobin levels for rats at the 100 mg dose, and a significant decrease in hemoglobin levels in rats at dose 300 mg and positive control rats (metformin); as we see a more or less significant decrease in the mean corpuscular hemoglobin concentration (MCHC) in metformin rats compared to the other doses tested.

We also notice a reduction in the number of white blood cells (WBC) in the positive control rats (metformin) *as well as* in the rats at the 500 mg dose, as we see that the number of lymphocytes in the healthy control rats is more numerous compared to other diabetic rats either under or without treatment. A significant reduction in the number of platelets in the metformin rats *as well as* in the 500 mg dose rats.

Table 10: Effect of aqueous extract of (100, 300 and 500 mg/kg) on hematological parameters of rats treated for 21 days

Parametres	Control (-)	100 mg	300 mg	500 mg	Metformin
RBC (10^6/µL)	7.56±0.148	8.40±1.13	7.84±0.38	7.75±0.56	7.63±0.83
Hemoglobin(g/dL)	15.25±0.63	16.18±1.92	11.27±6.27	15.34±1.40	13.83±0.58
Hematocrit (%)	39.85±0.91	42.9±5.61	41.45±1.89	40.72±1.88	41.6±0.9
MCV (fl)	52.65±2.19	51.08±2.48	52.75±3.98	51±2.32	51.16±2.30
MCH (pg)	20.25±1.20	19.32±1.19	18.92±1.60	19.82±0.76	18.2±1.70
CCMH (g/dl)	38.4±0.70	37.8±0.85	37.65±3.69	37.96±1.51	33.23±0.96
WBC (10^3/µL)	8.85±0.35	7.84±2.75	8.8±1.72	6.9±3.06	5.76±1.45
Gran (10^3/µL)	2.15±1.20	2.44±0.95	2.82±1.09	2.5±1.33	2.43±1.23
Lymp (10^3/µL)	6.15±0.91	4.82±2.18	4.72±0.41	3.46±1.49	3.2±1
Platelets (10^3/µL)	1011±15.55	922±142.60	1028.5±227.40	857.8±252.59	782±143.2

Values represent the mean ± SD, (n =5 group).

RBC: Red Blood Cell, **WBC**: White Blood Cell, **MPV**: mean platelet volume, **MCH**: Mean Corpuscular Hemoglobin, **MCHC**: Mean Corpuscular Hemoglobin Concentration. **Gran:** Granulocyte, **Lymph**: Lymphocyte

III.4.5. Biochemical parameters of tested rats

The values for the biochemical parameters in treated and control rats are presented in the **Figure 39**. The graph shows that the total cholesterol level of the healthy control rats is lower compared to the other diabetic rats which are increased a little, whereas the aqueous extract give best results in reducing the glycemia level in all the doses tested and better than Metformin.

According to the **Figure 39,** we see that the level of aspartate amino-taransferase (TGO) in healthy control rats is higher compared to other diabetic rats, and we see that the level of TGO is clearly reduced in rats' positive control (metformin) and decreased a little in the other rats at doses 100 mg, 300 mg, and 500 mg. Concerning the alanine aminotransferase (TGP) level, it was clearly reduced in the metformin rats and increased slightly in the 500 mg dose rats.

Eidi et *al.*, (2009) studied on antidiabetic effect of *Olea europaea* L. in normal and streptozotocin-induced diabetic rats they confirm the increase of the total cholesterol, aspartate amino transferase (AST) and alanine amino transferase (ALT).

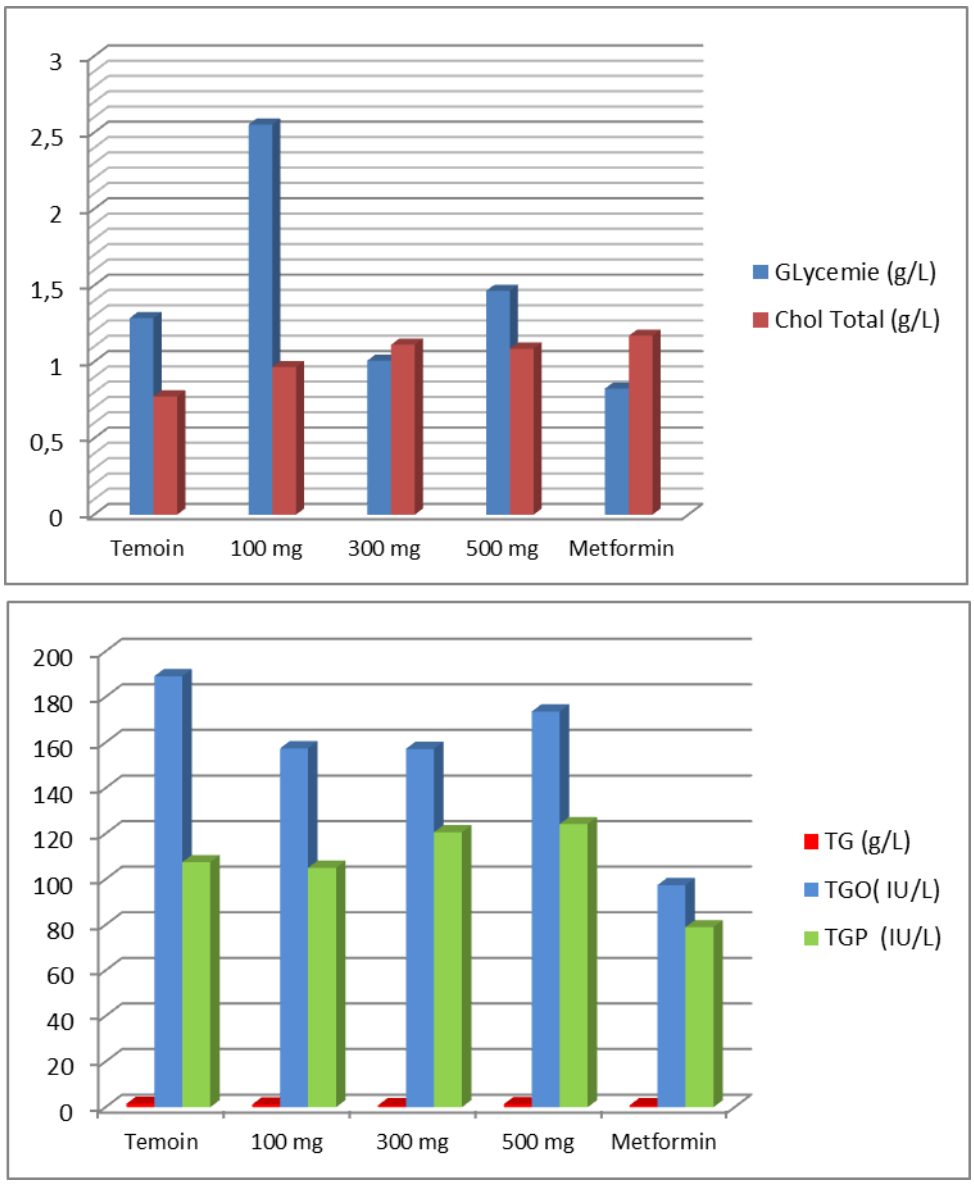

Figure 39: Effects of aqueous extract (100, 300 and 500 mg/kg) on biochemical parameters in rats treated for 21 days. Values represent the mean ± SD (n =5 group).

Olive leaves have been used in traditional medicine as hypoglycemic agent in many countries. In 2012 researchers reported that water decoction of olive leaf reduces blood glucose in normal and Alloxan diabetic rats **(Omer et *al.*, 2013)**. **Jemai et *al.*, (2008)** reported that *Olea europaea* L. have antidiabetic activity in normal and diabetic rats and their extract also decreased the serum glucose, total cholesterol, triglycerides, urea, uric acid, creatinine, aspartate amino transferase (AST) and alanine amino transferase (ALT). **Julio et *al.*, (2012)** also reported that hydroxytyrosol and Oleuropein from Olive leaves have antidiabetic and antioxidant activity while **Cumaoglu et *al.*, (2011)** showed that olive leaf extract may represent an effective adjunct therapy that normalizes glucose homeostasis in individuals with diabetes.

III.4.6. Tissue protein concentration

This graph shows a very significant reduction in the concentration of tissue proteins in the liver for the rats at the 100 mg and 300 mg doses, moreover at the dose of 500 mg is reduced but not in a significant way compared to the healthy control rats. On the other hand, we also notice the same modification in the concentration of tissue proteins in the pancreas.

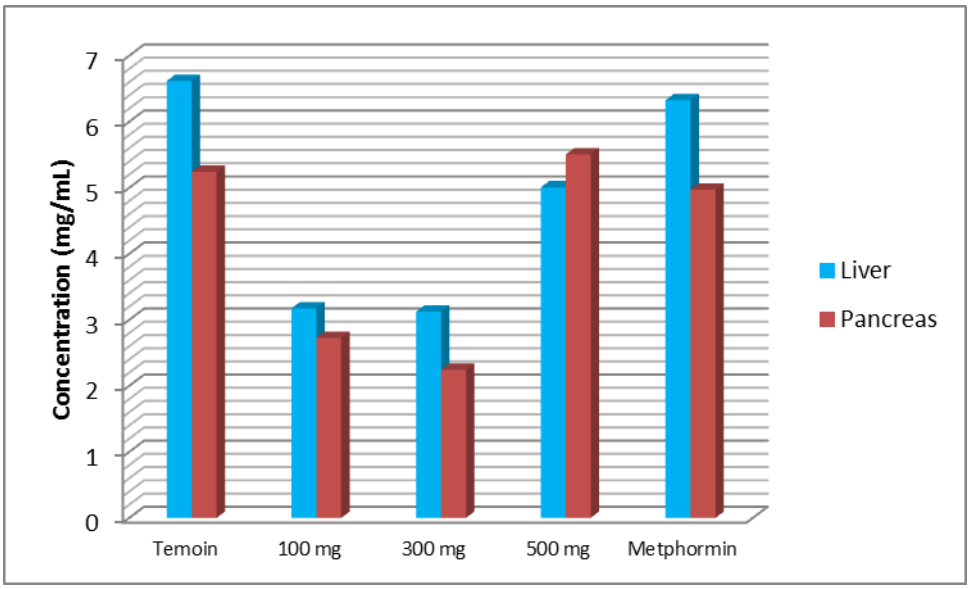

Figure 40: Protein tissue contents in liver and pancreas organs at different concentrations

III.4.7. Malondialdehyde (MDA) level

MDA concentrations were determined on cytosolic fractions of the organs: liver and pancreas. According to the graph below we observe that the tissue concentrations of MDA increase significantly in the liver of rats treated with doses 100 mg and 300 mg of our extract tested, while increase slightly in the liver in rats treated with dose 500 mg and the rats of positive control (metformin) in comparison to the negative control.

An increase in the pancreas's MDA level at the dose of 100 mg/kg, and slightly increases at 300 mg/kg of the extract tested, while at the dose of 500 mg/kg and Metformin rats we observed a decrease in MDA level in comparison to a negative control.

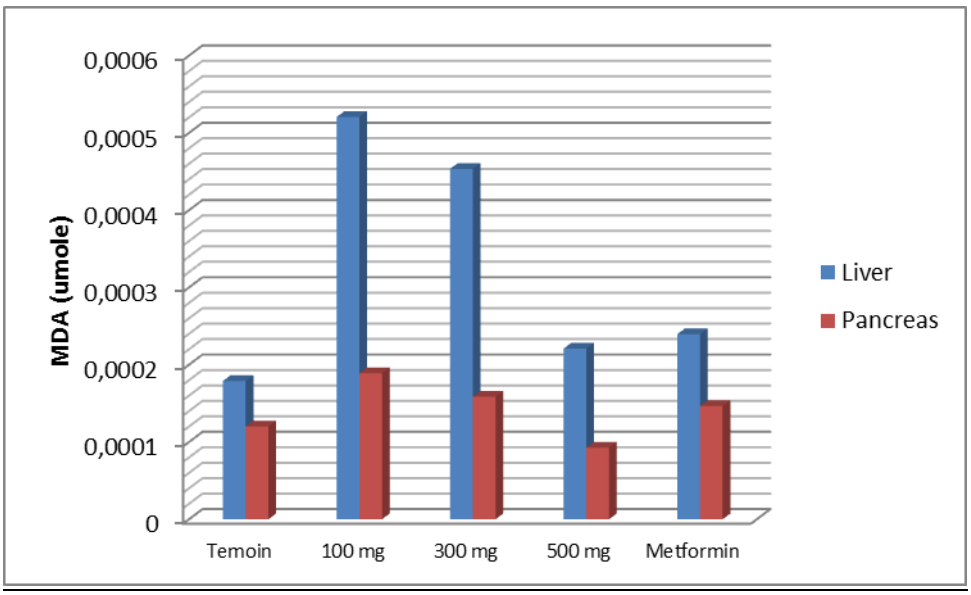

Figure 41: MDA concentrations in liver, pancreas and control group (Metformin)

Several previous studies on STZ-induced diabetes also reported similar results **(Alkreathy and Ahmad, 2020) (Haye et *al.*, 2022)**. These studies showed that the level of MDA in the tissues of the pancreas was significantly elevated as compared to the normal control group **(Gomathi et *al.*, 2013) (Fathy and dresse, 2015)**. Furthermore **(Jagtap and Patil, 2010)** and **(Abou Khalil et *al.*,2016)** reported that MDA value was increased in the plasma as well as the pancreatic tissue of diabetes-induced rats while it was significantly decreased by the treatment of diabetic animals with their plant extracts at 500 mg/kg.

III.4.8. Reduced glutathione (GSH) levels

Our results show that the concentration of reduced glutathione (GSH) in liver tissue is highly increased in rats treated with doses 100 mg, 500 mg and metformin, whilst a slight increase in liver GSH at the 300 mg compared to the negative control.

On the other hand, there was an increase in GSH pancreatic tissue in the different groups compared to the control group, but it was significant at the dose 300 mg.

