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THEME

Comprehensive Assessment of Medicinal Plants for Their Antidiabetic Properties: Connecting In Vivo and In Vitro Evaluations with Advanced Computational Analyses, Encompassing ADMET Prediction, Molecular Docking, and Molecular Dynamics Simulation.

Presented by:

GOUNNI Ahlem, TEREA Joutheima Malika, KADI Maroua

Jury Members:

Supervisor: Mr LANEZ Elhafnaoui	**Lecturer Professor, Université d'El Oued**
Co-Supervisor: Ms BOUDEBIA Ouafa	**Doctor, Université d'El Oued**

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“DEDICATION”

((وَ آخِرُ دَعْوَاهُمْ أَنِ الْحَمْدُ لِلَّهِ رَبِّ الْعَالَمِينَ))الحمد لله عند البدء وعند الختام ، فما تناهى درب ، ولا ختم جهد ، ولا تم سعي إلا بفضلهإلى من أبلغ الرسالة وأدى الأمانة ونصح الأمة إلى نبي الرحمة ونور العالمين

سيدنا خاتم الأنبياء الرسول محمد (صلّ الله عليه و سلم).

The trip was not short and should not be, the dream was not soon nor the road was fraught with facilities.

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And to the one who gave me for free, who taught me determination and perseverance, the source of hope and ambition to "***my father Ahmed*** " ... ❤️

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Ahlem

"DEDICATION"

وَآخِرُ دَعْوَاهُمْ أَنِ الْحَمْدُ لِلَّهِ رَبِّ الْعَالَمِينَ

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في النهاية أحمد الله سبحانه وتعالى الذي منَّ علينا بنعمة العقل والدين، وهو القائل في محكم التنزيل: “فَٱذۡكُرُونِيٓ أَذۡكُرۡكُمۡ وَٱشۡكُرُواْ لِي وَلَا تَكۡفُرُونِ”،وقد قال رسول الله صلى الله عليه وسلم: “مَنْ صَنَعَ إِلَيْكُمْ مَعْرُوفًا فَكَافِئُوهُ، فَإِنْ لَمْ تَجِدُوا مَا تُكَافِئُونَهُ فَادْعُوا لَهُ حَتَّى تَرَوْا أَنَّكُمْ قَدْ كَافَأْتُمُوه"

Maroua

Abstract:

Diabetes is a group of metabolic diseases characterized by chronic hyperglycemia caused by an imbalance in the secretion.

Insulin or its effect, or both disorders associated with serious disease complications associated with the ineffectiveness of the molecules of products used in its treatment. This prompted the WHO to use traditional medicine as an alternative solution. The induction of diabetes in the mouse model was achieved by injecting a fresh solution of aloxane intraperitoneum, and after a treatment period of 20 UMA, where weight and blood sugar were used in the case of fasting to evaluate the effectiveness of the treatment. In comparison between the different groups (the witness group, the healthy group treated, the group with diabetes and the group with diabetes treated) the results were shown in the mice with diabetes that were treated with a low blood sugar.

In the laboratory we relied on the characterization of essential oils by analysing the gas-mass spectrometer chromatography (GC/MS) and predicting in silico of the physical, chemical properties and the pharmacological and toxicity of Mp-Al compounds) (the four selected using servers: swissADME, admitSAR and Protox. The results showed that all of these compounds have drug-like properties.

EOs studied significantly increased α-amylase inhibition (%) with a corresponding increase in EO concentration. The effective inhibitory concentration (IC_{50} value) in which 50% of α-amylase activity was inhibited by *Ammudoucus leucotrichus* and *Mentha piperita* essential oils was obtained to be 7.013 and 1.310 μg/ml respectively, indicating a strong potential for slowing starch digestion by inhibition of α-amylase.

The results obtained by molecular docking confirm the results in the laboratory; they have negative values ΔG, which reflects the good convergence of bonds with the biological target the anti-diabetic effectiveness of these oils and makes it possible to be considered anti-diabetic drugs in the future.

Keywords:*Mentha piperita, Ammudaucus leucotrich*us, diabetes, swissADME,Aloxan, mice, wistar albinos, essential oil.

Résumé:

Le diabète est un groupe de maladies métaboliques caractérisées par une hyperglycémie chronique causée par un déséquilibre de la sécrétion.

L'insuline ou son effet, ou les deux troubles associés à des complications graves de la maladie associées à l'inefficacité des molécules de produits utilisés dans son traitement. Cela a incité l'OMS à utiliser la médecine traditionnelle comme solution alternative. L'induction du diabète dans le modèle de souris a été réalisée en injectant une solution fraîche d'aloxane intrapéritone, et après une période de traitement de 20 UMA, où le poids et la glycémie ont été utilisés dans le cas du jeûne pour évaluer l'efficacité du traitement. En comparaison entre les différents groupes (le groupe témoin, le groupe en bonne santé traité, le groupe diabétique et le groupe diabétique traité), les résultats ont été montrés chez les souris diabétiques qui ont été traitées avec un faible taux de sucre dans le sang.

Dans le laboratoire, nous nous sommes appuyés sur la caractérisation des huiles essentielles en analysant la chromatographie par spectromètre de masse gazeuse (GC/MS) et en prédisant en silicium les propriétés physiques, chimiques et la pharmacologique et la toxicité des composés Mp-Al) (les quatre sélectionnés à l'aide de serveurs : swissADME, admitSAR et Protox. Les résultats ont montré que tous ces composés ont des propriétés semblables à celles de la drogue.

Les EO ont étudié une augmentation significative de l'inhibition de l'α-amylase (%) avec une augmentation correspondante de la concentration d'EO. La concentration inhibitrice efficace (valeur IC50) dans laquelle 50 % de l'activité de l'α-amylase a été inhibée par les huiles essentielles *d'Ammudoucus leucotrichus* et de *Mentha piperita* a été obtenue à 7,013 et 1,310 μg/ml respectivement, ce qui indique un fort potentiel de ralentissement de la digestion de l'amidon par inhibition de l'α-amylase.

Les résultats obtenus par doying moléculaire confirment les résultats en laboratoire ; ils ont des valeurs négatives ΔG, ce qui reflète la bonne convergence des liens avec la cible biologique, l'efficacité antidiabétique de ces huiles et permet d'être considérés comme des médicaments antidiabétiques à l'avenir.

Mots clés:*Mentha piperita, Ammudaucus leucotrichus* , diabète, swissADME ,Aloxan, souris, wistar albinos, huile essentielle.

ملخص:

مرض السكري هو مجموعة من الأمراض الأيضية الذي يتميز بفرط سكر الدم المزمن الناتج عن خلل في إفرازالأنسولين أو تأثيره ، أو كلاهما من الاضطرابات المرتبطة بمضاعفات خطيرة المرض مرتبطة بعدم فعالية جزيئات المنتجات المستخدمة في علاجه. دفع هذا منظمة الصحة العالمية إلى استخدام الطب التقليدي كحل بديل. الدراسة ، تمت علاج داء السكري الناجم عن الألكوكسان في الفئران (wistar albinos) باستخدام االزيت الأساسي لكل من *Ammudaucus leucotrichus*و *Mentha piperita*.

الهدف من عملنا استكشاف الإمكانات المضادة للسكري ل*Ammudaucus leucotrichus* و *Mentha piperita*.من خلال التقييمات داخل الجسم الحي وفي المختبر. جمع الزيوت الأساسية والمستخلصات الخام واستخراجها باستخدم تحليلات السيليكون المتقدمة للكشف عن الآليات الجزيئية والفعالية وملامح السلامة للتطبيقات العلاجية المحتملة.

تم متحقيق تحريض مرض السكري في نموذج الفئران من خلال حقن محلول طازج من ألوكسان داخل الصفاق ، و بعد فترة علاج مدتها 20يو ًما ، حيث تم استخدام الوزن وسكر الدم في حالة صيام لتقييم فعالية العلاج. بالمقارنة بين المجموعات المختلفة (المجموعة الشاهدة، المجموعة السليمة ال ُمعالجة، المجموعة المصابة بداء السكري والمجموعة المصابة بداء السكري المعالجة) أظهرت النتائج في الفئران المصابة بداء السكري التي عولجت انخفا ًضا في ارتفاع السكر في الدم .

في المختبر اعتمدنا على توصيف الزيوت الأساسية بتحليل كروماتوغرافيا الغاز - مطياف الكتلة (GC/MS) و التنبؤ في السيليكون بالخصائص الفيزيائية والكيميائية والحرائك الدوائية والسمية لمركبات (Mp-Al)الأربعة المختارة باستخدام الخوادم: swissADME و admitSAR و Protox. أظهرت النتائج أن جميع هذه المركبات لها مردود خصائص شبيهة بالعقاقير.

زادت EOs المدروسة بشكل كبير من تثبيط α-amylase (٪) مع زيادة مقابلة في تركيز EO. تم الحصول على التركيز المثبط الفعال (قيمة IC50) الذي تم فيه تثبيط 50٪ من نشاط α-amylase بواسطة زيوت *Ammudoucus leucotrichus* و*Mentha piperita* الأساسية ليكون 7.013 و1.310 ميكروغرام / مل على التوالي، مما يشير إلى إمكانية قوية لتباطؤ هضم النشا عن طريق تثبيط α-amylase.

تؤكد النتائج التي تم الحصول عليها عن طريق الالتحام الجزيئي النتائج في المختبر؛ لديهم قيم سلبية ΔG، مما يعكس التقارب الجيد للروابط مع الهدف البيولوجي الفعالية المضادة للسكري لهذه الزيوت ويجعل من الممكن اعتبارها أدوية مضادة للسكري في المستقبل.

الكلمات المفتاحية:*Mentha piperita, Ammudaucus leucotrichu*، السكري ،swissADME.ألوكسان ، فئران ، wistar albinos،الزيت الأساسي .

ABREVIATIONS LIST

%: percentage

ΔG: Énergie libre de liaison

°C: Degrees Celsius

Å: Ångström

Abs: Absorbance

ADMET: Absorption,Distribution,Metabolism Excretion of Drugs and Toxicity

AFNOR: Association Française de Normalisation

ALP: Alkaline phosphatase

ALT: Alanine aminotransferase

CA: conversation agriculture's

CCM: chromatographie sur couche mince

CO2: Carbon dioxide

CRAPC: Centre de Recherche Scientifique et Technique en Analyses Physico Chimique

DL50: Dose létale 50

DT1: Type 1 diabetes

DT2: Type 2 diabetes

EDTA: Ethylenediaminetetraacetic acid

EOAI:essential oil from plant *Ammodaucus leucotrichus*

EOMP:essential oil from plant *Mentha Piperita*

FBS: Fasting blood sugar

FID: The International Diabetes Federation

GC/MS: Gas Chromatography-Mass Spectrometry

GD: gestational diabetes

GI: glucose intolerance

GLP-1: glucagon-like peptide-1 receptor antagonists

GRA: Granulocytes

GSH: Reduced glutathione

GST: glutathione-transferase

HCl: hydrochloric acid

HD: hydro distillation

HB: Hemoglobin

HPA: Human pancreatic alpha amylase

I: Inhibition

IC_{50}: Concentration causing 50% inhibition of an activity.

IFD: Induced Fit docking

IFG: includes moderate fasting hyperglycaemia

IR: Infrared Spectroscopy

K: connecting constant

KJ: Kilojoule

KOH: potassium hydroxide

L: liter

LYM: Lymphocytes

M: molar

MDA: Malondialdehyde

MDA: Malondialdehyde

MDS: Molecular Dynamics Simulation

Mg: Millgram

MHG: Microwave hydro-diffusion and gravity

MODY: Maturity Onset Diabetes of Young

MWHD: microwave-assisted hydro distillation

NS: non significative

OHWD: Ohmic heated water distillation

pH: hydrogen potential

pH: Potential of hydrogen

PLT: Platelets

R %: Percentage yield

RBC: Red blood cell

Screw: Visible

SD: steam distillation

SFE: Supercritical fluid extraction

SP: Standard Precision

THP: Treating Health Professional

TMK: Traditional Medical Knowledge

TM: Traditional Medicine

UA: Uric Acid

UV: Ultra-violet

VTRS: Valorisation and Technology of the Saharian Resources Laboratory

WBC: White blood cell

WHO: World Health Organization

α: Alpha

β: Beta

ΔG: Free binding energy

List of figures

List of figures

List of tables

List of tables

Summary

CHAPTER TWO: MEDICINAL PLANTS

GENERAL INTRODUCTION

Introduction

The chronic metabolic disorder diabetes mellitus is a fast-growing global problem with huge social, health, and economic consequences(Kaul et al., 2013).Diabetes is defined as a metabolic condition, characterized by chronic hyperglycemia associated with a lack of insulin secretion, insulin action, or both. During its development, diabetes can cause serious complications that affect the heart, vessels, eyes, kidneys and nerves. However, good disease control can significantly reduce the risk of complications (Fagot-Campagna et al., 2010). It is known as a serious long-term (or chronic) disease, and there are two main types of diabetes: type 1 diabetes and type 2 diabetes, the latter of which is the most common, and accounts for approximately 90% of the world's diabetes patients (Meseguem and OuladHammadi, 2021). In fact, the latter is a potentially fatal disease responsible in the world, each year, for nearly 4.0 million deaths (IDF., 2017). In Algeria, it is a public health problem. Its prevalence will be between 8 and 12% according to various epidemiological studies; it also represents the fourth cause of death (Chami et al., 2015).It shockingly affects hundreds of millions of people of all ages and social classes around the world, where it has become a global pandemic, generating significant and increasing direct and indirect medical costs, placing a heavy burden on patients (Hadj Ibrahim and Benbal, 2020). Patients with type 1 and type 2 diabetes are usually initially treated with diet and physical exercise. But if these measures are not enough to control blood sugar, drugs that are glucagon-like peptide-1 receptor antagonists (GLP-1), insulin, and oral hypoglycemic drugs such as sulfonylurea such as gliclazide that stimulates insulin secretion or regulated enzyme-regulating enzyme inhibitors such as carbos that acts as an inhibitor of α-amylase and α-glucosidase or a combination of these drugs (Erika F. Brutsaert., 2020).The past decades have been marked by an interest in the development of medicinal plants as a source of biologically active natural materials. Algeria has a rich heritage of medical and food agricultural resources traditionally used to treat many diseases, including diabetes (Kambouch et al., 2009). In Algeria, a large number of plants are used in traditional medicine, some of which are used to treat diabetes. The pharmacological ethnic approach is of great importance in this area. It makes it possible to identify anti-diabetic treatments and build a database of medicinal plants (Hamza, 2011).It is called the traditional Algerian Pharmacopoeia because it contains many traditional remedies that have continued to move orally from one generation to another without writing them, and this is what makes it tend to disappear. An effective way to preserve these traditional remedies is to integrate these plants and herbs into the modern health system by discovering them biochemically and using their molecules for treatment (Budjalal et al., 2013).We have among the medicinal

,plants,*Mentha piperita L*. (*peppermint*) is a popular plant whose oils are extensively utilized for a variety of applications. It is used in cosmeceuticals, personal hygiene products, meals, and pharmaceutical items for both flavoring and aroma qualities(Herro et al.,2010).and antimicrobial and antioxidant activities(Romero et al., 2005. Mimica et al., 2003).It is also used for culinary purposes. There is evidence that it has a positive effect on glycemia of the laboratory animal (Narendhirakannan et al.,2006). Also, *Ammodaucus leucotrichus*, known in Algeria as "*Moudrayga or El massoufa*", is a medicinal plant sold to herbalists in local markets, particularly in the Southern Algerian Sahara. The nomads collect the seeds and leaves for their own use, usually in the spring, when the fruits are ripe (khaldi et al., 2017). In the southern Algerian Sahara, the leaves of this plant are used as a flavoring herbal in teas and fruits are often used as a spice during culinary preparation. The leaves and seeds are consumed in the form of decoction or infusion for several therapeutic cases, such as blood pressure, chest pain, liver and digestive system ailments, gastroenteritis, as also for diabetes (Ziani et al., 2019). In Tassili (Algeria), the fruits and the leaves are commonly consumed in infusion (Halla et al., 2018). Other scientific evidence demonstrated that the essential oil seed of A. *leucotrichus* had an effective antioxidant and antimicrobial properties(Louail et al., 2016).Although this medicinal plant endemic to North African countries is known through several ethno- pharmacological surveys, however, few experimental studies have been performed about its pharmacological activity.Our goal from this study is to extraction, identification and explore the antidiabetic potential of the essential oil for both *Ammodaucus leucotrichus*and *Mentha piperita L* plants through assessments in vivo, in vitro and insilico.

FIRST PART:

BIBLIOGRAPHY

CHAPTER ONE:

DIABETES

1. Definition of diabetes:

Diabetes is defined as a metabolic disorder characterized by hyperglycemia (elevated bloodsugar levels) due to a deficiency in either insulin secretion, insulin action, or both (Alexis, 2014).

According to the World Health Organization, diabetes is a chronic disease caused by a metabolicdisease with multiple aetiologies. It is characterized by too high a level of glucose in the blood (hyperglycaemia). It is defined as a metabolic disorder of carbohydrates, fats and proteins caused bymany environmental and genetic factors (Klein, 2009).

2. Diagnosis:

The diagnosis of diabetes is mainly based on the measurement of fasting blood glucose and the hyperglycaemia caused (Arbouche, et al., 2012).

The diagnostic criteria for diabetes have changed over time, studies show a close relationship between the onset of complications and blood glucose levels (Duckworth et al., 2009; Beigi et al., 2010; Deborah,2019).

The criteria for diagnosing diabetes established by the WHO since 1998 are:

- Presence of symptoms of diabetes (polyuria, polydipsia, weight loss)
- A random blood glucose ≥11.1 mmol/l(2.00g/l).
- Fasting blood glucose (no caloric intake since at least 8 hours) is ≥ 7.0mmol/l(1.26g/l)
- Blood glucose ≥ 11.1 mmol/l (2.00 g/l) two hours after ingestion of glucose (75 g) during an HGPO.
- An HbA1c ≥ 6.5% by a validated method.
- The glucose regulation abnormality includes moderate fasting hyperglycaemia (IFG) and glucose intolerance (GI).

3. Classification:

There are generally two main types of diabetes, one associated with insulin deficiency, the other with a defect in the use of this hormone and other particular types (Cossette, 2001).

3.1.Type 1 diabetes:

Or insulin-dependent, juvenile, lean, ketosic diabetes (Defdaf and Benkahoul, 2019). It is caused by the destruction of the β cells of the pancreas, hence the inability of the affected person to secrete insulin. It is an autoimmune disease, caused by a dysregulation of the immune defences, responsible for a "self-destruction" of the ß cells that make insulin. This process begins several years before the onset of the disease. One of the markers of this autoimmune reaction is the presence in the blood of antibodies directed against Langerhans islets, clusters of β cells (Belabbes and Halaoune, 2018).

DT1 can be observed at any age, rare before 2 years old. The average age of onset is 10.6 years with a peak around puberty between 10-15 years. The 2 sexes are affected with a slight male predominance with a sex ratio of 1.06 (Guendouz and Hamza Zerigat, 2018).

3.2.Type 2 diabetes:

Non-insulin-dependent diabetes or diabetes of maturity, fatty, non-ketosic (Defdaf and Benkahoul, 2019). Type 2 diabetes (maturity diabetes) is a metabolic disease characterized by chronic hyperglycaemia, usually begins after the age of 40 and accounts for 90 to 95% of cases. Most of these patients are obese, and obesity in itself, causes a certain degree of lino-resistance, this heterogeneous (non-auto-immune) pathology results either:

- Insulin resistance, aggravated by overweight.
- A deficiency in insulin secretion of genetic origin, aggravated by chronichyperglycaemia (Mekhalfa and Assala, 2018).

Many people with type 2 diabetes are unaware of it for a long time because it can take several years before symptoms appear or are recognized. During this time, excess glucose in the blood causes damage to the body. The diagnosis is often only made when diabetes complications have already developed (Benyounes et al., 2021).

3.3.Other types of Diabetes:

3.3.1. Gestational diabetes:

Fasting blood glucose (GJ) between 0.92 g/L and 1.25 g/L in early pregnancy is not very predictive of gestational diabetes after 24 weeks. As a result, gestational diabetes (GD) is essentially diabetes that manifests itself during pregnancy. It can be classified as gestational diabetes managed without medication and sensitive to nutritional therapy A1GDM and gestational diabetes managed with drugs to obtain adequateglycaemiccontrol A2GDM (Quintanilla Rodriguez and Mahdy, 2019).

The causes of the DG are still unknown, but some speculate that HLA antigens may play a role, especially HLA DR2, 3, and 4; while some suggest that proinsulin can induce beta cell stress. Others believe that high concentrations of hormones such as progesterone, cortisol, prolactin,oestrogen and human placental lactogen that cause alterations and changes in insulin receptors, can affect beta cell function and insulin sensitivity (Sapra et al., 2019).

Women with DG have a 35 to 60% increased risk of developing diabetes mellitus 10 to 20 years after pregnancy (Quintanilla Rodriguez and Mahdy, 2019)

3.3.2. Monogenic diabetes:

Monogenic diabetes is a rare form of non-insulin-dependent and familial diabetes with a dominant autosomal mode of transmission, it is the consequence of a genetic defect that alters the function of beta cells, especially affects young people (< 25 years old).

More than 40 different genetic subtypes of monogenic diabetes have been identified, each with its own phenotype and transmission model, they include neonatal diabetes and, most often, maturity diabetes appearing in young MODY (Amed and Oram, 2016).

4. Physiopathology:

4.1.Physiopathology of DT1:

Type 1 diabetes (DT1) is caused by the autoimmune destruction of insulin-producing beta cells in thepancreatic islets (Kara and Hammadi, 2021). LTs produce antibodies against antigens expressed onthe surface of þ cells. The antibody-antigen reaction combined with the direct action of LT (Killers)leads to the destruction of these cells (Benguessoum et al., 2021).

4.2.Physiopathology of DT2:

Type 2 diabetes is a multifactorial disease. Hyperglycemia is due to an alteration in insulin secretion in response to glucose by the ß cells of the endocrine pancreas, and a decrease in tissue sensitivity mainly of skeletal muscles, adipose tissue and liver to the effects of insulin, which results in insulin resistance (Riant, 2009).

5. Risk factors:

5.1. Type 1 diabetes:

5.1.1.Genetic factors:

Genetic factors are involved in about a third of susceptibility to type 1 diabetes (Perlemuter et al., 2003). More than 20 different regions of the human genome represent a certain link with type 1 diabetes such as the region encoding for HLA on chromosome 6p21 and the region encoding for the insulin gene on chromosome 11p 15 (gene now called DSID2, or in English IDDM2). The types of HLA associated with diabetes vary according to the populations studied (Arfa et al., 2008).

5.1.2. Environmental factors:

Environmental factors play an important role in the onset and clinical expression of the disease. It has been shown that the absence of exposure to pathogenic organisms during the childhood period limits the maturation of the immune system and increases the susceptibility to developing autoimmune disease(Kekreja et al., 2002).

The role of viral infection in certain forms of type 1 diabetes has been proven by studies in which particles or autoimmunes from β cells have been isolated from the pancreas. Several

viruses were involved, including rubella virus, Epstein Barr virus and cytomegalovirus (Makhlouf and Chahboub, 2015).

Stress can advance the development of type 1 diabetes by stimulating the secretion of hyperglycemic hormones, and possibly by modulating immunological activity (Vialettes et al., 2006).

5.1.3. Diet:

Cow's milk introduced at weaning triggers insulitis and diabetes in animal models (Elliot et al., 1984; Knip et al., 2010).It has been shown that children who fed cow's milk at the beginning of their lives are more likely to develop type 1 diabetes than those who fed in the breast (Stuebe., 2007). SAB can cross the intestine wall of the newborn and reveal antibodies that can cross-react with constituents of β cells and damage them. Various nitrosamines, and coffee have been proposed as potentially diabetic factors (Williams., 2009).

The same applies to various dietary proteins (gluten for example) that can also play a role in the expression of type 1 diabetes (Knip et al., 2010).

5.1.4. Immunological factors:

Type 1 diabetes is a slow autoimmune disease mediated by lymphocytes, family studies have shown that the destruction of β cells by the immune system (autoantibodies directed against the pancreas) takes place over manyyears (Langlois., 2008).

Hyperglycaemiaand the classic signs of diabetes only appear when 80% of β cells have been destroyed,type 1 diabetes can be associated with other autoimmune conditions including thyroid disease, celiac disease, and certain forms of anemia (Makhlouf and chahboube., 2015).

5.2. Type 2 diabetes:

5.2.1 The age:

Regardless of the population studied, the prevalence of type 2 diabetes increases with age (French., 1990 and Gourdy., 2001; Hanis., 1983). The prevalence of diabetes increases steadily between 0 and 79 years old, but it is really from the age of 40 that its frequency exceeds 1% (0.68% in the age group 35-39 years and 1.27% in the age group 40-44 years old and then up to 13.96% in the age group 75-79 years)(Makhlouf and chahboube, 2015).

5.2.2 Genetic risk factor:

The presence of a type 2 diabetic in a family increases the risk of diabetes in other members of that family (Makhlouf and chahboube, 2015). This is in favor of genetic participation in the appearance of type 2 diabetes. In addition, twin-twin concordance studies, at least one of whom has type 2 diabetes, show a greater concordance in homozygotes (58%

to 80% according to the studies) than in heterozygotes (17% to 40%). This suggests significant genetic support for type 2 diabetes, but the lack of 100% concordance also suggests that this participation is dependent on other factors (Makhlouf and chahboube, 2015).

5.2.3 Obesity:

The level of obesity has long been known to be associated with an increased prevalence of type 2 diabetes (Bennett., 1992). The duration of obesity is an additional risk factor for obesity. In Pima Indians with a BMI greater than or equal to 30, the risk of diabetes increases by 24.8 per 1000 for those who have been obese for less than 5 years, to 35.2 per 1000 between 5 and 10 years and up to 59.8 per 1000 for those who have been obese for more than 10 years (Everhart., 1992). Epidemiological work carried out in Sweden showed that it was especially in the case of abdominal and visceral fat distribution that an obese had a significant risk of developing type 2 diabetes; this distribution is reflected by the ratio of waist circumference to hip circumference (Makhlouf and chahboube, 2015).

5.2.4 Disorders of insulin secretion:

Insulin secretion is ensured by the β cells of the pancreatic Langerhans islets. Under physiological conditions, insulin secretion is characterized by a so-called biphasic kinetics.

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).

5.2.5 Insulin sensitivity disorders:

The decrease in the effects of insulin on insulin-sensitive tissues (muscle tissues, adipose tissue, liver) causes a hyperproduction of glucose by the liver, explaining fasting hyperglycaemia and part of anteprandialhyperglycaemia(Henri., 2011).

Insulin resistance is therefore characterized at the level of peripheral tissues, in particular, the transport of glucose in the muscle and adipose tissue and the hepatic production of glucose. This insulin resistance is aggravated by hyperglycaemia and the excess of 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).

6. The complications of diabetes:

People with diabetes are exposed to various disabling and life-threatening health problems. Persistenthigh blood sugar can lead to serious diseases affecting the cardiovascular system,

eyes, kidneys andnerves. In addition, people with diabetes are more susceptible to infections (Gamouh and Keddisa., 2015).

6.1. Complications metabolic:

Various metabolic disorders that can lead to disorders of consciousness up to a coma are likely to occur in the diabetic patient. Two of them, hypoglycaemia and lactic acidosis, appear to be iatrogenic complications. The other two, diabetic ketoacidosis and hyperosmolar states result from a therapeutic insufficiency (insulin deficiency) or a lack of monitoring (plasma concentration abnormalities:Hyperglycaemiaorhypoglycaemia) (Blickle., 2010).

Acute complications related to an absence or poor adaptation of treatment (keto acid, hyperosmolar, hypoglycaemic coma), chronic hyperglycaemia is accompanied by long-term complications affecting many organs, especially the eye, kidney, nervous and cardiovascular systems (Chevenne and Fonfrède., 2001).

6.2.Chronic complications:

Chronic complications of diabetes include two components: macrovascular complications (coronaropathy, peripheral arterial disease and stroke) and microvascular complications (diabetic nephropathy, neuropathy and retinopathy), consequences of changes in the microvascular structure, causing structural and functional changes in the extracellular matrix that cause primary vascular and nerve complications, causing the synthesis of the extracellular matrix protein and capillary thickening of the basal membrane, which are the pathognomycharacteristics of diabetic microangiopathy (Kebir., 2018).

7. Treatment:

The main objective of the treatment of type 1 and type 2 diabetes is to prevent the occurrence of long-term complications; it is based on a balanced diet, adequate physical activity, maintaining a healthy weight and regulating blood glucose levels with oral antidiabetic drugs for type 2 and daily injections of insulin (for life) for type 1.

7.1. Insulin therapy:

Insulin therapy aims to prevent acute and chronic complications by achieving an almost normal glycemic balance and improving the patient's quality of life (Mohn et al., 2012).

Insulin can be administered according to various dosage regimens, but very few of these regimens have been studied in children whose diabetes had just been diagnosed. The choice of the type of insulin therapy will depend on the child's age, his lifestyle and of course the personal choice of the child/adolescent and his parents. The goal is to get as close as possible to physiology (Diabetes Canada Clinical Practice Guidelines Expert et al., 2018).

7.2.Antidiabetics:

The drug treatments currently available target the different metabolic abnormalities encountered in type 2 diabetes. Two major families of oral antidiabetics are known - insulin sensitizers and insulinsecretagogues (Tielmans et al., 2007).

The choice of antidiabetic drugs must be based on an assessment of the benefit/risk balance, integrating the cost of treatment, and promoting an individual approach focused on the diabetic patient (Scheen., 2015).

7.2.1.General view of the mode of action of Antidiabetics (Acarbose as an example):

- **Mode of action of Acarbose:**

Acarbose (marketed in Algeria as Acorbay 50mg) is a complex oligosaccharide that inhibits the activity of α-glucosidases from the brush border of the small intestine. It does not increase insulin secretion. The antihyperglycemic action of this drug results from the competitive and reversible inhibition of pancreatic α-amylase and α-glucoside hydrolases linked to the intestinal membrane (Youmbai and Sahraoui., 2021).

Pancreatic α-amylase transforms complex starches into oligosaccharides in the lumen of the small intestine, while α-glucosidases bound to the intestinal membrane transform oligosaccharides, trisaccharide's and disaccharides into glucose and other monosaccharides in the brush border of the small intestine.Most α-amylase inhibitors will interact with the active sites in a way similar to the substrate, the inhibition mechanism occurs through the direct blocking of the active centre of several subsites of the enzyme (Tormo et al., 1998).

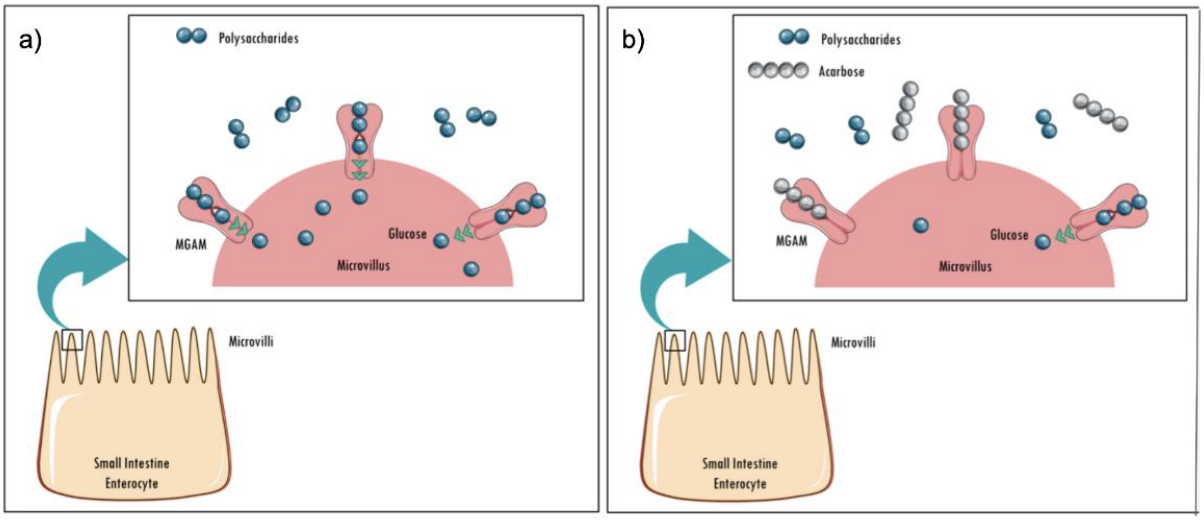


Figure 1. Mode of action of Acarbose. (Bischoff., 1991).

7.3.Herbal medicine:

Plants have been used for therapeutic purposes in most areas of pathology; this is what we call herbal medicine. The reasons for using plant products are generally the absence of side effects after the consumption of plants, the absence of chemicals and the economy of therapy (Hadj and Benbelal., 2020).

Diabetes has been treated by traditional Chinese medicine, by popular medicine and, in a more formalized way, by herbal medicine. Many experimental studies, conducted in vitro and in vivo, have clearly shown that plants contain hypoglycemic active ingredients (polyphenols, alkaloids, saponins, coumarins and terpenoids), there is no evidence-based argument to recommend the use of herbal medicine alone or in combination with conventional treatment to treat hyperglycemia, and its complications (Schlienger., 2014).

8. Regulation of glycaemia:

8.1.Carbohydrate Homeostatic:

Glucose moves between the site of its absorption (intestinal mucosa) or the site ofEndogenous production (liver) and the sites of mainly energy metabolism, including: brain, striatedmuscles, kidneys and adrenal glands. Hormonal factors, hypoglycaemia or hyperglycaemia, regulatethe evolution of blood sugar so that its concentration is as close as possible to its physiological value,these hormones include (Jean-Louis and Geneviève., 2011):

Substances secreted during hypoglycaemia: glucagon, cortisol, growth hormone and biogenicamines such as epinephrine and noradrenaline:

- The one that is secreted during periods of hyperglycaemia: insulin.
- Body and extracellular glucose concentrations that lead to glucose intolerance ordiabetes.

8.2.Regulation of glycemia by insulin:

Insulin, which is a hormone secreted by Langerhans beta cells, maintains carbohydrate homeostasisby acting on three target tissues: the liver, muscle and adipose tissue(Campbell et al., 2021).

The mechanism of secretion of this hormone when blood sugar rises involve an increase in glucose use by the β-pancreatic cell, an increased production of ATP and a decrease in the ADP/ATP ratio leading to the closure of dependent K+ATP channels. This leads to a cell depolarization that allows the opening of voltage-dependent Ca2+ channels. The increase in intracellular calcium, together with other second messengers (cAMP), stimulates the release of insulin. The effect of glucose on the closure of K+ channels can be mimicked by sulfonylureas (Ferré., 2005).

The entry of glucose into the cell represents the first step in controlling its use. There are seven different glucose transporters called GLUT1 to GLUT7, which transport glucose through the cell membrane by facilitated diffusion. GLUT 4 is the only glucose transporter that only comes into action in response to insulin (Ganong., 2005).

8.2.1. Synthèse et sécrétion de l'insuline:

Insulin is secreted by the β cells of the Langerhans islets of the pancreas, directly into the portal vein depending on the glycaemic level.The insulin gene codes for an mRNA that makes it possible to produce a pro hormone, pro insulin, The latter undergoes a maturation step in the Golgi apparatus, it will then be cleaved to give two hormones, the C peptide and insulin.Glucose acts as a regulator of insulin gene expression and on the degradation of the associated gene (Nazare., 2009).

8.2.2. Mechanism of action of insulin:

Insulin, the only hypoglycaemic hormone in the body, also controls lipid metabolism by stimulating lipogenesis and inhibiting lipolysis (Marshall and Bangert., 2005).

It is involved in the regulation of NO synthesis. (Nitrogen oxide) which is involved in inflammatory phenomena. It also reduces the production of free radicals induced by hyperglycaemia (Andreelli et al., 2006).

It also performs pleiotropic functions on protein metabolism (increased synthesis and inhibition of proteolysis), growth and control of apoptosis (Capeau., 2003).