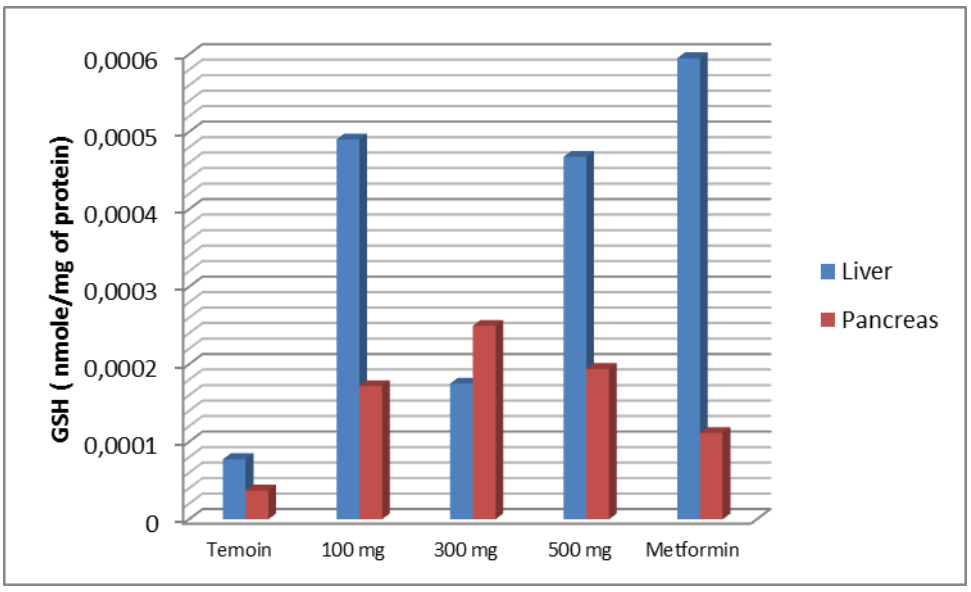

Figure 42: GSH concentrations in liver, pancreas and control group (Metformin)

Reduced glutathione (GSH) plays a multifactorial role in the antioxidant defense mechanism **(Sathishsekar MSc and Subramanian, 2005)**. It is a direct scavenger of free radicals, a necessary cosubstrate for GPx activity **(Ravi et *al.*, 2004)** and participates in the regeneration of oxidized vitamin E **(Dominguez et *al.*, 1998)**. Several studies support the hypothesis that during diabetes, chronic hyperglycemia increases the polyol pathway, the formation of AGEs and therefore the level of free radical production, which leads to an increase in GSH oxidation as well as a reduction in its regeneration **(Baynes and Thorpe 1999; Ou et *al.*, 1996)**. found similar results by **Ravi et *al.*, (2004)** and suggest that the decrease in the concentration of GSH in the liver and kidneys of rats made diabetic by alloxan is probably due on the one hand to an increase in its use by liver and kidney cells, and on the other hand to a reduction in the synthesis of GSH or an increase in its degradation during oxidative stress caused by diabetes. In another study **(Dominguez et *al.*, 1998)** indicate that the relative depletion of NADPH due to the activation of aldose reductase and the reduction of its production through the pentose cycle impairs the regeneration of GSH which leads to the exhaustion of this free radical scavenger.

III.4.9. Enzymatic activity of glutathione peroxidase

According to the following figure, we recorded a significant increase in the tissue content of glutathione peroxidase (GPx) in the liver of diabetic rats which we placed under different doses of our tested extract compared to healthy control rats, in addition a decrease in the concentration of GPx in the liver of meformin rats was recorded compared to healthy controls. We also noticed that the pancreatic GPx content a little increased in all batches of

diabetic rats except the 500 mg dose which remains almost equal to the GPx concentration of the healthy control batch.

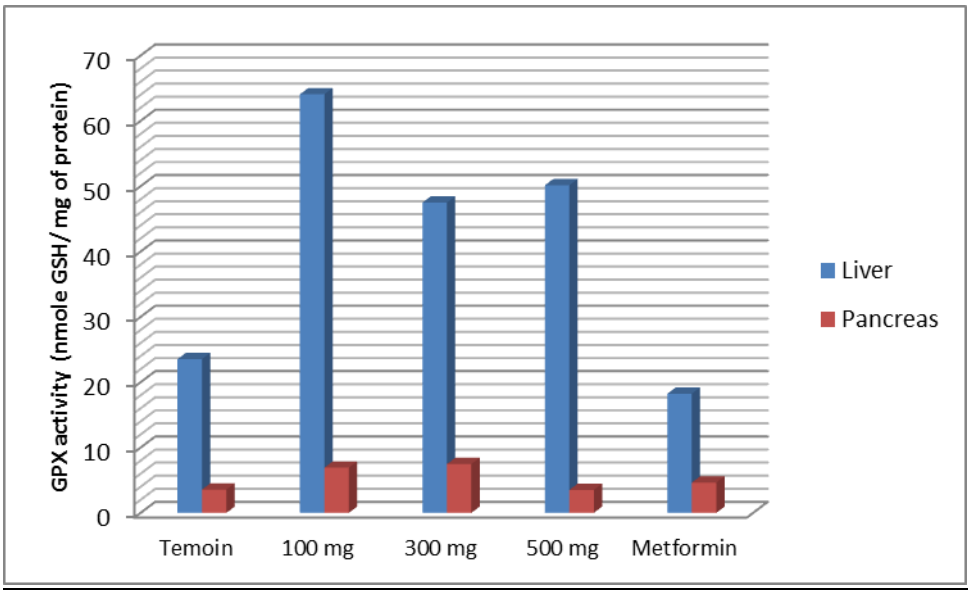

Figure 43: GPx concentrations in liver, pancreas and control group (Metformin)

Our results is better than the study of **Reggami et *al.*, (2021)** they had been obtained a value of GPx equal to 6.17±0.08 (mg of GSH consumed $min^{-1}mg^{-1}$ protein) of the aqueous extract of *A. herba-alba*, and this may be due to the synergetic effect of our mixture.

III.5. Histological examination of liver and pancreas

III.5.1. Effect of aqueous extract on rats liver histology

The histological section of the liver of the negative control rat showed an organized tissue architecture centered on a vein which is surrounded by liver cells (hepatocytes), these cells are separated by sinusoids.

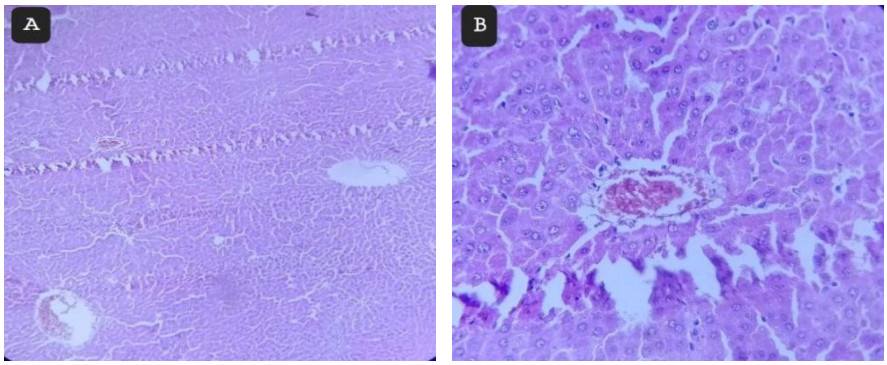

Figure 44: Histological sections of the liver of healthy control rat, (A): color HE×20, (B): color HE×40

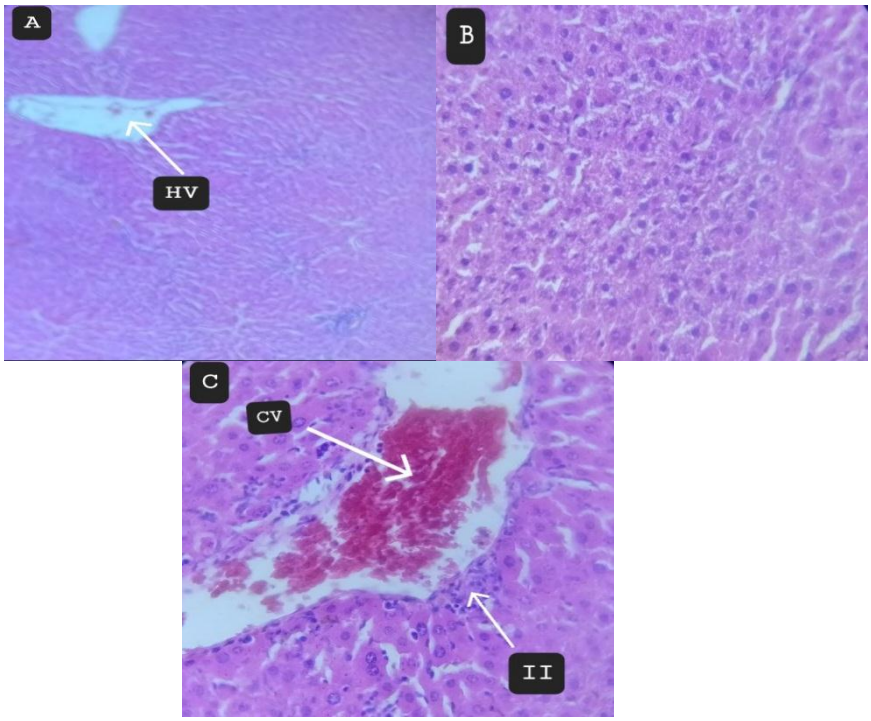

Figure 45: The effect of aqueous extract on the liver at dose 100 mg of the diabetic rats, (A): color HE×20, (B): color HE×20, (C): HE×40, **VH:** vascular hypertrophy, **CV:** vascular congestion**, II:** vascular infiltrate

In diabetic rats at a dose of 100 m/kg of the aqueous extract tested, we see that there is a clarification of the hepatocytes with micro-vacuolar cytoplasm; vascular congestion; and a lymphocytic infiltrate around the central vein.

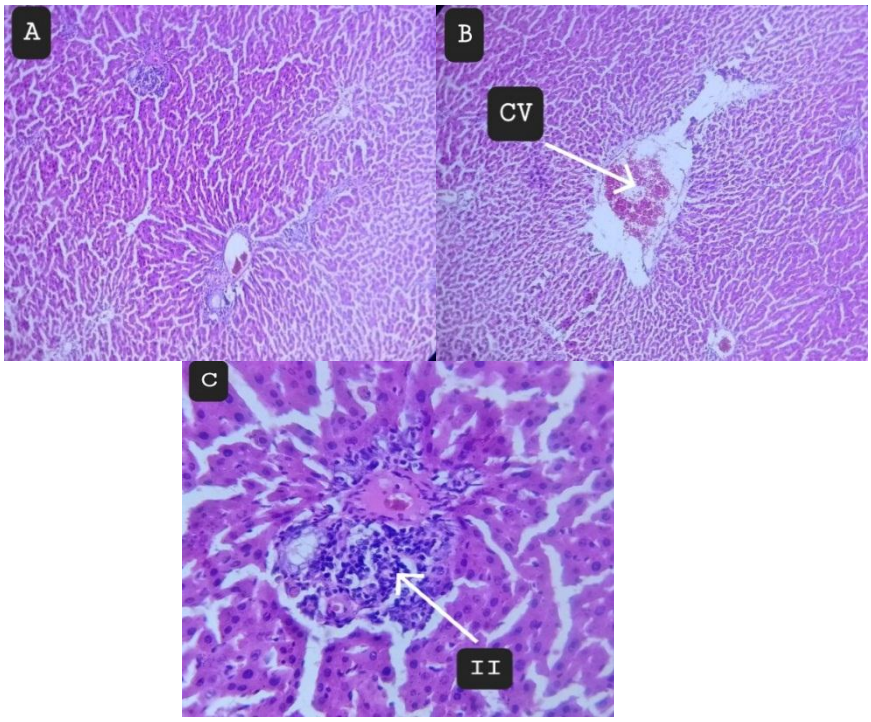

Figure 46: The effect of aqueous extract on the liver at dose 300 mg of the diabetic rats, (A): color HE×20, (B): color HE×20, (C): HE×40, **VH:** vascular hypertrophy, **CV:** vascular congestion**, II:** vascular infiltrate

The histopathological section of rats at a dose of 300 mg shows a lymphocytic infiltrate and vascular congestion with vascular hypertrophy.

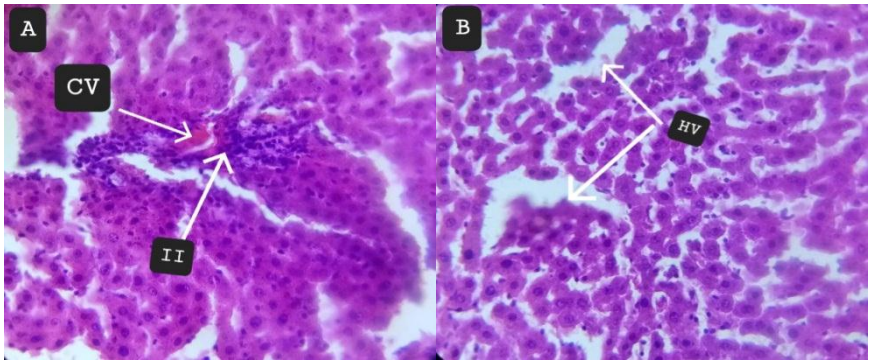

Figure 47: The effect of aqueous extract on the liver at dose 500 mg of the diabetic rats, (A): color HE×20, (B): color HE×40 **VH:** vascular hypertrophy, **CV:** vascular congestion**, II:** vascular infiltrate

Pathological analysis of the liver of rats at a dose of 500 mg shows a discreet dilatation of sinisoides with a discreet lymphocytic infiltrate.

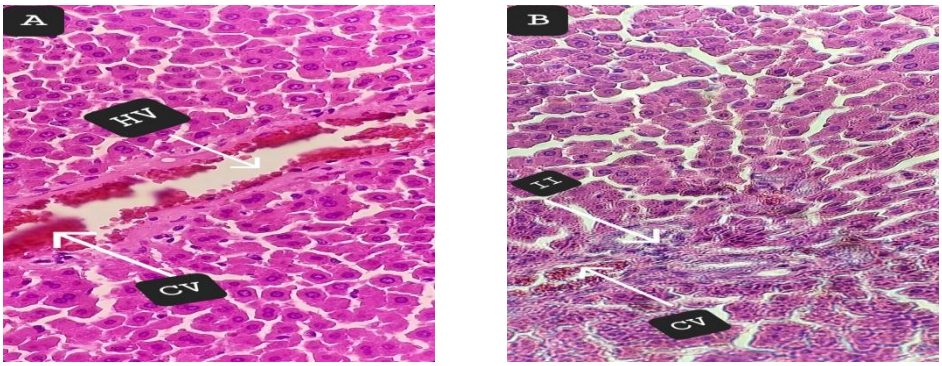

Figure 48: Histological sections of rat liver under metformin as positive control, (A): color HE×10, (B): color HE×40 **VH:** vascular hypertrophy, **CV:** vascular congestion**, II:** vascular infiltrate

Microscopic analysis of the histological section of positive control rats (metformin) shows that there is a similarity with the histological liver sections of rats at a dose of 500 mg.

The results of the study showed that the development of diabetes in rats led to obtaining histological changes in the liver of diabetic rats compared to the healthy control group. Histological sections of the liver of rats from the non-diabetic groups showed a typical architecture of hepatocytes, with normal sinusoids and a central vein.

The histological sections of the liver of diabetic rats revealed clear histopathological modifications especially in the group of rats treated with the 100 mg dose of our extract such as obvious bloody congestion with infiltration of lymphocytic inflammatory cells and dilated sinusoids compared to the negative control group, then we notice a progressive improvement in the group of rats at dose 300 mg, and this improvement is very clear in the group treated with the dose of 500 mg of the extract as in the group treated with metformin.

On the other hand, treatment with our extract showed a partial restoration of hepatocellular architecture with a remarkable improvement in diabetic rats at the 500 mg dose, compared to the group at the 100 and 300 mg doses which still show hepatocytes vacuolated and dilated sinusoids.

III.5.2. Effect of aqueous extract on rat's pancreas histology

Microscopic observation of the pancreas of rats from the negative control group shows normal morphology of the pancreas with islets of Langerhans of normal morphology and number.

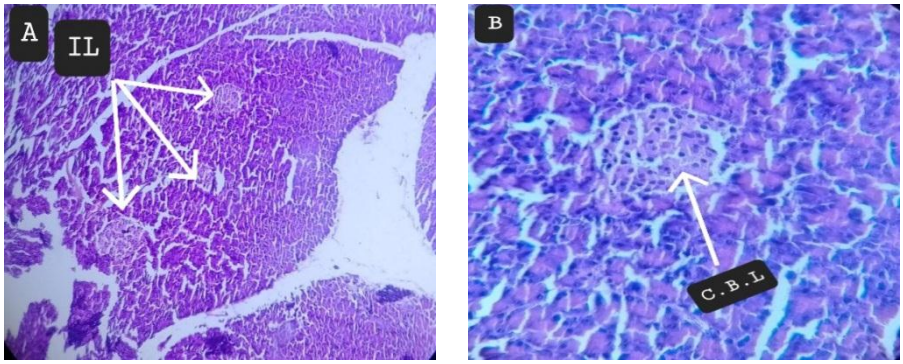

Figure 49: Histological sections of the pancreas of the healthy control rat, (A): color HE×20, (B): color HE×40, **CBL**: Langerhans beta cells

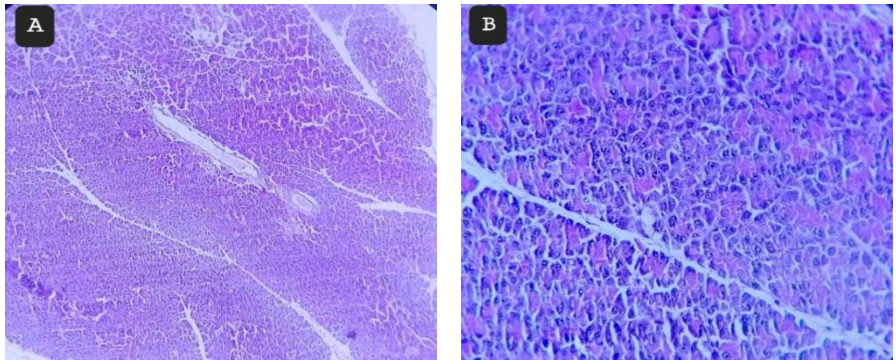

Figure 50: The effect of aqueous extract on the pancreas at dose 300 mg of the diabetic rats, (A): color HE×20, (B): color HE×40

We notice on the histological sections of the pancreas of rats at a dose of 100 mg a very significant rarefaction of the islets of Langerhans

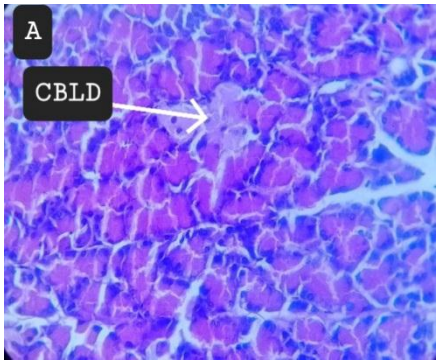

Figure 51: The effect of aqueous extract on the pancreas at dose 300 mg of the diabetic rats, A): color HE×40, **CBLD**: Langerhans beta cells destroyed

Microscopic observation of pancreatic sections from this group shows a rarefaction of the islets of Langerhans with atrophy of B cells.

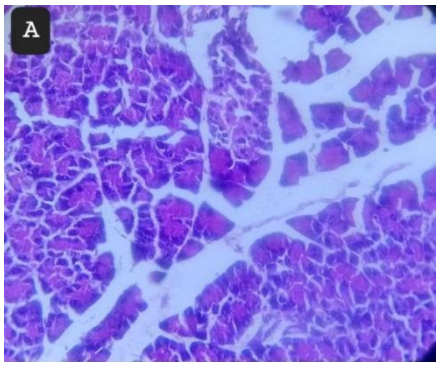

Figure 52: The effect of aqueous extract on the pancreas at dose 500 mg of the diabetic rats, (A): color HE×40

The histopathological analysis of sections of the pancreas of rats at a dose of 500 mg shows significant dystrophy of the islets of Langerhans which are more or less destroyed in appearance and very rare in number.

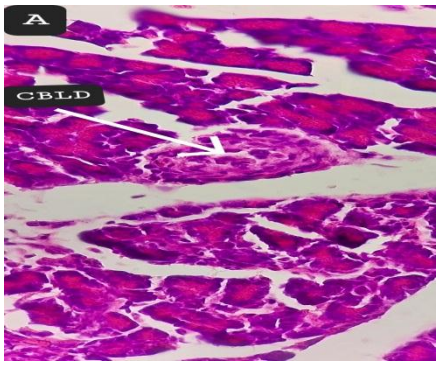

Figure 53: Histological section of the rat pancreas under metformin, (A): color HE×40, **CBLD**: Langerhans beta cells destroyed

The histopathological appearance of the pancreas of rats treated with metformin shows very rare islets of Langerhans with B cell dystrophy. The histological sections of the pancreas of rats from the non-diabetic groups showed a typical pancreatic architecture with islets of Langerhans of normal morphology and number. While the pancreas of treated rats revealed architectural changes in diabetic rats such as significant dystrophy of pancreatic cells with rarefaction of the islets of Langerhans and the absence of β cells especially in the group of rats treated with the dose of 100 mg of our aqueous extract, and even in the group of rats at a dose of 300 mg but in the 500 mg dose group we notice that there is the beginning of regeneration of islands with slightly destroyed beta cells.

These changes are marked while comparing with pancreatic sections of non-diabetic rats showing a typical histological organization. Moreover, treatment with our extract showed a small cell regeneration of the pancreas especially in rats treated with a dose of 500 mg, but this improvement remains insufficient and requires updating of research in this area.

A study investigated by **Ojewale et *al.*, (2014)** said that it can be speculated that the antioxidant nature of olive seed extracts might have contributed in the improvement of liver and kidney function tests and histopathological changes **(Ojewale et *al.*, 2014)**. It is previously established that alloxan administration had resulted in the generation of reactive oxygen species (ROS) through cyclic redox reactions of dialuric acid through metabolism **(Olayaki et *al.*, 2015)**. Thus, dialuric acid acts as a free radical producer and damages the pancreas, liver, and kidney. This generation of free radicals leads to oxidative stress resulting in cytotoxic effects in different organs **(Lenzen, 2008).**

It is evident from the current study that aqueous extract of our mixture reduced the alloxan-induced hepatic damage in diabetic rats. Previously, it is known that olive oil had reduced the hepatotoxicity induced by ethanol or acetaminophen by the reduction of oxidative stress due to the presence of phytochemicals such as gallic acid **(Ibrahim et *al.*, 2017)**.

III.5.3. Effect of ointment on rat's feet histology

The following figure shows that the histological aspect of feet in all groups is normal, *as well as* there is no change at all in the morphological skin of feet.

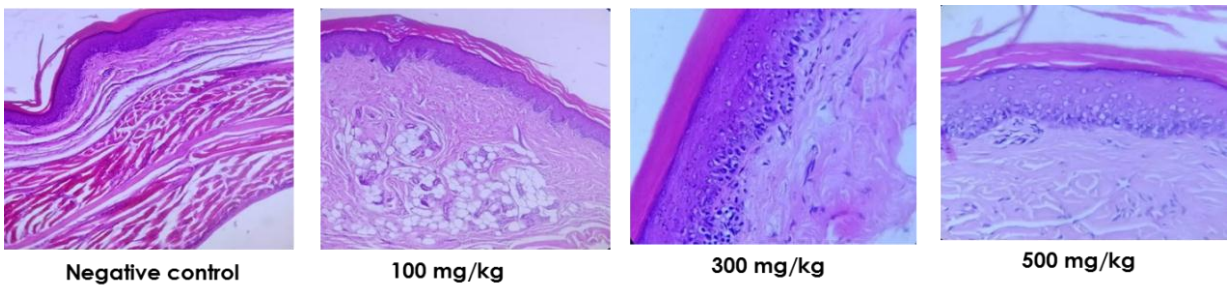

Figure 54: Effects of ointment on the feet at different doses HE×40

General Conclusion

In Algeria, traditional medicine is widespread and plays a major role in the treatment of diabetes. Knowing that diabetes constitutes a real scourge in Algeria, the number of studies in the search for new molecules capable of preventing or even delaying the appearance of complications linked to diabetes remains very limited.

Several treatments have been found for diabetes mellitus, oral antidiabetics and insulin are the main representatives. The complexity of the disease makes it difficult to control; research is being intensified to better understand the molecular mechanisms of the disease and to find other therapeutic targets and other more effective treatments.

In our study, we examined aqueous and methanolic extracts of a mixture of plant leaves: *Olea europaea* L., *Myrtus Communis* L., *Artemisia herba alba* L., *Commiphora myrrha* L., for their biological evaluation, namely the antioxidant activity, and in vitro, in vivo antidiabetic activity to formulate an ointment based on its plants. The phytochemical screening showed that the plant mixture extract contain phytochemicals as phenol, saponins, flavonoids, steroids, tannins, terpenoids and reducing compond with important quantitative value of total phenols and flavonoids which may contribute to the plant being a source of treatment for many diseases.

The *in vitro* study of extracts tested appeared essential antioxidant activities (DPPH method, reducing power and phosphomolybdem assay) which may suggest the effective treatment for many diseases associated with oxidative stress.

The hematological analysis concluded that the rats treated by plant mixture have a positive impact in the hypoglycemic which protect cardiovascular disease. In addition the positive influence of plant mixture aqueous extract, on restoring some biochemical markers *as well as* decrease of MDA and increases of GSH that demonstrate the beneficial effect of treatment against the physiologic and metabolic diseases.

The good protection capacity of our treatment was extended to microscopic level which shows their capacity in cells stabilization against pancreatic and liver injury induced by alloxane. We shed the powerful effect of plant mixture in our histological sections. This allows us to consider that this treatment has potential effect to limiting the histological alteration which associated with disease complications.

Perspective

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

- Detect all compounds (LC-SM) of our plant mixture aqueous extract
- Try to make extraction and the separation of these compounds
- Testing them in inflammatory, antibacterial and antifungal activities
- Testing anticancer action on different types of cancers
- Try to make a specific drug form plant mixture (*Olea europaea* L., *Myrtus Communis* L., *Artemisia herba alba* L., *Commiphora myrrha* L.).

References

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Abaza L., Talorete T.P.N., Yamada P., Kurita Y., Zarrouk M. and Isoda H. (2007). Induction of growth inhibition and differentiation of human leukemia HL-60 cells by a Tunisian gerboui olive leaf extract. Biosci Biotechnol Biochem .71:1306–1312.

Abaza L., Taamalli A., Nsir H. and Zarrouk Z.. (2015). Olive Tree (Olea europeae L.) Leaves: Importance and Advances in the Analysis of Phenolic Compounds Antioxidants 2015, 4, 682-698.

Abdul-Ghani R.A., **Loutfy N., Hassan A. (2009).** Myrrh and trematodoses in Egypt: an overview of safety, efficacy and effectiveness profiles. Parasitol Int.; 58:210–214.

Abdel-Hady H. (2019). Eman Ahmed El-Wakil, Eman Abdalla Morsi.Characterization of ethyl acetate and methanol extracts of *Commiphora myrrha* and evaluating *in vitro* anti-diabeticand anti-obesity activities. Journal of Applied Pharmaceutical Science. 9(09), 038-044.

Abou Khalil N.S., Abou-Elhamd A.S., Wasfy S.I., El Mileegy I.M., Hamed M.Y., Ageely H.M. (2016). Antidiabetic and Antioxidant Impacts of Desert Date (Balanites aegyptiaca) and Parsley (Petroselinum sativum) Aqueous Extracts: Lessons from Experimental Rats. Journal of Diabetes Research. 16:8408326.

Abdul-Ghani R.A., Loutfy N., Hassan A. (2009). Myrrh and trematodoses in Egypt: an overview of safety, efficacy and effectiveness profiles. Parasitol Int.; 58:210–214.

Ahrén B. (2007). Dpp-4 inhibitors. Best Practice & Research Clinical Endocrinology & Metabolism,; 21(4), 517-533.

Akrout A., El Jani H., Amouri S., and Neffati M. (2009). Screening of Antiradical and Antibacterial Activities of Essential Oils of Artemisia Campestris L., Artemisia Herba Alba Asso, & Thymus Capitatus Hoff. Et Link. Growing Wild in the Southern of Tunisia. Recent Research in Science and Technology, 2(1).

Aleksic J., Alexa A., Attwood T.K., Chue Hong N., Dahlö M., Davey R., Dinkel H., Förstner K.U., Grigorov I., Hériché J.K., Lahti L., MacLean D., Markie M.L., Molloy J., Schneider M.V., Scott C., Smith-Unna R., Vieira B.M. (2014). As part of the AllBio**:** An open science peer review oath. 3:271.

Aleksic V., Knezevic P. (2014). Antimicrobial and antioxidative activity of extracts and essential oils of Myrtus communis L journal homepage.

Aljaiyash A., Kasrati A., Jamali C. A., and Chaouch A. (2018). Effect of Cultivation on Chemical Composition and Bioactivities of Essential Oils from Artemisia herba-alba Asso Grown in Morocco. Biochemical Systematics and Ecology, 81: 74-79.

Alkreathy H.M., Ahmad A. (2020). Catharanthus roseus Combined with Ursolic Acid Attenuates Streptozotocin- Induced Diabetes through Insulin Secretion and Glycogen Storage. Oxidative Medicine and Cellular Longevity. 20:8565760.

Almaraz-Abarca N., Campos M.G., Ávila-Reyes J.A., et al. (2007). Antioxidant Activity of Polyphenolic Extract of Monofloral Honeybee-Collected Pollen from Mesquite (Prosopis juliflora, Leguminosae).J Food Compos Anal; 20 (2):119-124.

Amensour M., Bouhdid S., Fernandez-Lopez J., Idaomar M., Senhaji N. S., and Abrini, J. (2010). Antibacaterial activity of extracts of Myrtus communis against food-borne pathogenic and spoilage bacteria. International Journal of Food Properties, 13, 1215–1224.

American Diabetes Association (ADA). (2008). Diabetes Care. 31(55).

Amessis-Ouchemoukh N., Ibrahim M. Abu-Reidah, Quirantes-Piné R., Madani K., Segura-Carretero A. (2014). Phytochemical profiling, in vitro evaluation of total phenolic contents and antioxidant properties of Marrubium vulgare (horehound) leaves of plants growing in Algeria.Industrial Crops and Products. 61, 120-129.

Andrikopoulo N.K., Kaliora A.C., Assimopoulou A.N., Papageorgiou V.P. (2002). Inhibitory activity of minor polyphenolic and nonpolyphenolic constituents of olive oil against in vitro lowdensity lipoprotein oxidation. J Med Food. 5:1–7.

Ashry K.M., El-Sayed Y.S., Khamiss R.M., El-Ashmawy I.M. (2010). Oxidative stress and immunotoxic effects of lead and their amelioration with myrrh (Commiphora molmol) emulsion. Food Chem. Toxicol., 48, 236–241.

Aslam M.S., Ahmad M.S., Mamat A.S., Ahmad M.Z., Salam F. **(2016)**. An update review on polyherbal formulation: A global perspective. Syst Rev Pharm. 7(1):35-41.

Awad N.E., Seida A.A., Shaffie Z.E.N., El-Aziz A.M. (2012). Hypoglycemic activity of Artemisia herba-alba (Asso.) used in Egyptian traditional medicine as hypoglycemic remedy. J. Appl. Pharm. Sci.; 2:30–39.

B

Babou L., Hadidi L., Grosso C., Zaidi F., Valentão P., Paula A. (2016). Study of phenolic composition and antioxidant activity of myrtle leaves and fruits as a function of maturation 242:1447–1457.

Bahorun T., Gressier B., Trotin F., Brunete C., Dine T., Vasseur T., Gazin T.C., Pinkas M., Luycky M., Gazin M. (1996). Oxygen species scavenging activity of phenolic extact from Hawthorn fresh plant organs and pharmaceutical preparation. Arznein Forsch/Drug Res.P.16.