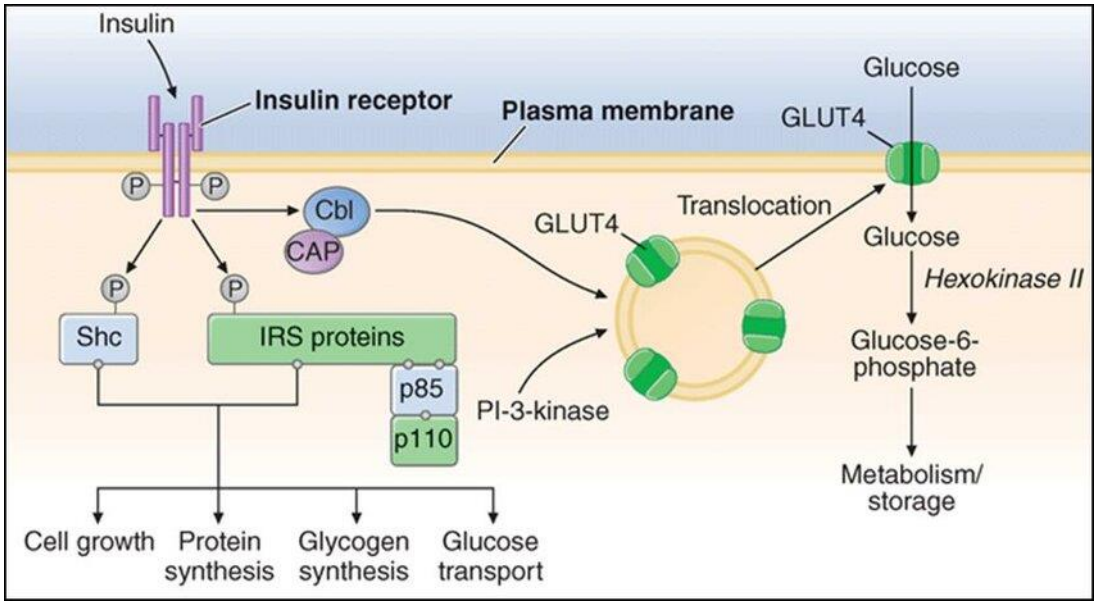


Figure 2. Mechanism of action of insulin. (Pravinkumar., 2018).

8.3.The effector organs of glycaemic regulation:

The constant maintenance of blood sugar implies a subtle balance between the entry and exit of glucose from the blood. Schematically, blood glucose has two origins. During a meal, the intestinal absorption of the glucose contained in the food bowl is responsible for a massive entry of glucose into the blood (Simon., 2016).

In post-absorbed conditions, the liver is generally considered the main glucose-producing organ in mammals (Bergman and Mittelman., 1998). The appearance of glucose in the bloodstream will mainly be detected by the pancreas. Its role in the regulation of carbohydrate homeostasis is crucial because of its secretion of hormones such as insulin or glucagon by the islets of Langerhans, which makes it possible to store glucose or, on the contrary, to use existing stocks depending on the metabolic state of the body (Duparc., 2012).

In addition, in the early 1990s, it was recognized that the kidney could play a crucial role, in nutritional conditions, in dogs, rats and humans (Minassian and Mithieux., 1994; Cersosimo et al., 1994; Ekberg et al., 1999). Studies in humans and rats have shown, under post-absorbing conditions, that renal glucose production represents about 25% of total production and its capture is about 20% on total glucose uptake (Stumvoll et al., 1995; Pillot et al., 2009).

Conversely, during the period of fasting, certain organs such as the liver and to a lesser extent the kidney and intestine will be able to produce glucose in order to avoid hypoglycaemia. Blood glucose can be consumed by all organs, it is the main source of energy for cells. Excess glucose will be stored in the liver and muscles as glycogen and in adipose tissue as lipids (Simon., 2016).

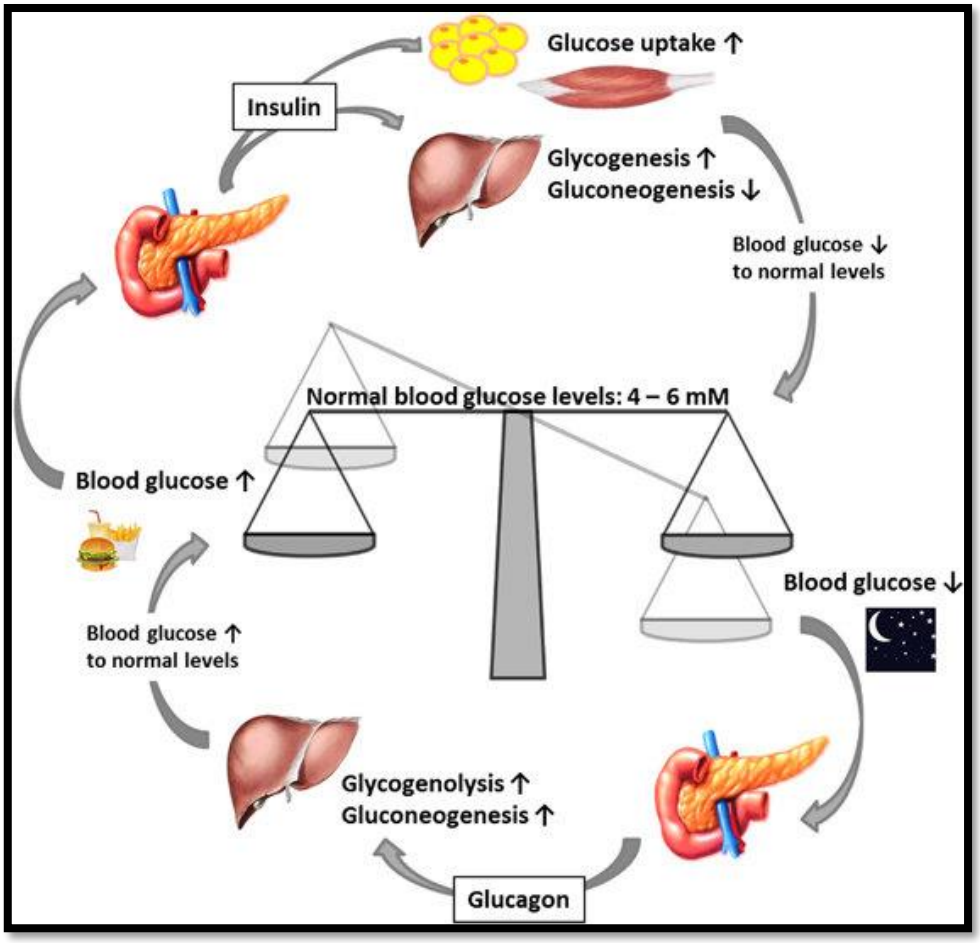


Figure 3. Pancreatic regulation of glucose hemostasis. (Pia et al., 2016).

8.4.Enzyme regulation of blood glucose:

8.4.1. Alpha amylase:

8.4.1.a. Structure of alpha amylase:

Alpha amylase is considered a glycoprotein with 478 amino acids and a molecular weight of 57.6 KDa. The first three-dimensional structure of experimentally determined alpha amylase indicates that alpha amylase has three characteristic domains: A, B and C (Burhan et al., 2003). These domains are linked by a polypeptide chain that is mainly hydrophobic residues (Banner et al., 1975).

- Domain A consists of a concentric alpha propeller cylinder containing 8 parallel beta sheets (Whintcomb and Lowe, 2007). It is composed of two residues of aspartic acid and a residue of glutamic acid that play an essential role in catalysis and are found in the active site of this field (Sarian et al., 2017).
- Domain B, it consists mainly of beta leaves and is adjacent to the cylindrical structure of domain A.
- Domain C consists of a barrel of eight anti-parallel β sheets (Alais et al., 2008).

- **Structure:**

Human pancreatic alpha amylase (HPA) is a monomer protein that binds calcium and chloride (Brayer et al., 1995). Since the sequence and structure of salivary alpha amylase are similar to those of pancreatic amylase, they are not treated separately. The HPA protein of 496 amino acids is composed of three domains.

Domain A (orange and red in color) is the largest of the 3 domains, and has a central β/a-baril parallel to eight strands (orange and red color; residues 1-99 and 169-404), the active site (Asp197, Glu233 and Asp 300) and a chloride binding site (residues Arg195, Asn298 and Arg337).

Domain B (sky blue color; residues 100-168) is the smallest of the 3 domains and is located on an extended loop between the third β and α-helix strand of the β-barril nucleus of domain A. It consists of a calcium binding site (Asn100, Arg158 and Asp167 resides; His201 from domain A).

Domain C (pink color; residues 405-496) occurs at terminal C and its function is not fully understood. It consists of an anti-parallel β structure and is connected to domain A (PDB ID 5td4; Zhang et al., 2016).

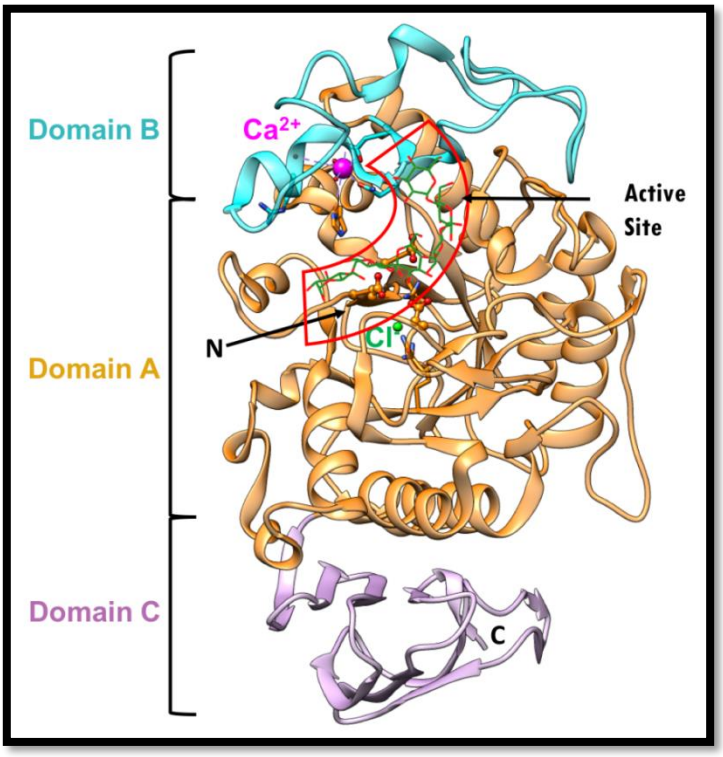


Figure 4. Ribbon diagram of the human pancreatic alpha amylase (HPA) and its subdomains in complex with individual structural subdomains highlighted in different colours.(PDB ID 5td4; Zhang et al., 2016).

8.4.1.b. Nomenclature:

- ✓ Codified name: EC 3.2.1.1.
- ✓ Common name: alpha-amylase.
- ✓ Other name (s): glycogenase, α-amylase; endo amylase; Taka-amylase A, maxilase.
- ✓ Systematic name: 1.4 - alpha -D-glucane-4-glucano hydrolase (Schamburg and Slzmann, 1991; Dauter et al., 1999; Nouadri., 2011).

8.4.1.c. The role of alpha-amylase:

The main source of carbohydrates for humans is the digestion of starch that begins in the mouth. The saliva contains α amylase, which hydrolyses all α glycosidic bonds (1- 4) except the most extreme and those close to connection points. When completely chewed food reaches the stomach, or the acidity inactivates α-amylase, the average length of the starch chains has increased from several thousand to less than eight units of glucose. The digestion of starch continues in the small intestine under the influence of β-amylase acting from a non-reductive end, and pancreatic α-amylase, which is similar to salivary α-amylase. This enzyme degrades starch into a mixture of maltose disaccharide, malttriose trisaccharide, formed of three glucose residues united by α (1-4) bonds and oligosaccharides called dextrins that have α (1-6) connections. These oligosaccharides are hydrolysed into monosaccharides by specific enzymes contained in the membranes of the brush border of the intestinal mucosa: an α (1-6) glucosidase, which detaches glucose residues from oligosaccharides one by one (Voet.D and Voet.J., 2005).

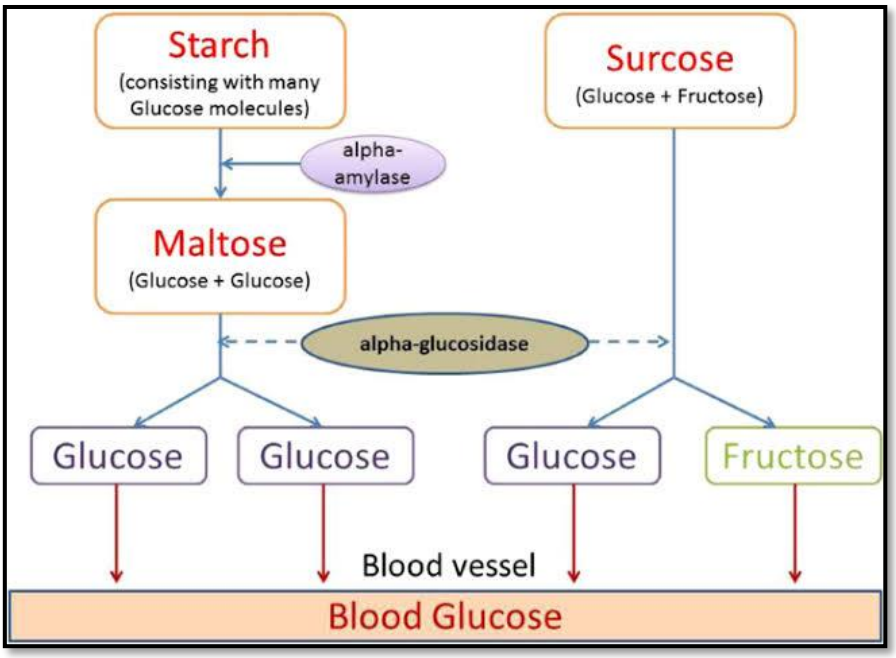


Figure 5. Hydrolysis of starch to glucose as catalyzed by alpha-amylase and alpha-glucosidase.(Yang et al., 2019).

8.4.1.d. Alpha-Amylase Mechanism of Action:

In the human diet, starch is the main source of energy. Food sugars and starches are divided intoglucose by alpha-glucosidase and alpha-amylase enzymes. Alpha-Amylase is found in saliva andpancreatic juice and is a member of the family of glycoside hydrolase 13 (GH13). α-Amylase is amineral enzyme that requires calcium ions that are important for structural integrity and is activatedby chloride ions. The AMY1 and AMY2 genes produce salivary and pancreatic alpha-amylaseenzymes, each containing 496 amino acids in a single polypeptide chain. Before the starch isabsorbed, it is hydrolyzed by salivary amylase and pancreatic in the mouth and small intestine.(Hmady and Kwang,2023).

These isoenzymes hydrolyze carbohydrate polymers into shorter oligomers such as maltose, α-limitdextrin, and malttriose by cleaving 1-4-α-glycosidic bonds. The salivary isoenzyme is partiallycleaved early into smaller oligomers (10-30%). When partially digested sugars enter the intestine,pancreatic amylase produced in the pancreas and secreted into the intestinal lumen hydrolyzes mostof them into smaller oligosaccharides. Subsequently, α-glucosidase located at the brush borderfurther hydrolyzes the α-amylase product in the small intestinal lumen into glucose,afterward,glucose transporters take the glucose from the intestinal mucosa and transport it into the bloodcirculation (Hamdy and Kwang., 2023).

CHAPTER TWO:

MEDICINAL PLANTS

1. A brief overview of medicinal plants

The beginnings of the medicinal plants' use were instinctive, as is the case with animals. In view of the fact that at the time there was not sufficient information either concerning the reasons for the illnesses or concerning which plant and how it could be utilized as a cure, everything was based on experience. Plants have provided man with all his needs in terms of shelter, clothing, food, flavors and fragrances as not the least, medicines. Plants have formed the basis of sophisticated Traditional Medicine (TM) systems that have been in existence for thousands of years and continue to provide mankind with new remedies. The ancient cultures are known for their systematic collection of information on herbs and their rich and well-defined herbal pharmacopoeias. Although some of the therapeutic properties attributed to plants have proven to be erroneous, medicinal plant therapy is based on the empirical findings of hundreds and thousands of years.(Beyene et al., 2016).

2. Introduction

Plants are an integral part of all living organisms of the earth, and medicinal plants are widely distributed worldwide. Since time immemorial, humans from all the cultures of the world have independently selected plants for food, shelter, clothing, and medicine. Plants were identified, according to their therapeutic properties and through trial and error, by the priests, shamans, herbalists, spiritual leaders and medicine men, and this practice is still a routine in many countries of Asia, Africa, the Middle East, Australia, and Latin America. Indeed, the widespread use of natural herbs and medicinal plants for curing and preventing diseases (nature's pharmacy) has been described in the ancient texts of the Vedas and the Bible (Hoareau and DaSilva 1999) and the Qur'an and the Ahadith. Duke, Duke, and duCellier (2008) even wrote a book entitled Duke's Handbook of Medicinal Plants of the Bible and cataloged "faith-based farmaceuticals."(Singh et al., 2012)

3. History of the Use of Plants as Medicines

One of the oldest records and considered the oldest written evidence of medicinal plants' usage goes back to about 5000 years ago and was written on Sumerianclay slabs containing a dozen recipes for drug preparations of over 200 different plants. Other records, which date back to around 2600 BC, came from ancient Mesopotamia and were recorded on clay tablets in cuneiform script There is evidence that medicinal plants have been used in early civilizations as demonstrated by the Chinese emperor Shen Nung (ca. 2500BC) who compiled descriptions of over 300 medicinal herbs and the Chinese materia medica has a long impressive history.Many of those herbs have been reported as medicines in different parts of the world such as ancient Egypt, Mesopotamia, and Europe. Opium is one of mankind's oldest

effective drugs and was used several millennia ago.The Assyrians, Babylonians, and Sumerians recorded herbal remedies in cuneiform inscriptions on numerous clay tablets and the Code of Hammurabi (18th century BC) contains many herbal medicines. The Egyptians recorded their medicinal knowledge in tomb illustrations and on papyrus dating from the Old Kingdom of Egypt. The 'Ebers Papyrus' (ca. 1550) was the most important of these recordings and contains over 600 prescription drugs of various plant species. European medicine is thought to have initiated with Hippocrates (460–377 BC) who compiled over 200medicinal plants which were classified by physiological action and he is considered the 'father of medicine'. Celsus (25 BC–50 AD), who was a famous medical writer, mentioned about 250 medicinal plants in his 'De re medica' book. who was a Greek military physician and considered the 'father of pharmacognosy', recorded the use of medicinal plants and wrote De Materia Medica in ca. 77 AD, which was used as a reference in Europe for more than a millennium and translated into several languages.It included over 900 drugs and most of them were of plant origin. Galen (130–200 AD), who was a renowned Greek physician and pharmacist, influenced the development of various scientific disciplines and documented the use of plants as medicines. Native Americans used plants as medicines for centuries and some anticancer drugs that are currently available to treat various cancers are derived from plants in North America such as the Pacific yew tree(Taxus brevifolia) from which the anticancer drug paclitaxel (Taxol®, 1) is derived. African traditional medicine has also been practiced for many centuries and it has diverse medical treatments for different diseases but it was poorly recorded. Structure of paclitaxel (Taxol®, derived from Taxus brevifolia). In the 17th century, one of the best herbal remedies was introduced into Europe; the use of the bark of the Cinchona tree which became a popular medicine to treat malaria. Its active ingredient quinine (2) was isolated two centuries later (1820). In the 18th century, the foxglove plant was used by Withering for treating dropsy and the active ingredient (digoxin) is currently being used for treating various heart conditions. The first 'pure' drugs became available during the 19th century. The alkaloids quinine (2, 1820), morphine (3, 1806), and ephedrine (4, 1887) were extracted from plants and chemists later on were able to disclose the chemical synthesis of these important alkaloids. A considerable number of drugs appeared in the second half of the 20th century that can treat diverse diseases as a result of the advancement in pharmaceutical science and the establishment of pharmaceutical companies. Although both synthetic and natural product-based drugs are currently used in curing diseases, numerous drugs in clinical use today are based on plant-derived natural products or their analogues.(Hassan, H. M. A., 2015).

4. Medicinal plants

A medicinal plant is any plant which, in one or more of its organs, contains substances that can be used for therapeutic purposes, or which are precursors for chemo-pharmaceutical semi-synthesis. (Yudharaj, P et al., 2016).

5. Classification of medicinal plants:

Approximately 5,000 species have specialized therapeutic benefit out of the 2,50,000 higher plant species on Earth, while over 80,000 species are known to have at least some medical potential.(Joy, P. P et al., 1998).

In general, medicinal plants are arranged according to their active principles in theirstorage organs of plants, particularly roots, leaves, flowers, seeds and other parts ofplant.(Lubbe et al., 2011).

Medicinal plants are classified in many ways Some of them are:

5.1.According to the usage

The herbs are classified in four parts: Medicinal herbs, culinary herbs, Aromatic herbs, Ornamental herbs. (Lubbe et al., 2011).

A. Medicinal Herbs

Medicinal herbs have curative powers and are used in making medicines because of their healing properties. (Lubbe et al., 2011).

B. Culinary Herbs

Are probably the mostly used as cooking herbs because of their strong flavors like mint, cinnamon, basil. (Lubbe et al., 2011).

C. Aromatic Herbs

A common usage for aromatic herbs is due to their fragrant foliage or blossoms. Aromatic plant oils are utilized in the food, beverage, cosmetic, toothpaste, and perfume sectors. For example, cumin, thyme, saffron, clove, and Rosemary.(Lubbe et al., 2011).

D. Ornamental Herbs

Ornamental herbs are used for decoration because they have brightly colored flowers and foliage like lavender, chives. (Lubbe et al., 2011).

5.2.According to the active constituents

There are five main groups into which the herbs are classified: Aromatic (volatile oils), Astringents (tannins), Bitter (phenol compounds, saponins, and alkaloids), Mucilaginous (polysaccharides), and Nutritive (food stuffs). (Lubbe et al., 2011).

A. Aromatic (volatile oils)

Many of these herbs have a nice scent, which is reflected in the name. They are widely utilized as fragrances, flavorings, and medicinal ingredients. There are two subcategories of aromatic herbs: nerviness and stimulants. such as garlic, ginger, and fennel.(IZOL et al., 2023).

B. Astringent Herbs

The tannins found in astringent herbs have the power to precipitate proteins, which tones, contracts, or "tightens" living tissue and aids in stopping discharges. Large quantities of them are toxic to the liver and have an impact on the urinary, circulatory, and digestive systems. They have antibacterial and analgesic properties. Red raspberry and peppermint, for instance.(Ashok et al., 2012).

C. Bitter Herbs

Bitter herbs are classified into four subcategories: laxative herbs (barberry), diuretic herbs (parsley), saponin-containing herbs (alfafa), and alkaloid-containing herbs (poppy family). Bitter herbs are termed due to the presence of phenols and phenol glycosides, alkaloids, or saponins.(Deshmukh et al., 2010).

D. Mucilaginous Herbs

The polysaccharides included in mucilaginous plants give them a slippery, mild flavor that tastes pleasant when diluted. For example, chia seeds, flaxseeds, aloe vera, and liquorice root.(Saju et al., 2021).

E. Nutritive Herbs

The nutritional value that these herbs add to the diet is the source of both their name and their classification. Apples, bananas, broccoli, cabbage, carrots, onions, pineapples, and so forth.(Hedges et al., 2007).

5.3.According to the period of life

A. Annual herbs: Finish their life cycle in a year begin with a seed (Annuals bloom for one season before dying). Herbs that grow year-round include saffron, chamomile, and basil.

B. Biennial herbs: Are plants that have two seasons and only bloom in the second (biennials have two seasons and only bloom in the second). once developed) and consist of (rosemary, fennel, and lavender).

C. Perennial herbs: grow for more than one season perennials live over winter and bloom each season) and include (Parsley, Mint, Thyme).(Miloradovich, 2012).

5.4.According to their taxonomy

Most of the medicinal plants belong to the following families

A. Medicinal plants of the Compositae family

Among all the groups, the Compositae family, dubbed the Daisy family, has the greatest number of therapeutic plants. This family of plants includes chamomile among its medicinal members.(Xu et al., 2017).

B. Medicinal plants of the Labiatae family

The Labiatae family, sometimes referred to as the mint family, is an extremely significant family of medicinal plants. This family of plants includes herbs and shrubs that frequently have fragrant scents. They are widespread in the Mediterranean region because some of them generate a lot of essential oil, which helps them endure the sweltering summer months. This family includes, among others, mints, rosemary, thyme, and lavender. (Carovic-StanKo et al., 2016).

C. Medicinal plants of the Umbelliferae family

Plants with the distinctive umbrella-arranged fruit belong to the Umbelliferae family, also known as the carrot family. Most of these plants provide an essential oil, which helps them to endure the sweltering summer months. The plant is actually cooled by the oil. This family includes, among other things, anise (*Pimpinella anisum*), fennel (*Foeniculum vulgaris*), and wild carrots (*Daucus carota*).(Redouan et al., 2020).

D. Medicinal plants of the Leguminosae family

Many native and naturally occurring plants that have been grown for food, fuel, and decoration make up the Leguminosae, or pea, family. Medicago sativa, alfalfa, and white and red clovers (*Trifolium repens and pratense*) are some of the plants that have therapeutic properties.(Benjamim et al., 2021).

E. Medicinal plants of the Rosaceae family

A lot of species of the Rosaceae, or rose family, are useful for medicine. The majority of these are bushes or trees with a range of characteristics. This family is well-known for its juicy and edible fruit trees and shrubs. The wood strawberry (*Fragaria moschata*), peach, almond, and apricot (*Prunus persica, amygdalus, and armeniaca*) are a few instances of this family.(Khan et al., 2016).

F. Medicinal plants of the Rutaceae and Solanaceae families

The Rutaceae or rue family is a small family that consists of cultivated fruit trees and medicinal herbs. Plants in this family include orange (*Citrus aurantium*), lemon (*Citrus limon*), grapefruit (*Citrus paradise*).(Panda et al., 2020).

G. Medicinal plants of the Cruciferae family

The Cruciferae or cress family is characterised by plant that have flowers with cross-like petals. This family groups a large group of medicinal plants that include Black mustard (*Brassica nigra*), White mustard (*Sinapisalba*), Wild radish (*Raphanus raphanistrum*).(Shankar et al., 2019).

H. Medicinal plants of the Liliaceae family

The lily family, Liliaceae, is made up of numerous plants with therapeutic properties. Rarely are they bushes; most are herbs. Aloe (*Aloe vera*), garlic (*Allium sativum*), garden onions (*Allium cepa*), and meadow saffron (*Colchium autunnale*) are a few examples of plants in this family.(Bozyel et al., 2020).

I. Medicinal plants of the Caryophyllaceae and Boraginaceae families

The Caryophyllaceae, or pink family group, of plants are characterized by four to five petals in their flowers, which are often pink or white in color. This family includes, among others, smooth rupture-wort (*Herniaria glabra*), nail wort (*Paronychia argentea*), sand spurrey (*Spergularia rubra*), common chickweed (*Stellaria media*), viscid sandwort (*Alsine tenuifolia*), and sandwort (*Arenaria serpyllifolia*).(Chandra et al., 2015).

J. Medicinal plants of the Ranunculaceae and Papaveraceae families

Bold blooms with five petals, often define the Ranunculaceae family, also known as buttercups. Pheasant's eye (*Adonis annuus*), larkspur (*Delphinium ajacis*), poppy anemone (*Anemone coronaria*), lesser celandine (*Ranunculus ficaria*), love in the mist (*Nigella damascena*), traveler's joy (*Clematis vitalba*), and evergreen traveler's joy (*Clematis cirrhosa*) are a few examples from this family.(Hao et al., 2022).

K. Medicinal plants of the Malvaceae and other families

The Malvaceae or mallow family groups those plants that have five-petalled flowers and a nutlet-like fruit. Examples include cotton (*Gossypium herbaceum*). (Das et al., 2019).

6. Sources of medicinal plants

The most popular places to find medicinal plants were homestead farms and roadside greenery, both of which were mentioned by 100% of THP.Markets, protected woodlands, and far-off forests were among others. The hardest places to find therapeutic herbs were wetlands. Every THPmember mentioned receiving TMKas a legacy from their parents and grandparents. The least common source of TMK was experimentation, which refers to the

haphazard selection and therapeutic application of plant species without first understanding their efficacy.(Ssenku, J. E et al., 2022).

7. Characteristics of Medicinal Plants

Medicinal plants have many characteristics when used as a treatment, as follow:

- **Synergic medicine:** The ingredients of plants all interact simultaneously, so their uses can complement or damage others or neutralize their possible negative effects.
- **Support of official medicine:**In the treatment of complex caseslike cancer diseases the components of the plants proved to bevery effective.
- **Preventive medicine:**It has been proven that the componentof the plants also characterize by their ability to prevent theappearance of some diseases. This will help to reduce the use ofthe chemical remedies which will be used when the disease isalready present i.e., reduce the side effect of synthetic treatment.(Rasool et al., 2012).

8. Factors affecting the harvesting and collection of medicinal plants

❖ **Cultivation**

Because of their superior medicinal worth and pharmacological activity, aromatic and medicinal plants are in higher demand on the market; for this reason, greater medical care and mechanical management are required. In the event that there is no scientific cultivation method accessible, then traditional cultivation techniques ought to be employed. If not, a method of cultivation must be developed through scientific research. The fundamentals of agricultural methods combined with a suitable rotation schedule and an environment that is suited to plant cultivation as needed by resources. When appropriate, conversation agriculture's (CA) capacity should lag behind abnormally high soil moisture and fertile matter increases.(Porwal et al., 2020).

✓ **Method of Propagation:**

a) Sexual or Seed Propagation

The process of spreading or seeding medicinal plants in suitable soil is known as sexual propagation. The two methods for showing seeds are the dibbling method (available for seeds of average size and weight; fennel, coriander, etc. are examples of seeds sown in holes) and the disseminating method (more suitable for small-sized seeds). Using this method, seeds, such as sesame and isapgol linseed, are freely dispersed over well-prepared soil for cultivation.(Porwal et al., 2020).

b) Asexual or Vegetative Propagation

When using the asexual method of vegetative propagation, a plant's stem or root is planted in a situation that encourages it to grow into a new plant. One of the benefits of asexual propagation is that the plants one grows and the plants from which it is generated are identical. Fruit seedlings (such as grapes, pomegranates, and lemons) may only be propagated vegetatively; plants begin to bear quicker than seedling trees; budding or grafting stimulates disease-resistant plant varieties; and subpar varieties may be disregarded. Plant components are sown in properly prepared soil to facilitate vegetative proliferation.Vegetativepropagationis done by sowing various parts of the plants in well-prepared soil. The following are examples of vegetative propagation:

- Bulbs: squill, garlic
- Corms: colchicum, saffron
- Tubers: jalap, potato
- Rhizomes: ginger, turmeric
- Runners: peppermint
- Suckers: mint, pineapple, banana, etc.(Porwal et al., 2020).

a. Factors affecting cultivation:

- **Soil**

The planet's surface layer, formed by the erosion of rocks, is represented as the soil. Plants and microbes combine to form weather strands, which results in the formation of soil. An appropriate quantity of organic matter, nutrients, and other components must be present in the soil. The ideal soil nick, pH, wet holding capacity, fertility, and soil effluent are all adjusted to meet the requirements of the selected curative plant. Generally speaking, using compost is essential to getting good harvests of medicinal plants. The ability of seeds to sprout, a plant's ability to remain erect, the strength and woodiness of the stem, the depth of the design, and the variety of blooms on the plan are all influenced by the soil. There are different kinds of soils, including Clay, Loamy, Silt loam, Sandy loam, Sandy soil and Chalky soil.(Shah, 2009).

- **Altitude, Temperature and Humidity**

Climate factors, for instance, day duration, rainfall and temperature, considerably influence the physical, chemical and biological standard of medicinal plants. The period of daylight, average rainfall, minimal average temperature, including day-timeandnightduration temperaturevariations, additionally impact thebiochemical pursuit of the plant.(Shah, 2009).

- **Rainfall and Irrigation**

Most of the plants need either proper arrangement for irrigationor sufficient rainfall for their favorable growth and development.

Throughout its several stages of growth, irrigation and drainage should be managed and maintained in accordance with the needs of each unique therapeutic plant taxon. Utilizing water for irrigation should improve local, regional, and/or national quality standards. It is important to exert supervision to ensure that the medicinal plants being grown are receiving the necessary amount of water. Aloe and acacia are two examples of xerophytes, which have the extra benefit of not needing irrigation or rainfall in order to grow such therapeutic plants.(EMEA, 2006).

- **Fertilizers**

A fertilizer is a mixture of organic and inorganic material unitized to increased soil fertility. There are various kinds of fertilizers.

1. **Biotic origin fertilizer:**fertilizer with a biotic origin Nitrogenous compounds and rich organic materials are often scarce in soil. This organic material is used as fertilizer only if it can supply the necessary ingredients.
2. **Green leaves manure:**Manure is fertile material that is mixed with clay. These furnish most of the essential nutrients required by the curative plants. This leads to a rise in crop productivity.
3. **Farmyard manure:**This isa combination oflivestock muck and remaining unutilised elements and husk and plants stalk fed to live stock.
4. **Composite manure:** This consists of a combination of delayed rotten and unutilized components of flora and fauna.
5. **Green manure:** This manure is made from non-woody crops that have been plowed under and mixed with the soil while it is still young. Most plants used as manure grow quickly. This soil has a good balance of organic and nitrogenous components. Additionally, it creates a shielding layer of soil that inhibits natural processes and erosion. Curative plant mass will rise by 30 50 % as a result.

6. **Bio-fertilizer:** Biological fertilizer when added to the soil, it functions as a biologically active byproduct of fungi, algae, and microorganisms that aids in nutrient delivery. These principally embrace Nitrogen adjusting bio-organisms. Some of the Bio-fertilizer is *Legume-bacteria* genus mutualism,*Azolla-anabaena mutualism*,nonsymbioticmicroorganism, a not secure coalition of nitrogen adjusting bio-organism, Eubacteria that isacynobacteria, a heterogeneousgroup of prokaryotic, principally photosyntheticorganism.
7. **Chemical fertilizers:**Nitrogen, phosphorous, potassium, calcium, magnesium and sulphur are used as macronutrients; and iron, manganese, zinc, boron, copper, molybdenum, carbon, oxygen are used as micronutrients.(Malik et al., 2011).

- **Pests and Pests Control**

Pesticides are artificially derived from natural and chemical sources, and they function well against pests in modest amounts. Pests are undesirable species of animal or plant. A variety of insects can transmit dangerous diseases including typhus and malaria. Certain insects, such *Gossypium, Zea mays, Triticum aestivum*, and *Oryza sativa*, severely damage or destroy valuable crops. Moreover, weeds like *Ambrosia artemisiiflora* and Toxicodendron radicans, as well as bacteria, fungi, and *Rattus rattus*, are frequent pests. To effectively design and implement pest management techniques that incorporate natural substances, it is crucial to comprehend the origins of the pest situation. Globally, the conventional pesticide industry is a massive one, with the majority of synthetic pesticides being used for agricultural or other uses.(MH et al., 2018).

❖ **General Methods of Pest Controls**

1. **Natural Controls**

The natural world is full of the example of prey predator associations. Each and every pest is more or less hamper in its rise by other rapacious organisms.

The main causes of natural insect controls are usually parasitic pests, predators, and diseases carried by the organism. However, using certain pesticides to eradicate a major pest on a crop may cause a secondary pest outbreak because natural enemies are destroyed, upsetting the balance between beneficial and destructive insects. Topographical influences from seasonal changes in temperature, precipitation, soil types, air moisture content, and other climate conditions also have an impact on insects and their host.(Kaluwa et al., 2012).

2. **Artificial Control**

Artificial controls of pest have been startby human. Artificial control can be classified as agriculture, chemical and biological controls as discussed below.