Bailey C.J. (1999). Insulin resistance and antidiabetic drugs. Biochemical Pharmacology, 58(10), 1511-1520.

Balakrishnan G., Agrawal R. (2014). Antioxidant activity and fatty acid profile of fermented milk prepared by Pediococcus pentosaceus. J Food Sci Technol. 51(12):4138-42.

Baldoni L., Tosti N., Ricciolini C., et al. (2006). Genetic structure of wild andcultivated olives in the central Mediterranean basin. Ann Botany. 98:935-42.

Batiha G.E.S., Wasef L., Teibo J. O., Shaheen H. M., Zakariya A. M., Akinfe O. A., and Papadakis M. (2023). *Commiphora myrrh*: a phytochemical and pharmacological update. Naunyn-Schmiedeberg's archives of pharmacology. 396(3); 405-420.

Batiha G.E., Wasef L., Teibo J.O., Shaheen H.M., Zakariya A.M., Akinfe O.A., Teibo T.K.A., Al-Kuraishy H.M., Al-Garbee A.I., Alexiou A., Papadakis M. (2023) *Commiphora myrrh*: a phytochemical and pharmacological update. Naunyn Schmiedebergs Arch Pharmacol. 396(3):405-420.

Baynes J.W. (1991). Role of oxidative stress in development of complications in diabetes. Diabetes. 40:405-412.

Baynes J.W., Thorpe, S.R. (1999). Role of Oxidative Stress in Diabetic Complications A New Perspective on an Old Paradigm. Diabetes. 48 (9): 1-9.

Bellamkonda R., Rasineni K., Singareddy S.R., Kasetti R.B., Pasurla R., Chippada A.R., Desireddy S. (**2011**). Antihyperglycemic and antioxidant activities of alcoholic extract of Commiphora mukul gum resin in streptozotocin induced diabetic rats. Pathophysiology.18(4):255-61.

Belmimoun A., Meddah B., Side Larbi K., Sonnet P. (2016). phytochemical study of zygophyllum album extract. International Journal of Engineering Technologies and Management Research. 4(5):1-10.

Belmimoun A., **Meddah B., Meddah A.T. and Sonne P. (2016).** Antibacterial and antioxidant activities of the essential oils and phenolic extracts of myrtus communis and zygophylum album from Algeria. Journal of Fundamental and Applied Sciences. 8(2):510.

Benchikh F., Amira S. and Benabdallah H. (2018). The Evaluation of Antioxidant Capacity of Different Fractions of Myrtus communis L. Leaves 22(5):1-14**.**

Benavente-Garcia J, Castillo J, Lorente A, Ortuno A, Del Rio JA (2000). Antioxidant activity of phenolics extracted from Olea europaea L. leaves. Food Chem. 68:457–462

Benmahdi H. (2000). Valorisation de certaines plantes médicinales à activité hypoglycémiante comme la coloquinte. Mémoire de magistère. Université Abou Bekr Belkaid, Tlemcen. Algérie. p. 111-114-157-158.

Benmeziane S., Salah Haddadin M., and AL-Domi H. (2023). Extraction Yield, Phytochemicals Analysis, and Certain in Vitro Biological Activities of Artemisia Herba Alba Extracts Siham. Jordan Journal of Agricultural Sciences.19;(2).

Bhat M., Zinjarde S.S., Bhargava S.Y., Kumar A.R., Joshi B.N. (2011). Antidiabetic Indian plants: a good source of potent amylase inhibitors. Evid Based Complement Alternat Med; 2011:810–815.

Bharskar G; **Siddheshwar S. (2022)**. Formulation and *In vitro* Evaluation of Topical Antimicrobial Preparation. Department of Pharmacy, Pravara Rural College of Pharmacy, Pravaranagar, India.15-10.

Bibi Sadeer N et al. (2020). The Versatility of Antioxidant Assays in Food Science and Safety-Chemistry, Applications, Strengths, and Limitations.MDPI.5.

Bisset N.G., Wichtl M. (1994). Herbal drugs and phytopharmaceuticals. Medpharm GmbH Scientific Publishers, Stuttgart, CRC Press, Boca Raton, P. 91–95.

Boskou D. (1996). Olive oil: chemistry and technology. AOCS Press, Champaign

Boligon A.A., Machado M.M., Athayde M.L. 2014. Technical evaluation of antioxidant activity. Journal of Medicinal Chemistry. 4(7): 517-522.

Boubacar S.A. (2005). Étude de la phytochimie et des activités biologiques de Combretum glutinosum Perr.ex DC (COMBRETACEA). Thèse de doctorat. Université de Bamako, Mali. P.60.

Boudjelal A., Siracusa L., Henchiri C., Sarri M., Abderrahim B., Baali F., Ruberto G. (2015). Antidiabetic Effects of Aqueous Infusions of Artemisia herba-alba and Ajuga iva in Alloxan-Induced Diabetic Rats. Planta Med. 81(9):696-704**.**

Bougandoura N. (2011). Pouvoir antioxydant et antimicrobien des extraits d'espèces végétales *Satureja calamintha* ssp nepta (nabta) et *Ajugaiva* L. (chendgoura) de l'Ouest d'Algérie. Mémoire de magistère. Université Abou Bakr Belkaid-Tlemcen. Algérie. P.37.

Bouhadjera K. (2005). Contribution à l'étude chimique et biologique de deux plantes médicinales sahariennes *Oudneya africana* R.Br. et *Aristida pungens* L. Thèse de doctorat, Université Abou Bekr Belkaid, Tlemcen. Algérie. p.58.

Boukhennoufa A., Benmaghnia S., Meddah B., and Meddah A.T.T. (2020). Antioxidant Activity of Extracts Formulated from Citrus Aurantium and Artemisia Herba Alba. European Journal of Biological Research, 10(4):343-351.

Bouldjadj R. (2009). Etude l'effet antidiabétique et antioxydant de l'extrait aqueux lyophilise d'artemesia herba alba asso chez des rats sains et des rats rendues antidiabétique par streptozotocine. Thèse de magistère en Biologie Cellulaire et Moléculaire. Constantine, P.111.

Bouzabata A., Casanova J., Bighelli A., Cavaleiro C., Salgueiro L., & Tomi F. (2016). The genus Myrtus L. in Algeria: composition and biological aspects of essential oils from M. communis and M. nivellei. Chemistry & Biodiversity, 13(6), 672-680.

Bradford, M. (1976). A rapid and sensitive method for the quantities of microgram quantities of protein utilizing the principle of protein-dye binding. Anal Biochem, 72:248–54.

Briante R., La Cara F., Febbraio F., Patumi M., Nucci R. (2002). Bioactive derivatives from oleuropein by a biotransformation on Olea europaea leaf extracts. J Biotechnol 93:109–119.

Braillard O., Gastaldi G., Favrod-Coune A. (**2017)**. Le Diabete type 2. Service de médecine de premier recours – DMCPRU – HUG- . Département de médecine communautaire, de Premier recours et des urgences.

Bruneton J. (1995). Pharmacognosy, phytochemistry and medicinal plants, English Translation by Hatton, C. K., Lavoisier Publishing, Paris, p 265.

Bruneton J. (2009). Pharmacognosie, Phytochimie, Plantes Médicinales, 4ème Edition, Revue et augmentée, Editions Médicales Internationales, Tec and Doc, Paris, France. p.128.

Buffet C., Vatier Masson C. (2010). Endocrinologie Diabétologie Nutrition Cahiers des ECN ISBN : 9782294701795. P446.

C

Cabarkapa A., Zivković L., Zukovec D., Djelić N., Bajić V., Dekanski D., Spremo-Potparević B. (2014). Protective effect of dry olive leaf extract in adrenaline induced DNA damage evaluated using in vitro comet assay with human peripheral leukocytes. Toxicol *In Vitro*. 28:451–456.

Castillo J.J., Alcaraz M., Benavente-García O. (2010). Antioxidant and radioprotective effects of olive leaf extract. In: Victor RP, Ronald RW (eds) Olives and olive oil in health and disease prevention. Academic Press, Oxford, P. 951–958.

Chakravarty N., Kellogg C., Alvarez J., Equils O., Morgan M. (2018). UV Protection by Natural Products: C. myrrha Oil Versus Sunscreen. J. Drugs Dermatol.17, 905–907.

Chaudhuri A., Sharma S. (2016). Evaluation of antidiabetic activity of polyherbal formulation in streptozotocininduced diabetic rats. UK Journal of Pharmaceutical Biosciences. 4(5):01-6.

Chiappetta A., Muzzalupo I. (2012). Botanical description. In: Olive germplasm. The olive cultivation, table olive and olive oil industryin Italy. MuzzalupoI, Ed. In Tech Open; P. 23-38.

Chigurupati S., Alharbi F. S., Almahmoud S., et al. (2021). Molecular docking of phenolic compounds and screening of antioxidant and antidiabetic potential of *Olea europaea* L. Ethanolic leaves extract. Arabian Journal of Chemistry. 14(11).

Cho N, Shaw J, Karuranga S, Huang Y, da Rocha Fernandes J, Ohlrogge A, Malanda B. (2018). IDF Diabetes Atlas: global estimates of diabetes prevalence for 2017 and projections for 2045. Diabetes Res Clin Pract. 138: 271–281.

Choi E., Park H., Lee J., and Kim, G. (2013). Anticancer, Antiobesity, and Anti-Inflammatory Activity of Artemisia Species In-Vitro. Journal of Traditional Chinese Medicine, 33(1): 92-97.

Cieśla L., Kryszeń J., Stochmal A., Oleszek W., Waksmundzka-Hajnos M. (2012). Approach to develop a standardized TLC-DPPHradical dot test for assessing free radical scavenging properties of selected phenolic compounds.Journal of Pharmaceutical and Biomedical Analysis. J Pharm Biomed Anal. 70:126-35.

Clara-Mihaela Ionescu (UGent), S Zlate and Robain De Keyser (UGent) (2006). An approach to control the blood glucose level in diabetic patients. International Conference on Automation, Quality, Testing and Robotics.1-2;22-27.

Cruz E.C., Andrade-Cetto A. (2015). Ethnopharmacological field study of the plants used to treat type 2 diabetes among the Cakchiquels in Guatemala. J. Ethnopharmacol.159:238–244.

Cumaoğlu A., Rackova L., Stefek M., Kartal M., Maechler P., Karasu C. (2011). Effects of olive leaf polyphenols against H_2O_2 toxicity in insulin secreting β-cells. Acta Biochim Pol; 58:45-50.

Dayem A.A., Choi H.Y., Kim J.H., Cho S.G. (2010). Role of oxidative stress in stem, cancer, and cancer stem cells. Cancers (Basel) 17;2(2):859-84.

De Leonardis A., Aretini A., Alfano G., Macciola V., Ranalli G. (2008). Isolation of a hydroxytyrosol-rich extract from olive leaves (Olea europaea L.) and evaluation of its antioxidant properties and bioactivity. Eur Food Res Technol 226:653–659.

Dekanski D., Janicijevic-Hudomal S., Tadic V., Markovic G., Arsic I., Mitrovic D. (2009). Phytochemical analysis and gastroprotective activity of an olive leaf extract. J Serbian Chem Soc 74:367–377.

Dellaoui H., Berroukche A., Terras M. (2018). Phytochemical study and evaluation of the antioxidant of Myrtus communis L. Fruit's Méthanolic extract. Etude phytochimique et évaluation du pouvoir antioxydant de l'extrait méthanolique de fruit du *Myrtus communis* L. PhytoChem & BioSub Journal. 12(2).

Demissew S. **(1993)**. **A** description of some essential oil bearing plants in ethiopia and their indigenous uses. J Essent Oil Res. 5:465–479.

Dilek Ozbeyli, **Ali Sen, Ozlem Tugce Cilingir Kaya, Busra Ertas, Sezgin Aydemir, Naziye Ozkan, Meral Yuksel, Goksel Sener. (2020).** Myrtus communis leaf extract protects against cerulein-induced acute pancreatitis in rats. J Food Biochem. 44(2):e13130.

Do Quy Diem., Artik Elisa Angkawijaya, Phuong Lan Tran-Nguyen, Lien Huong Huynh , Felycia Edi Soetaredjo, Suryadi Ismadji, Yi-Hsu Ju . **(2014)**. Effect of extraction solvent on total phenol content, total flavonoid content, and antioxidant activity of Limnophila aromatica Sep; 22(3):296-302.

Dohou N. (2004). Approche floristique, ethnobotanique, phytochimique et étude de l'activité biologique de *Thymeleae lythroïdes*, Thèse de doctorat, Maroc. p. 59.

Dominguez, Z., Ruiz, E., Gussinye, M., Carrascosa, A. (1998). Oxidative stress at onset and in early stages of type diabetes in children and adolescents. Diabetes care, 21: 1736-1742.

Drouin P., Blichle J.F., Charbonnel B., Eschwege E., Guillausseau P.J., Plouin P.F., Daninos J.M., Balarac N., Sauvanet J.P. (1999). Diagnostic et classification du diabèteè sucré les nouveaux critéres. Diabetes & Metabolism (Paris), 25, 72-83.

Ďuračková Z. (2010). Some current insights into oxidative stress. Physiol Res. 59(4):459-469.

E

Eidi A., Eidi M., Darzi R. (2016). antidiabetic effect of *olea europaea* l. in normal and diabetic rats. phytother res.; 23(3):347-50.

El Ansari W., Stock C., Mills C. (2013). Is alcohol consumption associated with poor academic achievement in university students. Int J Prev Med. 4(10):1175-88.

El Ashry, **E.S.H.; Rashed, N.; Salama, O.M.; Saleh, A. (2003).** Components, therapeutic value and uses of myrrh. Die Pharm.-Int. J. Pharm. Sci. 2003, 58, 163–168.

F

Faostat. (2021). Food and Agriculture Organisation of the United Nations Statistical Database; Statistical Division; FAO: Rome, Italy, Available.online: http://www.fao.org/statistics/en/.

Fleury M.B., Neudörffer A., Felim A., Blanco M., Monnet F.P., Largeron M. (2009). Métabolites de l'ecstasy et phénomènes de cytotoxicité. Annales Pharmaceutiques Françaises. 67(2):91-6.

Fahad T., Shameem I. (2018). Phytochemical and therapeutic potentials of murr makki (Commiphora myrrha): a review. International Journal of Advanced Research;8(9).

Fares R., Bazzi S., Baydoun S.E., Abdel-Massih R.M. (2011). The antioxidant and antiproliferative activity of the Lebanese Olea europaea extract. Plant Foods Hum Nutr 66:58–63.

Fathy S. M., Drees E. A. **(2015).** Protective effects of Egyptian cloudy apple juice and apple peel extract on lipid peroxidation, antioxidant enzymes and inflammatory status in diabetic rat pancreas. BMC Complementary and Alternative Medicine. 16(1):8–14.

Ferchichi H, Salouage I, Bacha S et al. (2011). Effect of *Myrtus communis* L. extracts on attenuation of liver normothermic ischemia-reperfusion injury. Int J Transplant Technol Res S3: 001, 2011.

Ferreira ICFR, Barros L, Soares ME, Bastos ML, Pereira JA. (2007). Antioxidant activity and phenolic contents of Olea europaea L. leaves sprayed with different copper formulations. Food Chem. 103:188–195.

Flohel G. (1984). Assays of glutathione peroxidase. *Methods Enzymol*. 105; 114-121.

Frank C.L.U. (1992). Toxicologie, Données générales, procédures d'évaluation, organes cibles, évaluation du risque. (Paris), 73-202.

Fu S., Arráez-Roman D., Segura-Carretero A., Menéndez J.A., Menéndez-Gutiérrez M.P., Micol V., Fernández-Gutiérrez A. (2010). Qualitative screening of phenolic compounds in olive leaf extracts by hyphenated liquid chromatography and preliminary

evaluation of cytotoxic activity against human breast cancer cells. Anal Bioanal Chem 397:643–654.

G

Gallo G. and Martino G. (2009). Red blood cell glutathione peroxidase activity in female nulligravid and pregnant rats. 7(1):7.

Gacem M.A., Ould El Hadj-Khelil A., Boudjemaa B., and Gacem H. (2020). Phytochemistry, Toxicity and Pharmacology of Pistacia lentiscus, Artemisia herba-alba and Citrullus colocynthis. Sustainable Agriculture Reviews 39, 57-93.

Gadir S.A., Ahmed I.M. (2014). *Commiphora myrrha* and *Commiphora Africana* essential oils. J. Chem. Pharm. Res., 6, 151–156.

Galicia-Garcia U., Benito-Vicente A., Jebari S., Larrea-Sebal A., Siddiqi H., Kepa B. Uribe, Ostolaza H., and Martín C. (2020). Pathophysiology of Type 2 Diabetes Mellitus.Int J Mol Sci. 21(17): 6275.

Georges Hennen. (2006). LIVRE Biochimie : approche bioénergétique et médicale; préfacé par Adrien Binet, Hennen, Georges; Binet, Adrien Paris : Dunod.

Giampieri F., Cianciosi D., Forbes-Hernández T.Y. (2020). Myrtle (*Myrtus communis* L.) berries, seeds, leaves, and essential oils: New undiscovered sources of natural compounds with promising health benefits. Food Frontiers, 1(3), 276-295.

Giannakopoulou E., Mitsopoulos G., Hagidimitriou M., Papageorgiou V., Komaitis M. (2011). Influence of cultivar, harvesting season and geographical origin ob phenolic content in leaves of Greek olive cultivars. Acta Hort 924:437–444

Goldsmith C.D, Vuong Q.V., Stathopoulos C.E., Roach P.D., Scarlett C.J. (2014). Optimization of the aqueous extraction of phenolic compounds from olive leaves. Antioxidant 3:700–712.

Gomathi D., Ravikumar G., Kalaiselvi M., Devaki K., Uma C. (2013). Efficacy of Evolvulus alsinoides (L.) L. on insulin and antioxidants activity in pancreas of streptozotocin induced diabetic rats. Journal of Diabetes & Metabolic Disorders;12(1):1–6.

Grbić M.L., Unković N. Dimkić I., Janaćković P., Gavrilović M., Stanojević O., Vukojević J. (2018). Frankincense and myrrh essential oils and burn incense fume against micro-inhabitants of sacral ambients. Wisdom of the ancients. J. Ethnopharmacol. 219, 1–14.

H

Halimi S. (2006). Metformin: 50 years old, fit as a fiddle, and indispensable for its pivotal role in type 2 diabetes management. Diabetes Metabolism. 32:555–6.

Hanem Mansour H.M.M., **Zeitoun A.A., Saeed Abdrabou H., El Enshasy H.A., Dailin D.J., Zeitoun M.A.A., and El-Sohaimy S.A. (2023)** Antioxidant and Anti-Diabetic Properties of Olive (*Olea europaea*) Leaf Extracts: *In Vitro* and *In Vivo* Evaluation (6):1275.

Hanus L.O., Rezanka T., Dembitsky V.M, et al. (2005). Myrrh—Commiphora chemistry. Biomed Pap Med Fac Univ Palacky Olomouc Czech Repub.; 149:3–27.

Happy A.A., Jahan F., Momen M.A. (2021). Essential Oils: Magical Ingredients for Skin Care. J. Plant Sci, 9, 54–64.

Harold E. Seifried, Susan M. Pilch. (2013). Antioxidants in Health and Disease. National Institutes of Health Library, Bethesda, Maryland. Nutrition in the Preventionand Treatment of Disease,Third Edition.

Harrigan R.A., Nathan M. S. and Beattie P. (2001). Oral agents for the treatment of type 2 diabetes mellitus: pharmacology, toxicity, and treatment. Annals of Emergency Medicine, 38(1), 68-78.

Haye A., Ansari M. A., Saini A., et al. (2022). Polyherbal formulation improves glucose lipid metabolism and prevent hepatotoxicity in streptozotocin induced diabetic rats: plausible role of IRS-PI3K-Akt-GLUT2 signaling. Pharmacognosy Magazine. 18(77):52–65.

Helal E.G., Mahmoud A., El-Badawy E.E., Kahwash A.A. (2005). Effect of *Commiphora myrrha* extract on some physiological parameters and histological changes in diabetic albino rats. Egypt. J Hosp Med.; 20:148–162.

Henri M. **(2011).** Obésité er insulino-résistance: Etude longitudinale avec un traceur du transport du glucose, le [125I]-6-déoxy-6-iodo-D-glucose. Thèse doctorat. Université de Grenoble.

Hollman P.C., de Vries J.H., van Leeuwen S.D., Mengelers M.J., Katan M.B. (1995). Absorption of dietary quercetin glycosides and quercetin in healthy ileostomy volunteers The American Journal of Clinical Nutrition 62:1276–1282.

I

Ingleton V.L. and Rossi J.A. (1965). Colorimetry of Total Phenolics with Phosphomolybdic-Phosphotungstic Acid Reagent. American Journal of Enology and Viticulture, 16, 144-158.

Ivo Oliveira, **Anabela Sousa, Jorge Sa´ Morais, Isabel C.F.R. Ferreira, Albino Bento, Letı´cia Estevinho, Jose´ Alberto Pereira. (2008**). Chemical composition, and antioxidant and antimicrobial activities of three hazelnut (Corylus avellana L.) cultivars Food and Chemical Toxicology 46, 1801–1807.

J

Jagtap A. G., Patil P. B. (2010). Antihyperglycemic activity and inhibition of advanced glycation end product formation by Cuminum cyminum in streptozotocin induced diabetic rats. Food and Chemical Toxicology;48(8-9):2030–2036.

Jemai H., Bouaziz M., Fki I., El Feki A., Sayadi S. (2008). Hypolipidimic and antioxidant activities of oleuropein and its hydrolysis derivative-rich extracts from Chemlali olive leaves. Chemico- Biological Interactions. 176:88–98.

Julio W., Tali G., Mona B., Yosef B.D., et al. (2012). Olive Leaf Extract as a Hypoglycemic Agent in Both Human Diabetic Subjects and in Rat. Journal of Medical Food.

Julkunen-Titto R. (1985). Phenolic constituents in the leaves of northern willows: methods for the analysis of certain phenolics. J Agric Food chem; 33 (2): 213- 217.

K

Kaeidi A., Esmaelli-Mahani S., Shebiani V., Abbasnejad M., Rasoulian B., Hajializadeh Z., Afrazi S. (2011). Olive (*Olea europeae* L.) leaf extract attenuates early diabetic neuropathic pain through prevention of high glucose-induced apoptosis: in vitro and in vivo studies. J Ethnopharmacol 136:188–196.

Kalam S., Singh R., Mani A., Patel J., Khan F.N. and Pandey A. (2012). Antioxidants: elixir of life. International Multidisciplinary Research Journal, 2(1), 18-34.

Kamil N.B. (2018). Al-Ghaban, N.M. Evaluation the effects of local exogenous application of a mixture of Myrrh and Sage oil on incisional wound healing on the skin of the face (Histological, Histochemical and Histomorphometrically study in rabbits). J. Pharm. Sci. Res. 2018, 10, 1030–1035.

Karagôzler A.A., Erdag B., Emek Y.G., Uygum D.A. (2008). Antioxidant activity and proline content of leaf extracts from *Dorystoechas hastate*. Food Chemistry, 111, 400-407.

Kelley D.E., Reilly J.P., Veneman T. and Mandarino L. (1990). Effects of insulin on skeletal muscle glucose storage, oxidation, and glycolysis in humans. American Journal of Physiology, 258(6), E923-E929.

Khan A.M., Qureshi R.A., Ullah F., Gilani S.A., Nosheen A., Sahreen S., Leghari M.K., Laghari M.Y., Rehman S., Hussain I., Murad W. (2011). Phytochemical analysis of

selected medicinal plants of Margalla Hills and surroundings. Journal of Medicinal Plants Research. 5(25): 6017-6023.

Korukluoglu M., Sahan Y., Yigit A., Karakas R. (2006). Antifungal activity of olive leaf (*Olea europaea* L.) extracts from the trilye region of Turkey. Annals Microbiol 56:359–362.

Koski R.R. (2006). Practical review of oral antihyperglycemic agents for type 2 diabetes mellitus. The Diabetes Educator, 32(6), 869-876.

Koul B., Taak P., Kumar A., Khatri T., and Sanyal I. (2017). The Artemisia genus: A review on traditional uses, phytochemical constituents, pharmacological properties and germplasm conservation. J. Glycomics Lipidomics, 7(1), 142.

L

Lee Chan Hee, Sung Ha Guyn, Eslami Moosa, Lee Se Young, Song Jae Y., Lee Sung Sill, Ha Jong K. (2007). Effects of Tween 80 pretreatment on dry matter disappearance of rice straw and cellulolytic bacterial adhesion. Asian-Aust. J. Anim. Sci., 20 (9): 1397-1401.

Lee-Huang S., Zhang L., Huang P.L., Chang Y.T., Huang P.L. (2003). Anti-HIV activity of olive leaf extract (OLE) and modulation of host cell gene expression by HIV-1 infection and OLE treatment. Biochem Biophys Res Commun 307:1029–1037.

Lenzen S., Ibrahim M.A.B., Wani F. A., and Rahiman S. (2008). "Hepatoprotective effect of olive oil and camel milk on "-e mechanisms of alloxan and streptozotocininduced diabetes," Diabetologia, vol. 51, no. 2, pp. 216–226.

Li P., Zhang L., Tian X., Xing J. (2014). Docosahexaenoic acid has an anti-diabetic effect in streptozotocin-induced diabetic mice. International Journal of Clinical and Experimental Medicine ;7(9):3021–3029.

Lien Ai Pham-Huy, Hua He. (2008). Chuong Pham-Huy Free radicals, antioxidants in disease and health Int J Biomed Sci. Jun;4(2):89-96.

Lorke D. (1983). A new approach to practical acute toxicity testing. Arch Toxicol; 54: 275-87.

Laouini S.E, Kelef A., Ouahrani M.R. (2018). Free Radicals scavenging activity and phytochemical composition of *Artemisia herba alba* extract growth in Algeria. ournal of Fundamental and Applied Sciences. 10(1), 268-280.

Lv L., Yan G.Y., Zhao Y.L., He X.J., Jiang X., Zhuo Y.Q., Cen, X.B. (2009). Investigation of the dermal sensitizing potential of traditional medical extracts in local lymph node assays. Exp. Biol. Med., 234, 306–313.

M

Mahajan S.M., Baviskar D.T., Chaudhari P.M. (2018). Antidiabetic activity of polyherbal formulation on alloxan induced diabetes. IOSR JPBS. 13:01-6.

Magnan C., Ktorza A. (2005). Production et sécrétion de l'insuline par la cellule β pancréatique Production and secretion of insulin by the pancreatic , β-cell EMC – Endocrinologie. 2(4); 241-264.

Makker K., Agarwal A., Sharma R. (2009). Oxidative stress & male infertility**.** Indian J Med Res. 129(4):357-67.

Makris D., Boskoub G., Andrikopoulos N.K. (2007). Polyphenolic content and in vitro antioxidant characteristics of wine industry and other agri-food solid waste extracts. J Food Comp Anal 20:125–132.

Malekpour A., Dehghani S., Zahedi S., Eskandari F. (2012). Effects of the hydro-ethanol extract of myrtus communis l. on blood glucose level and histopathological changes in alloxan-induced diabetic rats. Middle-East J Scient Research 12: 517-522.

Manthena S.V., Arutla R., Mohan P.C., Prusty B. (2017). Evaluation of antidiabetic and antioxidant activity of root extracts of herbal mixture (Catharanthus roseus, Leucas linifolia). IOSR JPBS.12(6):29-36.

Massoud A., El Sisi S., Salama O. (2001). Preliminary study of therapeutic efficacy of a new fasciolicidal drug derived from Commiphora molmol (myrrh). Am J Trop Med Hyg 65:96–99.

Memelink J., Verpoort R., Kijine J.W. (2001). Organization of jasmonate responsive gene expression in alkaloid metabolism. Trends in Plant Science, 6, 212-221.

Mezouar D., Aissaoui M., Benmessaoud A., Boucif Lahfa F.. (2022). Phytochemical analysis and alpha-amylase inhibitory property of olive (Olea europaea L.) leaves extracts Dounia. J Nat Prod Res App 2022, 1 (3): 56-67.

Mezouar M ., Lahfa F.B, Djaziri R, Boucherit-Otmani Z. (2014). Évaluation de l'activité antioxydante de *Berberis vulgaris* L. 12:297-301.

Mohamed A. E.H.H., El-Sayed M., Hegazy M.E., Helaly S.E., Esmail A.M., and Mohamed N.S. (2010). Chemical constituents and biological activities of Artemisia herba-alba. Records of Natural Products, 4(1).

Reza M., S. A., Mahnaz, K., Mannan, H., Maryam J. and Abbas H. (2010). Comparison of antioxidant activity and total phenol contents of some date seed varieties from Iran. Iranian Journal of Pharmaceutical Research, 9(2): 141 – 146

Molina-Alcaide E, Yáñez-Ruiz DR. (2008). Potential use of olive by-products in ruminant feeding: a review. Anim Feed Sci Technol 147:247–264.

Morales M.T., Calvente J.J., Aparicio R. (1996). Influence of olive ripeness on the concentration of green aroma compounds in virgin olive oil. Flav Frag J 11:171–178

Motamed S.M., Naghibi F. (2010). Antioxidant activity of some edible plants of the Turkmen Sahra region in northern Iran. Food Chemistry, 119, 1637-1642.

Mouchem M.F.Z. (2015). Contribut à l'etude des huiles essentielles de l'armoiseblanche de trois localités de l'ouest algérien (Ras-Elma, el-Aricha et Mécheria) et leurs effets antimierobiens, Thèse de Doctorat, Univ. Djillali libes de Sidi Bel-Abbes (Algerie), 131 p.

Mourtzinos I., Salta F., Yannakopoulou K., Chiou A., Karathanos V. (2007). Encapsulation of olive leaf extract in β-cyclodextrin. J Agric Food Chem 55:8088–8094

Moussa S. (2008). Oxidative stress in diabetes mellitus. Romanian J biophys. 18:225–236.

Mulla M., Attar S., Nithore N., Parkar R. (2022). Formulation and evaluation of herbal ointment containing Neem and Karanj oil, International journal of creative research thoughts (IJCRT); 10(5):200-205.

N

N'Guessan K., Kadja B., Zirihi G., Traoré D., Aké-Assi L. (2009). Screening phytochimique de quelques plantes médicinales ivoiriennes utilisées en pays Krobou (Agboville, Côted'Ivoire). Science of Nature. 6(1): 1-15.

Nathalie Kirschvink. (2008). Marianne Raes, Claude Saegerman Impact of a natural bluetongue serotype 8 infection on semen quality of Belgian rams in 2007 November 2009182(2):244-251.