Table 1 Demonstrates control techniques andMethods involved

Controlling techniques	Methods involved
mechanical control	Involves manual labor along with different devices for collection anddestruction of pest, by burning, hand picking and trapping of insects, destruction of eggs, larva and pupae etc.
Agricultural control	Croprotation, deep ploughing,change in environment, use ofsystemic insecticide.
Chemical controls	Use of pesticide, herbicides, antifeedants,biopesticides

- **Collection of medicinal plant:**

No matter what type of the crude drug and area of collection, there cannot be two opinions that the drugs are collected suitably when they contain maximum quantity of secondary plant metabolites. The collection of medicinal plant affected by few important things as: weather of the collected plant, plant age, daily session sunlight windy, and the phase of the physical growth of the plant part utilized. The collection factors influence quality and quantity of the potent active component of plants: weather of the collected plant for example the leaves of *Mentha*, used for culinary propose, Eucalyptus species, a cash crop & Spearmint leave, used for memory enhancer during spring season contain a high content of volatile oils, while during hot & in cold weather the content of these oils become lessen. The medicinal plant age for examples cinchona tree species from rubiaceae have high amount of the alkaloid Quinine, a medication used to treat malaria and baesiosis and Cinchonine, antipyretic properties compound in their barks at their early age about 6 to 9 years old. The stage of Physiological development of medicinal plant used: for examples the leaves of (Digitalis, Tobacco & Senna) that are accumulate when they are in complete physiological maturity phase and the seeds of black cumin (*Nigella sativa*) are collected when they are white & black in color.(Tariq et al., 2013 ; Porwal et al., 2020).

- **Harvesting:**

Harvesting is an important operation in cultivation technology, as it reflects upon economic aspects of the crude drugs. An important point which needs attention over here is the kind of

drug to be harvested and the standard quality which it requires to attain.It is important to collect medicinal plants during the best weather or time of day to ensure that the final herb product is of the lowest possible quality and that medicinal plant material is assembled. the time between harvests determined by the natural item to be used.Careful statistics regarding the suitable temporal arrangement of harvest is commonly obtainable in national pharmacopeia, printed authentic literature, latest official monographs, and vital reference books. Still, it is standard that the content of bioactive phytochemical depends with the phase of plant maturity.Rather than considering the entire vegetative quantity of the targeted therapeutic plants, the optimal harvesting period should be based on the average concentration of bioactive components. When harvesting, take care to ensure that no undesirable materials, weeds, or toxic plants are mixed in with the harvested plant material.(Pandey et al., 2017).

Harvesting curative herbs should be done in the simplest possible conditions; avoid condensation, rain, or very high levels of moisture. Harvesters, cutting tools, and other equipment need to be cleaned and adjusted to various materials and soil contamination.The harvested rough curativeplant stuffs ought to be shift quickly in good, withered places. Harvested plant can be transferred in clean vessel, dry pouches, caravan, hoppers or different airyvessels and shift to the process supplying. All vessels used at harvest ought to beunbroken clean and free from foreign matter by accidently harvested curativeplants and different contamination.(Pandey et al., 2007).

When a vessel isn't in use, it needs to be kept in good condition and kept out of the way of pests like birds, rats, and insects. Any mechanical harm or compression of the unprocessed medicinal plant ingredients as a consequence for instance, from packing to much should be prevented. Throughout the harvest, post-harvest inspections, and processing, rotted medicinal plant materials should be identified and disposed of to prevent microbial contamination and the deterioration of raw medicinal plant material. Restoring some of the traditional and profitable medicinal plants requires the use of well-known aromatic and curative plants.(Pandey et al., 2017).

- **Drying:**

When medicinal plant materials are prepared for use in dry form, the moisture content of the material should be kept as low as possible in order to reduce damage from mould and other microbial infestation.Drying can be done in two ways, either by natural or artificial drying.(Safarov et al., 2016).

1. **Natural drying:**

Natural drying can be achieved by direct sun-light or shade. Mainly we preferred shade drying because it helps to retain its natural color of the drug (e.g. digitalis, clove, senna) and the volatile principles of the drug (e.g. peppermint). We can also dry drug directly if it quitestable to the temperature and sunlight (e.g. gumacacia, seeds and fruits).(Safarov et al., 2016).

2. **Artificial Drying:**

a) Tray Drying:

If the plants without volatile oils and completely stable to heat or which require deactivation of enzymes, then tray dryers used for drying. In tray drying hot air of the specific temperature is flow through the dryers and this make easy the removal of moisture content of the plants (e.g. belladonna roots, cinchona bark, tea and raspberry leaves and gums are dried by this method).(Ahmed et al., 2013).

b) Vacuum Drying:

Tannic acid and digitalis leaves are sensitive to higher temperature thus used vacuum for drying.

c) Spray Drying:

Few plants which are highly reactive to atmospheric conditions and temperature are dried.(Ahmed et al., 2013).

❖ **Storage:**

1) Storage facilities for medicinal material should be well aerated, dry and protected from light, and, when necessary, be supplied with air conditioning and humidity control equipment as well as facilities to protect against rodents, insects and livestock.
2) The floor should be tidy, without cracks and easy to clean. Medicinal material should bestored on shelves which keep the material a sufficient distance from the walls; measures shouldbe taken to prevent the occurrence of pest infestation, mould formation, rotting or loss of oil; andinspections should be carried out at regular intervals.
3) Continuous in-process quality control measures should be implemented to eliminate substandard materials, contaminants and foreign matter prior to and during the final stages of packaging. Processed medicinal plant materials should be packaged in clean, dry boxes, sacks, bags or other containers in accordance with standard operating

procedures and national and/or regional regulations of the producer and the end-user countries.

4) Materials used for packaging should be non-polluting, clean, dry and in undamaged condition and should conform to the quality requirements for the medicinal plant materials concerned.Fragile medicinal plant materials should be packaged in rigid containers.
5) Dried medicinal plants/herbal drugs, including essential oils, should be stored in a dry, well- aerated building, in which daily temperature fluctuations are limited and good aeration is ensured.
6) Fresh medicinal plant materials should be stored at appropriate low temperatures, ideally at 2-8°C; frozen products should be stored at less than -20°C.
7) Small quantity of crude drugs could be readily stored in air tight, moisture proof and light proof container such as tin, cans, covered metal tins or amber glass containers.
8) Wooden boxes and paper bags should not be used for storage of crude drugs. (Small et al., 2007).

Plant *Mentha Piperita*

1. **Definition of plant *Mentha Piperita***

Peppermint is a hybrid herb known by the scientific name *Mentha piperita* that was created by combining two types of plants:pointed mint and water mint.It has a distinctive smell and a burning aromatic flavor, but it gives a feeling of freshness.The plant was first identified by Carl Linnaeus in1753.Mint is a green plant with violet-purple flowers.Its green roots have a distinctive square shape. The plant has reddish stems with a greenish-purple color underneathThis plant grows to a height of about 2 and a half feet (76 cm). It is hardy and can survive freezing conditions. Mint plants prefer full sunlight, but they can also be planted in shady areas.It is a perennial plant, which means it can live for more than two years. If conditions are right, the plant will return year after yearPapers.(Linnaeus, 1753)

2. **Botanical taxonomy of*Mentha Piperita*:**

- **Scientific name:***Mentha Piperita*.
- **Synonym:***Peppermint*.
- **Common name:***Peppermint*.

Table 2 Botanical classification of*Mentha Piperita*

Species	*Mentha*
Generates	*Piperita*
Family	Lamiaceae
Order	Lamiales
Class	Mangnoliopsida
Branch	Mangnoliophyta
Reign	Plant

3. **Morphological description of*Mentha Piperita:***

It is a herbaceous rhizome plant, 30 cm long and up to 90 cm high. Its stem is smooth, and in its cross section it appears square in shape. RhizomeA denuded, fibrous, fleshy rhizome, spreading densely. The leaves are 4-9 cm long and 1.5-4 cm wide, and are dark green with red veins, with a sharp apex and jagged, serrated tips.The leaves and stem often have small hairs. Its flowers are purple, 6-8 mm long. The crown is divided into four sections with a diameter of 5 mm. They are formed in the form of whorls around the stem. They are thick and have sharp waves.This plant blooms from mid-summer etcetera . The number of chromosomes in it is variable , since it is a diploid of the chromosome set (2N) and the number of chromosomes

is recorded, 66, 72, 84, 120 (Flora et al., 2014) . *Mentha Piperita* is a fast-growing plant until buds appear on it, it becomes rapidly spreading .(Blamey et al., 1989)

Figure 6. Photograph of*Mentha Piperita*.

4. Geographical distribution of*Mentha Piperita*:

Mint is grown in Europe, Asia, North Africa and North America. In Australia, the Galapagos Islands and New Zealand...United States in the Great Lakes region, where it has been observed since 1843.It is also widespread in Algeria (In the north it is more abundant than in the south).

5. Traditional uses of *Mentha Piperita*:

It is used as an appetite stimulant and drunk as a healthy drink with a natural flavour. *Peppermint* contains volatile essential oil at a rate of about 1%, and it contains menthol at a rate of 50-60%, and other substances such as acetaldehyde and acid.Valerico limonino menton is also called medicinal mint.Among the Egyptians, it is recommended against vomitingand among the Romans it was usedas a flavoring for wine and sauces, but also in relieving headaches and stomach disorders ,it works to calm intestinal spasms, digestive spasms, nausea, and bloating. It is also an anthelmintic and tonic for the nervous system.(Duband et al., 1992)

6. Previous studies on plants *Mentha Piperita*:

Microbial biofilms have become increasingly problematic in the food processing and medical industries where they cause food and surface contamination. Biofilms have also been implicated as the cause of serious infections in humans as their occurrence makes it difficult to treat common infections and the likelihood of recurrent infections is high. Due to emerging resistance, conventional control methods are fast becoming ineffective. In this study, the use of a selection of commercial plant extracts is investigated. The inhibitory effects of eight herbal extracts on the development of microbial biofilms was investigated against clinical and reference strains of *Pseudomonas aeruginosa* and *Candida albicans*. The antimicrobial activity was investigated on the planktonic forms using the minimum inhibitory concentration assay. The extracts that showed the highest antimicrobial activity against the two test organisms were *Echinacea angustifolia* (coneflower), *Mentha piperita* (peppermint) and *Rosmarinus officinalis* (rosemary) with minimum inhibitory concentration values between 0.38 and 2.5 mg/ml. The crystal violet assay was used to assess the effect of pre-treating a surface with plant extracts on cell attachment and the extent of biofilm development following exposure to extracts (biofilm biomass). Most of the extracts reduced microbial colonization by at least 50%. In contrast, preformed biofilms were less responsive to the majority of extracts, thus growth inhibition was more difficult to achieve. *Mentha piperita* was the only extract that showed some antibiofilm activity against both pathogens.(Sandasi et al., 2011)

- **In another study**

The chemical composition, anti-inflammatory, cytotoxic and antioxidant activities of essential oil from leaves of *Mentha piperita* (MEO) grown in China were investigated. Using GC-MS analysis, the chemical composition of MEO was characterized, showing that it was

mainly composed of menthol, menthone and menthy acetate. MEO exhibited potent anti-inflammatory activities in a croton oil-induced mouse ear edema model. It could also effectively inhibit nitric oxide (NO) and prostaglandin E2 (PGE2) production in lipopolysaccharide (LPS)-activated RAW 264.7 macrophages. The cytotoxic effect was assessed against four human cancer cells. MEO was found to be significantly active against human lung carcinoma SPC-A1, human leukemia K562 and human gastric cancer SGC-7901 cells, with an IC_{50} value of 10.89, 16.16 and 38.76 µg/ml, respectively. In addition, MEO had moderate antioxidant activity. The results of this study may provide an experimental basis for further systematic research, rational development and clinical utilization of peppermint resources.(Sun, Z et al., 2014)

Plant *Ammodaucus leucotrichus*

1. **Definition of plant *Ammodaucus leucotrichus*:**

Coss. & Dur. is a small annual plant, 10-12 cm high, glabrous with erect, finely striated stems. The leaves are finely dissected and slightly fleshy. The flowers are grouped in umbels of 2 to 4 branches with 5 free petals. The fruit is a diachene, 6-10 mm, long and is covered with dense silky white hairs. The plant has a strong smell of anise. It usually flowers in early spring (February to April). The plant is common in the Algerian Sahara; its presence is also mentioned in the Canary Islands. (El Haci et al., 2021).

The traditional medicine uses *A. leucotrichus* to treat a wide range of ailments. We have previously identified *perillaldehyde and limonene* as the primary components in our essential oil from the fruit portion *of A. leucotrichus*. (El Haci et al., 2021).

2. **Botanical taxonomy of *Ammodaucus leucotrichus:***

- **scientific name:** *Ammodaucus leucotrichus.*
- **Synonym:***Hairy cumin.*
- **common name:***Kûmun-Sûfi*. (MAYOU et al., 2018).

Table 3 Botanical taxonomy of *Ammodaucus leucotrichus*

Species	*Leucotrichus*
Genus	*Ammodaucus*
Family	Apiaceae
Order	Araliales
Class	Rosopsida
Branch	Magnoliophyta
Kingdom	Plants

3. **Morphological description of *Ammodaucus leucotrichus*:**

Morphologically, it is a small yearly wild and cultivated plant from 10 to 12 cm tall with fine and little fleshy leaves and white flowers grouped in umbels of 2, until 4 branches with 5 free petals. The fruit is diachene 6 to 10 mm long and has a dense, soft and white hair ; in fact, it is known in some areas as hairy cumin.(Abderrezag et al., 2021).

(A)

(B)

Figure 7. Photographs of a plant*Ammodaucus leucotrichus.* (Chatelain et al., 2021).

4. Geographical distribution of *Ammodaucus leucotrichus*:

Ammodaucus which belongs to this family is found as endemic genus in North Africa Saharan and Sub-Saharan countries including Algeria, Morocco, Tunisia and Egypt from this genus, the only species growing wild in Algeria is *leucotrichus Coss*& Dur *subsp*

leucotrichus. Other subspecies was described in Canary Island by Bultrán which was *leucotrichus Coss&Dur subsp*. nanocarpus.(Mohammedi et al., 2018).

5. Traditional uses of *Ammodaucus leucotrichus:*

Ammodaucus leucotrichus, known in Algeria as "*Moudrayga or El massoufa*", is a medicinal plant sold to herbalists in local markets, particularly in the Southern Algerian Sahara. The nomads collect the seeds and leaves for their own use, usually in the spring, when the fruits are ripe. In the southern Algerian Sahara, the leaves of this plant are used as a flavoring herbal in teas and fruits are often used as a spice during culinary preparation. The leaves and seeds are consumed in the form of decoction or infusion for several therapeutic cases, such as blood pressure, chest pain, liver and digestive system ailments, gastroenteritis, as also for diabetes.(Idm'hand et al., 2020).

6. Previous studies on plants *Ammodaucus leucotrichus*:

leucotrichus plant was harvested in the Algerian Sahara Desert, region known for the characteristic climate. This plant showed a yield in essential oil higher than 2.5%, being Perilla aldehyde the major compound, accounting for 85% of its composition. EOALF revealed antioxidant, antibacterial and also anti-infl ammatory activity, being these bioactivities related to the presence of *Perilla aldehyde* and *li-monene* (the second major compound present in EOALF).Considering the interest of the cosmetic industry for natural bioac-tive ingredients, tests concerning the evaluation of EOALF behavior when incorporated in a base cream have been performed. From the obtained results, it was possible to conclude that the bioactivity of the EOALF was evidenced during 28 days, even a decreasing pattern was observed with time. This was perceived right after the incorporation, pointing out possible interferences with the base cream matrix. In this context, the need to provide stabilization, and long-lasting effect was identified, which can be achieved through microencapsulation of the EOALF. Even so, the individual bioactive properties of EOALF pointed out the interest to exploit this essential oil as a natural cosmeceutical ingredient.(Halla et al., 2018).

- **In another study:**

comparative content and chemical composition analysis of EOs from the root, stem/leaf, flower and fruit of A. *leucotrichus subsp.leucotrichus* growing wild in the Algerian desert has been performed. Results highlighted that the pattern of specialized metabolites was different in both organs. Results have shown a considerable variation in the EO content and

composition between organs. Oil yields from these different parts varied from 0.52 to 3.04 % (w/w, based on dry weight) and contained complex mixtures of up to 89 diverse compounds. The main chemicals found in the extracts of A.*leucotrichus* organs were (Z)-falcarinol, β bisabolene, myristicin, methyl eugenol, α-pinene, muurola-4,10(14) dien-1-β-ol, ge-ranyl acetate, perillaldehyde, and limonene.TheEOs analyzed here have shown a great diversity in their composition that affects the major and minor constituents. In any case, these data make A. *leucotrichus* a good candidate to study the biosynthetic regulation of specialized compounds in relation to biotic and/or abiotic constraints.We may conclude that different parts of A. *leucotrichus* could be a useful source of bioactive EOs and bio-volatiles that have the potential to be exploited in the pharmaceutical sector based on the chemical composition and diversity of these compounds.(Neghliz-Benabdelkader et al., 2021).

CHAPTER THREE:

ESSENTIAL OIL

1. Definition

According to AFNOR (Association Française de Normalisation): "these are products obtained from raw materials of plant origin, either by steam training, or by mechanical processes from the fresh epicarp of certain citrus fruits, or by distillation and which are then separated from the aqueous phase by physical processes"(Bruneton et al., 1999).

For some authors, it is important to distinguish essential oil and gasoline (Carette et al., 2000).

- Essence: natural secretion developed by the plant organism, contained in various types of producing organs, variable according to the part of the plant considered.
- Essential oil: is a natural extract of raw materials of vegetable origin,obtained by distillation by water vapour, i.e. the essential oil is the distilled gasoline

2. Distribution and location

Essential oils exist almost only in higher plants (Khia et al., 2014). Their chemical composition is of great complexity, which makes them specific because each essential oil actually includes several very elaborate and very different aromatic substances. Essential oils can be stored in all parts of the plant (flowers, leaves, fruits, seeds, bark, stems, etc.) with a greater amount in the upper parts (flowers and leaves) (Aboughe Angone et al., 2015).

Essential oils are produced in the cytoplasm of secretory cells and generally accumulate in specialized glandular cells, located on the surface of the cell and covered with a cuticle, The accumulation of these secondary metabolites is usually done at the level of specialized histological structures, often located on the surface of the plant such as secretory hairs, secretory pockets and secretory channels for asteraceae(Teucher et al., 2003).

3. Physico-chemical properties of essential oils

Essential oils are made up of aromatic molecules of very low molecular weight (Degryse et al., 2008). They are liquid at room temperature but also volatile, which differentiates them from so-called fixed oils. They are fat-soluble and soluble in common organic solvents as well as in alcohol, water vapor-drainable but very poorly soluble in water (Couic-Marinier et al., 2013).

They have a density generally lower than that of water and a high refractive index (Desmares et al., 2008). They are mostly colored: e.g. reddish for cinnamon oils and a variety of thyme, pale yellow for sclary sage and rosemary oils. They are alterable and sensitive to oxidation; therefore, their conservation requires darkness and moisture (Couic-Marinier et al., 2013).

4. Chemical composition and biosynthesis of essential oils

The study of the chemical structure of essential oils reveals that they are complex and prominently altered mixtures of components belonging exclusively to two groups characterized by distinct biogenetic origins: terpene compounds such as monoterpene and cysciterpine; and aromatic compounds derived from phenylpropane, less frequent such as cinnami alcohol. They can also contain various products of decomposing processes involving non-volatile components such as acids, alcohols, aldehydes, esters, etc.) (Bakkali et al., 2008; Couic-Marinier et al., 2013). Biosynthesis of the components of these essential oils takes two pathways using either mephalonic acid or chichimic acid respectively for the the turbinoids and phenelpropanoids as intermediates (Singh et al., 1990).

5. Toxicity of essential oils

As with a drug, for each essential oil there is a balance between the benefit and the risk that must also be considered depending on the subject. The cutaneous application of HEs containing furocoumarins and pyrocoumarins (Citrus oil) or even their oral intake, can cause, under the prolonged effect of the sun, erythematous reactions that may promote carcinogenesis (Bakkali et al., 2008).

Also, the oral absorption of HEs rich in monoterpenes over long periods of time can inflame and eventually deteriorate nephrons (the functional units of the kidney). This is called nephrotoxicity.

In addition, the use of HEs in local application, in perfumery or cosmetics, can generate irritations, allergies or even photosensitization. This is the case with the essential oil of Thyme, Oregano, Sarriette (oils rich in thymol or carvacrol) which are known for their irritating and aggressive power(Bakkali et al., 2008).

Essential oils are complex mixtures of molecules; whose structure and chemical properties make it possible to identify common characteristics (lipophilic, low molecular weight) ensuring good diffusion in the body. Despite the few studies on these molecules, it is necessary to retain their toxic power. In particular their hepatotoxicity, neurotoxicity and skin toxicity recently. Their very varied use constitutes a high potential for intoxication, whether accidental or by incorrect use.(Lucette ,2001).

6. Biological activities of essential oils

The virtues of essential oils have been known and used for a long time, but this use was based on traditional practices and applications without precise scientific bases. Nowadays, their use is made on a scientific and rational basis since a lot of research has focused on the

antimicrobial and antioxidant activities of the essential oils of aromatic plants. Essential oils are known to have antiseptic and antimicrobial properties. Many of them have anti-toxic, anti-venomous, antiviral, antioxidant, and antiparasitic properties. More recently, they are also recognized as anti-cancer properties.

In herbal medicine, they are used for their antiseptic properties against infectious diseases of bacterial origin, for example against endocanal bacteria (Billerbeck ,2008), or at the level of the vaginal microflora (Duarte,2007), and of fungal origin against dermatophytes. However, they also have cytotoxic properties (Bakkali,2008). That thereforebring them closer to antiseptics and disinfectants as broad-spectrum antimicrobial agents.

In phytosanitary fields, essential oils or their active compounds could also be used as protective agents against phytopathogenic fungiand in agri-food, against microorganisms that contaminate foodstuffs (Smith,2002).

7. Essential oils extraction methods:

7.1.Classical and Conventional Methods:

Numerous techniques exist for extracting essential oils. Many sections of the world continue to overuse the timid technology related to the processing of essential oils, despite their obvious relevance. The approximately conventional and widely used techniques include hydro distillation (HD), steam distillation (SD), solvent extraction, evaporation, cohobation, and maceration(Rassem et al., 2016).

7.1.1. Hydro distillation (HD):

Hydro distillation is a traditional method for removal of essential oils. Water or hydro distillation is one of the oldest and easiest methods. Being used for the extraction of essential oils. Hydro distillation normally used to isolation essential oils from the aromatic and medicinal plant. The conventional method for the extraction of essential oils is hydro distillation (HD), in which the essential oils are evaporated by heating a mixture of water or other solvent and plant materials followed by the liquefaction of the vapors in a condenser. The setup comprises also a condenser and a decanter to collect the condensate and to separate essential oils from water, respectively. The principle of extraction is based on the isotropic distillation. In fact, at atmospheric pressure and during extraction process (heating), water or other solvent and oils molecules. Hydro distillation (HD) is a variant of steam distillation, which is bespoke by the French(Rassem et al., 2016).

There are three types of hydro distillation: with water immersion, with direct vapor injection and with water immersion and vapor injection. The distillation time depends on the

plant material being processed. Prolonged distillation produces only a small amount of essential oil, but does add unwanted high boiling point compounds and oxidation products(Rassem et al., 2016).

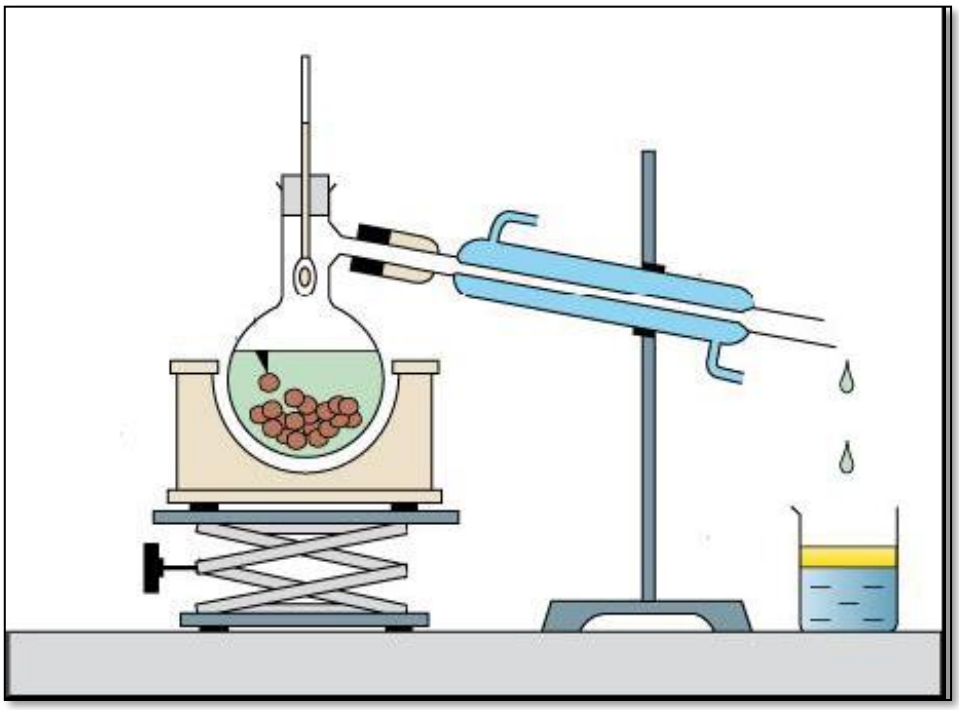


Figure 8. The schematic subsidize apparatus for hydro distillation. (Rassem et al., 2016).

7.1.2. Steam Distillation:

One kind of distillation (a separation or extraction procedure) for a plant that is temperature-sensitive, such naturally occurring aromatic chemicals, is steam distillation. Vacuity distillation has rendered this once-common laboratory technique for organic compound purification outdated.(Machado et al., 2022).

The plant materials charged in the alembic are subjected to the steam without maceration in water. The injected steam passes through the plants from the base of the alembic to the top. Steam distillation is a method where steam flows through the material as shown in Figure 9.(Machado et al., 2022 ; Rassem et al., 2016).

The raw materials pores are broken up and the essential oil is released by the action of this steam. The desired essential oil and vapor are produced by the system. The essential oil is then collected when this vapor is further condensed. The idea behind this method is that the volatile components, whose boiling temperatures range from 150 to 300 °C, can evaporate at a temperature that is similar to that of water if the combined vapor pressure reaches the

ambient pressure at roughly 100 °C. In addition, this method can also be used under pressure, depending on how tough it is to extract the essential oils.(Machado et al., 2022).

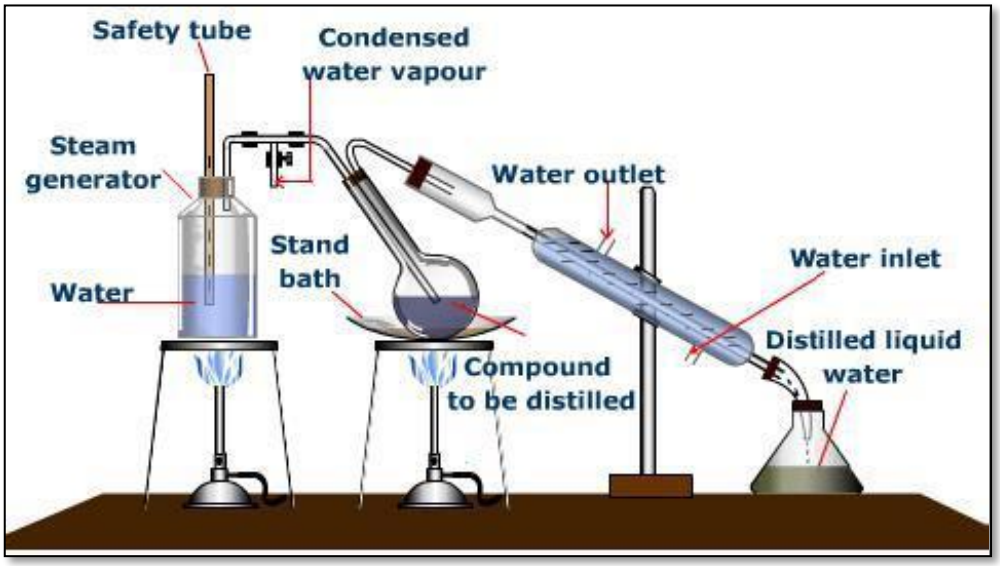


Figure 9. The schematic subsidize apparatus for Steam distillation(Rassem et al., 2016).

7.1.3. Solvent extraction:

Solvent extraction, also known as Liquid–liquid extraction or partitioning, is a method to separate a compound based on the solubility of its parts. This is done using two liquids that don't mix, for example, water and an organic solvent (Figure 10).(Rydberg, 2004).

In the Solvent Extraction method of Essential Oils recovery, an extracting unit is loaded with perforated trays of essential oil plant material and repeatedly washed with the solvent. Solvent extraction is used in the processing of perfumes, vegetable oil, or biodiesel. Solvent extraction is used on delicate plants to produce higher amounts of essential oils at a lower cost. The most frequently applied sample preparation procedure in plant material analysis. The quality and quantity of extracted mixture are determined by the type of extra heat applied because of the method is limited by the compound solubility in the specific solvent used. Although the method is relatively simple and quite efficient, it suffers from such disadvantages as long extraction time, relatively high solvent consumption and often unsatisfactory reproducibility.(Rydberg, 2004).

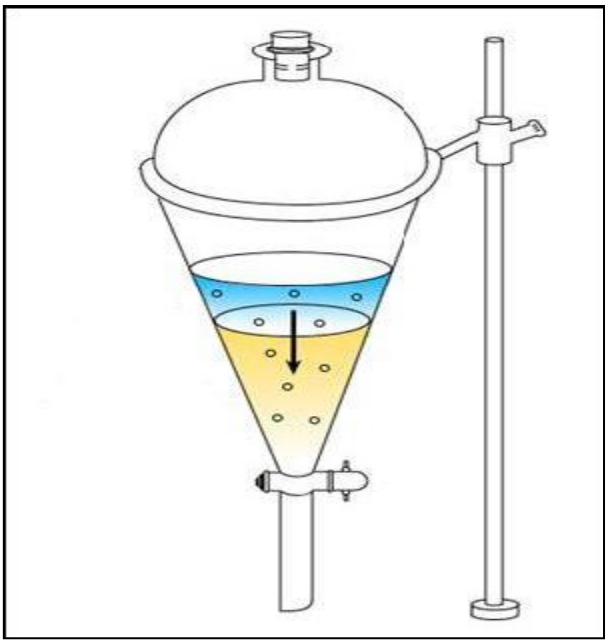


Figure 10. Solvent Extraction (Rassem et al., 2016).

7.1.4. Soxhlet Extraction:

A Soxhlet extractor is a piece of laboratory apparatus invented in 1879 by Franz von Soxhlet. The primary purpose of its invention was to extract a lipid from a solid (Figure 11). When the target molecule has a restricted solubility in a solvent and the impurity is insoluble in that solvent, a Soxhlet extraction is typically utilized.(Gavahian et al., 2019).

It allows for unmonitored and unmanaged operation while efficiently recycling a small amount of solvent to dissolve a larger amount of material. Soxhlet extraction involves solid-liquid contact for the removal of one or several compounds from a solid by dissolution into a refluxing liquid phase. In a conventional soxhlet device, the solid matrix is placed in a cavity that is gradually filled with the extracting liquid phase by condensation of vapors from a distillation flask. When the liquid reaches a preset level, a siphon pulls the contents of the cavity back into the distillation flask, thus carrying the extracted analyses into the bulk liquid.(Gavahian et al., 2019).

Repeat this process until the extraction is almost all completed. Using Soxhlet extraction has various benefits. Reintroducing the sample to new solvent parts on a regular basis is particularly crucial. Analyze extraction from the matrix is improved by this process, which also keeps the solvent from being saturated with extractable material.(Gavahian et al., 2019).

Moreover, the temperature of the system is close to the boiling point of the solvent. This excess energy in the form of heat helps to increase the extraction kinetics of the system. Soxhlet extraction has several disadvantages, including it requires several hours or days to perform; the sample is diluted in large volumes of solvent, and due to the heating of the distillation flask losses due to thermal degradation and volatilization have been observed.(Gavahian et al., 2019).

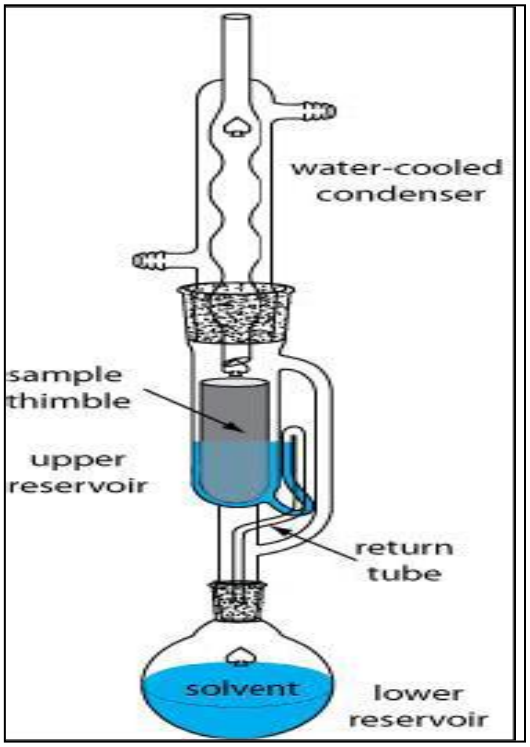


Figure 11. Soxhlet Extraction (Rassem et al., 2016).

7.1.5. Cold Pressing method:

The term cold pressed theoretically means that the oil is expeller pressed at low temperatures and pressure. Cold pressed method is one of the best methods to extract essential oils. This process is used for most carrier oils and many essential oils. This process ensures that the resulting oil is 100% pure and retains all the properties of the plant (Figure 12).(Cakaloglu et al., 2018).

The raw material is batch processed using a mechanical extraction technique that minimizes heat. Scarification is another term for the cold pressed technique. Essential oils from plants, flowers, seeds, lemon, and tangerine oils are mostly extracted using the cold pressed process.(Cakaloglu et al., 2018).

In this process, the outer layer of the plants contains the oil are removed by scrubbing. Then the whole plant is pressed to squeeze the material from the pulp and to release the essential oil from the pouches. The essential oil rises to the surface of the material and is separated from the material by centrifugation.(Rassem et al., 2016; Cakaloglu et al., 2018).

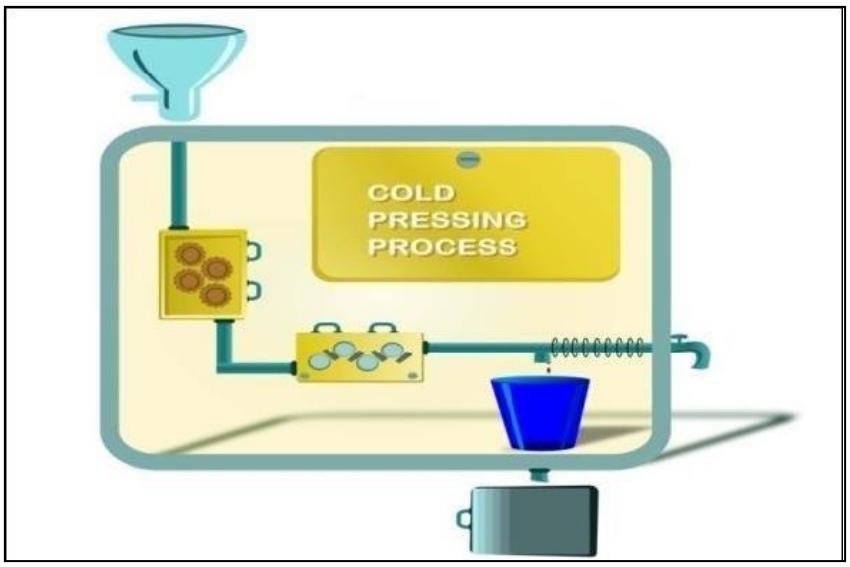


Figure 12. Cold Pressing Method (Rassem et al., 2016).

7.2.Innovative Techniques Of Essential Oils Extraction (Non-Traditional):

Innovative/ non-conventional techniques are "green" in concept. Various innovative extraction methods have been proposed to overcome the limitations of traditional methods. These are less time-consuming, environmentally friendly, improve yields and quality of essential oils with less utilization of solvents and energy, and help avoid toxic chemicals. (Murti et al., 2023).

7.2.1. Supercritical Fluid Extraction:

Supercritical fluid extraction (SFE) is a sophisticated method of isolating one component from a combination utilizing a supercritical fluid as an extracting solvent composed of gases or liquids. Their dissolving power is controlled by temperature and/or pressure. However, it is employed to isolate those chemical classes of volatile oils, which cannot be advisable to extract through the steam distillation method. The schematic representation of SFE system is shown in Figure 13. (Murti et al., 2023).

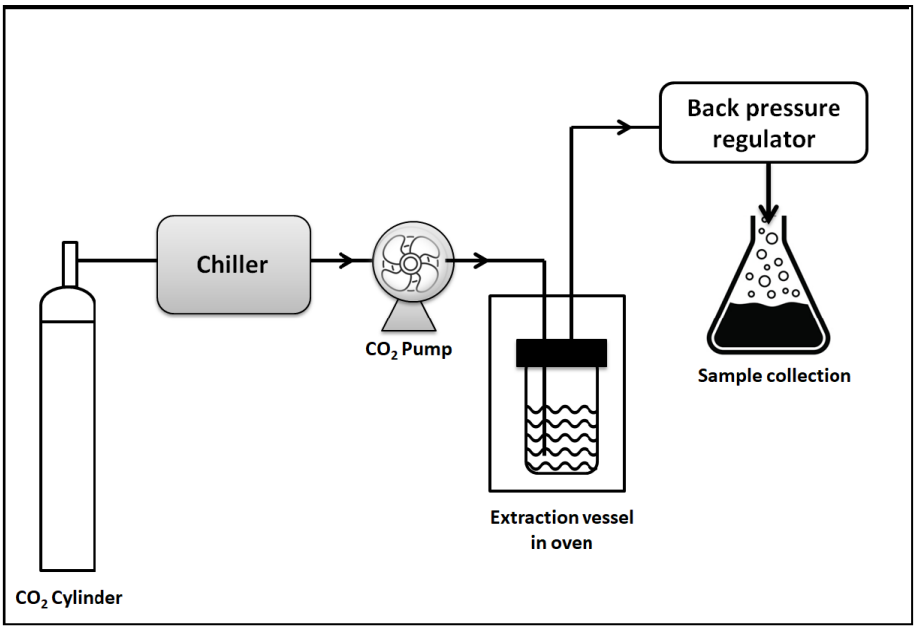


Figure 13. Schematic representation of the supercritical fluid extraction (SFE) system.(Murti et al., 2023).

Carbon dioxide (CO2) is the most widely used solvent due to a number of useful factors, including its low critical pressure (73.8 bar) and temperature (31.1 °C), as well as its nonflammable, noncorrosive, safe, affordable, and very pure availability. It can be readily isolated from plant material using the press release**.** (Murti et al., 2023).

One drawback is that CO2 is nonpolar, so it cannot extract polar analytes alone but can be used as a supercritical fluid with co solvents such as dimethyl ether, ethane, ethylene ethanol, freons, methanol, nitrous oxide, propane, ethylene, etc**.** (Murti et al., 2023).

Benefits of this method include providing a high-quality range with advanced biological and functional properties compared to other products obtained by using the hydro distillation method. This method is environmentally friendly because the nontoxic solvents used in the extraction process leave no harmful residue. The extracting solvent can be quickly recovered from the extract because of its high volatile nature**.** (Murti et al., 2023).

High boiling point components are easily removed at low temperatures, which make them appropriate for thermolabile components. The main drawback of this approach is its system's complexity, which drives up equipment costs**.** (Murti et al., 2023).

7.2.2. Subcritical Liquid Extraction :

SLE takes place when liquid reaches a pressure greater than Pc (critical pressure) but less than Tc (critical temperature). The schematic representation of the SLE system is shown in Figure 14**.** (Murti et al., 2023; Diaz-Reinoso et al., 2023).

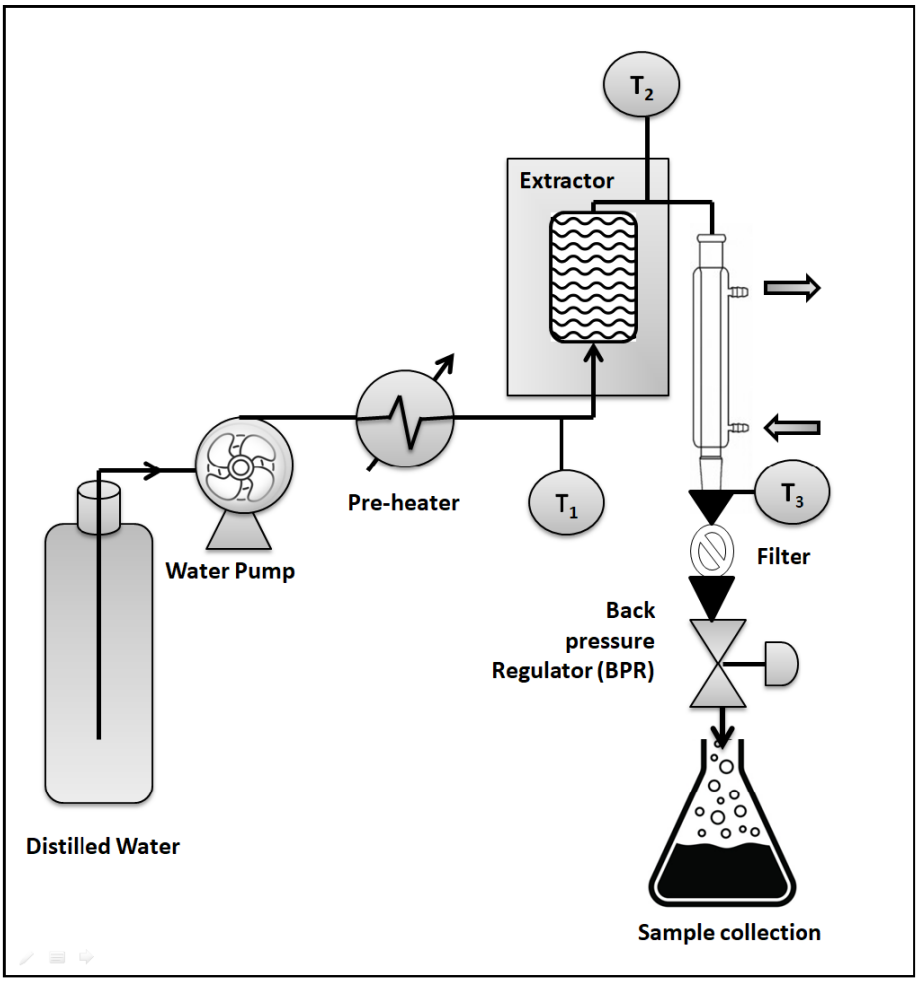


Figure 14. Schematic representation of the subcritical liquid extraction (SLE) system. (Murti et al., 2023).

The solvents used to extract essential oils in this process are H2O and CO2. The fluid's subcritical condition has several advantages: decreased density, lower viscosity, and improved gas-liquid diffusivity. This extraction process is thought to be the finest substitute approach for thermolabile components because it is carried out at a low working temperature. This method takes 15 minutes to finish as opposed to other conventional methods. (Diaz-Reinoso et al., 2023).

7.2.3. Microwave-Assisted Hydro distillation :

This microwave-assisted hydro distillation (MWHD) technique is used to heat the solvent in place of regular electric heating. This approach works by changing the polarity of water and then heating it with microwaves. Electromagnetic energy is converted into heat energy utilizing microwave energy directly generated through molecular interaction between aromatic plants (materials) and the electromagnetic field(Moradi et al., 2018).

These waves may cause some structural alterations within the plant cells. The schematic representation of the MWHD system is shown in Figure 15. Heat and mass transfer occur in the same direction, i.e., from the inner cells to the outside. The factors affecting the efficiency of this technique include time, temperature, physicochemical properties of the extracted compounds, dielectric properties of the sample mixture, and solvent type**.**

This technique is applied in industry and, given the right circumstances, offers a very flexible instrument that can be employed on a variety of plant materials. This technique has quick extraction times while using less solvent. Because it releases less CO2 into the atmosphere, it is an environmentally benign process. This technique protects against thermolabile substances, and its effectiveness is primarily dependent on the dielectric constants of water and plant material, respectively.(Murti et al., 2023; Moradi et al., 2018).

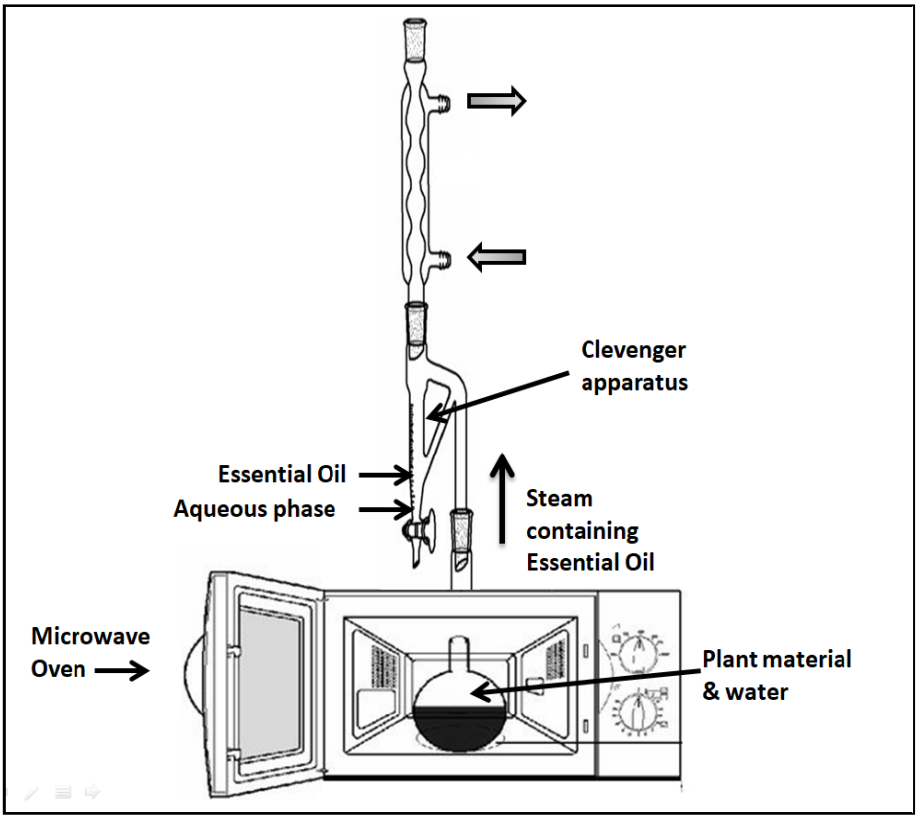


Figure 15. Schematic representation of the microwave-assisted hydro distillation (MWHD) system(Murti et al., 2023).

7.2.4. Ultrasound-Assisted Extraction :

The UAE method permits highly selective and escalation of volatile oils to get separated from plant material. The principle behind this method is that it develops cavitations of some tiny bubbles within the solvent system due to the passage flow of ultrasound waves which usually allow a more significant percentage of a solvent system within the plant material that enhances the surface area(Jadhav et al., 2023).

Plant raw materials are immersed in water or another solvent (such as methanol or ethanol) and subjected to ultrasound (Figure 16), this technique involves extracting essential oil components from leaves, seeds, and flowers**.** A number of factors, including ultrasonic frequency, ultrasonic treatment length, immersion time, extraction temperature, ultrasonic duty cycle, characteristics, and plant material size, have a major impact on the quality and yield percentage of essential oils (Jadhav et al., 2023).

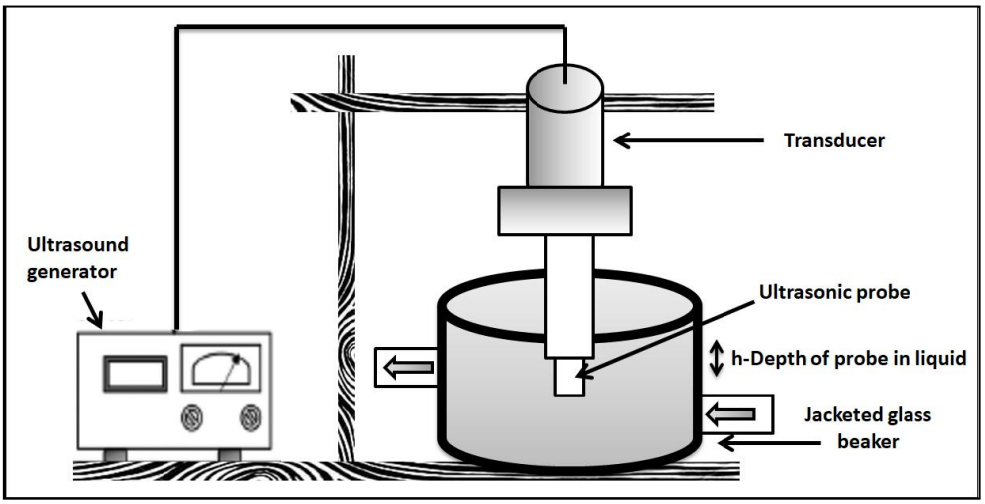


Figure 16. Schematic representation of the ultrasound-assisted extraction (UAE) system(Murti et al., 2023).

7.2.5. Solvent-Free Microwave Extraction :

This procedure combines the utilization of microwave heating energy with dry distillation. This technique uses the moisture in the plant material as a solvent (Filly et al., 2014).

The herbal ingredients are soaked with water for around two hours prior to the extraction process. The materials that have been moistened are microwaved, and the essential oils are collected using a condenser. When comparing microwave heating to traditional methods, the pressure and thermal stress created within the plant tissues being treated may result in their disruption more quickly. The instrument's panel, which was embedded within, managed the radiation power, pressure, and temperature (Figure 17)(Filly et al., 2014).

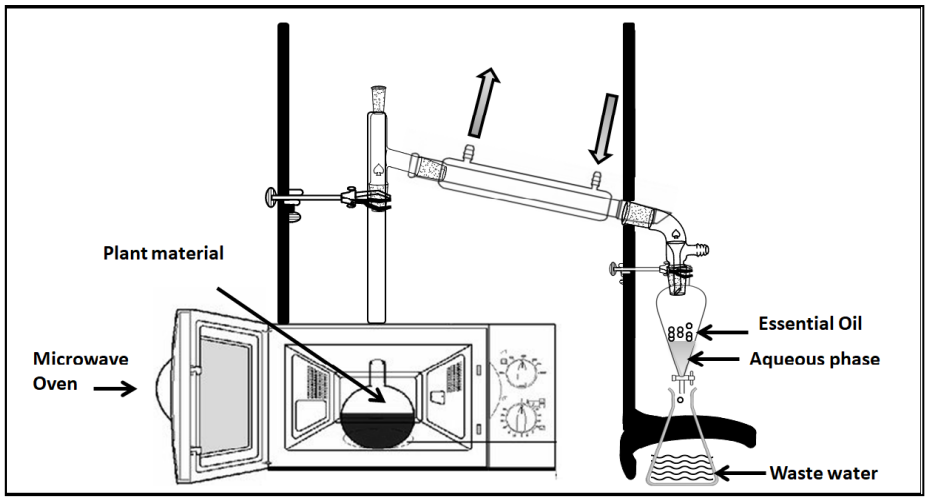


Figure 17. Schematic representation of the solvent-free microwave extraction (SFME) system(Murti et al., 2023).

7.2.6. Microwave Hydro-diffusion and Gravity:

Microwave hydro-diffusion and gravity (MHG) is one of the novel green methods of extracting volatile oils. This technology utilizes microwaves and earth gravity to harvest and extract volatile oils that hydro diffuse from the inner cell areas to the exterior of the plant material. The schematic representation of the MHG extraction system is shown in (Figure 18). It is typically carried out at atmospheric pressure with no solvent added. It was designed for small-scale experimentation and processing (Asofiei et al., 2017).

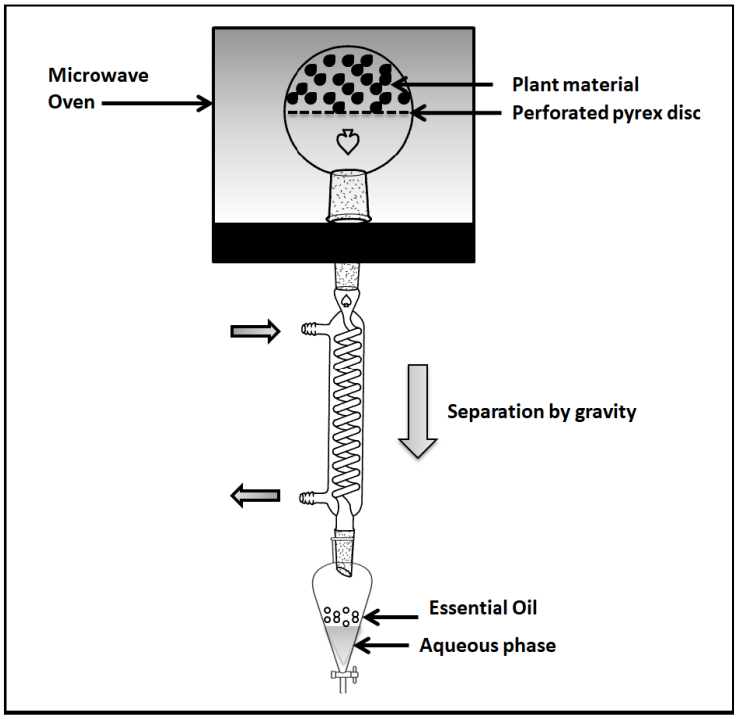


Figure 18. Schematic representation of the microwave hydro-diffusion and gravity (MHG) extraction system (Murti et al., 2023).

7.2.7. Ohmic Heated Water Distillation :

Ohmic heated water distillation (OHWD) is a revolutionary process for isolating essential oils that use ohmic or Joules' heating, and it requires less power (per mL). Controlling treatment homogeneity necessitates the most accurate modeling inputs. The schematic representation of the OHWD extraction system is shown in (Figure 19), The heating rate is proportional to the square of the electric field strength and the conductivity of the medium.(Gavahian et al., 2015).

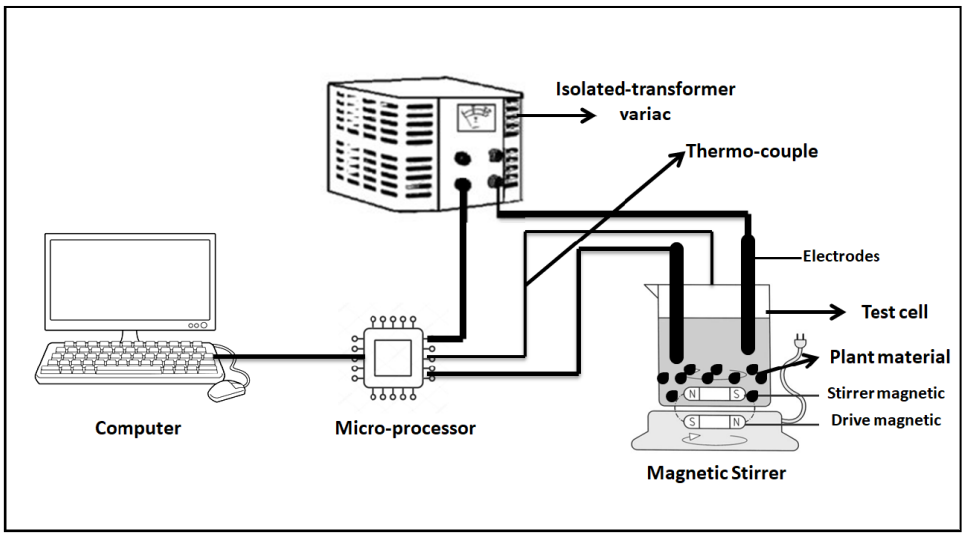


Figure 19. Schematic representation of the Ohmic heated water distillation (OHWD) extraction system (Murti et al., 2023).

7.2.8. Solar Distillation :

Through cost savings and a decrease in environmental pollutants, solar energy benefits the agricultural industry. Using renewable energy sources like sunlight, new technology has been created to increase the efficiency of the distillation process. About the same amount of heat energy is needed using this process per unit weight of plant material. In solar distillation, many components such as an oil separator, distillation still, condenser, focus concentrator, and Scheffler fixed steam receiver are utilized (Figure 20)(Hussain et al., 2022).

The amount of energy available for the distillation process is determined by the sun intensity and the solar distillery's thermal and optical efficiency. It is a low-cost method for extracting essential oils from medicinal plants(Hussain et al., 2022).

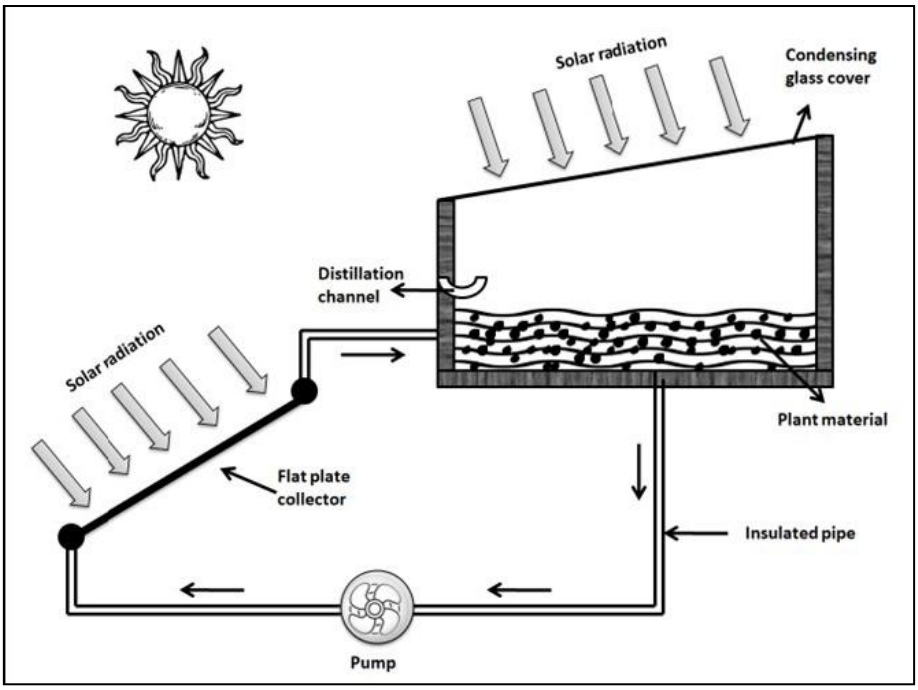
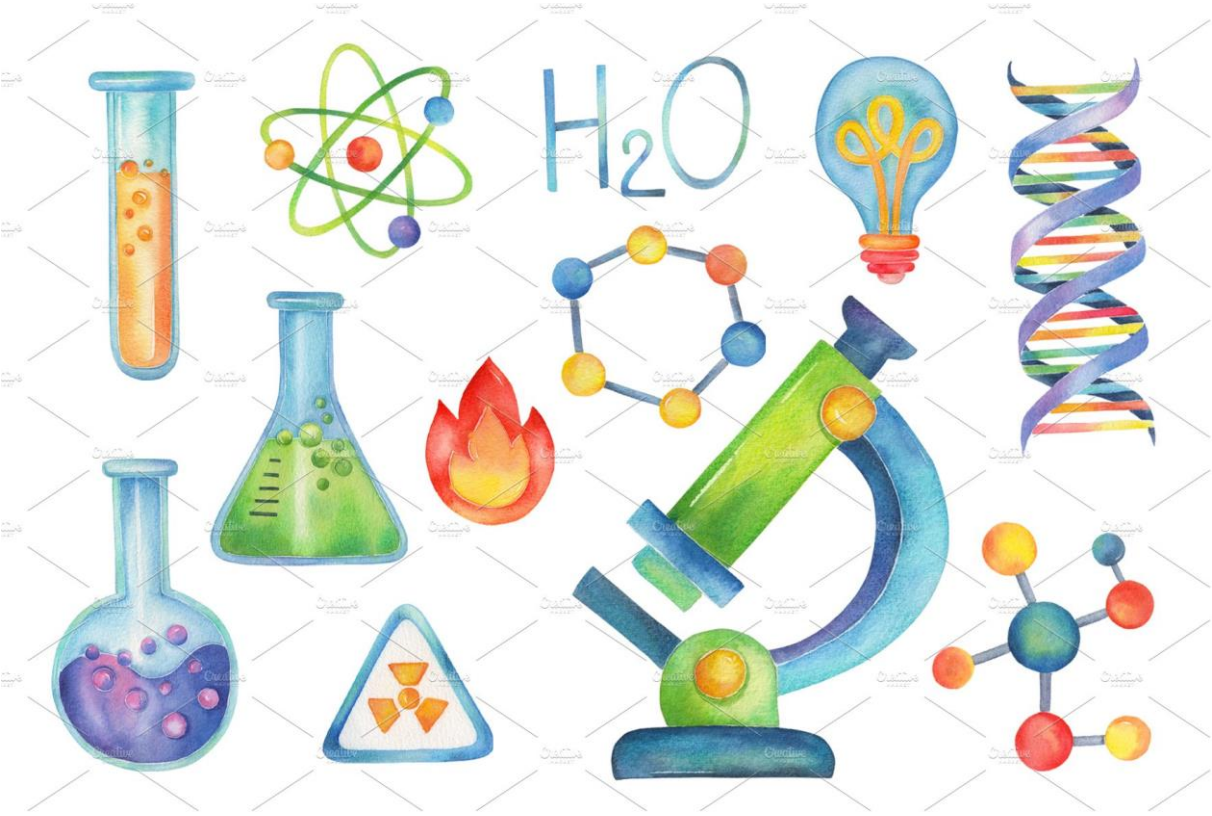


Figure 20. Schematic representaiton of the solar distillation (SD) extraction system (Murti et al., 2023).

SECOND PART:

EXPERIMENTAL

CHAPTER ONE: MATERIALS AND METHODS



1.Introduction:

This study was conducted collaboratively between two specialized laboratories: the Valorisation and Technology of the Saharian Resources Laboratory (VTRS) at the Faculty of Exact Sciences, Department of Chemistry, University of El Oued, and the Centre de Recherche Scientifique et Technique en Analyses Physico-Chimique (CRAPC) in Ouargla, Algeria. The VTRS lab facilitated essential oil extraction, conducted *in vivo* and *in vitro* assays, and also carried out *in-silico* assays. Meanwhile, CRAPC handled the precise GC/MS analysis of the extracted oils. This collaborative effort ensured thorough and accurate experimentation, combining expertise and resources from both institutions.

2.Plant Material:

2.1. *Ammudaucus leucotrichus:*

The plant *Ammudoucus leucotrichus* was harvested at different time intervals during the month of June 2023 in a desertic forest region located in southeastern Algeria, specifically in the province of El Oued. The area exhibits the following specifications:

- Geographic coordinates: 33 degrees north, 6 degrees east.
- Astronomical location: Latitude 32 degrees north.
- Altitude above sea level: 82 meters.
- Distance from sea level: 230 kilometers.
- Bioclimatic character: Desertic.

2.2. *Mentha piperita:*

During various time intervals in May 2023, the peppermint plant (*Mentha piperita*) was harvested in a desertic woodland area located in the southeastern region of Algeria. Specifically, in Elbayadahcity, which falls under the jurisdiction of the El Oued province. The region possesses the following specifications:

- Geographical coordinates: 33°N, 6°E.
- Astronomical location: Latitude 33°N.
- Elevation above sea level: 84 meters.
- Distance from sea level: 310 kilometers.
- Bioclimatic type: Desertic.

3.Chemicals and reagents:

Alpha Amylase:

A solution of alpha-amylase (commercially marketed as Megamylase with a specific activity of 3000 (U. CEIP/cp) at a concentration of 0.5 mg/ml) was prepared in a phosphate saline buffer (pH = 6.4).

Acarbose:

Different concentrations of acarbose solution (commercially marketed as Acorbay 50 mg) were prepared in a phosphate saline buffer (pH = 6.4).

Substrate:

As a substrate, soluble starch was utilized. 250 mg of starch was dissolved in 25 ml of distilled water at a temperature of 50 to 70°C under agitation.

Lugol:

The Lugol solution (KI/I_2; Sigma-Aldrich Chemical) was obtained commercially from Sigma-Aldrich and utilized as a staining reagent in the study. Stored at room temperature in a dark container.

Phosphate Buffer Solution:

The phosphate buffer saline solution was meticulously prepared using sodium dihydrogen phosphate and disodium hydrogen phosphate (Sigma Aldrich) in conjunction with double-distilled water and KCl. The pH was meticulously maintained at 6.4 through the utilization of this phosphate buffer.

4.Materials and Methods:

4.1. Essential Oils Extraction:

4.1.1. Apparatus:

The essential oil extraction process required the use of specialized equipment to ensure accuracy and efficiency. In this study, the following apparatus was utilized:

- ✓ Adventurer – Pro AV53 sensitive balance
- ✓ Heating flask
- ✓ Refrigerant
- ✓ Clevenger apparatus
- ✓ Separating funnel

- ✓ Rotary evaporator

The Clevenger apparatus, a key component in the extraction process, is depicted in Figure 21

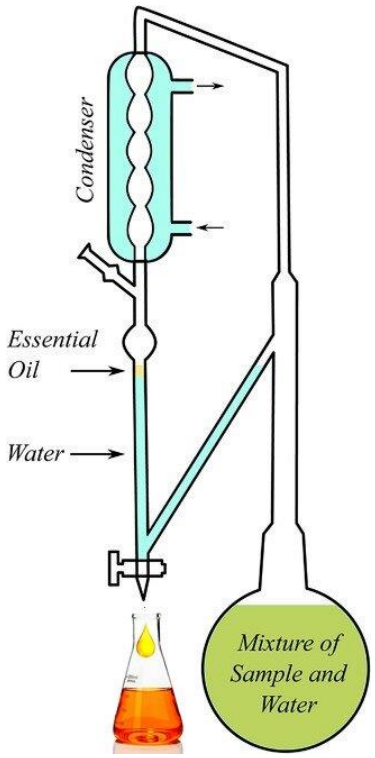


Figure21. Schematic representation of the Clevenger apparatus used in the essential oil extraction process(Biswa et al., 2023)

4.1.2. Procedure:

The procedure for essential oil extraction involved a series of meticulous steps to ensure optimal results. The extraction process was conducted as follows:

- ✓ Cleaning of the plant sample to remove any impurities.
- ✓ Weighing the plant sample to a quantity of 100 g using a sensitive balance.
- ✓ Washing the plant sample with water to prevent burning, followed by placement in a heating flask.
- ✓ Addition of small pieces of boiling regulator to the flask.
- ✓ Direct heating of the mixture at a temperature of 100 degrees Celsius for three and a half hours.
- ✓ Conducting the steam distillation process and subsequent separation of oil and water phases using liquid-liquid separation with diethyl ether.
- ✓ Drying of the organic oily phase with anhydrous sodium sulphate.
- ✓ Filtration of the dried oil phase through filter paper to remove diethyl ether particles.
- ✓ Evaporation of the filtered oil phase using a rotary evaporator to remove any remaining organic solvent.

- ✓ Storage of the obtained essential oil in small brown bottlesand refrigeration at a temperature of 5°C.

4.2. Yield of Essential Oil Extraction:

Two distinct methodologies are commonly employed to ascertain the yield of essential oil extraction, each offering unique perspectives on the efficiency of the process.

4.2.1. Volumetric Yield - Mass-based: (Naima et al., 2019)

This method entails an assessment of the mass of the utilized plant material intended for essential oil extraction, juxtaposed against the volume of the resultant oil. The yield is then computed utilizing the following Equation 1:

$$R_{EO} = \frac{V_{EO}(ml)}{m_0(g)} \times 100 \quad (1)$$

Where:R_{EO}: Essential oil yield, m_0: Mass of the utilized plant sample, V_{EO}: Volume of the extracted essential oil

4.2.2. Mass-based Yield - Mass-based: (Larbi et al., 2018)

Alternatively, the essential oil extraction yield is defined as the quotient of the mass of the extracted essential oil and the mass of the plant material utilized. This yield is calculated using Equation 2:

$$R_{EO} = \frac{m_{EO}(g)}{m_0(g)} \times 100 \quad (2)$$

Where:R_{EO}: Essential oil yield, m_0: Mass of the utilized plant sample, m_{EO}: Mass of the extracted essential oil

These methodologies have been systematically applied to derive essential oil yields for all examined plant specimens.

4.3. Characterization of Essential Oils:

The characterization of essential oils comprises two fundamental components: physicochemical properties and Gas Chromatography-Mass Spectrometry (GC/MS) analysis.

4.3.1. Physicochemical Properties:

Here, we delve into the fundamental traits of essential oils, including their relative density, acidity, ester content, and refractive index. These properties offer valuable insights into the composition and behavior of essential oils, helping us understand their chemical makeup and how they interact with their environment (Atti-Santos et al., 2005).

- *Relative Density (AFNOR NF T75-111 Standard):*

At 20°C, 1 mL of the essential oil is measured using a pipette, and its mass is then determined. The procedure is repeated for distilled water, and density is calculated using the following Equation 3(Valarezo et al., 2015):

$$d = \frac{m_{EO}}{m_{H_2O}} \quad (3)$$

Where: m_{EO}: Mass of the extracted essential oil, m_{H_2O}: Mass of the distilled water

- *AcidityIndex (AFNOR NF T75-111 Standard):*

To determine the acid value, representing the concentration of free acids in 1 g of the essential oil, a titration method with potassium hydroxide (KOH) solution is employed(Sahoo et al., 2007).

Initially, a small aliquot (0.5 mL) of the essential oil is mixed with 2 to 3 drops of phenolphthalein indicator in a small vessel. Subsequently, titration is performed with 0.5 N KOH solution until the appearance of a faint pink colour, indicating complete neutralization of the acids. The acid value is then calculated using the following Equation 4.

$$I_a = \frac{56.11 \times V \times C}{m} \quad (4)$$

Where: V: the volume of the KOH solution, C: Concentration of KOH, m: the mass of the essential oil

- *Ester Index (AFNOR NF T75-111 Standard):*

The determination of free acids resulting from ester hydrolysis within the essential oil involves a titration process utilizing 0.5 N potassium hydroxide (KOH) solution(Alajtal et al., 2018).

- Begin by placing 0.5 mL of the essential oil into a small vessel.
- Add 1 mL of 0.5 N potassium hydroxide (KOH) solution to the vessel to initiate the titration process.
- Place the mixture in a gas-evacuated water bath for a specified duration to facilitate reaction.
- After cooling, introduce 0.5 mL of distilled water and add 3 drops of phenolphthalein indicator to the mixture.
- Titrate the excess potassium hydroxide (KOH) using 0.5 N hydrochloric acid (HCl) until a colour change is observed, indicating neutralization.

- Quantify the volume of hydrochloric acid (HCl) required to neutralize the excess potassium hydroxide (KOH).
- Calculate the ester content using the following Equation 5:

$$I_e = \frac{2805}{m}(V_0 - V_1) - I_a \quad (5)$$

Where: V_0 (ml): Volume of hydrochloric acid (HCl) without essential oil, V_1 (ml): Volume of hydrochloric acid (HCl) in the presence of essential oil, I_a: Acid value, I_e: Ester value and m: Mass of the essential oil sample.

- *Refractive Index (AFNOR NF T75-111 Standard):*

The refractive index of an essential oil is defined as the ratio between the sine of the angle of incidence and the sine of the angle of refraction of a light ray, with a specific wavelength, transitioning from air into the essential oil, while the latter is maintained at a constant temperature (Singh, 2002)

The refractive index of the essential oil is directly measured using a refractometer at a reference temperature 20°C.

4.3.2. Gas Chromatography-Mass Spectrometry (GC/MS) analysis:

The coupling (GC/MS) technique stands as the most frequently employed method within the field of essential oils, facilitating the concurrent separation, identification, and quantitative measurement of the various constituents present in extracted oils.

- *Principle:*

The principle is founded on the varying affinities of compounds within the mixture towards two phases: a stationary phase and a mobile phase. This technique relies on the distribution of constituents between a stationary phase and a gas phase. The stationary phase comprises a silicone-based liquid that permeates an inert and granular solid material, housed within a typically coiled steel or glass column measuring 1 to 3 meters in length and 2 to 4 millimetres in diameter. The mobile phase consists of an inert carrier gas such as nitrogen, helium, or argon.

The column is maintained at a high temperature via a furnace. Under the influence of temperature, constituents vaporize and become separable. The basis of separation lies in the discrepancy of partition coefficients of volatile compounds between the stationery and gas phases. A detection system generates a signal at the exit of each molecule from the column, manifesting as the recording of peaks corresponding to each constituent.

Gas chromatography is coupled with a mass spectrometer (MS); this coupling relies on computerized comparison of the spectrum of an unknown peak with one or more reference libraries, enabling its identification.