Nees D., Engler G. A. (1883). https://fr.wikipedia.org/wiki/Arbre_%C3%A0_myrrhe. 30.06.2024.

Nigam, M., Atanassova, M., Mishra, A. P., Pezzani, R., Devkota, H. P., Plygun, S., selehi, B., Setzer, W. N., & Sharifi-Rad, J. (2019). Bioactive Compounds and Health Benefits of Artemisia Species. Natural Product Communications, 14 (7), 1934578-19850354.

O

Ohnishi M., Matuo T., Tsuno T., et *al*.(2004). Antioxidant activity and hypoglycemic effect of ferulic acid in STZ-induced diabetic mice and KK-Aymice. BioFactors . 2004; 21(1-4):315 319.

Ojewale A, Adekoya A, Faduyile A, Yemitan O, and Odukanmi A. (2014). "Nephroprotective activities of ethanolic roots extract of Pseudocedrela kotschyi against oxidative stress and nephrotoxicity in alloxan-induced diabetic albino rats,"British Journal of Pharmacology and Toxicology.; 5(1); 26–34.

Oki T., Masuda M., Furuta S., Nishibia Y., Terahara N. and Suda I. (2002). Involvement of anthocyaninsand other phenolic compounds in radical-scavenging activity of purple-fleshed sweet potato cultivars. Food and Chemical Toxicology, 67: 1752 – 1756.

Olayaki L.A , IrekpitaJ.E , Yakubu M.T , and Ojo O.O. **(2015).** "Methanolic extract of Moringa oleifera leaves improves glucose tolerance, glycogen synthesis and lipid metabolism in alloxan-induced diabetic rats," Journal of Basic and Clinical Physiology and Pharmacology, vol. 26, no. 6, pp. 585–593.

Omer S.L. Al-Janabi, Amer M.S, Khayri M.H. (2013). Effects of the extracts of Olive and Morus Alba Leaves on Experimentally STZ Induced Diabetes in Male Rats. International Journal of Science and Research.

Osterwalder U., Herzog B. (**2009**). Sun protection factors: world wide confusion. British Journal of Dermatology. 161(s3), 13-24.

Oyedeji F.O., **Hassan G.O. and Adeleke B.B. (2011).** Hydroquinone and Heavy Metals Levels in Cosmetics Marketed in Nigeria. Trends in Applied Sciences Research, 6: 622-639.

Özcan M. M., Al Juhaimi F., Ahmed I.A.M., Babiker E.E., and Ghafoor K. (2020). Antioxidant activity, fatty acid composition, phenolic compounds and mineral contents of stem, leave and fruits of two morphs of wild myrtle plants. Journal of Food Measurement and Characterization, 14, 1376–1382.

Özcan O. (2017). Mersin bitkisinin (Myrtus communis subsp. communis) termal deri yanığı üzerine etkisi (Master's thesis, Sağlık Bilimleri Enstitüsü).

Özkan A.M.G., and Güray Ç.G. (2009). A mediterranean: Myrtus communis L.(myrtle). Plants and Culture: Seeds of the Cultural Heritage of Europe, Morel JP, Mercuri AM (eds.). Edipuglia: Bari, 159-168.

P

Palmieri B., Sblendorio V. (2007). Oxidative stress tests: overview on reliability and use. Part I. Eur Rev Med Pharmacol Sci. 11(5):309-42.

Panjeshahin R., **Azadbakht M., Akbari N. (2016).** Antidiabetic Activity of Different Extracts of Myrtus Communis in Streptozotocin Induced Diabetic Rats. Rom J Diabetes Nutr Metab Dis. 23(2):183-190.

Parasuraman S., Ching T. H., Leong C. H., Banik U. (2019). Antidiabetic and antihyperlipidemic effects of a methanolic extract of Mimosa pudica (Fabaceae) in diabetic rats. Egyptian Journal of Basic and Applied Sciences; 6 (1):137–148.

Parvaiz M., Hussain K., Shoaib M., William G., Tufail M., Hussain Z., Gohar D., Imtiaz S. (2013). A review: therapeutic significance of olive (*Olea europaea* L; oleaceae family). Global J Pharm 7(3):333–336.

Pereira J.A., Pereira A.P.G., Ferreira I.C.F.R., Valentão P., Andrade P.B., Seabra R., Estevinho L., Bento A. (2006). Table olives from Portugal: phenolic compounds, antioxidant potential and antimicrobial activity. J Agric Food Chem 54:8425–8431.

Preethi K.C., Kuttan G., and Kuttan R. (2006). Anti-tumour activity of Ruta. Asian Pac J Cancer Prev. 7(3):439-43.

Prieto P., Pineda M., Aguilar M. (1999). Spectrophotometric quantitation of antioxidantcapacity through the formation of a phosphomolybdenum complex: specific application to the determination of vitamin E. Analytical Biochemistry, 269, 337-341.

Q

Quezel P. and Santa S. (1963). Nouvelle flore de l'Algérie et des regions désertiques méridionale. Tome II Edition. CNRS. Paris.

Quintin Ernst Muhl, **(2011)**. Elsa S. Du Toit, Petrus Johannes Robbertse Adaptability of *Moringa oleifera* Lam. (Horseradish) Tree Seedlings to Three Temperature Regimes.

R

Rafiq S., Huma N., Pasha I., Sameen A., Mukhtar O., Khan M.I. (2015). Chemical Composition, Nitrogen Fractions and Amino Acids Profile of Milk from Different Animal Species. Asian-Australas J Anim Sci. l;29(7):1022-8.

Rahman K. (2007) Studies on free radicals, antioxidants, and co-factors. Clin Interv Aging.2(2):219-36.

Rao R.M., Khan Z.A., Shah A.H. (2001). Toxicity studies in mice of Commiphora molmol oleo–gum–resin. J Ethnopharmacol. 76:151–154.

Raverot G., Weiss J., Park S.Y., Hurley L., Jameson J.L. (2005). Sox3 expression in undifferentiated spermatogonia is required for the progression of spermatogenesis. Dev Biol. 2005 Jul 1; 283(1):215-25.

Ravi K., rojasekaran S., Subramanian S. (2005). Antihyperlipidemic effect of Eugenia Jambolana seed Keinel on STZ induced diabetic rats. Food chemical Toxicologie43:1433-1439.

Riyazullah Kavitha S. and Ravi M.. (2021). Formulation of a polyherbal cream and screening for its anti-inflammatory activity. World Journal of Pharmaceutical and Life Sciences. 7(10),101-105.

Rodier M. (2001). Définition et classification du diabète. Médecine Nucléaire—Imagerie fonctionnelle et métabolique, 25, 5-18.

Rohman A., Riyanto S., Yuniarti N., Saputra W. R., Utami R. and Mulatsih, W. (2010). Antioxidant activity, total phenolic, and total flavaonoid of extracts and fractions of red fruit (Pandanus conoideus Lam) International Food Research Journal 17: 97-106.

S

Sumbul S., Ahmad M.A., Asif M., Saud I., Akhtar M. (2010). Evaluation of Myrtus communis Linn. berries (common myrtle) in experimental ulcer models in rats. Hum Exp Toxicol. 29(11):935–944.

Sumbul S., Ahmad M.A., Asif M., Akhtar M., Saud I. (2012). Physicochemical and phytochemical standardization of berries of Myrtus communis Linn. J Pharm Bioallied Sci. 4(4):322–326.

Sabry O.M.M. (2014). Review: beneficial health effect of olive leaves extracts. J Nat Sci Res 4:1–9

Sakthivel M., Halith Mohamed S., Karthikeyan R., Kaviya M., Kiruthika M., Kowsalya S., Krishnapriya R. (2023). Formulation and Evaluation of Herbal Ointment Containing Neem and Turmeric Extract. Int. J. Pharm. Sci. Rev. Res., 78(2),134-139.

Salah M.B., Abdelmelek H., Abderraba M. (2012). Study of phenolic composition and biological activities assessment of olive leaves from different varieties grown in Tunisia. Med Chem 2(5):107–111.

Salehi B., Ata A., Anil Kumar N V., et al. (2019). Antidiabetic potential of medicinal plants and their active components. Biomolecules; 9(10):p. 551.

Salwa H.N. Al-Rubae'I and Abass K. Al-Musawi. (2013). An evaluation of antioxidants and oxidative stress in Iraqi patients with thyroid gland dysfunction. Global Journal of Biochemistry and Biotechnology. 1(1), 057-065.

Samarth R.M., Panwar M., Kumar M., Soni A., Kumar M., Kumar A. (2008). Evaluation of antioxidant and radical-scavenging activities of certain radioprotective plant extracts. Food Chem 2008; 106 (2): 868-873.

Samet I., Han J., Jlaiel L., Sayadi S., Isoda H. (2014). Olive (Olea europaea) leaf extract induces apoptosis and monocyte/macrophage differentiation in human chronic myelogenous leukemia K562 cells: insight into the underlying mechanism. Oxidative Med Cell Longevity 2014:1–16

Sathishsekar D., et Subramanian S. (2005). Antioxidant properties of Momordica Charantia (bitter gourd) seeds on Streptozotocin induced diabetic rats. AsiaPac J Clin Nutr,14 (2):153-158.

Sato H., Genet C., Strehle A., et al. (2007). Anti-hyperglycemic activity of a TGR5 agonist isolated from Olea europaea. Biochemical and Biophysical Research Communications.;362(4):793–798.

Sharififar F., Moshafi M.M., Mansouri S.H., Khodashenas M., Khoshnoodi M. (2007). *In vitro* evaluation of antibacterial and antioxidant activities of the essential oil and methanol extract of endemic Zataria multiflora Boiss. Food Control 18 800–805.

Schlienger J.L. (2014). Diabète et phytothérapie: les faits. Médecine des Maladies Métaboliques, 8(1), 101-106.

Schulze R. (1956). Einige Versuche und Bemerkungen zum Problem der handelsüblichen Lichtschutzmittel. Parfum und Kosmet; 37: 310.

Seddik K., Nadjet I., Abderrahmane B., Daoud H., Lekhmici A. (2010). Antioxidant and Antibacterial Activities of Extractsfrom Artemisia Herba Alba Asso. Leaves and Some Phenolic Compounds. Journal of Medicinal Plants Research. 4(13); 1273–1280.

Sedef N.E.l., Karakaya S. (2009). Olive tree (*Olea europaea*) leaves: potential beneficial effects on human health. Nutr Rev. 67:632–638.

Selin Ş. and Bilgin M. (2017). Olive tree (*Olea europaea* L.) leaf as a waste by-product of table olive and olive oil industry.

Shameem, I. (2018). Phytochemical & therapeutic potentials of Murr makki (*Commiphora myrrha*). Indian J. Appl. Res, 8, 102–104.

Shodehinde S. A., Oboh G. (2013). Antioxidant properties of aqueous extracts of unripe Musa paradisiaca on sodium nitroprusside induced lipid peroxidation in rat pancreas in vitro. Asian Pacific Journal of Tropical Biomedicine.; 3(6):449–457.

Shokoohi H, Boniface KS, Zaragoza M, Pourmand A, Earls JP. (2017). Point-of-care ultrasound leads to diagnostic shifts in patients with undifferentiated hypotension. Am J Emerg Med. 2017 Dec;35(12):1984.e3-1984.e7.

Siham Benmeziane, Malik Haddadin, and Hayder AL-Domi. (2023). Extraction Yield, Phytochemicals Analysis, and Certain in Vitro Biological Activities of Artemisia Herba Alba Extracts.19;2.

Sileshi Dubale, Dereje Kebebe, Ahmed Zeynudin, Negera Abdissa, and Sultan Suleman (2023). Phytochemical Screening and Antimicrobial Activity Evaluation of Selected Medicinal Plants in Ethiopia J Exp Pharmacol.; 15: 51–62.

Simonis-Bik AM, Nijpels G, van Haeften TW, Houwing-Duistermaat JJ, Boomsma DI, Reiling E, van Hove EC, Diamant M, Kramer MH, Heine RJ, Maassen JA, Slagboom PE, Willemsen G, Dekker JM, Eekhoff EM, de Geus EJ, 't Hart LM. (2010). Gene variants in the novel type 2 diabetes loci CDC123/CAMK1D, THADA, ADAMTS9, BCL11A, and MTNR1B affect different aspects of pancreatic beta-cell function. Diabetes. 59(1):293-301.

Singh H, Arora S, Mani M, Mahaur KK, Chandra P. (2014). Development of multicomponent formulation of herbal drugs for evaluation of Antidiabetic activity. Pharm Lett. 6(1):219-23.

Soheila Moein, Mahmoodreza Moein, Narjes Ebrahimi, Fatemeh Farmani, Jelveh Sohrabipour and Reza Rabiei. (2015). Extraction and determination of protein content and antioxidant properties of ten algae from Persian Gulf. 6(2), 29-38.

Soler RC, Espín JC, Wichers HJ (2000). Review oleuropein and related compounds. J Sci Food Agric.80:1013–1023.

Somova LI, Shode FO, Ramnanan P, Nadar A. (2003). Antihypertensive, anti-atherosclerotic and antioxidant activity of triterpenoids isolated from *Olea europaea*, subspecies Africana leaves. J Ethnopharmacol. 84:299–305.

Soulama S., Sanon H.O., Meda R.N.T., Boussim J.I. (2014).Teneur en Tanins de 15 ligneux fourrgers de bourkina faso. Afrique Science. 10(4): 180-190.

T

Taamalli A, Arráez-Román D, Barrajón-Catalán E, Ruiz-Torres V, Pérez-Sánchez A, Herrero M, Fernández-Gutiérrez A (2012). Use of advanced techniques for the extraction of phenolic compounds from Tunisian olive leaves: phenolic composition and cytotoxicity against human breast cancer cells. Food Chem Toxicol.50:1817–1825.

Talhaoui N, Taamalli A, Gomez-Caravaca AM, Fernandez Gutierrez A, Segura-Carretero A. (2015). Phenolic compounds in olive leaves: analytical determination, biotic and abiotic influence, and health benefits. Food Res Int 7:92–108.

Tavafi M, Ahmadvand H, Toolabi P. (2012). Inhibitory effect of olive leaf extract on gentamicin-induced nephrotoxicity in rats. Kidney Dis 6:25–32.

Tepe B., Sokmen M., Sokmen A., Daferera D., Polissiou M. et al. (2005). Antimicrobial and antioxidative of the essential oil and various extracts of Cyclotrichium origanifolium (Labill.) Manden. & Scheng.Journal of Food Engineering.; 69(3):335-342.

Togola A. (2002). Étude de la phytochimie et de l'activité antipaludique *d'Alchornea cordifolia* Schmach. (Euphorbiaceae).Thèse de doctorat. Université de Bamako, Mali. p. 38-42.

Tsimidou M.Z., Papoti V.T. (2010). Bioactive ingredients in olive leaves. In: Preedy VR, Watson RR (eds) Olives and olive oil in health and disease prevention. Elsevier Inc., Amsterdam, pp 349–356.

Tuck K.L., Hayball P.J. (2002). Major phenolic compounds in olive oil: metabolism and health effects. J Nutr Biochem 13:636–644.

U

Upadhyay P., Sadhu A., Purohit S., Singh P.K., Shivakumar S. **(2015).** Effect of a novel polyherbal formulation on diabetes induced memory deficits in rats. Clin Exp Pharmacol.5:194.