- *Apparatus:*

The identification of the chemical constituents of our essential oils was performed using a gas chromatographic system (HP 5890-SERIE II) equipped with an HP5 MS capillary column (30 meters in length, 0.25 mm internal diameter, and 0.25 µm film thickness) coupled with a mass spectrometer (HP-MSD 5972).

N_2 was employed as the carrier gas for the analysis of the two essential oils. Spectra were recorded at an emission energy of 70 eV, and spectral analysis of the compounds was conducted by comparison with their counterparts using the WILEY275 (Chiu et al., 1982; Guinaudeau et al., 1975; Kiryakov, 1968; Shamma, 1972)spectral libraries.

- *Procedure:*

The carrier gas (N_2) is introduced at a flow rate of 1 mL/min, with the injected volume of the essential oil being 1 µL in split injection. The injector and detector temperatures are set at 250°C and 320°C, respectively. The oven temperature is programmed to initially reach 60°C and held for 8 minutes, then gradually increased to 250°C at a rate of 2°C/min, maintained isothermally at 250°C for 15 minutes, and finally elevated to 300°C at a rate of 10°C/min.

4.4. *In vivo* antidiabetic activities:

4.4.1. Animals Care:

The experimental cohort comprised thirty-five (35) Wistar Albino rats, initially weighing between 100 and 150 grams and of uniform age, with favourable physiological status. Procured from the Institute Pasteur in Algiers, these animals were domiciled in a dedicated animal facility within the Faculty of Natural Sciences and Life at the University of Eloued, where they were subjected to standardized environmental conditions: a temperature regimen of 25 ± 2 °C and a light-dark cycle of 12 hours/24 hours. Housing arrangements involved plastic cages, accommodating five rats each and furnished with sawdust substrates, replenished biweekly throughout the experimental duration. Regular assessments of body weight and blood glucose levels were conducted weekly to monitor the animals' physiological parameters.

4.4.2. Induction of Experimental Diabetes in Rats:

Diabetes induction in the rat model was achieved through intraperitoneal Alloxan administration in conjunction with a freshly prepared solution of Alloxan monohydrate

(Sigma) at a dosage of 150 mg/kg body weight, following an overnight fasting period. Alloxan was dissolved in a physiological saline solution (NaCl 0.9%). Conversely, healthy control rats received an equivalent volume of intraperitoneal buffer (NaCl 0.9%). Subsequently, to mitigate the risk of hypoglycaemia induced by Alloxan, the experimental rats were provided with glucose-enriched water, initially comprising a 20% glucose solution on the first day, followed by a 5% glucose solution on the subsequent two days. This precautionary measure was essential to avert potential fatality resulting from Alloxan-induced hypoglycaemia in rats. Diabetes status confirmation was attained 72 hours post-injection through blood glucose measurement using a glucometer, with only rats exhibiting blood glucose levels exceeding 14 mmol/l deemed diabetic and thus included in the experimental cohort.

4.4.3. Experimental Design:

Following diabetes induction, the experimental groups were maintained under identical conditions. Subsequently, the animals were randomly allocated seven groups, each comprising five rats: Group 01 (control), consisting of rats fed a standard diet; Group 2(Diabetic group), comprising diabetic rats fed a standard diet; Group 3 (Diabetic + Acarbose),comprising diabetic rats administered Acarbose alongside a standard diet,Group 4 (Diabetic + EOMP), consisting of diabetic rats administered 10 µL of essential oil from plant *Mentha Piperita* alongside a standard diet; Group 5 (Diabetic + EOAI), comprising diabetic rats administered 10 µL of essential oil from plant *Ammodaucus leucotrichus* alongside a standard diet;Treatment initiation commenced 72 hours post-diabetes induction and persisted for a total duration of 15 days. and Group 6 (EOMP),we gave the essentialoil from plant *Mentha Piperita* to the rats without causing diabetes, which means the rats were healthy and not infected, Finally Group 7(EOAI), alsowe gave the essentialoil from plant *Ammodaucus leucotrichus*to the mice without causing diabetes.

4.4.4. Sacrifice and Collecting Blood and Organs:

At the conclusion of the treatment period, the animals underwent a fasting period of 16 hours before being anesthetized with 94% chloroform inhalation and subsequently euthanized by decapitation. Blood glucose levels were measured during the sacrifice process. Blood samples were collected into EDTA tubes for hematological analysis and into tubes without anticoagulants for biochemical analysis. The latter samples were centrifuged at 3000 rpm for 10 minutes to obtain serum, which was then stored at -4 °C for subsequent biochemical

analysis, including assessments of glycemia, urea, creatinine, triglycerides, uric acid, TGO, and TGP levels.

Following euthanasia, the liver, pancreas, and kidneys were meticulously dissected, and adipose tissue was carefully stripped from these organs. The isolated organs were then weighed and rinsed in a 0.9% NaCl solution. Subsequently, organ specimens were prepared for the detection of oxidative stress factors, including Malondialdehyde (MDA) and Reduced Glutathione (GSH). Notably, Pancreatic sectors, liver sectors, and also kidney sectors from each rat within each experimental cohort were immersed in a formalin solution to facilitate tissue preservation and subsequent histological analysis.

4.5. *In Vitro* alpha-amylase inhibition assay:

Inhibition of α-amylase activity was carried out as described by E. Deveci et al (Deveci et al., 2020), with nuanced adjustments. Briefly, A starch solution (1% w/v) was prepared by stirring 1g starch in 100 ml of 20 mM of phosphate buffer (pH 6.9) containing 6.7mM of sodium chloride. The enzyme solution was prepared by mixing 50 mg of α-amylase in 100 ml of 20 mM of phosphate buffer (pH 6.9 with potassium chloride) to 200 µl of appropriate dilutions (1 - 20µg/mL) of each essential oil ,500 µl of alpha amylase solution was added and the mixture was incubated at 37 ºc for 10 min. To the reaction mixture 100 µl (1%) starch solution was added and incubated at 37ºC for 10 min. The reaction was stopped by addition of 25 µL HCl (0.1 M) and 100 µL Lugol solutions and kept it in a boiling water bath for 5 minutes and cooled to room temperature. The reaction mixture was then diluted to 2 ml with distilled water, and absorbance measured at 545 nm with UV–Vis spectrometer, (Shimadzu 1800, Japan) using a cell of 1 ml with an optical path length of 1 mm. For each concentration, blank tubes were prepared by replacing the enzyme solution with 500 µL in distilled water. The experiment was repeated using acarbose as a standard antidiabetic drug (positive control). The experiments were repeated thrice using the same protocol. The alpha-amylase inhibitory activity was expressed as percentage inhibition. The percentage inhibition was calculated using the following Equation 6:

$$Percentage\ inhibition\ (\%) = \left(1 - \frac{A}{B}\right) \times 100 \quad (6)$$

Where, A represents the absorbance of negative control (enzyme activity in the absence of the standard inhibitor or extract). B is the absorbance of test assay (enzyme activity in the presence of only the extract or only the standard inhibitor).

4.6. *In-Silico* analysis:

4.6.1. Software

Computational simulations, including Induced Fit Docking, molecular dynamics studies, and MM-GBSA calculations, were conducted employing the Glide module, Induced Fit Docking module, Prime module, and the Desmond module within the Maestro version 11.7 user interface of the Schrödinger suite (Small-Molecule Drug Discovery Suite 2021-4, Schrödinger, LLC, New York, NY, 2021) (Schrödinger, 2015). The simulations were executed on a DELL Intel(R) Core(TM) i9-13900HX CPU @ 2.20 GHz processor, equipped with 32.0 GB RAM, and operated on a 64-bit Linux Ubuntu 18,04.1 LTS operating system.

4.6.2. ADMET and drug-likeness evaluation:

Drug candidates should possess favourable ADMET properties and ideally non-toxic. Therefore, the major identified compounds from the essential oils extract were evaluated of their ADME profile, including physicochemical, lipophilicity, water solubility, pharmacokinetics, drug-like nature, medicinal chemistry, and several other parameters using SwissADME (Daina et al., 2017; Riyadi et al., 2021) module provided in SIB (Swiss Institute of Bioinformatics) webserver (https://www.sib.swiss). Furthermore, the toxicity aspect of designed compound was also predicted using ProTox (Banerjee et al., 2018)webserver (https://comptox.charite.de/protox3/).

4.6.3. Docking setup:

- *Ligands preparation:*

The three-dimensional configurations of the major compounds isolated from *Cotula cinerea* and *Origanum Majorana L* essential oilswere obtained from the National Library of Medicine (NCBI) database (Kim et al., 2016), accessible through the NCBI website (https://pubchem.ncbi.nlm.nih.gov/).

Ligand preparation involved an energy optimization process to derive the most energetically favourable conformations for each compound. Utilizing LigPrep module within the Schrödinger suite(Schrödinger, 2024), this optimization procedure ensured the attainment of the lowest energy state for the studied drugs, including Montbretin A (a co-crystallized ligand). The ionization states were established at a pH of 7.0 ± 2.00, as computed by the Epik classic module, while maintaining specified chirality and generating relevant tautomeric forms. Furthermore, partial atomic charges were computed using Optimized Potentials for Liquid Simulations OPLS4 force field(Lu et al., 2021).

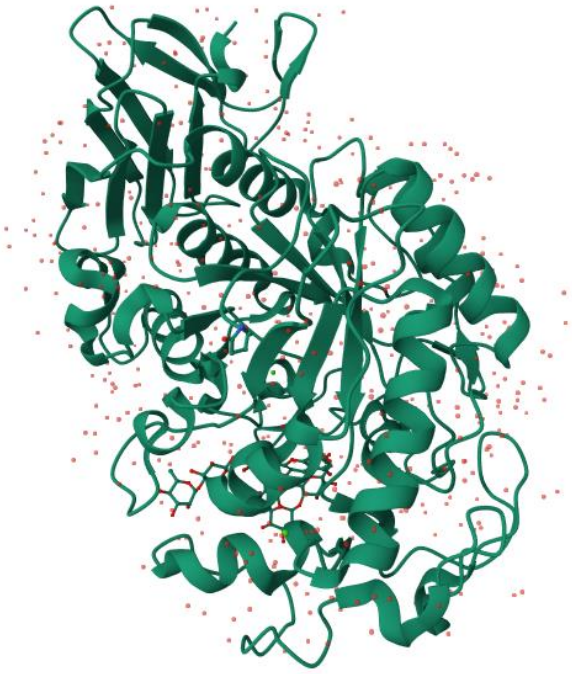
- *Receptor Preparation:*

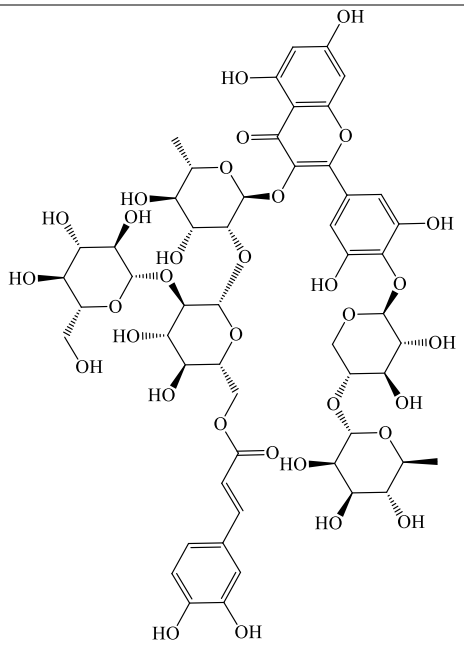
The crystallographic data for Human pancreatic alpha-amylase (Table 4) (PDB ID: 4W93) (Williams et al., 2015) was retrieved from the Protein Data Bank (http://www.rcsb.org) (Rose et al., 2017), adhering to specific parameters such as a resolution of 1.35Å and R value-free of 0.211. Processing of the protein structure was executed through the "protein preparation Workflow" module within the Schrödinger suite (Madhavi Sastry et al., 2013), involving consecutive stages of import and processing, review and modification, and refinement.

In the initial stage, the Prime tool was employed to address missing residues and side chains, maintaining the pH of PROPKA at 7.0 ± 2.00. Subsequent steps included the optimization and assignment of hydrogen bonds, along with the removal of water molecules beyond 8 Å. Restrain minimization utilizing the Optimized Potentials for Liquid Simulations (OPLS4) force field was performed to achieve a low-energy state for the protein (Lu et al., 2021). This phase of protein preparation signifies an energy optimization methodology, presenting the protein in its energetically favourable state for subsequent *in-silico* studies.

The "Receptor grid generation" panel facilitated the creation of a grid encompassing the active site of the protein, delimited by the co-crystallized ligand Montbretin A. Default parameters were maintained, and the grid centre was generated at the coordinates X = -6.65; Y = 6.99; Z = -20.65.

Table 4. Target receptor information chosen for docking studies

Human pancreatic alpha-amylase	**Detaille's**	
	PDB ID	4W93
	Mutation	No
	Resolution (Å)	1.35
	R-Value Free	0.211
	R-Value Work	0.198
	R-Value Observed	0.198
	Organism	Homo sapiens
	Expression System	Komagataella pastoris
	Space Groupe	P 2_1 2_1 2_1
	Sequence Length	496

Co-Crystallized Ligand	Detaille's	
	Name	Montbretin A
	Formula	$C_{53}H_{64}O_{33}$

- *Molecular Docking:*

Computational simulations, including Induced Fit Docking, molecular dynamics studies, and MM-GBSA calculations, were conducted employing the Glide module, Induced Fit Docking module, Prime module, and the Desmond module within the Maestro version 11.7 user interface of the Schrödinger suite (Small-Molecule Drug Discovery Suite 2021-4, Schrödinger, LLC, New York, NY, 2021) (Schrödinger, 2015). The simulations were executed on a DELL Intel(R) Core(TM) i9-13900HX CPU @ 2.20 GHz processor, equipped with 32.0 GB RAM, and operated on a 64-bit Linux Ubuntu 18,04.1 LTS operating system.

The molecular docking tool employed for all docking studies was Glide (Grid-based Ligand docking with Energetics), a module within the Schrödinger suite (Yang et al., 2021). The prepared ligands underwent docking onto the specified protein site utilizing the Glide module, in Standard Precision (SP) modes (Friesner et al., 2006).

- *Induced Fit Docking (IFD):*

The Induced Fit docking (IFD) module of the Maestro molecular modelling suite has been noted as a reliable and effective docking approach to consider flexibility in both ligands and the binding pocket residues in the binding pocket of target receptors(Khelil et al., 2020). During the IFD process, Glide/SP (Standard Precision) was performed for each ligand, the Prime refinement step specifically addressed the side chains of residues within a 5 Å radius of the ligand. Noteworthy is the retention of a maximum of 20 poses for each ligand.

- *Molecular Dynamics Simulation (MDS):*

The best compound in each plant exhibiting the highest IFD docking score was chosen for Molecular Dynamics Simulation (MDS). Recognizing the limitations of ligand docking in representing the biological system under aqueous conditions(Korb et al., 2012), a 100 ns simulation time for MDS was executed using the Desmond module within the Schrödinger suite(Bowers et al., 2006).

The MDS protocol comprised three essential steps: system builder, minimization, and MDS. In the system builder phase, the protein and ligand complex were selected and immersed in a biological environment. The transferable intermolecular potential with 3 points (TIP3P) solvent model, with the boundary condition maintained in an orthorhombic box form throughout the process with dimensions of 10 x 10 x 10 Å(Akbar et al., 2022). The OPLS-3e force field was consistently applied(Roos et al., 2019). The neutralization of model was conducted by addition of counter ions when needed and 0.15 M of NaCl salt was included to mimic the physiological state.

Subsequently, the NPT ensemble was utilized for energy minimization, maintaining pressure and temperature at 1.0132 bar and 300 K, respectively. Finally, MDS was conducted for the minimized protein-ligand complex (Halder et al., 2023).

- *Free Energy (MM-GBSA) Calculation:*

Upon completion of the dynamic simulation, an assessment of the free binding energy between the protein and the ligand was systematically undertaken utilizing the Prime MM-GBSA module within the Maestro molecular modelling suite(E. Wang et al., 2021). The calculation of ligand binding affinities was accomplished through the Molecular Mechanics/Generalized Born Surface Area ΔG (MM-GBSA ΔG) metric applied to the optimized Receptor-Ligand Complex. This computation, facilitated by the VSGB solvation model and the OPLS4 force field, stands as a methodologically rigorous approach for the comprehensive evaluati

on of molecular interactions and binding strengths (Gorla et al., 2021).

CHAPTER TWO: RESULTS AND DISCUSSION

1. Introduction:

This chapter delves into the findings regarding the extraction yield, characterization, and comprehensive exploration of the anti-diabetic properties inherent in two essential oils derived from the aerial constituents of *Ammudoucus leucotrichus* and *Mentha piperita*. The overarching aim of this investigation was to assess the potential anti-diabetic efficacy exhibited by essential oils as significant agents in the domain of novel pharmaceutical development.

The elucidations provided herein furnish intricate insights into the chemical constitution of the essential oils, thereby enabling their utilization in the *in-silico* study. This involved the application of advanced computational techniques such as Induced Fit Docking (IFD) and Molecular Dynamics Simulation (MDS) for each compound, capabilities not feasible in traditional *in vitro* and *in vivo* studies.

2. Extraction Yield:

Figure 22 illustrates the yields of essential oils obtained through hydrodistillation of the aerial parts of *Ammudoucus leucotrichus* and *Mentha piperita*. variety of Eloued.

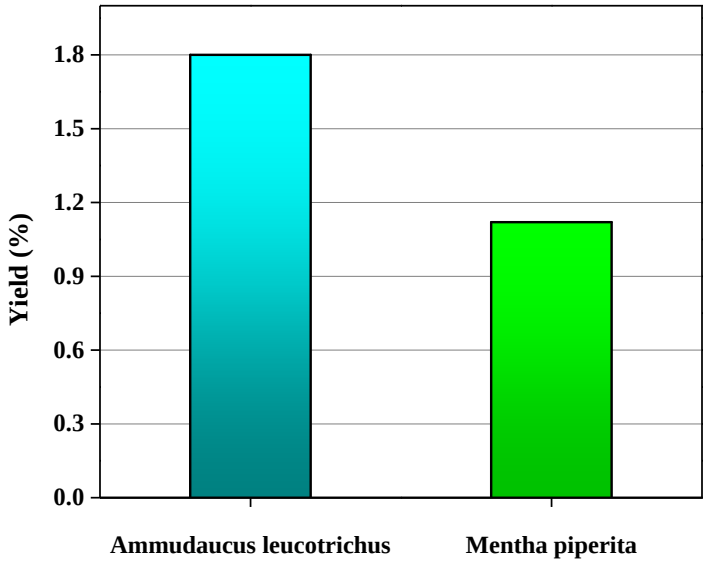


Figure 22. The yields of extracted essential oils

We observe that *Ammudoucus leucotrichus* exhibits a higher essential oil yield than *Mentha piperita* (1.80% and 1.12% respectively). However, the difference between the two yields is non-significant.

Nevertheless, findings reported by (Manssouri et al., 2020) indicate lower yields of essential oils extracted by hydrodistillation from *Ammudoucus leucotrichus*at ambient temperature.

Similar results to ours were, however, obtained by (Naima et al., 2019) for the essential oil extracted from the same variety using the same method.

Furthermore, significantly higher yields were achieved by (Samber et al., 2015) during the hydrodistillation of *Mentha piperita*.

These variations in results can be explained by the fact that essential oil yields are influenced by several factors during extraction: either factors related to the plant (species, variety, chemical composition, etc.) or factors associated with experimental conditions (extraction process, extraction duration, etc).

3. Chemical composition of essential oils:

3.1. Organoleptic characteristics:

Through the conducted work, it has been revealed that the essential oil extracted from the studied plants exhibits the following (Table 5) organoleptic properties:

Table 5. The organoleptic characteristics of essential oils

Plant	Smell	Aspect	Colour
Ammudoucus leucotrichus	A strong smell	liquid at room temperature	Bleu
Mentha piperita	A pleasant fragrance	liquid at room temperature	Light-yellow

3.2. Physicochemical Properties:

The physicochemical properties were meticulously determined according to the standards of the French Association for Standardization (AFNOR) using established methodologies to measure relative density, refractive index, acidity index, and ester index, as depicted in the following Table 6.

Table 6. The physicochemical properties of essential oils

Plant	Relative Density	Refractive Index	Acidity Index	Ester Index
Ammudoucus leucotrichus	0.887	1.4268	4.30	40.91
Mentha piperita	0.933	1.4601	4.46	41.88

By comparing these results to those obtained by (Naima et al., 2019) (density 0.953, refractive index 1.474, acidity index 4.93 and ester index 45.96) and (Mimica-Dukić et al., 2003) (density: 0.834 ± 0.02, 0.835 ± 0.02, refractive index, at 25°C 1.4596 ± 0.03, 1.4622 ± 0.04), and considering that the refractive index was measured at a temperature of 23°C, we

can conclude that these values are in accordance with the standards described by AFNOR (Afnor, 1982).

3.3. Gas Chromatography-Mass Spectrometry (GC/MS) analysis:

The analysis focused on the essential oil constituents extracted from two specific plant species using gas chromatography-mass spectrometry (GC/MS). Mass spectra corresponding to each chromatographic peak were juxtaposed with spectra from relevant scientific literature and the Weily electronic database for mass spectra (Horai et al., 2010). Retention indices were employed to ascertain compound identities. Moreover, utilizing the identical non-polar HP5 stationary phase in the gas chromatography column facilitated the preservation of consistent peak numbers and elution sequences for the compounds under investigation.

Figure 23 and Figure 24 depict the chromatograms illustrating the essential oil compositions of *Ammudoucus leucotrichus* and *Mentha piperita*, respectively, as obtained through gas chromatography-mass spectrometry (GC/MS) analysis:

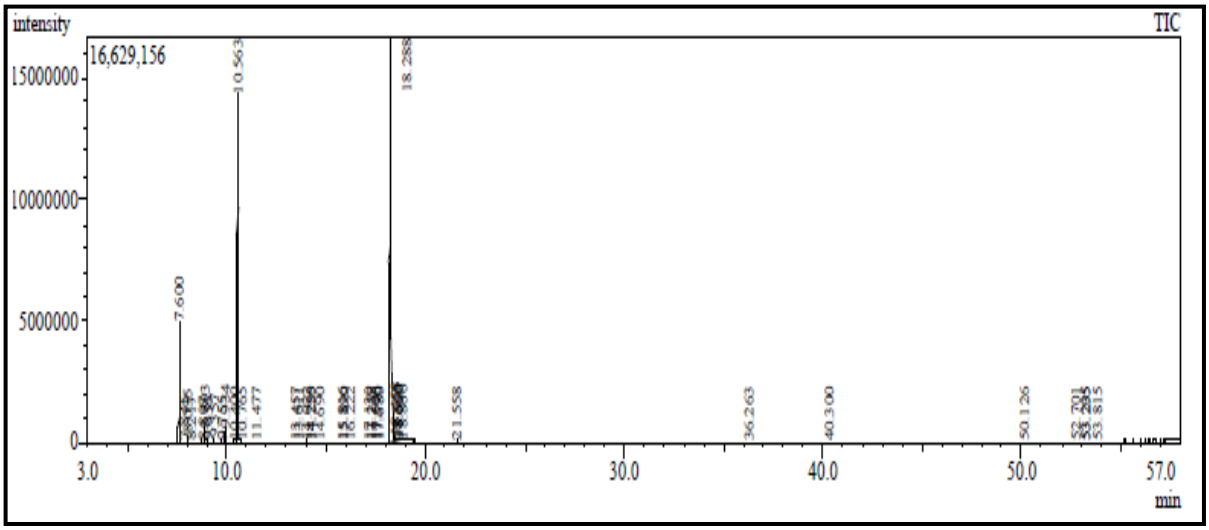


Figure 23. Chromatogram of the essential oil of *Ammudoucus leucotrichus*plant obtained by GC/MS

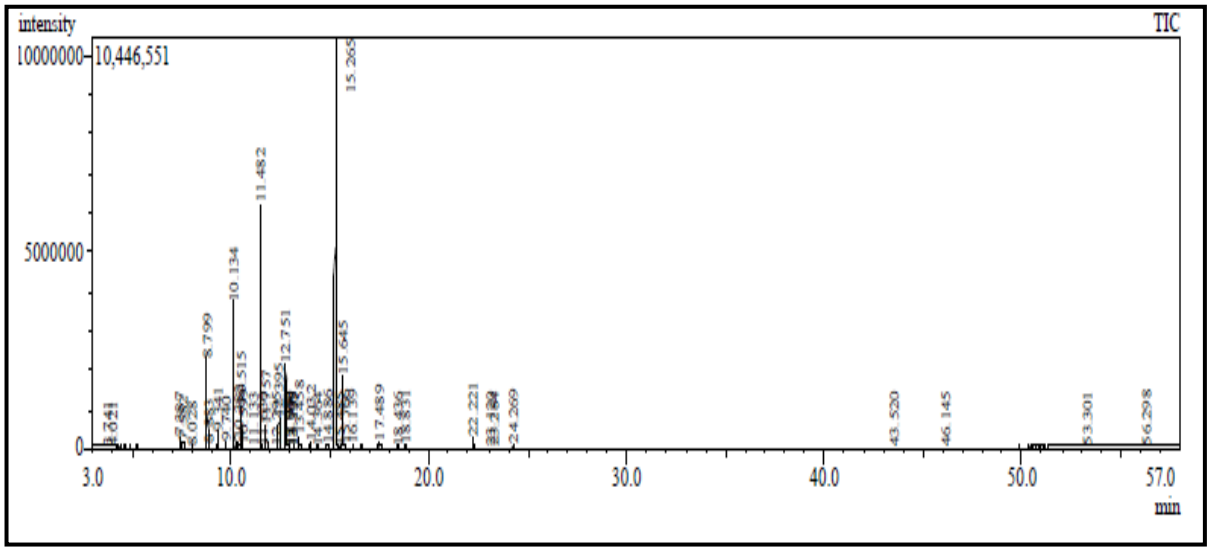


Figure 24. Chromatogram of the essential oil of *Mentha piperita* plant obtained by GC/MS

Upon scrutinizing the two chromatograms, notable resemblances are evident, with discernible bands interspersed with overlapping ones. Generally, distinct regions can be identified, delineating three segments:

1. The initial segment, delimited within the time frame of (4 min to 12.4 min), hosts several bands denoting the presence of hydrocarbon monoterpenes.
2. The succeeding segment, spanning from (12.4 min to 24 min), encompasses the predominant bands, indicative of oxygenated monoterpenes prevalent in the plant's essential oil.
3. The final segment, spanning (24 min to 32.5 min), exhibits minimal banding, suggesting a negligible presence of hydrocarbon sesquiterpenes in the plant's essential oil.

The pertinent compounds in each essential oil, extracted from *Ammudoucus leucotrichus*and *Mentha piperita*, have been identified and collated as follows

3.3.1. *Ammudoucus leucotrichus:*

The hydrodistillation extraction of *Ammudoucus leucotrichus*yielded a Bleu oil with a yield of 1.80%. Gas chromatography-mass spectrometry (GC/MS) analysis identified 24 compounds, collectively constituting 99.91% of the oil's composition (as presented in Table 7).

The aromatic oil derived from *Ammudoucus leucotrichus*comprises 64.8% oxygenated monoterpenes and 34.86% hydrocarbon monoterpenes. Notably, perillaldehyde predominates at 64.66%, succeeded by D-Limonene at 26.99%, with a minor presence of α-pinene at 5.8%. At concentrations below 1%, additional compounds were detected, such as β-pinene at 0.66%,

β-Ocimene at 0.65%, camphene at 0.4%, and minimal quantities (0.08%) of both methylperillate and bornylacetate.

Table 7. Essential oil constituents of *Ammudoucus leucotrichus*identified by GC/MS

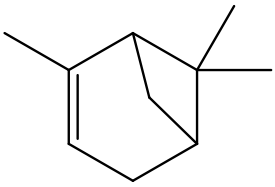
No	Compounds	IR_{Exp}	IR_{Ref}	(%)
01	**Alpha-Pinene**	**932**	**925**	**5.8**
02	Alpha-Fenchene	945	934	0.02
03	Camphene	946	941	0.4
04	Thuja-2,4(10)-diene	953	948	0.04
05	Sabinene	969	969	0.03
06	Beta-Pinene	974	972	0.66
07	Myrcene	988	989	0.15
08	Alpha-Phellandrene	1002	1003	0.04
09	Beta-Ocimene	1009	1022	0.65
10	para-Cymene	1020	1024	0.06
11	**D-Limonene**	**1024**	**1029**	**26.99**
12	1,3,8-p-Menthatriene	1108	1122	0.02
13	Alpha-Campholenal	1122	1127	0.02
14	trans-Pinocarveol	1135	1141	0.05
15	Pentyl-Benzene	1152	1147	0.03
16	Myrtenal	1195	1198	0.01
17	Verbenone	1204	1212	0.02
18	Cumin aldehyde	1238	1244	0.02
19	Carvone	1239	1249	0.02
20	Linalool acetate	1257	1257	0.03
21	**Perilla aldehyde**	**1269**	**1285**	**64.66**
22	Bornyl acetate	1287	1291	0.08
23	Benzyl isobutanoate	1297	1305	0.03
24	Methyl perillate	1392	1399	0.08
	Total		**99.91**	

Our findings are consistent with those reported by (Baser et al., 1993; Louail et al., 2016; Sonboli et al., 2012). However, when comparing our results with other studies, some differences in the quantitative measurement of the main components of the essential oil and the nature of the identified components were observed. For instance, studies by (Louail et al.,

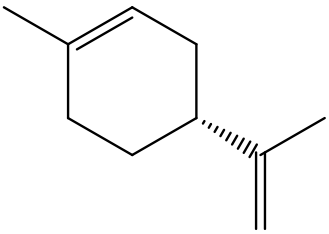
2016; Moulay et al., 2014)indicated the presence of a small quantity of α-pinene, which does not align with our results.

Additionally, (Moulay et al., 2014)mentioned that the essential oil of *Ammudoucus leucotrichus* primarily contained perillaldehyde (88.7%) and limonene (8.26%), contrasting with our findings where perillaldehyde predominates and limonene is less prominent. According to(Louail et al., 2016), the tested *Ammudoucus leucotrichus*oil contained 59.12% perillaldehyde and 23.89% limonene. Hence, the origin of the oil from Algeria (specifically Eloued region)serves as a source of the active component (perillaldehyde), commonly utilized in the fragrance and cosmetics industries due to its scent (S. Y. Wang & Chen, 2010).

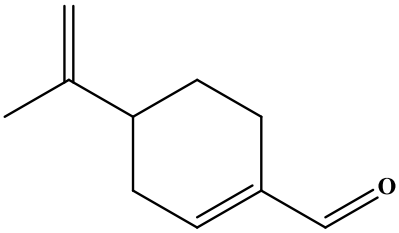
For a visual representation of the top major compounds (more than 1%) and their structures, refer to Figure 25, which depicts the chemical structures and IUPAC names of the predominant constituents identified in the essential oil of *Ammudoucus leucotrichus*.



2,6,6-trimethylbicyclo[3.1.1]hept-2-ene
(Alpha-Pinene)



(R)-1-methyl-4-(prop-1-en-2-yl)cyclohex-1-ene
(D-Limonene)



4-(prop-1-en-2-yl)cyclohex-1-enecarbaldehyde
(Perilla aldehyde)

Figure 25. Chemical structures and IUPAC names of the top major compounds identified in the essential oil of *Ammudoucus leucotrichus*

3.3.2. Mentha piperita:

The application of hydrodistillation for extracting the essential oil from *Mentha piperita*, commonly referred to as peppermint, yielded a light-yellow oil with a 1.2% yield. Analysis revealed the presence of 34 chemical compounds (as presented in Table 8), constituting 98.24% of the total oil composition.These compounds were categorized into 70.3%

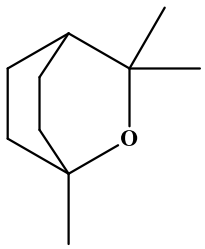
oxygenated monoterpenes, 3.6% hydrocarbon monoterpenes, 0.18% oxygenated sesquiterpenes, and 22.96% other compounds. Notably, three primary compounds were identified, each exceeding 20%: trans-Sabinene hydrate (27.51%), Linalool acetate (21.90%), and Pulegone (20.77%).

Table 8. Essential oil constituents of *Mentha piperita* identified by GC/MS

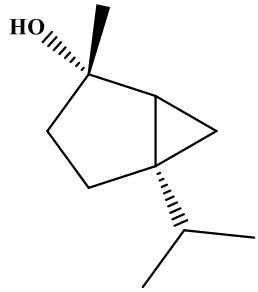
No	Compounds	IR_{Exp}	IR_{Ref}	(%)
01	Alpha Pinene	924	932	0.46
02	Camphene	940	946	0.22
03	Sabinene	968	969	0.22
04	β Pinene	971	974	0.91
05	Myrcene	988	988	0.58
06	Alpha Terpinene	1015	1014	0.05
07	para-Cymene	1023	1020	0.04
08	Limonene	1027	1024	0.46
09	**1,8-Cineole**	**1030**	**1026**	**16.08**
10	trans-beta-Ocimene	1037	1044	0.41
11	δ Terpinene	1058	1054	0.15
12	Terpinolene	1088	1086	0.10
13	**trans-Sabinene hydrate**	**1101**	**1098**	**27.51**
14	3-Octanol, acetate	1123	1120	0.03
15	trans-Sabinol	1141	1137	0.03
16	**Menthone**	**1154**	**1148**	**1**
17	iso-Menthone	1165	1158	0.07
18	**Borneol**	**1167**	**1165**	**1.66**
19	l neoiso' Isopulegol	1173	1167	0.11
20	Terpinen-4-ol	1178	1174	0.22
21	**Alpha-Terpineol**	**1192**	**1186**	**2.54**
22	neoiso-Dihydrocarveol	1230	1126	0.22
23	**Pulegone**	**1243**	**1233**	**20.77**
24	**Linalool acetate**	**1257**	**1254**	**21.90**
25	Lavandulyl acetate	1292	1288	0.33
26	Piperitenone	1347	1340	0.06
27	Linalool isotretinoin	1383	:1373	0.48
28	Caryophyllene (E)	1424	1417	0.79
29	Alpha Humulene	1459	1452	0.12
30	Germacrene D	1488	1484	0.25
31	gamma Cadinene	1521	1513	0.04
32	Elmole	1555	1548	0.18
33	Cinnamaldehyde	1591	1599	0.19
34	Khusimone	1600	1604	0.06
	Total		**98.24**	

Furthermore, α-Terpineol, Menthone, and Borneol were among the compounds present in concentrations exceeding 1%.Our findings are largely consistent with certain other studies, albeit with variations observed in the proportions of major compounds. For instance, (Gavahian et al., 2015) reported significant levels of 1,8-Cineole (7.2%), Menthone (24.7%), and Pulegone (4.5%).Similarly, (Smaoui et al., 2016)obtained results in alignment with ours,

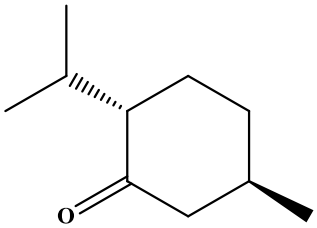
revealing predominant compound percentages of 1,8-Cineole (2.8%), Menthone (33%), and Pulegone (1.6%).



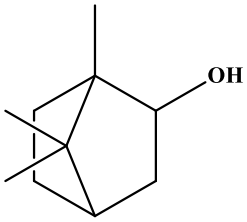
1,3,3-trimethyl-2-oxabicyclo[2.2.2]octane
(1,8-Cineole)



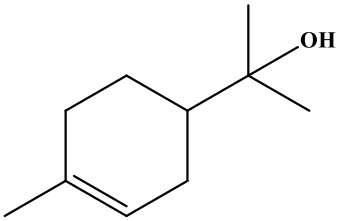
(2*S*,5*R*)-5-isopropyl-2-methylbicyclo[3.1.0]hexan-2-ol
(trans-Sabinene hydrate)



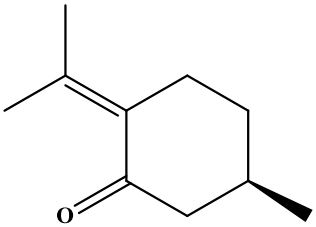
(2*S*,5*R*)-2-isopropyl-5-methylcyclohexanone
(Menthone)



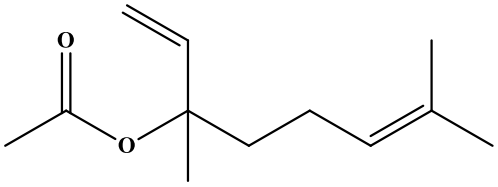
1,7,7-trimethylbicyclo[2.2.1]heptan-2-ol
(Borneol)



2-(4-methylcyclohex-3-en-1-yl)propan-2-ol
(alpha -Terpineol)



(*R*)-5-methyl-2-(propan-2-ylidene)cyclohexanone
(Pulegone)



3,7-dimethylocta-1,6-dien-3-yl acetate
(Linalool acetate)

Figure 26. Chemical structures and IUPAC names of the top major compounds identified in the essential oil of *Mentha piperita*

However, discrepancies were noted in the analysis conducted by (Ahmad et al., 2014), where the presence of Menthol at 34.82% differed from our findings.In a separate study by (Hossain et al., 2014) on peppermint plants cultivated in Oman, the detection of Eucalyptol,

absent in our study, was noteworthy.For an illustrative overview of the major compounds identified, Figure 26, displaying the structures and IUPAC nomenclature of these constituents.

4. *In vivo* antidiabetic activities:

4.1. Hematological parameters

The hematological parameters results for all experimental groups are displayed in the table below. In comparison to the control group, the alloxan in the diabetic group resulted in a very highly significant decrease ($p<0.001$) in lymphocytes (LYM), a significant decrease ($p<0.05$) in granulocytes (GRA), and a highly significant decrease ($p<0.01$) in platelets (PLT).On the other hand, the results show no significant variation in the number of leucocytes (WBC), erythrocytes (RBC), and hemoglobin (HGB) level. However, treatment with the essential oil of *Ammodaucus leucotrichus* (Diabetic+EOAl group) significantly restored the platelet count in comparison to the diabetic group.

Table 09. Plasma concentration of hematological parameters of different experimental groups

	WBC ($\times10^9$/L)	**LYM** ($\times10^9$/L)	**GRA** ($\times10^9$/L)	**HGB** (g/dL)	**RBC** ($\times10^{12}$/L)	**PLT** ($\times10^9$/L)
Control	6.81±0.73	4.43±0.51	3.9±0.1	11.93±0.37	7.19±0.06	919.6±26.2
Diabetic	4.06±1^{NS}	2.46±0.5^{c}	1.5±0.5^{a}	12.46±0.45^{NS}	7.52±0.05^{b}	475.0±25^{b}
Diabetic+ EOMP	4±1^{NS}	2.5±0.5^{NS}	2.03±0.95^{NS}	10.33±1.06^{NS}	6.82±0.18^{a*}	466.0±6^{c} ^{NS}
Diabetic+ EOAl	3.63±0.63^{a} ^{NS}	2.23±0.68^{b} ^{NS}	1.23±0.68^{a} ^{NS}	12.43±0.45^{NS}	7.70±0.13^{a} ^{NS}	861.3±35.5^{a} **
EOMP	6.1±1.01 ^{NS}	4.5±0.5^{NS}	2.1±0.8^{NS}	12.7±1.04 ^{NS}	7.73±0.37 ^{NS}	604.6±93^{a}
EOAl	5.6±0.52 ^{NS}	2.9±0.1^{b} ^{NS}	3.2±0.72 ^{NS}	12.86±1.25^{NS}	8.14±0.41 ^{NS}	518±234^{NS}

Comparison with diabetic group: * p <0.05; ** p <0.01; *** p <0.001, comparison with control group: a p <0.05; b p <0.01; c p <0.001, (NS) non significatif.

4.2. Biochemical parameters

- ***Glycemia and parameters of liver function***

The following table displays the concentration of glucose and parameters of liver function results for all the experimental groups. Where, alloxan causes a highly significant increase of glycemia (FBS) in diabetic group $p<0.01$, compared to the control group, and when compared to the diabetic group, there is a highly significant decrease in the (Diabetic+ Acarbose) and (Diabetic+EOMP) groups, and a very highly significant decrease in the (Diabetic+ EOAl)group.

Alloxan caused a significant increase in aspartate aminotransferase (AST) and alanine aminotransferase (ALT) compared to the control group, and when compared to the diabetic group, the aspartat aminotransferase value changed non-significantly in the (Diabetic+ Acarbose) and (Diabetic+EOAl) groups, and decreased significantly in the (Diabetic+EOMP) group.Meanwhile, alanine aminotransferase decreased highly significantly in the (Diabetic+ Acarbose) group and significantly in the (Diabetic+EOMP) and (Diabetic+EOAl) groups.

Alkaline phosphatase (ALP) was affected by a very highly significant increase in the diabetic group compared to the control group, and compared to the diabetic group, its value decreased highly significantly in the (Diabetic+ Acarbose) and (Diabetic+EOMP) groups,significantly in the (Diabetic+EOAl) group.

Table 10. Concentration of Glucose and parameters of liver function of different experimental groups

	FBS(g/L)	**AST** (U/L)	**ALT** (U/L)	**ALP** (IU/L)
Control	0.65±0.05	242.3±0.57	85.3±3.05	370.6±11
Diabetic	1.47±0.04^{c}	310.3±21.9^{a}	127.0±7^{a}	843.3±3.5^{c}
Diabetic+Acarbose	0.74±0.04^{NS} **	315.6±14.2^{a} ^{NS}	88±2 ^{NS**}	319.6±70.5^{NS**}
Diabetic+EOMP	0.89±0.02^{a**}	249.0±9^{NS*}	77.0±14.7 ^{NS*}	300±44 ^{NS**}
Diabetic+ EOAl	0.79±0.02^{NS} ***	297.0±2$^{c\ NS}$	78.0±10^{NS*}	232.6±160 ^{NS*}
EOMP	0.39±0.01^{a}	313±3^{c}	131.0±1 ^{b}	223.6±102 ^{NS}
EOAl	0.30±0.01^{b}	386±5.29 ^{c}	122.0±2 ^{b}	206±79.2 ^{NS}

Comparison with diabetic group: * $p<0.05$; ** $p<0.01$; *** $p<0.001$, comparison with control group: a $p<0.05$; b $p<0.01$; c $p<0.001$, (NS) non significatif.

- ***Parameters of kidneys function and triglycerides***

The values of urea, creatinine (Crea) and Uric Acid (UA) increased significantly in the diabetic group compared to the control group. While, when compared with diabetic group the urea value decreased highly significantly in (Diabetic+EOMP) and (Diabetic+EOAl) groups. As for triglycerides (TG) value increased very highly significantly in the diabetic group compared to the control group, and decreased highly significantly in the (Diabetic+ Acarbose) group, decreased very highly significantly in the (Diabetic+EOMP) and (Diabetic+EOAl) groups compared to the diabetic group.

Table 11. Parameters of kidney functionand triglycerides ofdifferent experimental groups

	Urea(g/L)	**Crea** (mg/L)	**UA** (mg/L)	**TG** (g/L)
Control	0.2±0.04	3±0.0	14.67±1.52	0.37±0.02
Diabetic	0.39±0.03^{a}	4±0.0^{a}	21±1^{a}	1.03±0.03^{c}
Diabetic+Acarbose	0.39±0.027^{NS}	3±0.0^{NS}	16.67±5.5 ^{NS}	0.54±0.07^{NS*}
Diabetic+EOMP	0.21±0.01^{NS**}	3±0.0 ^{NS}	18±1 $^{a\ NS}$	0.46±0.02 ^{NS***}
Diabetic+ EOAl	0.16±0.02^{NS**}	2±0.0^{NS}	18±0.0^{NS*}	0.43±0.02 ^{NS***}
EOMP	0.74±0.04^{b}	3±0.0 ^{NS}	17±1 ^{NS}	0.55±0.05 ^{NS}
EOAl	0.18±0.03^{NS}	3±0.0 ^{NS}	21±1 ^{a}	0.60±0.0^{b}

Comparison with diabetic group: * p <0.05; ** p <0.01; *** p <0.001, comparison with control group: a p <0.05; b p <0.01; c p <0.001, (NS) non significatif.

4.3. Oxidative stress parameters

- ***Pancreas***

Alloxan caused in the pancreas a very highly significant increase in the level of MDA and a very highly significant decrease in the concentration of GSH compared to the control group. Acarbose treatment affected a very highly significant decrease in the concentration of GSH compared to the control group, and compared to the diabetic group, the treatment with the essential oils of *Mentha piperita* and *Ammodaucus leucotrichus*contributed to the decrease of MDA and the increase of GSH concentration.

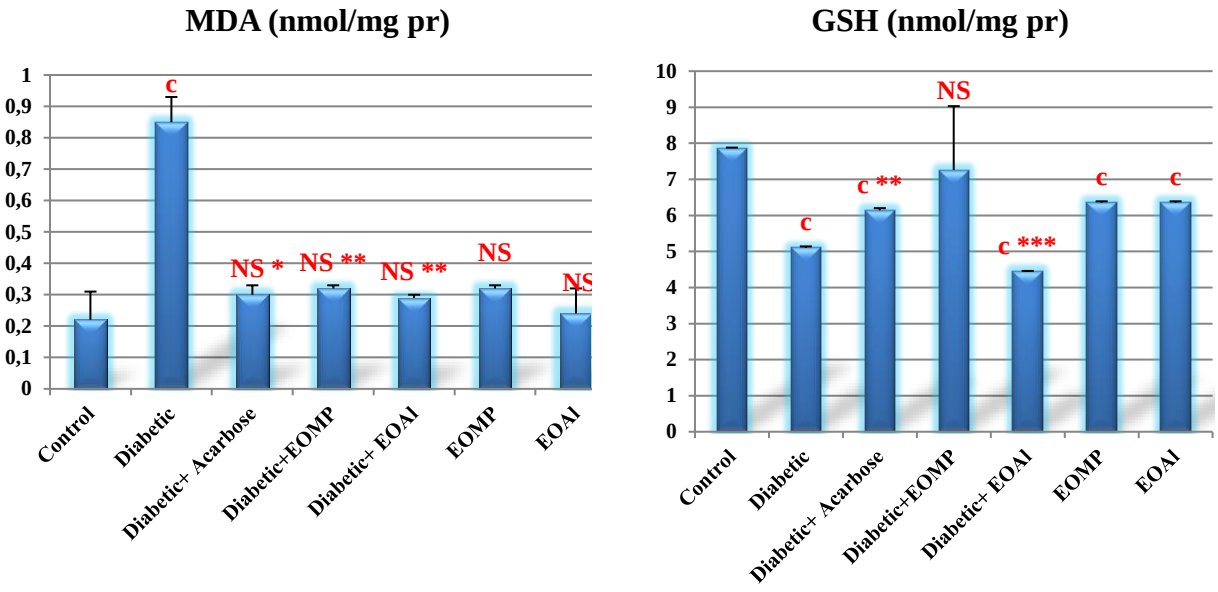


Figure 27. Oxidative stress parameters in the pancreas of control and experimental groups. Comparison with diabetic group: * p <0.05; ** p <0.01; *** p <0.001, comparison with control group: a p <0.05; b p <0.01; c p <0.001, (NS) non significatif.

- ***Liver***

Oxidative stress of liver results indicated that alloxan caused a significant increase in MDA level and a very highly significant decrease in GSH concentration compared to the control group. However, acarbose contributed to a significant decrease in the level of MDA a very highly significant increase the concentration of GSH compared to the diabetic group. The treatment with the essential oils of *Mentha piperita* and *Ammodaucus leucotrichus*significantly improved MDA level, GSH concentration compared to the diabetic group.

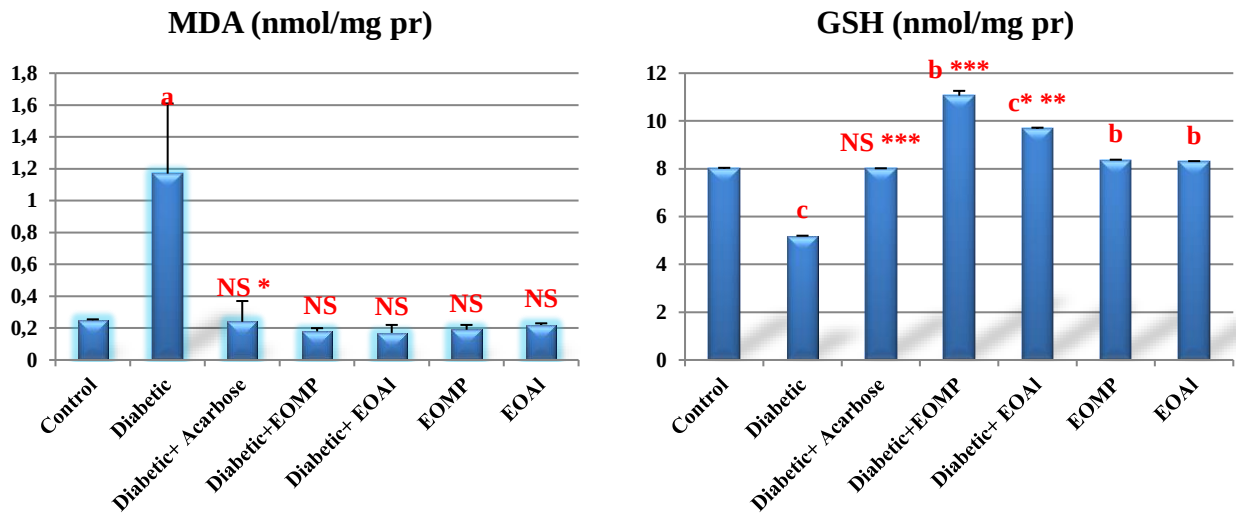


Figure 28. Oxidative stress parameters in the liver of control and experimental groups. Comparison with diabetic group: * p <0.05; ** p <0.01; *** p <0.001, comparison with control group: a p <0.05; b p <0.01; c p <0.001, (NS) non significatif.

- ***Kidneys***

In the kidneys, alloxan did not affect the level of MDA, but it caused a significant decrease in the concentration of GSH compared to the control group. Acarbose treatment caused a no significant increase in MDA level, a significant decrease in GSH concentration compared to the control group. And compared to the diabetic group, the essential oils contributed to a highly significant increase in the concentration of GSH.

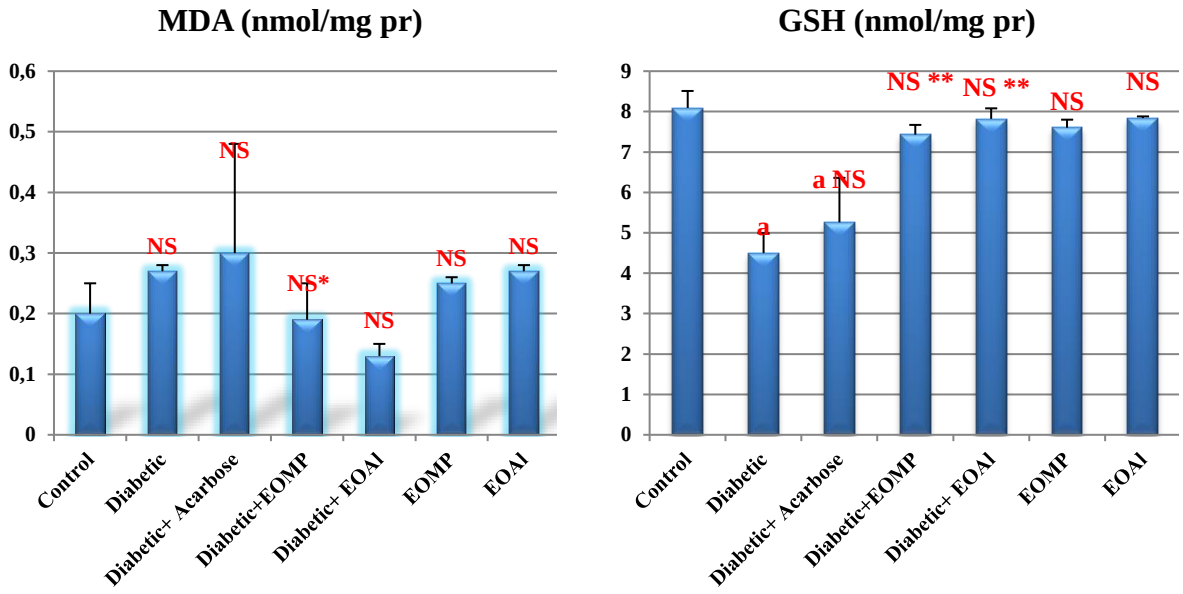


Figure 29. Oxidative stress parameters in the kidney of control and experimental groups. Comparison with diabetic group: * p <0.05; ** p <0.01; *** p <0.001, comparison with control group: a p <0.05; b p <0.01; c p <0.001, (NS) non significatif.

4.4. Histopathological studies

- ***Pancreas***

The control group's pancreatic tissue was shown under a microscope to be normal. Comprised of pancreatic acini and Langerhan's islet, which is divided by thin, flexible connective tissue and displays islet cells grouped in an acinar and trabecular pattern with a tiny, central nucleus and an abundance of eosinophilic cytoplasm. Islets are spherical in shape and include a lot of β-cells, which are normally rounded and have unique round nuclei.The pancreatic tissues of the diabetic group displayed irregularly shaped islets with a degraded outer layer of connective tissue, as well as reduced islet size and number of β-cells.The tissue of the group treated with acarbose showed a similar appearance to the tissue of the diabetic group.The pancreatic tissues in the essential oil-treated groups showed a significant improvement, including the restoration of the islets of Langerhans' size, regular and increased number of islets cells, islets form, and the surround connective tissue sheet.

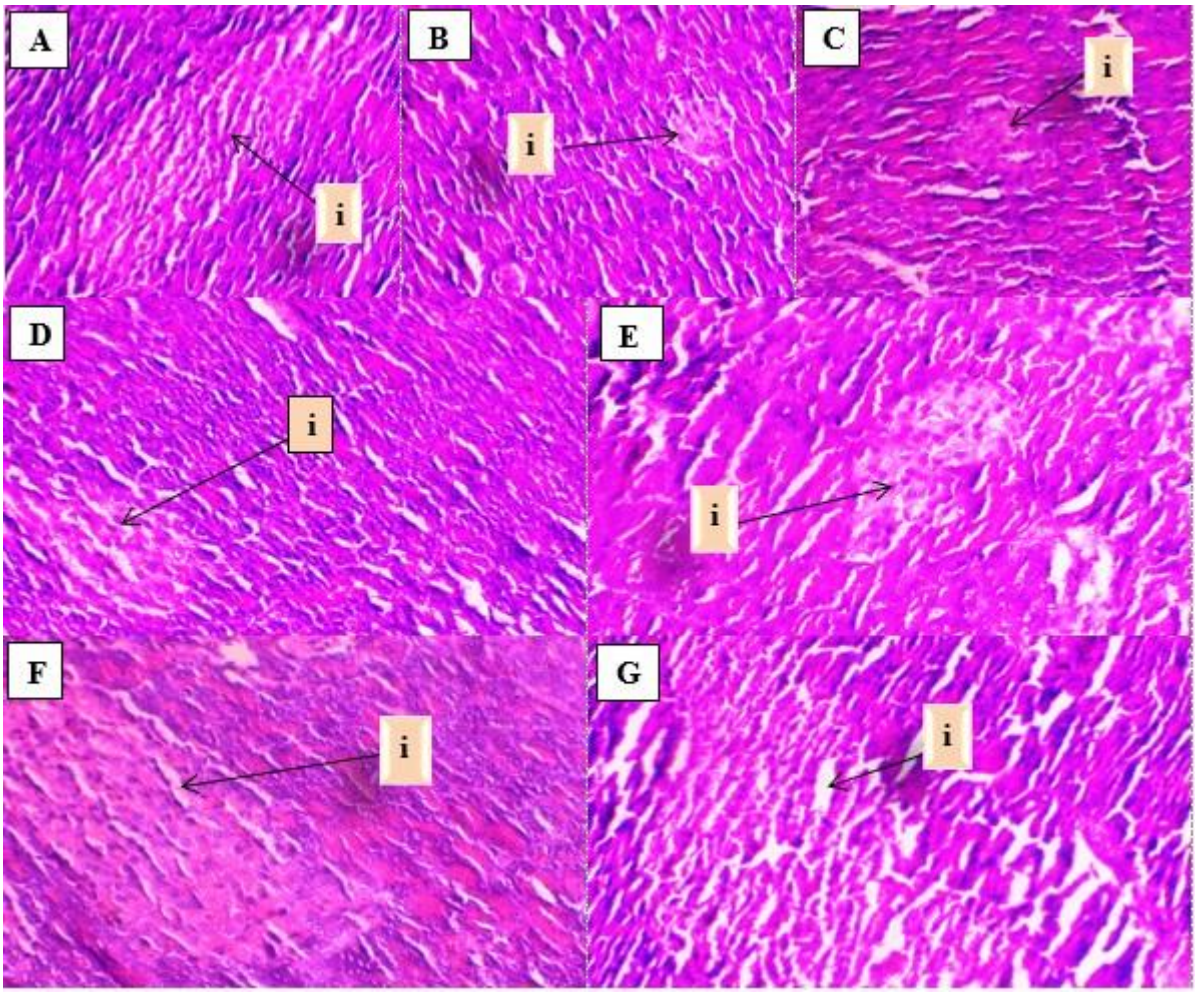


Figure 30. Microscopic observation of a histological section of pancreas in different experimental groups, Control group (A), diabetic group (B), Diabetic+ Acarbose group (C), Diabetic+EOMP group (D), Diabetic+ EOAl group (E), EOMP group (F) and EOAl group (G), (i) islets of Langerhans, magnification x40.

- ***Liver***

Microscopic photos of liver tissue showed a normal structure of hepatocytes with a normal appearance in the control group, while the diabetic group revealed pathological changes manifested in the appearance of immune cells (lymphocytes, polynuclear) with mild inflammatory infiltration in addition to focal lobular necrosis. The liver tissues of rats treated with acarbose and essential oils had a healthy architecture and a normal appearance similar to the liver of the control rats.

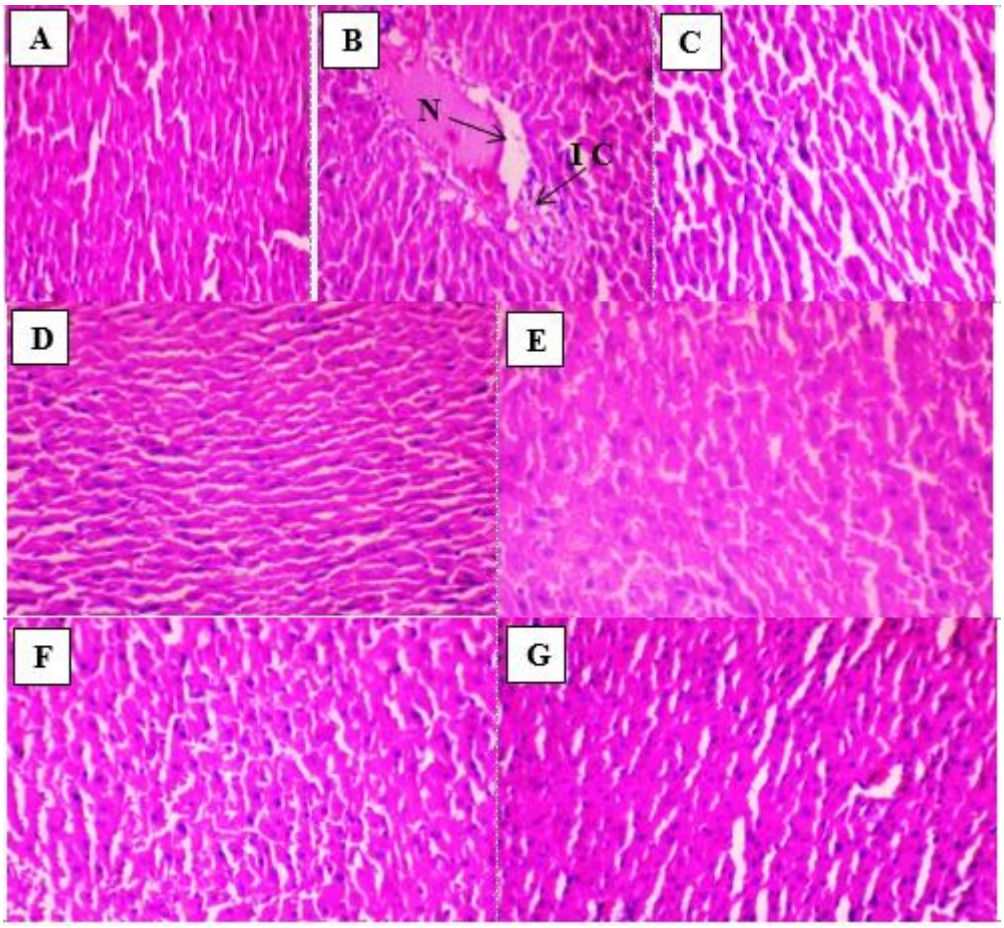


Figure 31. Microscopic observation of a histological section of liver in different experimental groups, Control group (A), diabetic group (B), Diabetic+ Acarbose group (C), Diabetic+EOMP group (D), Diabetic+ EOAl group (E), EOMP group (F) and EOAl group (G), (I C) immune cells, (N) necrosis, magnification x40.

- ***Kidneys***

Microscopic images of rat kidneys show a normal architecture and normal appearance, in addition to the absence of immune cells, no inflammatory infiltration, and no cell necrosis in the tissues of all groups of rats studied.

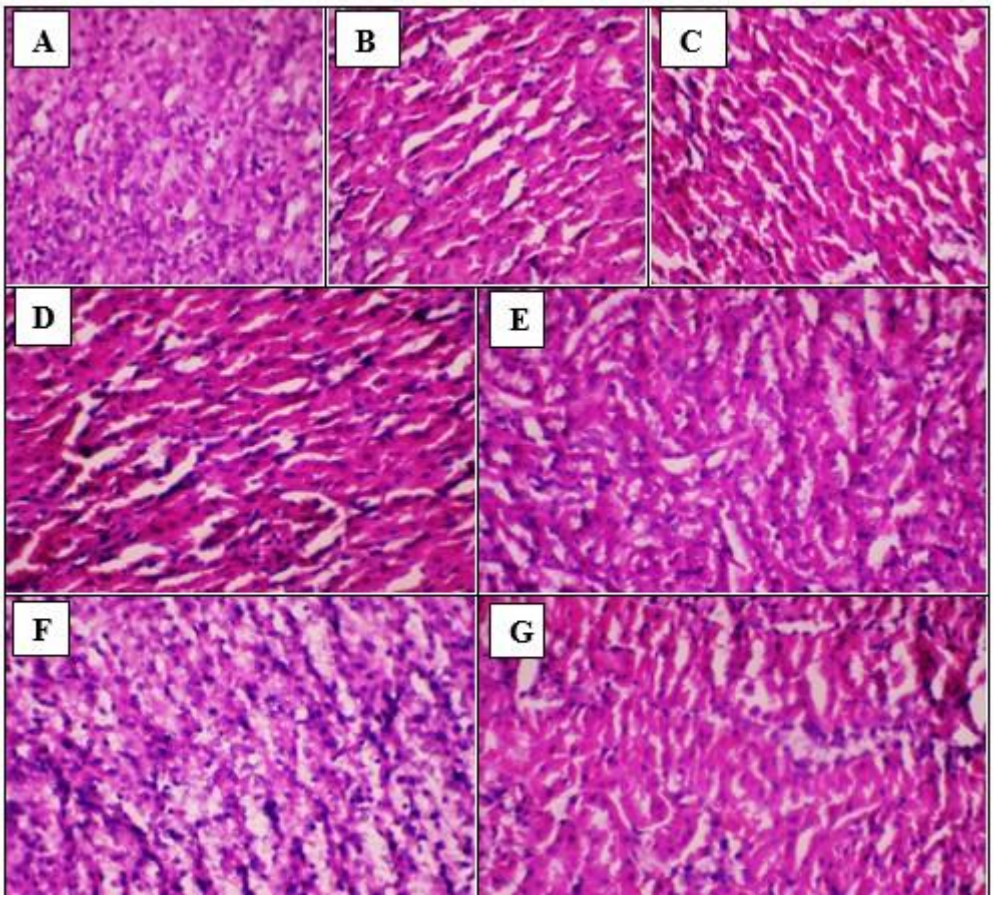


Figure 32. Microscopic observation of a histological section of kidney in different experimental groups, Control group (A), diabetic group (B), Diabetic+ Acarbose group (C), Diabetic+EOMP group (D), Diabetic+ EOAl group (E), EOMP group (F) and EOAl group (G), magnification x40.

Alloxan is the dominant substance utilized for inducing experimental diabetes. The loss of the pancreatic islet beta cell caused by alloxan results in persistent diabetes mellitus in several animals. Alloxan is specifically targeted towards the islet beta cell, causing minimal impact on other structures (Furman., 2017).

In our study, alloxan caused a decrease in lymphocytes and granulocytes, as well as a decrease in platelets. Lymphocytes and granulocytes function as phagocytes that eliminate foreign material. The presence of hyperglycemia in uncontrolled diabetes mellitus leads to the oxidation of membrane proteins, resulting in a decrease in white blood cells. This oxidation process promotes the generation of lipid peroxides, which in turn causes leukolysis(Mahmoud et al., 2013).

An abnormally low or high platelet count can result in excessive bleeding or the production of blood clots, respectively. The latter can impede or block blood vessels that provide blood to

the brain and/or heart, resulting in a stroke or heart attack, respectively. Insulin can directly regulate platelet function via a functional insulin receptor found on platelets and the absence of insulin due to alloxan in the diabetic rat group led to a decrease in platelets(Kakouros et al., 2011).

Alloxan is a toxic chemical that affects pancreatic β cells and the liver. It works by attacking sulfhydryl groups, chelating, altering enzyme and metabolic processes, altering the way electrolyte membranes are transported, increasing lipoperoxidation, and reducing antioxidant defenses (Gargouri et al., 2016).

The results show an increase in blood sugar and liver enzymes in the diabetic group. The observed disruptions in alloxan-treated rats validated that diabetes mellitus is a metabolic ailment defined by elevated blood sugar levels caused by deficiencies in insulin synthesis and efficacy. The main process of hyperglycemia in diabetes mellitus involves excess synthesis and reduced use of glucose by the tissues (Adesokanet al., 2010).

Whereas ALT and AST are cytosolic enzymes, ALP is an enzyme that is membrane bound. These enzymes are concentrated in the liver and are only detected in considerable amounts in the serum when the cell membrane ruptures or becomes leaky. An indicator of liver cell injury is an increase in the blood level of certain intracellular enzymes or a reduction in their tissue level (Gargouri et al., 2016).

Elevations in plasma levels of urea, creatinine, and uric acid are caused by diabetic hyperglycemia and are thought to be important indicators of kidney damage. The current study's results indicate that the diabetic group's serum urea, creatinine, and uric acid levels have significantly increased(Ikewuchi et al., 2017).

High glucose (blood sugar) levels are a symptom of diabetes. Insulin facilitates the conversion of glucose into glycogen, a form that can be stored. It also aids in the liver's glycogen storage. The body will utilize glucose to produce fatty acids if the liver contains an excessive amount of glycogen. Triglycerides are produced by the acids, they can accumulate in fat cells and contribute to body fat when they are released into the circulation. However, because the body is producing more triglycerides and is unable to eliminate fat from the blood, the danger of renal failure has made it difficult to control blood fat levels. These issues eventually result in elevated levels of triglycerides(Alexopoulos et al., 2019).

Acarbose acts as an inhibitor of some complex glycolytic enzymes found in the intestine, as it inhibits pancreatic alpha-amylase and membrane-bound alpha-glucosidase - including intestinal glucoamylase, sucrase, maltase, and isomaltase, thus preventing the breakdown of complex starches, oligos, trisaccharides, and disaccharides into simple absorbable sugars.

Acarbose reduces the absorption of dietary carbohydrates and the subsequent increase in blood glucose levels postprandial(Mao et al., 2014).In this study, acarbose contribute to reducing blood sugar and all the problems associated with it, such as decreases in lymphocytes and granulocytes, liver and kidney function alterations.

The principal processes responsible for the antidiabetic effect of the Essentail Oils were found to be the inhibition of alpha-glucosidase and alpha-amylase and the decrease in gluconeogenesis. EOs work through a variety of mechanisms, such as scavenging free radicals, regulating antioxidant enzymes, and reducing lipid peroxidation, to exert their hypoglycemic properties, which are linked to their capacity to increase glucose uptake, hinder glucose production, and have antioxidant effects. Owing to the varied chemical makeup of phytocompounds present in specific plants, these outcomes highlight the potential of EOs as adjuvant therapy in the treatment of diabetes mellitus(Bungau et al., 2023).

The studied plant's essential oils played a major role in lowering blood sugar levels; *Mentha piperita* and *Ammodaucus leucotrichus* both had hypoglycemic results of 0.89g/l and 0.79 g/l, respectively, which was an incredible outcome. This involves modifying the way the liver and kidneys function, low triglycerides levels, and the level of stress in the pancreas, liver, and kidneys. According to the data, most of the characteristics in the groups who received only essential oils were similar to those in the control group, indicating that the treatment was extremely safe.

It was found in this study that the blood glucose levels of the diabetic rats were elevated. However, the glycemia of the diabetic rats treated with *Mentha piperita* essential oil was also shown to be significantly reduced, supporting the results of (Narendhirakannan et al., 2006), who assessed the effects of *Mentha piperita* on diabetic rats. These results demonstrate the potential benefits of using peppermint juice in the treatment, prevention, or treatment of diabetes and associated complications among children born to diabetic mothers. Additional research in the literature indicates that taking plants or antioxidant-rich foods can help prevent the harm caused by diabetes (Volpato et al, 2007, Kumar et al., 2010).

Linalool is among the compounds found in the essential oil of the *Mentha piperita* plant. Where, previous research indicates the effective antioxidant and antidiabetic properties of linalool. Linalool was discovered to have beneficial effects on glucose metabolism in animal models of diabetes. Hence, the suppression of carbohydrate breakdown in the digestive system by α-amylase inhibition is seen as a potential method to regulate diabetes. Prior research has shown that linalool in the essential oil of lemon grass has the ability to efficiently suppress the activities of α-amylase and α-glucosidase(Peana et al, 2002, Ke et al., 2020).

Limonene is among the compounds found in the essential oil of the *Ammodaucus leucotrichus* plant. It has been shown in previous studies that limonene reduces high blood sugar and alleviates complications associated with diabetes (Bacanlı et al, 2017, More et al., 2014).

5. *In Vitro* Anti-Diabetic Activity:

5.1. Alpha-amylase inhibitory activities (IC_{50}):

The *in-vitro* antidiabetic potential of the two essential oils obtained from *Ammudoucus leucotrichus*and *Mentha piperita*was investigated using α-amylase inhibition model and the results are as shown in Table 12. From the linear plots of *Ammudoucus leucotrichus*and *Mentha piperita* concentration (µg/mL) against percentage α-amylase inhibition (Figure 33),the studied EOs significantly increased α-amylase inhibition (%) with corresponding increase in EO concentration. The effective inhibitory concentration (IC_{50} value) at which 50% of α-amylase activity was inhibited by the *Ammudoucus leucotrichus*and *Mentha piperita*essential oils was obtained to be 7.013 and 1.310µg/mL respectively, indicating a powerful potential to slowdown starch digestion via α-amylase inhibition.

The inhibitory potential of acarbose, a standard type 2 antidiabetic drug, on α-amylase activity was also analysed in the present study to serve as positive control. The result obtained showed that the two essential oils inhibited α-amylase with more degree (about 5.36-fold more), with *Mentha piperita*essential oil show a more degree, compared to acarbose (IC_{50} = 11.166µg/mL) (Figure 33).

Metabolism of carbohydrate (complex sugar or polysaccharide) by hydrolases (such as α-amylase, α-glucosidase, maltase etc.) leads to release of simple sugars such as glucose and thus boosts blood sugar level rise immediately after consumption of a carbohydrate-rich food (Anigboro et al., 2018); inhibitors of α-amylase, amongst others, are thus very useful in preventing sudden rise in blood glucose level especially in type 2 diabetic patients, following their consumption of carbohydrate-rich food substances (Adefegha &Oboh, 2012; Avwioroko et al., 2020). The relatively inhibitory prowess of the two studied compounds as α-amylase inhibitor compared to acarbose is a desirable property in the present study. This is because previous studies had reported that the extremely high inhibitory effect of acarbose against α-amylase eventually leads to excessive delay of starch hydrolysis in gastrointestinal tract thereby causing abdominal discomfort and flatulence in diabetic patients who take the drug (Malekaneh & Zarban, 2011).