V

Vaghasiya Y., Chanda S. (2007). Screening of methanol and acetone extracts of fourteen Indian medicinal plants for antimicrobial activity. Turk. J. Biol. 31, 243–248. 2Dohou N., Yamni K., Tahrouch S., Idrissi Hassani L.M., Badoc A., Gmira N. 2003.

Valko M., Leibfritz D., Moncol J., Cronin M.T., Mazur M., Telser J. (2007). Free radicals and antioxidants in normal physiological functions and human disease. Int J Biochem Cell Biol.;39(1):44-84.

Van Vuuren S., Viljoen A. (2011). Plant-based antimicrobial studies–methods and approaches to study the interaction between natural products. Planta Med.;77(11):1168-82.

W

Wang, H.X. and T.B. Ng, (1999). Natural products witha hypoglycemic, hypotensive, hypocholesterolemic, antiatherosclerotic and antithrombotic activities. Life Sci., 65: 2663-2677.

Weckbercker, G., Cory, J.G. (1988). Ribonucleotide reductase activity and growth of glutathione-depleted mouse leukemia L1210 cells in vitro. *Cancer Letters*,40 (3)257-264.

Wichtl M. (1994). Herbal drugs and phytopharmaceuticals, Medpharm. GmbH Scientific Publishers, Birkenw aldstrasse.; 44:650–652

Williamson EM. (2001). Synergy and other interactions in phytomedicines. Phytomedicine. 8(5):401-9.

WOH. **(2022)**. Wourld health organization Protocole National de Diagnostic et de Soins (PNDS).15/11/2022.

Y

Yagi, K. (1976). Simple fluorimetric essay for lipoperoxide in blood plasma. Biochem Med. Vol. 15: 212-216.

Réggami Y., **Benkhaled A., Boudjelal A., Berredjem H., Amamra A., Benyettou H., Larabi N., Senator A., Siracusa L. and Ruberto G.. (2021).** *Artemisia herba-alba* aqueous extract improves insulin sensitivity and hepatic steatosis in rodent model of fructose-induced metabolic syndrome. Arch Physiol Biochem.127(6):541-550.

Ying Xiong; Joachim D Uys; Kenneth D. (2011). S- Glutathionylation: From Molecular Mechanisms to Health Outcomes.15(1):233-70.

Younsi F., **Trimech R., Boulila A., Ezzine O., Dhahri S., Boussaid M. and Messaoud C. (2016).** Essential Oil and Phenolic Compounds of Artemisia herba-alba (Asso.): Composition, Antioxidant, Antiacety lcholinesterase, and Antibacterial Activities. International Journal of Food Properties, 19:1425–1438.

Z

Zatout F, Benarba B, Bouazza A, Babali B, Bey N and Morsli A. (2021). Ethnobotanical investigation on medicinal plants used by local populations in Tlemcen National Park (extreme North West Algeria). Botany 42, e69396, 2021.

Zarzuelo A., Duarte J., Jimenez J., Gonzales M. (1991). Utrilla Vasodilator effect of olive leaf. Planta Med 57:417–419.

Zadák Z., Hyspler R., Tichá A., Hronek M., Fikrová P., Rathouská J., Hrnciariková D., Stetina R. (2009). Antioxidants and vitamins in clinical conditions. Physiol Res. 58 Suppl 1:S13-S17.

Zhishen J., Mengcheng T., Jianming W. (1999). The determination of flavonoid contents inmulberry and their scavenging effects on superoxide radicals. Food Chemistry. 64(4): 555-559.

Zhou X., Seto S.W., Chang D., Kiat H., Razmovski- Naumovski V.R., Chan K., Bensoussa A. (2016). Synergistic effects of Chinese herbal medicine: A comprehensive review of methodology and current research. Front Pharmacol.7: 201.

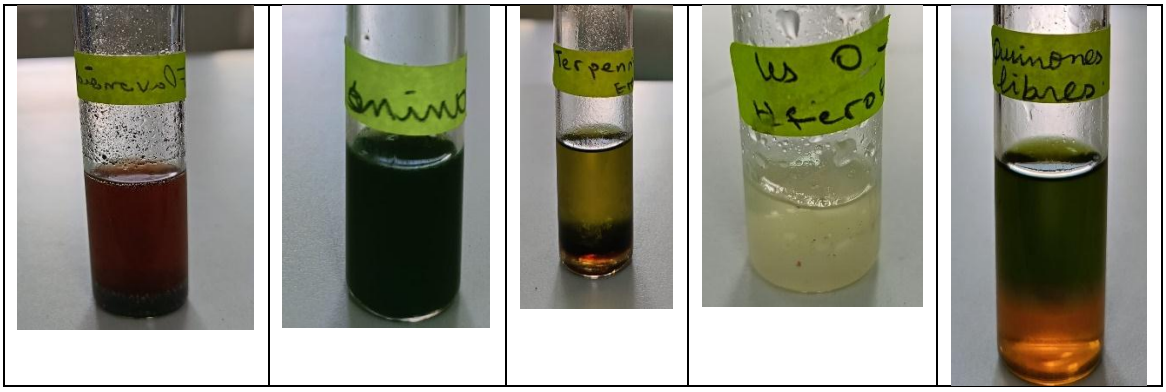
Zhou Q., Liu Y., Tang Y., Shokoohinia Y., Chittiboyina A.G., Wang M., Avonto C. (2019). Identification of potential skin sensitizers in myrrh. Cosmetics, 6, 47.

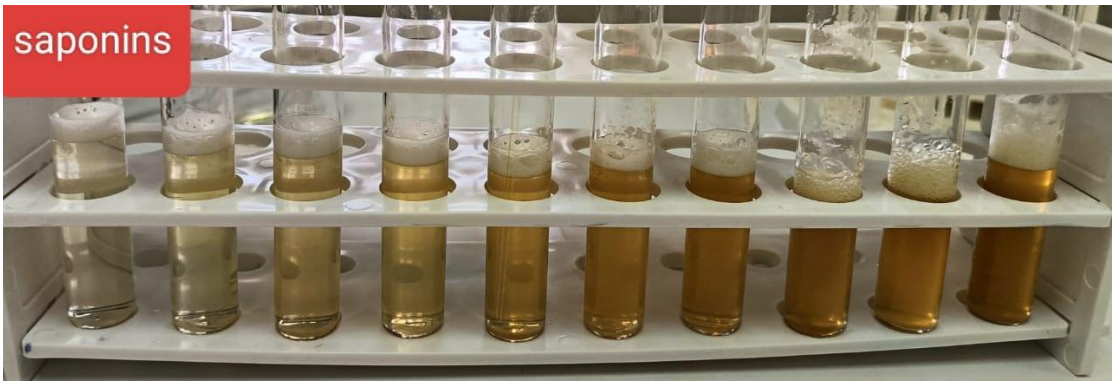
Ziane N. (2014). Valorisation des Ressources Végétales ; Thème Contribution à l'étude de l'activité hypoglycémiante des extraits de Pistacia atlantica Desf de la réserve nationale d'El-Mergueb (M'sila) –Algérie.

Annexes

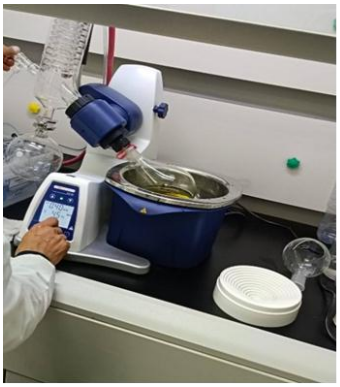
Results of phytochemical screening

Flavonoids	Tannins	Terpenoids	O- heteroside sterolic	Free quinones


Quinones
libres


saponins

Laboratory materials used

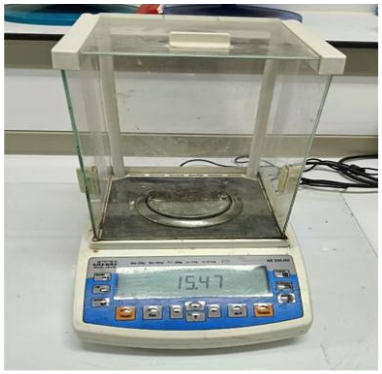


Evaporator rotatif

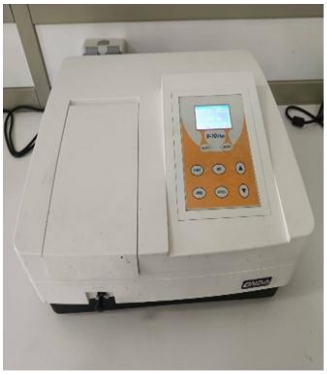
Etuve

Centrifugeuse

Marie bath



Analytique balance



Spectrophotometre UV-VIS

Microscope optique OPTIKA

pH metre

الجمهورية الجزائرية الديمقراطية الشعبية
REPUBLIQUE ALGERIENNE DEMOCRATIQUE ET POPULAIRE Demande N°:2083

INSTITUT NATIONAL ALGERIEN
DE LA PROPRIETE INDUSTRIELLE

المعهد الوطني الجزائري للملكية الصناعية

REQUETE EN DELIVREANCE D'UN BREVET D'INVENTION
طلب منح براءة الإختراع

1 Natrure de la demande de protection
طبيعة الطلب

Brevet d'invention براءة الإختراع [x] | Demande divisionnaire طلب جزئي | Certificat d'addition شهادة الإضافة

Extension de la demande internationale PCT
الإمتداد عبر طلب دولي

2 INFORMATION SUR LE DEPOSANT
معلومات حول مقدم الطلب — 71

Dénomination: إسم الشركة: Université d'El oued

Forme juridique: الطبيعة القانونية: EPCSCP

Secteur d'activité: قطاع النشاط التجاري: service

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3 CODE DU MANDATAIRE
رمز الوكيل — 74

Nom du mandataire: إسم الوكيل: //////////

4 INFORMATIONS SUR 'INVENTEUR
معلومات حول المخترع — 72

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5 DONNEES RELATIVES A LA PRIORITE
بيانات الأولوية — 30

Date: تاريخ: ////////// | Numéro: رقم الأولوية: //////////

Pays d'origine: البلد الأصلي: //////////

6 TITRE DE L'INVENTION
عنوان الإختراع — 54

Composition d'un compliment alimentaire à effet antidiabétique

7 DOMAINE TECHNIQUE DE L'INVENTION
المجال التقني للإختراع — 51

//////////

8 DONNEES RELATIVES AU DEPOT
بيانات الإيداع

Date: تاريخ: 05 JUIN 2024 | Heure: الوقت:

Numéro: رقم الإيداع: 240568

9 DONNEES RELATIVES A LA DEMANDE INTERNATIONAL
بيانات الطلب الدولي — 72

Date: تاريخ: | Heure: الوقت:

Numéro: رقم:

10 DECHEANCE
إبطال

La déchéance d'un brevet d'invention intervient en cas de non-acquittement, à la date anniversaire du dépôt, des taxes de maintien en vigueur,

يسقط الحق على ملكية براءة الإختراع
في حالة عدم تسديد الرسوم السنوية المستحقة

CADRE RÉSERVÉ À L'INAPI
إطار خاص بالمعهد

inapi
BOUKHEMAL Sofia
Service Dépôt
Chef de Département DTP

SIGNATURE (CACHET)
ختم / توقيع

مدير الجامعة
أ.د عمر قرحاتي

4	INFORMATIONS SUR L'INVENTEUR 2 معلومات حول المخترع	72

Nom et Prénom: الإسم واللقب	Hani Mohamed Laid
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4	INFORMATIONS SUR L'INVENTEUR 3 معلومات حول المخترع	72

Nom et Prénom: الإسم واللقب	Fellah khadidja
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دليل مشروع

للحصول على شهادة براءة اختراع
في إطار القرار الوزاري 1275

ديسمبر
2022

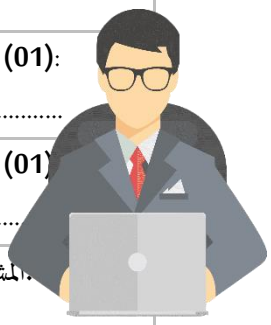
CNCSIIU — MHESR — ALGERIA

بطاقة معلومات

حول فريق الاشراف وفريق العمل

1- فريق الاشراف:

فريقالاشراف	
المشرفالرئيسي (01):	التخصص: شمسة أحمد خليفة
المشرفالرئيسي (01):	التخصص: .....
المشرفالمساعد:	التخصص: فلاح خديجة



2- فريق العمل:

فريق المشروع	التخصص	الكلية
الطالب: هقي غفران	بيوكيمياء تطبيقية	كلية علوم الطبيعة والحياة
الطالب: هاني محمد العيد	بيوكيمياء تطبيقية	كلية علوم الطبيعة والحياة
الطالب:	.....	.....

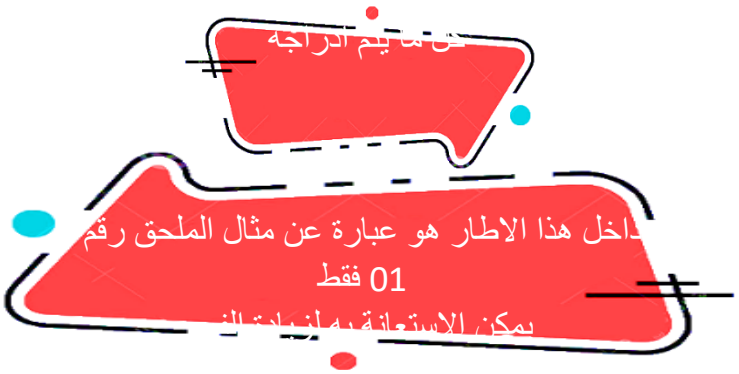
فهرس المحتويات

فهرس المحتويات

مقدمة

بغرض تمكين الطلبة حاملي المشاريع المبتكرة، ومن باب تسهيل عملهم في صياغة وكتابة براءات اختراعهم والانتقال من مجرد أفكار إلى اختراع متكامل بتفاصيل ومعلومات توضح الجوانب الابتكارية للمشروع ومدى جاذبيته وسلامة التسلسل المنطقي للأفكار تقترح اللجنة الوطنية التنسيقية لمتابعة الابتكار وحاضنات الأعمال هذا الملف الذي يعتبر بمثابة دليل للطالب لصياغة مذكرته ضمن القرار الوزاري رقم: 1275 شهادة – براءة اختراع .

سيتم تقديم الشروحات اللازمة للدليل بالاعتماد على مثال بسيط جدا من باب تقريب الفهم أكثر للطالب، هذا المثال يتعلق بمشروع إنتاج عصير طبيعي انطلاقا من فواكه طبيعية لم تخضع للمبيدات أو أسمدة كيميائية.



المحور الأول

تقديم المشروع

المحور الأول

تقديم المشروع

1. فكرة المشروع (الحل المقترح)

هنا اكتب مشروعك في بعض الاسطر متناولا فيها :

- ✓ مجال النشاط (خدمات، صناعي، تطبيقات حديثة، فلاحي، تجاري ...)
- ✓ كيف بدأت الفكرة وكيف تطورت ؟
- ✓ ما الذي سوف تقوم به؟
- ✓ كيف سيكون ذلك؟
- ✓ من الذي سينجز ذلك؟
- ✓ أين سيتم إنجازه ؟

مجال نشاطنا يتمثل في الصناعات الغذائية
(مكمل غذائي طبيعي باستخدام نباتات طبية)

1. بدأت فكرة المشروع من خلال دراسة توصلت إلى أن كل شخص يشرب ما يقارب 23 لتر عصير في السنة، وان 2 من 3 أشخاص يفضلون المكمل الغذائي الطبيعي على باقي المكملات الغذائية الكيميائية، وأن 2 من 10 أشخاص يقبلون على شراء المكمل الغذائي الطبيعي لدواعي صحية.
2. سنقوم بإنتاج المكمل الغذائي.
3. يتم ذلك من خلال انجار وحدة إنتاجية تعتمد على احدث التكنولوجيا في المجال، وبالاعتماد على مواد أولية (نباتات طبية مصنفة).
4. تم اختيار منطقة (.....) لقربها من مصادر النباتات الطبية الجبلية والطبيعية والخالية من المواد الكيميائية.