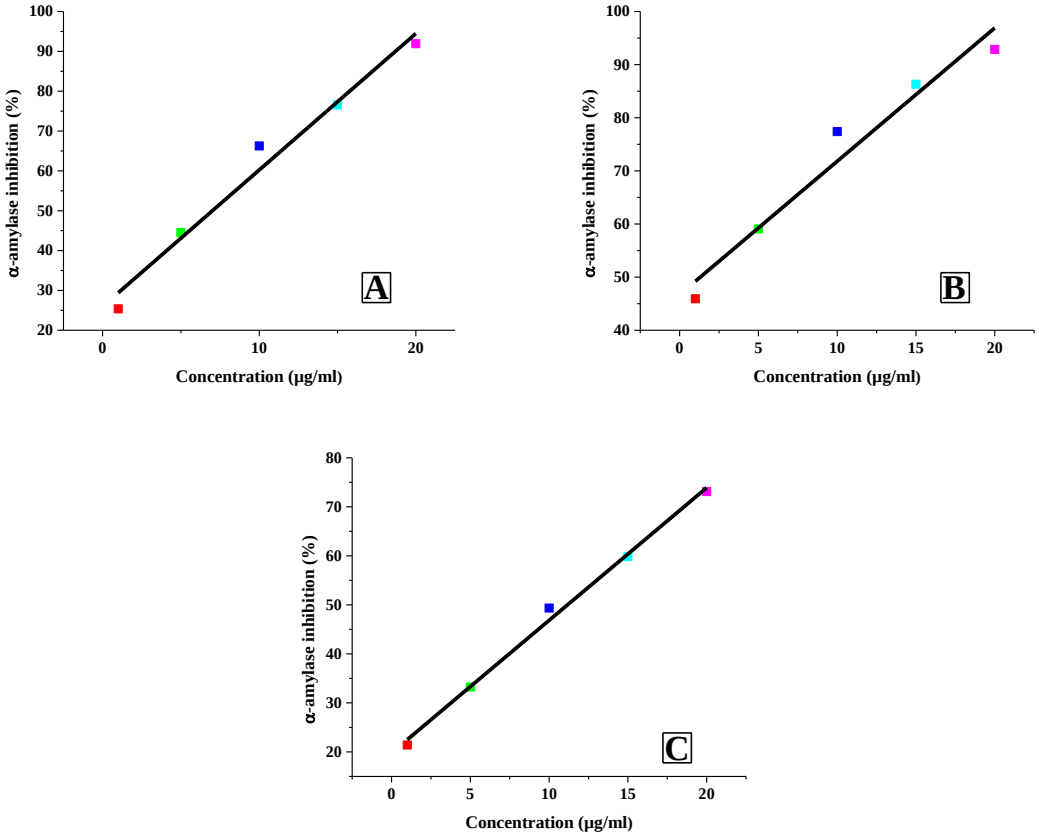


Figure 33. Linear regression of the inhibition of α-amylase activity by the essential oils of: *Ammudoucus leucotrichus*(A), *Mentha piperita*(B) and Acarbose (C)

Table 12.*In vitro*Anti-diabetic activity of the essential oils extracted from *Ammudoucus leucotrichus*and *Mentha piperita*by α-amylase inhibitory assay

Concentration (µg/mL)	**Percentage of α-Amylase Inhibition**		
	Ammudoucus leucotrichus	*Mentha piperita*	Acarbose
1	25.352	45.918	21.405
5	44.503	59.073	33.239
10	66.242	77.399	49.353
15	76.549	86.305	59.829
20	91.908	92.867	73.112
IC_{50} Values	**7.013µg/Ml**	**1.310µg/Ml**	**11.166µg/mL**

Hence, it is believed that the moderately high α-amylase inhibitory potential of*Ammudoucus leucotrichus*and *Mentha piperita*essential oils could help in control of diabetes by diminishing the absorption of glucose, in the case of *Ammudoucus leucotrichus*would help to prevent sudden rise in blood glucose level in diabetic patients following consumption of a carbohydrate diet. Some researchers also suggested that essential

oils extract from plant materials displayed the anti-diabetic activity against amylase(Adefegha et al., 2017; Ibrahim et al., 2019; Sahin Basak & Candan, 2010).

5.2. Alpha-amylase molecular binding interaction:

One of the effective techniques for monitoring alterations in protein conformation during its interaction with ligands is absorption spectroscopy (Benesi & Hildebrand, 1949; B. L. Wang et al., 2020). Usually, when measured at the same wavelength range, the absorption peak(s) of a free protein and that of the protein-ligand complex are expected to differ; such difference could be attributed to the alteration in the native structure of the protein due to its binding interaction with the ligand.

5.2.1. Binding constants:

The gradual decrease in the absorption values of the α-amylase solution by increasing essential oils and acarbose concentrations can be exploited to calculate the binding constant by applying the following Equation 7(Benesi & Hildebrand, 1949):

$$\frac{A}{A_0 - A} = \frac{\varepsilon}{\varepsilon_0 - \varepsilon} + \frac{\varepsilon}{\varepsilon_0 - \varepsilon} \frac{1}{K_b \times C} \quad (7)$$

Where A_0 and A are the absorbencies of the α-amylase and its complexes with the studied essential oils respectively, while ε_0 and ε are respectively their extinction coefficients, and C represents the concentration of EOsand acarbose (mmol.L^{-1}), K_b refers to the binding constant (L.mol^{-1}).

A plot of *A/(A₀ - A)* versus *1/C* (Figure 34) gave a slope of $\varepsilon/(\varepsilon_0 - \varepsilon)K_b$ and a 'y' intercept equal to $\varepsilon/(\varepsilon_0 - \varepsilon)$, where K_b is the ratio of the slope to the y intercept.

5.2.2. Binding free energy:

The binding free energy change was calculated using the following Equation 8:(Laraoui et al., 2023)

$$\Delta G = -nRT \ln K_b \quad (8)$$

Where ΔG is the binding free energy in KJ.mol^{-1}, R is the gas constant, 8.32 J.$mol^{-1}K^{-1}$ and T is the absolute temperature, 298K.

The negative values of ΔG indicate the spontaneity of the α-amylase and the essential oils interaction, whereas its magnitude indicates the strong binding between the protein and the

studied compounds(Gil et al., 2002). The obtained values of binding constants and their corresponding free binding energies are summarized in Table 13.

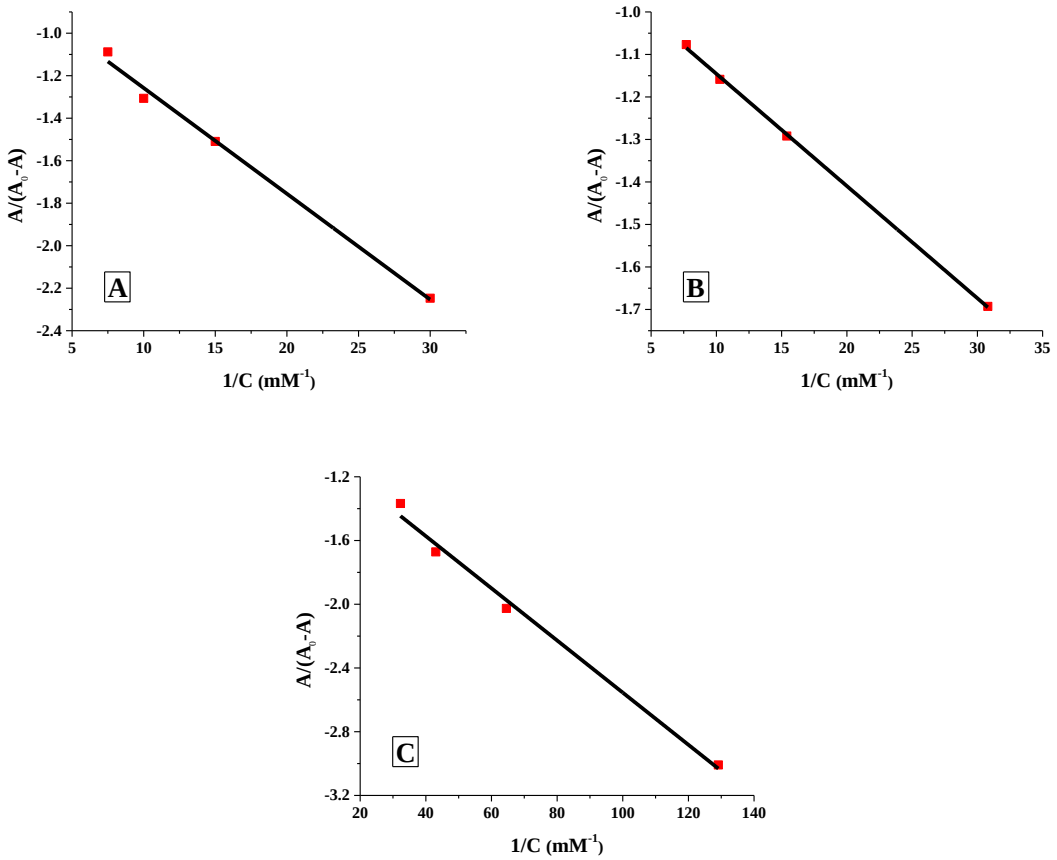


Figure 34. Plots of A/(A_0 - A) versus 1/[*Ammudoucus leucotrichus*] (A), 1/[*Mentha piperita*] (B) and 1/[Acarbose] (C) used to calculate the binding constants

Table 13. Binding constant and binding free energy values for *Ammudoucus leucotrichus*, *Mentha piperita*and acarbose with alpha-amylase

Adduct	K_b (M^{-1})	-ΔG (KJ.mol^{-1})
α-amylase_*Ammudoucus leucotrichus*	1.53 × 10^4	23.88
α-amylase_*Mentha piperita*	3.33 × 10^4	25.82
α-amylase_Acarbose	2.68 × 10^4	25.28

6. In-Silico analysis:

6.1. ADMET and drug-likeness prediction:

Modern drug discovery involves assessment of competence of the dynamic molecules and their strength to reach target site in bioactive form, which involves cellular, animal and human clinical trials which are highly priced and encumbered with risks (Ranjith & Ravikumar,

2019; Ranjith D & Viswanath S, 2019). Presently computer aided drug development encouraged the estimate of absorption, distribution, metabolism and excretion of drugs (ADME), they postulate anticipatory and dependable data very quickly and compliment for experimental approaches (Ranjith & Ravikumar, 2019; Sliwoski et al., 2014). It has been determined that the initial appraisal of ADME properties in the discovery period diminishes remarkably the fraction of pharmacokinetics related failures in the clinical phase (Hay et al., 2014; Ranjith & Ravikumar, 2019).

In the present study we evaluated the ADME properties of the major compounds present in both essential oils using SwissADME web tool. A total of 4 potent phytoconstituents were analysed to study general characteristics (Table 14), Physicochemical properties (Table 9), lipophilicity and water solubility characteristics (Table 10&11), pharmacokinetic parameters (Table 12), drug likeness rule and bioavailability score (Table 13) and medicinal chemistry properties (Table 14), respectively, the six compounds are compared to the standard drug (Acarbose). General characteristics of the studied compounds revealed all the compounds having molecular weight less than 500 Da, which is a good prime property to be called as drug likeness of the small molecules and they still have a low molecular weight than the Acarbose.

Table 14. General characteristics of the phytoconstituents of essential oils

SI. No	Compounds	Molecular formula	Canonical SMILES	Molecular weight (g/mol)
1	trans-Sabinene hydrate	$C_{10}H_{18}O$	CC(C12CCC(C2C1)(C)O)C	154.25
2	Linalool acetate	$C_{12}H_{20}O_2$	C=CC(OC(=O)C)(CCC=C(C)C)C	196.29
3	Perilla aldehyde	$C_{10}H_{14}O$	O=CC1=CCC(CC1)C(=C)C	150.22
4	D-Limonene	$C_{10}H_{16}$	CC1=CCC(CC1)C(=C)C	136.23
5	Acarbose	$C_{25}H_{43}NO_{18}$	CC1C(C(C(C(O1)OC2C(OC(C(C2O)O)OC3C(OC(C(C3O)O)O)CO)CO)O)O)NC4C=C(C(C(C4O)O)O)CO	645.60

Table 15. Physicochemical properties of the phytoconstituents of essential oils

Properties	trans-Sabinene hydrate	Linalool acetate	Perilla aldehyde	D-Limonene	Acarbose
Num. heavy atoms	11	14	11	10	44
Num. arom. heavy atoms	0	0	0	0	0
Fraction Csp3	1.00	0.58	0.50	0.60	0.92
Num. rotatable bonds	1	6	2	1	9
Num. H-bond acceptors	1	2	1	0	19
Num. H-bond donors	1	0	0	0	14
Molar refractivity	46.90	60.17	47.32	47.12	136.69
TPSA (Å²)	20.23	26.30	17.07	0.00	321.17

Table 16. Lipophilicity characteristics of the phytoconstituents of essential oils

Properties	trans-Sabinene hydrate	Linalool acetate	Perilla aldehyde	D-Limonene	Acarbose
iLOGP	2.47	3.08	2.16	2.72	0.63
XLOGP3	2.12	3.93	3.13	4.57	-8.53
WLOGP	2.19	3.24	2.49	3.31	-8.56
MLOGP	2.45	2.95	2.10	3.27	-6.94
SILICOS-IT	2.44	2.98	2.64	2.97	-7.69
Consensus Log Po/w	2.33	3.24	2.50	3.37	-6.22

Table 17. Water Solubility characteristics of the phytoconstituents of essential oils

Small molecules	ESOL				Ali				SILICOS-IT			
	Log S (ESOL)	Solubility		Class	Log S (Ali)	Solubility		Class	Log S (SILICOS-IT)	Solubility		Class
		mg/ml	mol/L			mg/ml	mol/L			mg/ml	mol/L	
trans-Sabinene hydrate	-2.07	1.33e-0	8.59e-3	S	-2.18	1.03e-0	6.67e-3	S	-1.91	1.92e-0	1.24e-2	S
Linalool acetate	-3.14	1.43e-1	7.30e-4	S	-4.18	1.29e-2	6.58e-5	MS	-2.52	5.97e-1	3.04e-3	S
Perilla aldehyde	-2.61	3.68e-1	2.45e-3	S	-3.16	1.04e-1	6.96e-4	S	-1.83	2.22e-0	1.48e-2	S
D-Limonene	-3.50	4.33e-2	3.18e-4	S	-4.29	6.93e-3	5.09e-5	MS	-2.26	7.54e-1	5.53e-3	S
Acarbose	2.13	8.61e+4	1.33e+2	HS	2.56	2.32e+5	3.60e+2	HS	6.40	1.62e+9	2.51e+6	S

Table 18. Pharmacokinetics parameters of the phytoconstituents of essential oils

Proprieties	trans-Sabinene hydrate	Linalool acetate	Perilla aldehyde	D-Limonene	Acarbose
GI absorption	High	High	High	Low	Low
BBB permeant	Yes	Yes	Yes	Yes	No
P-gp substrate	No	No	No	No	Yes
CYP1A2 inhibitor	No	No	No	No	No
CYP2C19 inhibitor	No	No	No	No	No
CYP2C9 inhibitor	No	No	No	Yes	No
CYP2D6 inhibitor	No	No	No	No	No
CYP3A4 inhibitor	No	No	No	No	No
Log Kp (Skin Permeation) (cm/s)	-5.74	-4.71	-4.99	-3.89	-16.29

Table 19. Druglikeness rule and bioavailability score of the phytoconstituents of essential oils

Proprieties	trans-Sabinene hydrate	Linalool acetate	Perilla aldehyde	D-Limonene	Acarbose
Lipinski	Yes; 0 violations	Yes; 0 violations	Yes; 0 violations	Yes; 0 violations	No; 3 violations: MW>500, NorO>10, NHorOH>5
Ghose	No; 1 violation: MW<160	Yes	No; 1 violation: MW<160	No; 1 violation: MW<160	No; 4 violations: MW>480, WLOGP<-0.4, MR>130, #atoms>70
Veber	Yes	Yes	Yes	Yes	No; 1 violation: TPSA>140
Egan	Yes	Yes	Yes	Yes	No; 1 violation: TPSA>131.6
Muegge	No; 2 violations: MW<200, XLOGP3>3.5	No; 1violation: MW<200	No; 2 violations: MW<200, Heteroatoms<2	No; 2 violations: MW<200, Heteroatoms<2	No; 5 violations: MW>600, XLOGP3<-2, TPSA>150, H-acc>10, H-don>5
Bioavailability score	0.55	0.55	0.55	0.55	0.17

Table20. Medicinal Chemistry properties of the Phytoconstituents of essential oils

Proprieties	trans-Sabinene hydrate	Linalool acetate	Perilla aldehyde	D-Limonene	Acarbose
PAINS	0 alert	0 alert	0 alert	0 alert	0 alert
Brenk	0 alert	1 alert: isolated_alkene	1 alert: isolated_alkene	1 alert: isolated_alkene	1 alert: isolated_alkene
Leadlikeness	No; 1 violation: MW<250	No; 2 violations: MW<250, XLOGP3>3.5	No; 1 violation: MW<250	No; 2 violations: MW<250, XLOGP3>3.5	No; 2 violations: MW>350, Rotors>7
Synthetic accessibility	2.82	2.75	3.19	3.46	7.34

Lipophilicity property of the compounds portrays an important role for molecular discovery activities in multifarious domains. The quantitative descriptor of the lipophilicity is the partition coefficient P of a given molecule between *n*-octanol and water system (Daina et al., 2014). Because of its amphiphilic nature, *n*-octanol is considered a good mimic of phospholipid membrane characteristics (Liu et al., 2011). Multifarious algorithms are accessible to compute log Po/w, which rely on factual methodologies. The classic log P predictors branched in to two division, first ones split molecular structures into molecular fragments includes fragmental approach e.g. KLOGP (Klopman et al., 1993), KOWWNIN (Meylan & Howard, 2000) oratomic approach e.g. ALOGP (Ghose et al., 1998; R. Wang et al., 1997), XLOGP (Cheng et al., 2007; Moriguchi et al., 1994). The second division gathers the topological methods in which, the molecules description is related to its topology being as count or flags for specific atoms, groups or structural properties e.g.MLOGP(Brenk et al., 2008; Moriguchi et al., 1992), the prediction attained by manifold linear regression trained on large molecular data sets. The SILICOS-IT is a hybrid technique which combines both molecular fragments and topological parameters, which confide on 27 fragments and 7 topological descriptors, it was disciplined on 23,455 molecules with experimental *n*-octanol/water partition values (Daina et al., 2014). The version three of the XLOGP atomic model is established on a system of 87 fragments and two corrective factors. If the input structures are similar to a reference compound, the fragments differentiating them are treated and the corresponding log P contributions added to the reference structure log P value (Cheng et al., 2007). Lipophilicity estimated as consensus Log P, which is the average value of all Log P evaluated with various lipophilicity criteria, determined acarbose as most lipophilic whereas trans-Sabinene hydrateas least lipophilic and water solubility of the small molecules ranged from highly water soluble (Acarbose) to water soluble (trans-Sabinene hydrate).

The SwissADME model returns “Yes” or “No” if the compound under examination has greater probability to be a substrate or non-substrate of P-gp or inhibitor or non-inhibitor of Cytochrome P450 isoenzymes (CYP1A2, CYP2C9, CYP2C19, CYP2D6 and CYP3A4).

The pharmacokinetics and drug likeness performed using SwissADME showed a low level of GI absorption and BBB permeant with D-Limonenewhile a high absorption detected with trans-Sabinene hydrateand Linalool acetate. All the compounds present in the essential oils are not the substrates for P-gp except contrarily to the Acarbose (Table 18), so they are not susceptible to the efflux mechanism performed by this transporter which is used by many tumours cell lines as a drug-resistance mechanism(Ranjith & Ravikumar, 2019)

All of the small molecules returned as non-inhibitors for inactivation for CYP isoenzymes. The skin permeability coefficient (Log Kp), a multiple linear regression, the more negative the log Kp (with Kp in cm/s), the less skin permeant is the molecule. Among the phytoconstituents, trans-Sabinene hydrate(-5.74) is the least permeant andD-Limonene(-4.13) is highly permeant respectively. This SwissADME section gives access to five different rule-based filters, with diverse ranges of properties inside of which the molecule is defined as drug-like. The Lipinski (Pfizer) filter is the pioneer rule-of-five implemented and with the Ghose (Amgen), Veber (GSK), Egan (Pharmacia) and Muegge (Bayer) methods. Multiple estimations allow consensus views or selection of methods best fitting the end-user's specific needs in terms of chemical space or project-related demands. Any violation of any rule described here appears explicitly in the output panel. All the four compounds followed the filtered rule invoked in the SwissADME; the violation shown by the molecules are minimal.

SwissADME interpretation posts 0 PAINS alert of the 4 studied compounds. Brenk considered compounds that are smaller and less hydrophobic and not those defined by "Lipinski's rule of 5" to widen opportunities for lead optimization. This was after exclusion of compounds with potentially mutagenic, reactive and unfavourable groups such as nitro groups, sulphates, phosphates, 2-halopyridines and thiols (Brenk et al., 2008). All the compounds examined flouted Brenk's rule with only one alert, all the compounds failed Lead-likeness criteria due to their molar weight.

In silico toxicity study aims to help in optimizing compounds regarding their toxicity proprieties. The study could offer an important improvement to the awareness of the full perspective of virtual screening for the identification of target compounds with negligible or no toxicity, which may open a path for the selection of novel nontoxic phytoconstituents present in *Ammudoucus leucotrichus* and*Mentha piperita*essential oilswith high antidiabetic activity. In silico toxicity study of the chosen compounds was performed using the ProTox-II web server (Drwal et al., 2014). It aims to predict hepatotoxicity (Dili), carcinogenicity (Carcino), immunotoxicity (Immuno), mutagenicity (Mutagen), cytotoxicity (Cyto), median lethal dose (LD_{50}), and toxicity class (TC).

According to in silico toxicity profiles presented in Table 21, the toxicity class of all the phytoconstituents was detected to be equal to 5 except the Linalool acetatewhich predicted to be 6. Also, all the compounds were nontoxic in all the type of toxicities. Acarbose the standard of type 2 diabetes was predicted to be immunotoxin with a toxicity class of 6.

Table21. In silico toxicity profiles of the studied compounds

Molecule	Dili	Carcino	Immuno	Mutagen	Cyto	LD_{50} (mg.Kg^{-1})	TC
trans-Sabinene hydrate	Inactive	Inactive	Inactive	Inactive	Inactive	2000	4
Linalool acetate	Inactive	Inactive	Inactive	Inactive	Inactive	12000	6
Perilla aldehyde	Inactive	Inactive	Inactive	Inactive	Inactive	1720	4
D-Limonene	Inactive	Inactive	Inactive	Inactive	Inactive	4400	5
Acarbose	Active	Inactive	Active	Inactive	Inactive	24000	6

6.2. Molecular Docking Study:

In the current study , the binding interaction of all the major compounds presented in *Ammudoucus leucotrichus* and*Mentha piperita*essential oilscompounds with α-amylase was further investigated by carrying out a molecular docking analysis of the binding interaction between human pancreatic α-amylase with compounds: Perilla aldehyde, D-Limonene, alpha-Pinene, Pulegone, Menthone, trans-Sabinene hydrate, alpha-Terpineol, Borneol, Linalool acetate, 1,8-Cineole **(**the major phytochemicals present in the two plants with yield > 1)using Maestro version 11.7 user interface of the Schrödinger suite (Small-Molecule Drug Discovery Suite 2021-4, Schrödinger, LLC, New York, NY, 2021) (Schrödinger, 2015).

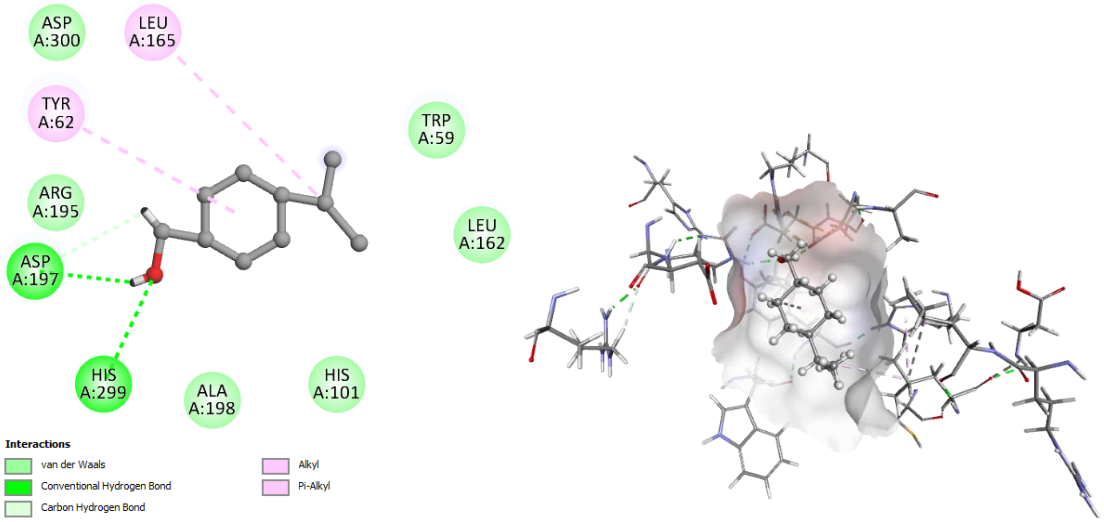
Each compound was docked with human pancreatic α-amylase (HPA) to understand their interactions at the enzyme's active site. Acarbose, a standard type 2 antidiabetic drug and HPA inhibitor, was likewise docked with HPA to serve as positive control. Of all the major compounds present in *Ammudoucus leucotrichus* and*Mentha piperita*essential oils, the binding affinities of them for α-amylase were comparable to that of acarbose (IFD score = –5.259 kcal/mol) (Table 22).This result indicates that the plant is a rich source of compounds which, either individually or synergistically, resulted in the decrease of α-amylase activity as observed in the *in vitro* and *in vivo* experimental α-amylase inhibition study. Also, the interactions were spontaneous as observed from the negative Gibb's free energy values (Avwioroko et al., 2020).

From studies on the X-ray crystal structure and enzyme kinetics of HPA, it was found that HPA active site is characterized by the existence of three special amino acid residues namely ASP-197, GLU-233 and ASP-300 which contribute to the overall catalytic action of the enzyme towards starch hydrolysis(Anigboro et al., 2021). ASP-197 acts as a catalytic nucleophile during starch hydrolysis,GLU-233 residue acts as acid–base catalyst, while ASP-300 plays the role of optimizing the orientation of the substrate(Anigboro et al., 2021).

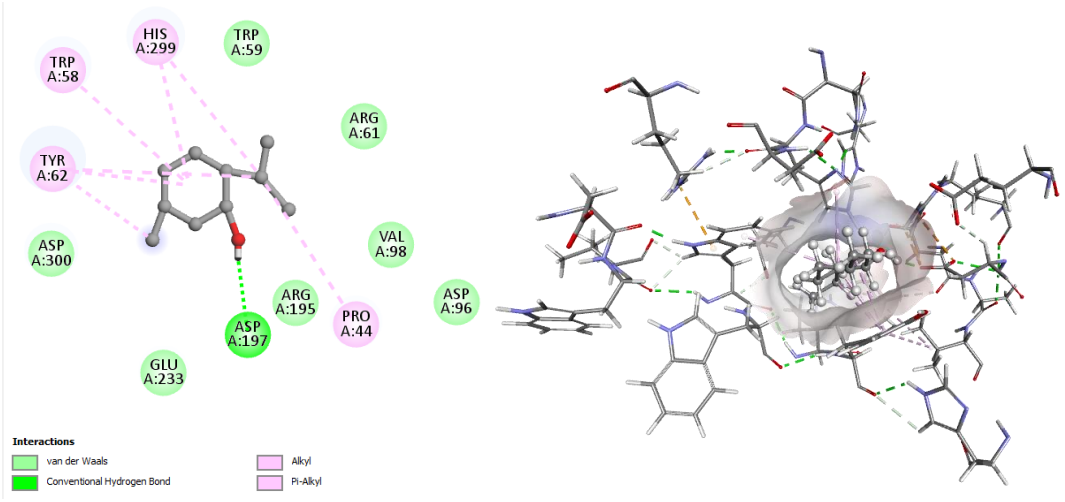
Table22. Docking score of the studied compounds

Compound	Rigid Docking Score (Kcal/mol)	IFD scores (Kcal/mol)
Perilla aldehyde	-5.569	-5.828
D-Limonene	-5.052	-5.794
alpha-Pinene	-5.033	-4.994
Pulegone	-4.944	-7.651
Menthone	-4.505	-7.069
trans-Sabinene hydrate	-4.365	-6.669
alpha-Terpineol	-4.185	-6.162
Borneol	-3.884	-5.636
Linalool acetate	-3.706	-5.094
1,8-Cineole	-2.986	-4.816
Acarbose	-3.213	-5.259

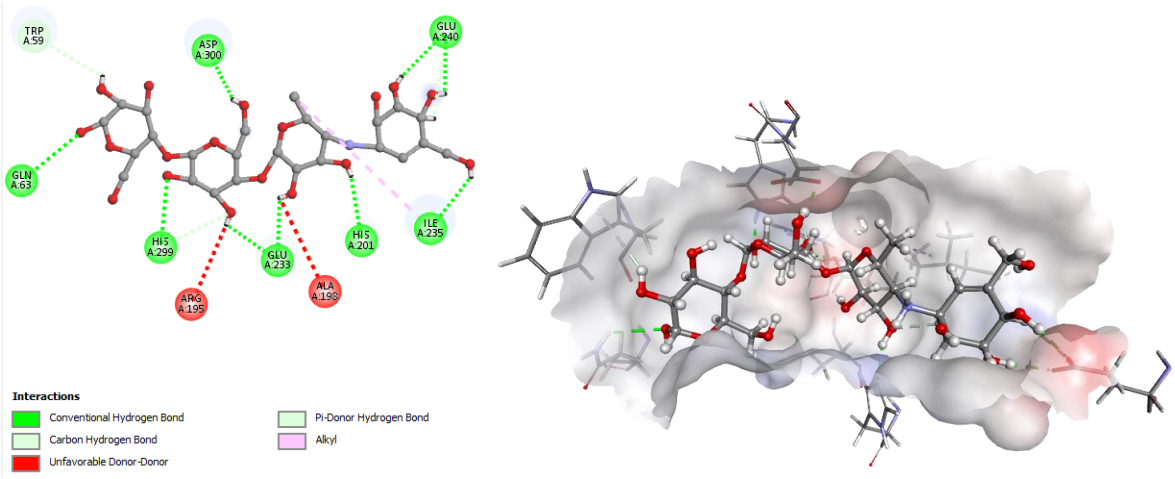
The results in Figure 35 revealed that the best two compounds in all the plants interacted with a similar set of amino acid residues at the active site of the protein just as the standard HPA inhibitor did. It is noticed in their interaction with the enzyme's key catalytic residues (ASP-197, GLU-233 and ASP-300). compoundPerilla aldehydein addition to the standard (acarbose) showed interaction with the three key amino acids involved in catalysis at HPA active site, whereas Pulegoneshowed interaction with two of the catalytic triads (GLU-197 and ASP-197). This perhaps denotes that the inhibitory effect contributed by Pulegoneto the overall inhibitory effect of the other compounds may be that of non-competitive inhibition whereas that of other compound (Perilla aldehydethe compound with the best binding affinity) may be that of competitive mode of inhibition like that of acarbose. Overall, the *in-silico* analysis of the ligand protein interaction of these compounds with amino acid residues present in the α-amylase active site domain (such as TRP-58, TRP-59, TYR-62, GLN-63, ASP-197, GLU-233, ASP-300 etc.) gave credence to the inhibitory effect of the compounds reported in this study. Furthermore, it also confirmed the possibility of the binding interaction between α-amylase and the phytoconstituents of *Ammudoucus leucotrichus* and*Mentha piperita* essential oilsinvestigated and observed using spectroscopic methods reported. The best ligand-binding poses in the catalytic domain of HPA obtained after docking as well as the amino acid residues involved in the interaction are as illustrated in Figure 35.



Docking complex and interaction plot for compound Perilla aldehydewith HPA



Docking complex and interaction plot for compound Pulegonewith HPA



Docking complex and interaction plot for Acarbose with HPA

Figure 35. Molecular interactions of studied compounds & Acarbose with humane pancreatic α-amylase

6.3. Molecular Dynamics Simulation:

Molecular Dynamics Simulation (MDS) investigations were conducted for the premier compound show the best IFD score, Pulegone, in complex with α-amylase. The principal objective of these MDS endeavours was to subject the ligand-receptor complex to physiological conditions, a feat unattainable through the confines of molecular docking (Dumitrică & James, 2007). Throughout the MDS protocol, a trajectory frame was generated at intervals of 100 picoseconds(Tu et al., 2008), accumulating a total of 1000 frames over the course of a 100-nanosecond simulation.Analysis encompassed metrics such as 'Root Mean Square Deviation (RMSD), and 'Root Mean Square Fluctuation (RMSF). Specifically, RMSD values were computed by aligning the frames of the protein and ligand-protein complex with the reference frame, respectively. These analytical approaches provide a detailed understanding of the structural stability and dynamic fluctuations exhibited by the Acarbose-α-amylase complex throughout the simulation period(Schreiner et al., 2012).

The Root Mean Square Deviation (RMSD) values for the complex consistently fall within the acceptable range of 0 – 2 Å. Examination of the analysed complex revealed no significant conformational alterations; however, an initial minor deviation was discerned in the vicinity of 8 – 20 nanoseconds, and a subtle drift was observed approximately between 45 – 55 nanoseconds. Beyond these periods, the complex demonstrated marked stability throughout the entirety of the simulation process, with no notable occurrence of major conformational changes.(Figure 36).

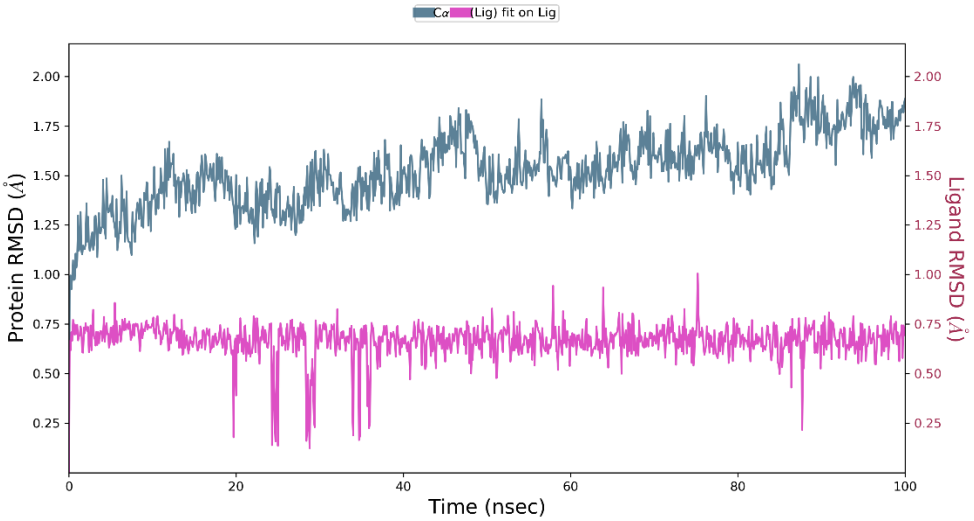


Figure 36.RMSD plot of trans- thujone and α_amylase complex

The Root Mean Square Fluctuation (RMSF) analysis provides insights into local variations along the protein chain, facilitating the identification of residues contributing to structural fluctuations within the complex(Dey et al., 2021). In the examined complex, the observed fluctuation variation remained below 3 Å. Major fluctuations (2.20-2.63 and 1.81-2.96Å) were observed in the C-terminal and loopregions (223-225 and 363-366), respectively, which are positioned awayfrom the binding pocket of alpha amylase. Except for the loop regions, the RMSFvalues of most residues are less than 1.5 Å, indicating that theresidue conformation is relatively stable during the simulation.A graphical representation of the RMSF plot depicting the specific ligand interactions with amino acid residues in the protein is presented in Figure 37.