2. القيم المقترحة :

يمكن أن تنشأ القيم المقترحة أو المقدمة للزبائن من خلال العناصر التالية:

- ✓ الحداثة: تلبية احتياجات جديدة كلياً لم تكن هناك عروض مماثلة لها في السابق.
- ✓ الأداء:أن يكون أداء المنتج أو الخدمة أعلى أو مساوي لتوقعات العميل.
- ✓ التكييف: المرونة في التعديل والتغيير لتكييف المنتجات والخدمات تبعا للاحتياجات المحددة للعملاء.
- ✓ إنجاز المهمة: مساعدة العميل على انجاز مهام محددة.

- ✓ التصميم:جعل التصاميم تتوافق مع رغبات وظروف العميل.
- ✓ خفض التكاليف: مساعدة العملاء على خفض تكاليفهم.
- ✓ الحد من المخاطر: تقليص احتمال تعرض العملاء للمخاطر لدى شرائهم المنتجات أو الخدمات بتقديم ضمانات.
- ✓ سهولة الوصول: جعل المنتجات متاحة للعملاء الذين لم يكن بإمكانهم من قبل الوصول إليها.
- ✓ الملاءمة/سهولة الاستخدام: جعل الأشياء سهلة بسيطة الاستخدام.

في مثالنا يمكن أن تخلق القيمة المضافة من خلال ما يلي:

1. يعتبر مكمل غذائي طبيعي وصحي انطلاقا من نباتات طبية محلية
2. تكلفة إنتاج منخفضة من خلال الاعتماد على تكنولوجيا متطورة

3. فريق العمل (المخترعين):

هنا تتحدث عن فريق العمل على المشروع من خلال :

- ✓ تحدث عن فريق عملك (إذا كنت لست بمفردك في هذا المشروع) مهاراتهم وأدوارهم في براءة الاختراع، (المهارات والمؤهلات العلمية، الدورات التدريبة المتحصل عليها .....).
- ✓ التنظيم المناسب (توزيع المهام والمسؤوليات).
- ✓ طرق التفاعل والتواصل بين الفريق.

يتكون فريق المشروع (براءة اختراع) من الآتي :

1. الطالب 01:.....، تخصص .....، قام بدورات تدريبية في مجال .....
2. الطالب 01:.....، تخصص .....، قام بدورات تدريبية في مجال .....
3. يتمثل دور الطالب 01 في تسيير المشروع والبحث على الأسواق والتسويق

4. أهداف المشروع:

تحتاج في هذا الجزء إلى تحديد الأهداف الابتكارية لبراءة الاختراع

نسعى الى ان نصبح المنتج رقم واحد للعصير الطبيعي في الجزائر خلال السنوات الخمسة الأولى

والوصول الى حصة سوقية تقدر ب35 بالمئة (يتم تقدير الحصة السوقية حسب القدرة الإنتاجية) من اجمالي ما ينتج في الجزائر من العصائر الطبيعية

5. <u>جدول زمني لانجاز براءة الاختراع:</u>

- ✓ كيفية تقسيم الهدف النهائي لبراءة الاختراع إلى مهام فردية.
- ✓ تحديد الوقت اللازم لكل مهمة.
- ✓ تحديد النتائج الرئيسية لكل مهمة.

الشهر أو الأسبوع

			1	2	3	4	5	6	7
1		البحث في قواعد البيانات الخاصة ببراءات الاختراع وجمع المعلومات	✓	✓					
2		الشروع في الاختبارات المخبرية لإعداد النموذج الأولي		✓	✓				
3		تجريب النموذج الأولي		✓	✓	✓			
...		تجربة النموذج الأولي خارج المخابر			✓	✓	✓		
ن		تسجيل براءة الاختراع من اجل الحصول على رقم الإيداع والحماية الصناعية						✓	
...		متابعة عملية الحصول على براءة الاختراع وتصحيح ملاحظات الممتحنين من **inapi**							✓

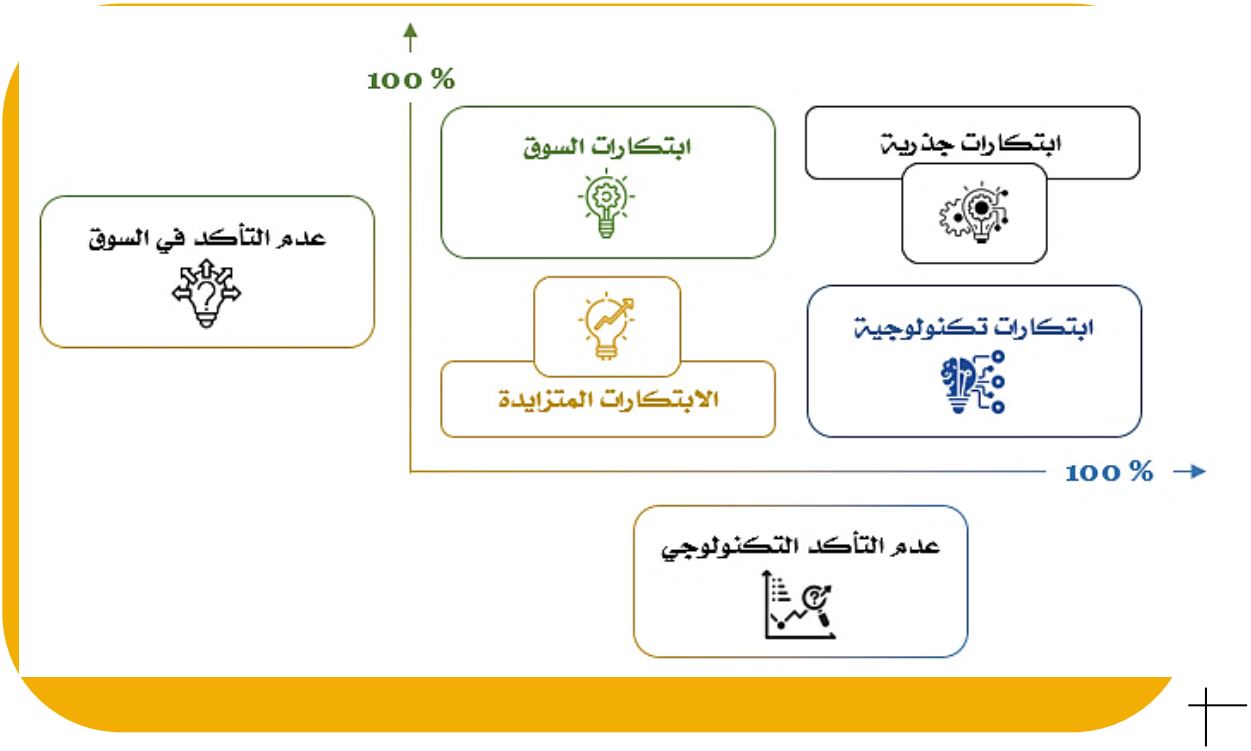
الأعمال

المحور الثاني

الجوانب الابتكارية

1. طبيعة الابتكارات :

ينبغي أن يحدد هنا طبيعة الابتكارات المعتمدة في براءة الاختراع :



2. مجالات الابتكارات :

من خلال الحالات السابقة يمكن ان يشمل الابتكار المجالات التالية :

- ✓ عمليات جديدة (زيادة الربحية من خلال زيادة كفاءة العمليات).
- ✓ تجارب جديدة (بيع المزيد لشرائح العملاء الحاليين عن طريق تغيير السياق (السياقات).
- ✓ الميزات الجديدة (تقديم منتجات أو خدمات محسّنة).
- ✓ العملاء الجدد (عرض النطاق المعتاد من المنتجات أو الخدمات لشرائح العملاء الجدد).
- ✓ عروض جديدة (إنشاء - أو على الأقل إدخال - منتجات مبتكرة).
- ✓ نماذج جديدة (تغيير نموذج العمل، اعتماد "نظام" آخر لتوليد القيمة).

المثال في الملحق رقم 01

المحور الثالث

وصف براءة الاختراع

المحور الثاني: وصف براءة الاختراع

- عنوان براءة الاختراع : العنوان يضبط جيد وفي حدود 10 كلمات ولايحتوي على اختصارات

..... صناعة مكمل غذائي من نباتات طبية ذات التأثير المضاد لمرض السكري"

.....

- ملخص براءة الاختراع (250 كلمة)

.....

.....

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.....تصنيع وتسويق منتجين

مبتكرين لمرضى السكري:

مكمل غذائي لتخفيض السكري.

3. المنتجات:

- مكمل غذائي:

يتكون من مزيج فريد من الأعشاب الطبيعية التي تم اختبارها سريريًا لإثبات فعاليتها في خفض مستويات السكر في الدم.

4. مميزات المشروع:

منتجات مبتكرة وفعالة.

تلبية احتياجات السوق غير الملبيّة.

فرص تجارية واعدة.

5. الخطوات التالية:

إكمال أبحاث السوق والتطوير.

الحصول على الموافقات التنظيمية.

تصنيع المنتجات.

تسويق المنتجات.

6. التحديات:

المنافسة.

اللوائح التنظيمية.

التسويق.

7. الفرص:

سوق كبير ومتنامٍ لمرضى السكري.

طلب متزايد على المنتجات الطبيعية.

إمكانية التوسع إلى أسواق جديدة.

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- الميدان التقني الذي ينتمي إليه الاختراع (مثلا ميدان العلوم، البيولوجيا، البيو تيكنولوجي ، ميدان التكنولوجيا، الطاقات المتجددة ،.....الخ)

.....ن تنتمي براءة الاختراع الى المجال التقني لصناعات الدوائية و الغذائية

المجال: صناعات دوائية و غذائية

التخصص الفرعي: تصنيع مكملات غذائية لمرضى السكري

المنتجات:

- مكمل غذائي من اعشاب طبيعية طبية محلية لخفض نسبة السكر في الدم

المعدات : معدات مخبرية، مواد أولية طبيعية، معدات التصنيع

المنتجات المقدمة: مكمل غذائي.

المجال المستهدف: مرضى السكري

- الحالة التقنية السابقة (براءات الاختراع السابقة التي تدخل في نفس مجال براءة اختراعنا)

..... نظرا للانتشار الواسع لداء العصر -مرض السكري- الذي دخل تقريبا كل البيوت الجزائرية، وهذا مما أدى إلى تكثيف البحوث والاجتهادات المخبرية حول طرق العلاج الناجعة والوقوف ضد المضاعفات الأليمة التي يسببها هذا المرض، ولعل من بين هذه المنتجات المكمل الغذائي المعروف ب RHB(رحمة ربي) الجزائري الصنع إذ لم يكن موجها لمرضى السكري فقط، على عكس منتوجنا الذي اعتمدنا فيه على مزيج من النباتات الطبية المعروف كل منها في فعاليتها في التخفيف من معاناة مرضى السكري فهو موجه خصيصا لهذه الشريحة بالضبط.....

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- الغرض (الهدف) من الاختراع

.....

..... يهدف هذا الاختراع إلى تقديم حلول فعالة وآمنة للمساعدة في خفض نسبة السكر في الدم .

تلبية احتياجات السوق غير الملبية: يركز المشروع على تصنيع وتطوير منتجات جديدة فريدة من نوعها لم يتم اختبارها سابقا لعلاج مرض السكري.

تحقيق ربح : مشروع تصنيع منتجات لمرضى السكري مربحا، حيث يعاني الملايين من الاشخاص حول العالم من هذا المرض و الكل يبحث على طرق فعالة وآمنة و طبيعيا لتخفيف من هذا المرض بعيدا عن مضاعفات الأدوية الكيماوية.

خلق فرص عمل : يساهم المشروع في خلق فرص عمل جديدة في مجالات البحث و التطوير والإنتاج والتسويق.

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- تقديم جوهر الاختراع

النموذج الأولي التجريبي

النموذج الأولي التجريبي هو نسخة أولية تم صنعها من المنتج أو الخدمة والتي تستخدم كأساس في التطوير للوصول الى المنتج النهائي الذي سيطبق في السوق رسميا.

- ✓ في هذا الجانب يقدم صاحب المشروع نموذج اولي ملموس (يقدم للجنة)، كما يمكنه تصوير مقطع فيديو أو مجموعة من الصور تثبت وصوله إلى إتمام النموذج الأولي .
- ✓ كما يمكنه تقديم شرح للمراحل الأساسية المتبعة للوصول الى النموذج الأولي.

✓ يمكن لأصحاب مشاريع التطبيقات والمنصات الرقمية عرض نموذج أولي للتطبيق الكترونيا.

- عندما يتعلق الأمر بجهاز

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- عندما يتعلق الأمر بطريقة عملية (الإنتاج أو المعالجة)

.....

.....

.....

- عندما يتعلق الأمر بمنتوج (مركب، مزيج، تشكيل....)

.....

.....

.....

- عندما يتعلق الأمر بتطبيق جديد لجهاز معروف أو طريقة معروفة أو مادة معروفة

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.....

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.....

- عندما يتعلق الأمر بتركيبة جديدة لعناصر معروفة أو غير معروفة

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8. شرح الأشكال و الرسومات: (دون وضعها في الوصف)

يتم شرح الأشكال وفق ترقيمها وشرح كل رقم على حدى وتبيان دوره وفعاليته في براءة الاختراع

رقم 01

مكمل غذائي يعمل على تخفيض نسبة السكر في الدم يتميز بتركيبته الفريدة من نوعهت بخليط من نباتات مميز

رقم 02

علبه زجاجية معتمة تحوي على 45 ml من المرهم الخاص بمرضى سكري تتميز ببساطة الشكل و تصميم

رقم03

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7.طريقة واليية عمل الجهاز المخترع او المادة المخترعة

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المحور الرابع

المطالب

المطلب الرئيس يتمثل في القيمة الإضافية والميزة التي جاء بها اختراعنا مقارنة بباقي الاختراعات الأخرى

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2. ***المطالب المستنبطة من المطلب الرئيسي والتي تميز اختراعنا*** 1- هنا يعاد كتابة العنوان كما هو مذكور في الصفحة الاولى.

2- وفقا للمطلب الأول هذا الاختراع يتميز بكونه منتج طبيعي مستخلص من نباتات طبية

3- وفقا للمطلب الأول هذا الاختراع يتميز بسهولة التحضير (نباتات محلية)

4- وفقا للمطلب الأول هذا الاختراع يتميز بسهولة استخدامه

5- وفقا للمطلب الاول هاذا الاختراع يتميز بتكلفته المقبولة

6- وفقا للمطلب الأول هذا الاختراع يتميز باستهدافه في نفس الوقت خفض نسبة السكر في الدم.

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قائمة الملاحق

مثال عن براءة اختراع

دليل مشروع

للحصول على شهادة مؤسسة ناشئة
في إطار القرار الوزاري 1275

ديسمبر
2022

CNCSIIU — MHESR — ALGERIA