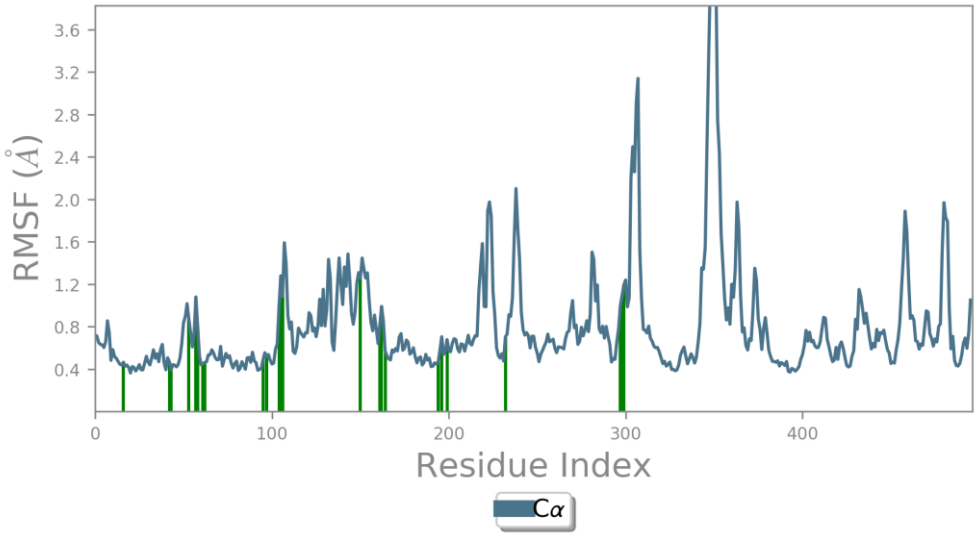


Figure 37.RMSF plot of trans- thujone and α_amylase complex

Within the investigated complex, notable hydrogen bond interactions were observed involvingHIS299, ASP197, and TRP58. Additionally, water-mediated hydrogen bond interactions were formed, encompassing the same residues, as visually depicted in Figure 38.

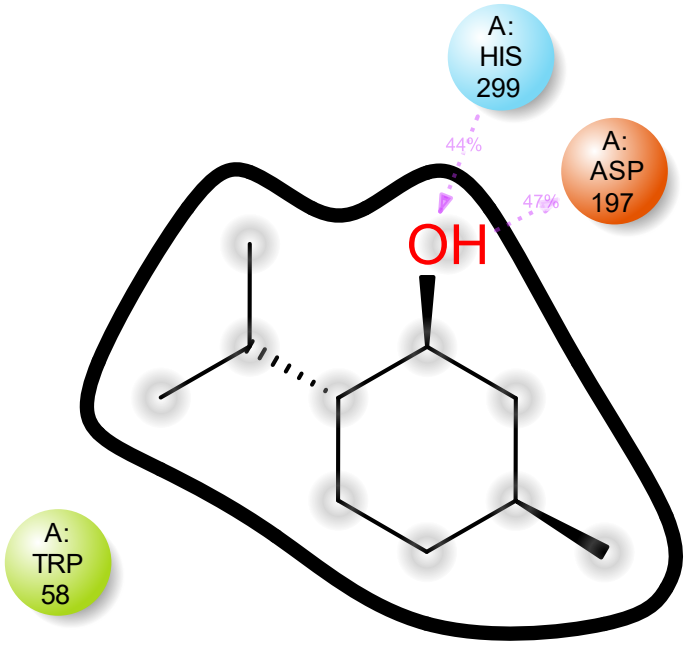


Figure 38. Interaction diagram of protein-ligand after MDS

The analysis of Protein-ligand contact serves to elucidate the temporal continuity of interactions between the ligand and protein amino acids throughout the simulation. A numerical representation is employed, where a value of 0.5 denotes that a specific interaction was sustained for 50% of the simulation duration. Conversely, a value exceeding 1 suggests that protein amino acids may establish multiple contacts of the same subtype with the ligand(Hatmal & Taha, 2017).

In the context of the investigated complex, the interaction values for the residues GLU240, HIS201, ASP197, GLN63, GLU233, and THR163 are recorded as 1.25, 1, 1.75, 1, 0.60, and 1.5, respectively. These values signify varying degrees of sustained interactions between the ligand and corresponding amino acid residues. The graphical representation of the protein-ligand contact histograms for the complex is depicted in Figure 39for visual elucidation.

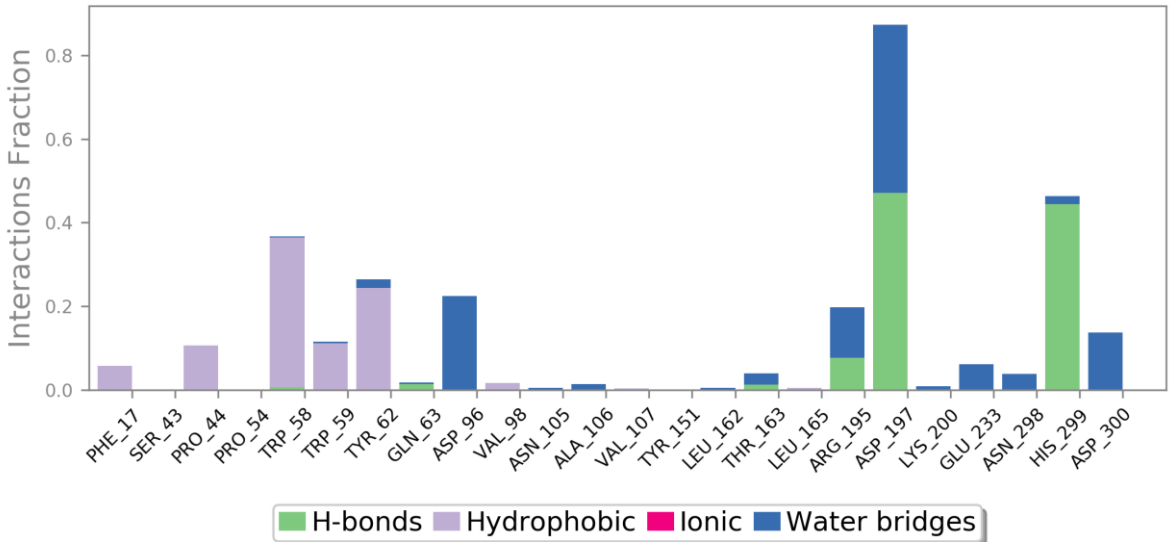


Figure 39. Histogram of protein-ligand complex

To comprehensively investigate the interactions between the ligand and alpha-amylase receptor, two supplementary panels were introduced by Schrödinger post Molecular Dynamics Simulation (MDS), as depicted in Figure 40. The upper panel provides insights into the total number of specific contacts established by the protein with the ligand throughout the trajectory. Conversely, the lower panel delineates the residues engaged in interactions with the ligand in each trajectory frame.

Notably, certain residues exhibit multiple specific contacts with the ligand, visualized through varying shades of orange on the plot, in accordance with the scale positioned to the right of the diagram. This nuanced representation captures the dynamic nature of the interactions, offering a detailed portrayal of the residues that consistently contribute to the ligand-receptor interface. The incorporation of these supplementary panels enhances the granularity of our understanding of the molecular dynamics and specific contacts governing the ligand-receptor interaction over the course of the simulation.

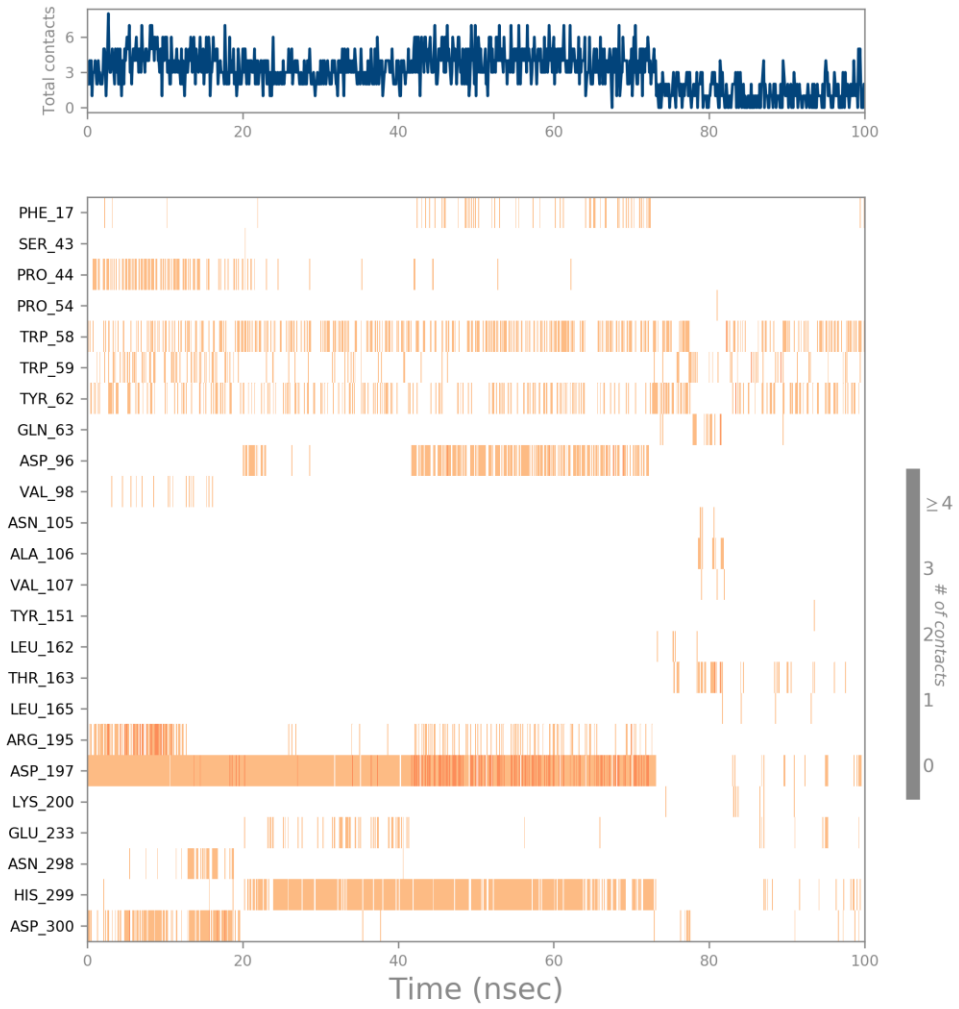


Figure 40. Details of the protein ligand contact

6.4. Free Energy (MM-GBSA) Calculation:

To evaluate the binding efficacy of the preeminent compound, trans- thujone, with the alpha-amylase protein, the Prime-MM-GBSA method was applied to compute the binding free energies. The establishment of stable complexes is evidenced by favourable binding interactions between the compound and the protein. The determination of binding free energy entails the consideration of various energy components, encompassing van der Waals (vdW), lipophilic (lipo), Generalized Born electrostatic solvation (Solv GB), Coulomb, and hydrogen-bonding (hbond) energies. The discrete contributions of these energy components to the overall binding free energy of the trans- thujone-alpha-amylase complex are delineated in Table 23.

Table 23. Energy components of the studied complex

Component	Energy (kcal/mol)
ΔG Bind	-61.14
ΔG Bind Coulomb	-30.03
ΔG Bind Solv GB	48.10
ΔG Bind vdW	-54.91
ΔG Bind lipo	-20.51
ΔG Bind hbond	-3.79

Notably, ΔG Bind is calculated at -61.14 kcal/mol, indicating a robust favourable binding interaction.Individual positive contributions include Coulombic energy at -30.03 kcal/mol, van der Waals interactions at -54.91 kcal/mol, lipophilic energyat -20.51 kcal/mol, and hydrogen-bonding energy at -3.79 kcal/mol. However, solvation energy through Generalized Born model contributed negatively at 48.10 kcal/mol. This collective interplay of energy components underscores the strong binding affinity observed in the complex.

CONCLUSION

Conclusion:

In recent years, scientific research has been interested in plant compounds intended for use in the field of plant protection. Molecules from the called natural plants are a very important source of medicines.

This thesis focused on the characterization of both the *Ammudaucus leucotrichus* and *Mentha piperita* plants, and its biological activity in the organism and in the in vitro in addition to predicting within in silico by molecular docking and molecular dynamics.

The results showed that Essential oilsof two plants(*MP, AL*) has many interesting biological activities, including antidiabetics, making it a potentially beneficial compound for medicine and pharmacy.It is clear from this work that after injection into the mice (wistar albinos) by aloxan as a result of the induction of diabetes, which is later treated by essential oils from the two medicinal plants (*MP, AL*), this revealed the anti-hyperglycemic effect through a regenerative effect on Langerhans cells leading to an improvement in insulin secretion.

A phytochemical study conducted revealed that the qualitative dose of the two plants shows this richness in polyphenols, flavonoids, tannins, saponins and terpenoids that can be at the origin of the regenerative effect of plants and thus their anti-diabetic effect.Laboratory tests have revealed that essential oils for both medicinal plants (*MP, AL*) have significant anti-diabetic activity by inhibiting alpha-amylase activity.Molecular docking tests within the *in-silico* confirmed the results of in vitro tests by showing that compounds of two plant essential oils (*MP, AL*) bind to the active sites of the enzyme involved in diabetes.

Toxicity prediction have shown that the essential oil compounds of both *Ammudaucus leucotrichus* and *Mentha piperita* are not toxic, enhancing its potential as a therapeutic compound. The results of this study could help guide future research on its use as a potential drug to treat various diseases.

Perspective

The results of this study opened new research prospects on the use of *MP, AL* essential oils for the treatment of diseases such as diabetes. Further studies can be conducted to better understand the basic molecular mechanisms of the biological activity of oils, including the effect on other enzymes involved in these diseases.

In addition, the study of the structure of essential oils (*MP, AL*) can be enhanced to improve its vital activity by designing their more effective isotopes. Silicon methods can be used to predict the biological activity of these isotopes before they are synthesized and tested in the laboratory. Clinical studies may also be considered to assess their effectiveness in humans and to create an ideal therapeutic dose.

Finally, since the study and some other studies also revealed that the essential oils of the plants *Ammudaucus leucotrichus* and *Mentha piperita* are able to use them against other diseases such as cancer, infections, bacteria ... So, it will be very interesting to discover and develop it.

REFERENCES

References:

References Bibliography

- Abderrezag, N., Sánchez-Martínez, J. D., Louaer, O., Meniai, A. H., & Mendiola, J. A. (2021). Optimization of pressurized liquid extraction and in vitro neuroprotective evaluation of Ammodaucus leucotrichus. Untargeted metabolomics analysis by UHPLC-MS/MS. *Molecules*, *26*(22), 6951.
- Aboughe Angone, S., Aworet Samseny, R.R.R., et Eyele Mve Mba, C. (2015). Quelques propriétés des huiles essentielles des plantes médicinales du Gabon. Phytothérapie, 13: 283–287.
- Ahmed, N., Singh, J., Chauhan, H., Anjum, P. G. A., & Kour, H. (2013). Different drying methods: their applications and recent advances. *International Journal of food nutrition and safety*, *4*(1), 34-42.
- Alais , C ; Linden , G ; Miclo , L . (2008). Production and characterization of alpha amylase.Biochimie alimentaire .(6) :67-71
- Alexis, Guerin-Dubourg. (2014) Etude des modifications structurales et fonctionnelles del'albumine dans le diabète de type 2 : identification de bio marqueurs de glycoxydation et defacteurs de risque de complications vasculaires. Thèse de doctorat: biochimie. AmphithéâtreCharpak, Saint Denis : université de la Réunion, 170 .
- AMED, S. & ORAM, R. 2016. Maturity-Onset Diabetes of the Young (MODY): making the right diagnosis to optimize treatment. Canadian journal of diabetes, 40, 449- 454.
- Andrali SS, Sampley ML, Vanderford NL, Ozcan S. Glucose regulation of insulin gene expression in pancreatic beta-cells. Biochem. J, 2008, 415:1–10.
- Arbouche, Z., Lezzar, A., Salah-Mansour, A., & Zinai, S. (2012). Le transfert des insulines humaines vers les analogues de l'insuline entraîne une amélioration de l'HbA1c et une réduction des hypoglycémies chez les patients diabétiques de type 2: données de la cohorte algérienne de l'étude A1chieve. Médecine des Maladies Métaboliques, 6(6), 511-518.
- Arfa L., Abid A., Kéfi R., Nouira S. (2008). Base génétique du diabète. XI éme congrès de la Société Tunisienne de médecine interne .www.stmi.org.tn. Janvier 2011.
- Ashok, P. K., & Upadhyaya, K. (2012). Tannins are astringent. *Journal of pharmacognosy and phytochemistry*, *1*(3), 45-50.

- Asofiei, I., Calinescu, I., Gavrila, A. I., Ighigeanu, D., Martin, D., & Matei, C. (2017). Microwave hydrodiffusion and gravity, a green method for the essential oil extraction from ginger-energy considerations. *University Politehnica of Bucharest Scientific Bulletin Series B: Chemistry and Materials Science*, *79*(4), 81-92.
- Bakkali, F., Averbeck, S., Averbeck, D., and Idaomar, M. (2008). Biological effects of essential oils. Rev. Food Chem. Toxicol, 46: 446–475.
- Banner , D.W , Bloomer , A, C, Petsko , G.A, Phillips ,D,C, Pogson , C,I, Wilson , I. A.....et Priddle , J.D. (1975). Structure of chicken muscle triose phosphate isomerase determined crystallographically at 2.5 A resolution : using amino acid sequence data .Nature , 255(5510), 609- 614.
- Benjamim, J. K. F., da Costa, K. A. D., & Santos, A. S. (2021). Chemical, botanical and pharmacological aspects of the Leguminosae. *Pharmacognosy Reviews*, *14*(28), 106-120.
- Belabbes R., et Halaoune H., (2019): Etude épidémiologique du diabète de type I dans la wilaya de Médéa. Université de DR Yahia Fares de Médéa, pp: 3-7.
- Benyounes A., Boucena R., et ChiroufA., (2021): Prévalence de l'obésité abdominale et du syndrome métabolique chez les diabétiques à Guelma. Université de Guelma, pp: 25-27.
- Bergman R.L. et Mittelman S.D.,(1998). Central role of the adipocyte in insulin resistance. J Basic Clin Physiol Pharmacol. 9(2-4): 205-21.
- Beyene, B., Beyene, B., & Deribe, H. (2016). Review on application and management of medicinal plants for the livelihood of the local community. *Journal of Resources Development and Management*, *22*(1), 33-39.
- Bischoff, H. (1991) Effect of Acarbose on Diabetic Late Complications and Risk Factors – New Animal Experimental Results. Akt Endokr Stoffw 12, 25-32.
- Blamey, M. & Grey-Wilson, C. Flora of Britain and Northern Europe 1989
- Blickle,J. Attali, Z. Barrou, P. Brocker, N. De rekeneire, C. Verny, M. Leutenegger. (1999). Le diabète du sujet âgé. Diabète et Métabolisme, 25, 84-93.
- Bozyel, M. E., Merdamert-Bozyel, E., Benek, A., Canlı, K., Altuner, E. M., Christov, I., ... & Efe, R. (2020). Ethnomedicinal uses of colchicaceae and Liliaceae taxa in Turkey. *Advances in Scientific Research: Engineering an d Architecture. St. Kliment Ohridski University Press, Sofia*, 649-660.
- Bruneton, J. (1999). Pharmacognoise. Phytochimie, plantes médicinales. Ed: TEC et DOC. Lavoisier.

- Burhan A, Nisa U , Gokhan C , Omer C , A shabil A , Osmanc G. (2003). Enzymatic properties of novel thermostable , thermophilic , alkalineand chelator resistant amylase from an alkaliphic Bacillus sp . Isolate ANT-6 . Process Biochem .38 , p: 1397 -1403.
- Cakaloglu, B., Ozyurt, V. H., & Otles, S. (2018). Cold press in oil extraction. A review. *Ukrainian food journal*, (7, Issue 4), 640-654.
- Campbell JE, Newgard CB. Mechanisms controlling pancreatic islet cell function in insulin
- Capeau J .Voies de signalisation de l'insuline: mécanismes affectés dans l'insulino-résistance . Médecine Sciences .2003, 19, (8–9): 834–839.
- Carette, A.S. (2000). La lavande et son huile essentielle. In Besombes C., 2008. Contribution à l'étude des phénomènes d'extraction hydrothermomécanique d'herbes aromatiques. Applications généralisées. Thèse de doctorat. Université de La Rochelle, 289p.
- Carović-StanKo, K., PeteK, M., Grdiša, M., Pintar, J., Bedeković, D. A. L. I. B. O. R., Herak Ćustić, M., & Satovic, Z. (2016). Medicinal plants of the family Lamiaceae as functional foods-a review. *Czech journal of food sciences*, *34*(5), 377-390.
- Chandra, S., & Rawat, D. S. (2015). Medicinal plants of the family Caryophyllaceae: a review of ethno-medicinal uses and pharmacological properties. *Integrative Medicine Research*, *4*(3), 123-131.
- Chatelain, C., Chambouleyron, M., Charrier, M., Léger, J. F., & Perret, M. (2021). Révision du genre Ammodaucus (Apiaceae) en Afrique du Nord. *Candollea*, *76*(2), 191-200.
- Chevenne, D., & Fonfréde, M. (2001). Actualité sur les marqueurs biologiques du diabète. Immunoanal. Biol. Spec. 16. P 215-229.
- Chiba S . (1988). Amyl glycosidase . In . Handbook of Amylases and related enzymes (The Amylase Research Society of Japan ,éd).Pergamum Press , Oxford , U.K,pp104-1
- COSSETTE, J. 2001. Impact précoce du diabète sur la fonction des neurones glutamatergiques du cerveau. Université du Québec à Trois-Rivières.
- Couic-Marinier, F., and Lobstein, A. (2013). Composition chimique des huiles essentielles. Actual. Pharm, 52: 22–25.
- D.C, Lowe M.E. (2007). "Human pancreatic digestive enzymes " .Digestive Diseases and Sciences , 52 , p.1-17

- Das, U., & Islam, M. S. (2019). A review study on different plants in Malvaceae family and their medicinal uses. *Am. J. Biomed. Sci. Res*, *3*(2), 94-97.
- Dauter, Z. Dauter, M. Brzozowski, .A.M. Christensen, .S. Borchert, T.V, Beier, .L Wilson Delluc G. Delluc B. & Roques M. 2003. - La nutrition prehistorique. In Ellipses. p. 223
- Defdaf D., et Benkahoul L., (2019): Contribution à l'Etude De L'effet Antidiabétique D'un Extrait Aqueux De Deux Plantes (Oppuntia ficus indica L. et Nigellasativa L.) Issue De La Région De –Batna- Sur Des Souris (Wistar albinos) Diabétiques à L'Alloxane. Université Mohamed Khider de Biskra, p: 3.
- Degryse, A.C., Delpla, I., et Voinier M.A. (2008). Risques et bénéfices possibles des huiles essentielles. Atelier santé environnement -IGS- EHESP, 87p.
- Desmares, C., Laurent, A., et Delerme C. (2008). Recommandations relatives aux critères de qualité des huiles essentielles. AFSSAPS. Anatole, France, 18p.
- Deshmukh, D., Baghel, V. S., Shastri, D., Nandini, D., & Chauhan, N. S. (2010). Plants as bitter. *International Journal of Advances in Pharmaceutical Sciences*, *1*(4).
- DIABETES CANADA CLINICAL PRACTICE GUIDELINES EXPERT, C., WHERRETT, D. K., HO, J., HUOT, C., LEGAULT, L., NAKHLA, M. & ROSOLOWSKY, E. 2018. Type 1 Diabetes in Children and Adolescents. Canadian journal of diabetes, 42 Suppl 1, S234-S246.
- Diabetologia. 1984;26(4):297-299. doi:10.1007/bf00283653.
- Díaz-Reinoso, B., Rivas, S., Rivas, J., & Domínguez, H. (2023). Subcritical water extraction of essential oils and plant oils. *Sustainable Chemistry and Pharmacy*, *36*, 101332.
- Duband F, Carnat AP, Carnat A et al. [Aromatic and polyphenolic composition of infused peppermint, Mentha piperita L.]. Ann Pharm Fr.;50:146-155. 1992
- Duparc Thibaut .(2012).Communication inter-organes dans le contrôle du métabolisme glucidique : Mise en évidence de l'implication du monoxyde d'azote et de l'apeline dans l'hypothalamus.Thèse de doctorat. Université Toulouse III - Paul Sabatier. P 7,8,11, 17,18,21,24.
- EL HACI, I. A., MAZARI, W., & Fawzia, A. B. (2021). Effect of Ammodaucus leucotrichus Coss. & Dur. Essential Oil on the Viability of Erythrocytes and its Antiradical Activity Assessment. *Journal of Natural Product Research and Applications*, *1*(02), 45-53.

- Elliott RB, Martin JM. Dietary protein: a trigger of insulin-dependent diabetes in the BB rat?
- EMEA. (2006). Guideline on Good Agricultural and Collection Practice (GACP) for Starting Materials of Herbal Origin.
- Ferré, P. (2005).Action et sécrétion de l'insuline : double jeu pour les canaux potassiques. Médecine/ Sciences, 21 (8-9) : 694-6.
- Fery ,F., et Paquot, N. (2005). Etiopathogènie et physiopathologie du diabète de type 2.Revue Médicale de Liège ,60 (5) : 361-368.
- Filly, A., Fernandez, X., Minuti, M., Visinoni, F., Cravotto, G., & Chemat, F. (2014). Solvent-free microwave extraction of essential oil from aromatic herbs: From laboratory to pilot and industrial scale. *Food chemistry*, *150*, 193-198.
- Gamouh Ch. et Kedissa S. , (2015), Etude comparative des différents paramètres biochimiques chez les diabétiques de type 1 et de type 2 , Mémoire présenté en vue de l'obtention du Diplôme de master, Université des Frères Mentouri Constantine, P 1-5 .
- Ganong ,W. (2005).Physiologie médicale. De Boeck Supérieur.849 p.
- Gavahian, M., Chu, Y. H., & Mousavi Khaneghah, A. (2019). Recent advances in orange oil extraction: An opportunity for the valorisation of orange peel waste a review. *International Journal of Food Science & Technology*, *54*(4), 925-932.
- Gavahian, M., Farhoosh, R., Farahnaky, A., Javidnia, K., & Shahidi, F. (2015). Ohmic-assisted hydrodistillation of essential oils from Mentha piperita: a comparison with conventional hydrodistillation. *Iranian Food Science and Technology Research Journal*, *11*(3), 236-246.
- Gianfrancesco F,Ivan C,Giovanni C,Matteo F,Angela I,Mario P,Antonello ERi,Alessandro S,and Silvano GC,.2020.Current Pharmacological Treatment of Type 2 Diabetes Mellitus in Undocumented Migrants: Is It Appropriate for the Phenotype of the Disease?, Int J Environ Res Public Health, 17(21): 8169,.
- Gourdy P. (2013). Diabetes and oral contraception. Best Pract Res Clin Endocrinol Metab.27(1): 67-76.
- Guendouz A., et Hamza Zerigat I., (2018): Etude comparative des différents paramètres lipidiques chez les diabétiques de type 1 et 2 et enquête ethnobotanique des plantes médicinales antidiabétiques dans l'ouest Algérien. Université Abdelhamid Ibn Badis-Mostaganem, pp: 5-10.

- HADJ, B .H et BENBELAL, O.(2020). Study of the antidiabetic effect of the aqueous extract of the leaves of Moringa oleifera L. in Wistar rats made diabetic by streptozotocin. Master's thesis. Université de Ghardaïa. P20.
- 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
- Halla N, Heleno SA, Costa P, Fernandes IP, Calhelha RC, Boucherit K, Rodrigues AE, Ferreira IC, Barreiro MF. Chemical profile and bioactive properties of the essential oil isolated from Ammodaucus leucotrichus fruits growing in Sahara and its evaluation as a cosmeceutical ingredient. Ind Crop Prod. 2018;119:249–54.
- Halla, N., Heleno, S. A., Costa, P., Fernandes, I. P., Calhelha, R. C., Boucherit, K., ... & Barreiro, M. F. (2018). Chemical profile and bioactive properties of the essential oil isolated from Ammodaucus leucotrichus fruits growing in Sahara and its evaluation as a cosmeceutical ingredient. *Industrial crops and products*, *119*, 249-254.
- Hamdy ,K. ,Kwang, H B.,(2023). New Insights into the Latest Advancement in α-Amylase Inhibitors of Plant Origin with Anti-Diabetic Effects ,Department of Biotechnology,Yeungnam University, Gyeongsan 38541, Republic of Korea,12(16), 2944.
- Hao, D. C., Xu, L. J., Zheng, Y. W., Lyu, H. Y., & Xiao, P. G. (2022). Mining therapeutic efficacy from treasure chest of biodiversity and chemodiversity: Pharmacophylogeny of Ranunculales medicinal plants. *Chinese Journal of Integrative Medicine*, *28*(12), 1111-1126.
- Hassan, H. M. A. (2015). A short history of the use of plants as medicines from ancient times. *Chimia*, *69*(10), 622-622.
- Hedges, L. J., & Lister, C. E. (2007). Nutritional attributes of herbs. *Crop and Food Research Confidential Report*, (1891), 1-89.
- 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.
- Herro E, Jacob SE. Mentha piperita (peppermint). Dermatitis. 2010 Nov-Dec;21(6):327-9. PMID: 21144345.
- Hussain, Z., Munir, A., Liu, J., & Sultan, M. (2022). Experimental study on solar distillation system for oil extraction from eucalyptus plant leaves. *Thermal Science*, *26*(5 Part A), 3897-3909.

- Idm'hand, E., Msanda, F., & Cherifi, K. (2020). Medicinal uses, phytochemistry and pharmacology of Ammodaucus leucotrichus. *Clinical Phytoscience*, *6*(1), 6.
- İZOL, E., YAPICI, İ., & TARHAN, A. (2023). Medicinal Aromatic Plants and Phytochemicals from the Perspective of Biochemistry. *Medicinal, Aromatıc Plants and Phytochemicals, ed. İzol E., Yılmaz MA, Haspolat YK, Orient Publications*, 1-10.
- Jadhav, J. J., Jadeja, G. C., & Desai, M. A. (2023). Ultrasound-assisted hydrodistillation for extraction of essential oil from clove buds–a step towards process improvement and sustainable outcome. *Chemical Engineering and Processing-Process Intensification*, *189*, 109404.
- Jeans Louis B. , Geneviève D. , (2011), biochimie médicale marqueurs actuels et perspectives , 2ème édition , P 214,146
- Joy, P. P., Mathew, S., & Skaria, B. P. (1998). Kerala Agricultural University Aromatic and Medicinal Plants Research Station Odakkali. Asamannoor PO Ernakulam District, Kerala, India.
- Kaluwa, K., & Kruger, E. (2012). Natural pest and disease control handbook. *KwaZulu-Natal Department of Agriculture and Rural Development Private Bag X9059 Pietermaritzburg*, *3200*.
- Kara S., et Hammadi D., (2021): Synthèse bibliographique sur la physiopathologie du diabète. Universite Mouloud Mammeri de Tizi Ouzou, p: 18. Benguessoum R., Khaled S., et Doubali B., (2021): Les caractéristiques physiologiques et pathologiques du diabète type 1 et type 2.Université des Frères Mentouri Constantine, pp: 24 - 47.
- Kaul, K., Tarr, J.M., Ahmad, S.I., Kohner, E.M., Chibber, R. (2013). Introduction to Diabetes Mellitus. In: Ahmad, S.I. (eds) Diabetes. Advances in Experimental Medicine and Biology, vol 771. Springer, New York, NY. https://doi.org/10.1007/978-1-4614-5441-0_1
- Kebir. N ;Aichouni .A . (2018) . Mémoire doctorat. Propriétés de lait de chamelle cru sur les profils glucidique et lipidique des rats wastar rendus diabétique par l'alloxane
- Khaldi A, Meddah B, Moussaoui A, Sonnet P. Anti-mycotoxin effect and antifungal properties of essential oil from Ammodaucus leucotrichus Coss. & Dur. On Aspergillus flavus and Aspergillus ochraceus. J Essent Oil Bear Pl. 2017;20(1):36–44.
- Khan, M. Q., & Shinwari, Z. K. (2016). The ethnomedicinal profile of family Rosaceae; a study on Pakistani plants. *Pak. J. Bot*, *48*(2), 613-620.

- Khia, A., Ghanmi, M., Satrani, B., Aafi, A., Aberchane, M., Quaboul, B., Chaouch, A., Amusant, N., et Charrouf, Z. (2014). Effet de la provenance sur la qualité chimique et microbiologique des huiles essentielles de Rosmarinus officinalis L. du Maroc. Phytothérapie, 12: 341–347.
- Klein M .2009. Relations entre le diabète sucré de type 2 et l'amyloidose chez le .Thèse d'état en vitrine .Univ de Toulouse, France.17-88.
- Knip M., Virtanen S., Seppa K., Llonen J. (2010). Dietary Intervention in Infancy and Later Signs of Beta-Cell Autoimmunity. N Engl J Med; 363: 1900-8.
- Kukreja A., Maclaren NK.(2002). NKT Cells and Type-1 diabetes and the "Hygiene Hypothesis" to explain the rising incidence rates diabetes. Technology & Therapeutics., 4(3): 323- 33.
- Langlois A., (2008). Optimisation de la revascularisation des ilots pancréatiques au cours de la transplantation, approche génétique ou pharmacologique ? Thèse Doctorat en sciences de la vie et santé. Université Louis Pasteur. Strasbourg. France.
- Linnaeus, C. Species Plantarum 2: 576–577. 1753
- Louail, Z.; Kameli, A.; Benabdelkader, T.; Bouti, K.; Hamza, K.; Krimat, S. Antimicrobial and antioxidant activity of essential oil of Ammodaucus leucotrichus Coss. & Dur. Seeds. J. Mater. Environ. Sci., 2016, 7, 2328-2334.
- Lubbe, Andrea, and Robert Verpoorte. "Cultivation of medicinal and aromatic plants for specialty industrial materials." Industrial crops and products 34.1 (2011): 785-801.
- lucette couderc V(2001),Toxicite des huiles essentielles,l'université paul-sabatier de toulouse, p :3-55 .
- Machado, C. A., Oliveira, F. O., de Andrade, M. A., Hodel, K. V. S., Lepikson, H., & Machado, B. A. S. (2022). Steam distillation for essential oil extraction: An evaluation of technological advances based on an analysis of patent documents. *Sustainability*, *14*(12), 7119.
- Magnan C, Ktorza A. (2005).Production and secretion of insulin by the pancreatic β-cell. EMC Endocrinologie. 2: 241-264.
- Makhlouf S., Chahboube S. (2015). Evaluation des factures de risque chez les diabétiques au niveau de Ain defla. Mémoire de Master. Régulation endocriniennes et physiopathologies. Khemis Meliana: UniversitéEl djilali Bouamama de Khemis Meliana, 58 p.

- Malik, A. A., Sanghmitra Suryapani, S. S., & Javed Ahmad, J. A. (2011). Chemical vs organic cultivation of medicinal and aromatic plants: the choice is clear.
- Marshall, S.M. The pancreas in health and in diabetes.Diabetologia.,2020 , 63 :1962–1965.
- MAYOU, N. S., MEDJOURI, M., & YOUMBAI, A. *Activités biologiques des polysaccharides hydrosolubles d'Ammodaucus leucotrichus (Sahara septentrional Algérien)* (Doctoral dissertation).
- Mekhalfa S., et Assala F., (2018): Evaluation de l'activité antidiabétique et inhibitrice de l'alpha amylase pancréatique et de la xanthine oxydase de la plante Fraxinus Angustifolia de la région de Béjaia.Université Mohammed Seddik Ben Yahia – Jijel, pp: 4-10.
- Mentha piperita- Peppermint - Flora of Northwest Europe". 2014. Retrieved 29 December 2014
- MH, S. K., & Srinivas, M. P. (2018). Pests attacking medicinal and aromatic plants in India: a review. *Journal of Entomology and Zoology Studie*, *6*(5), 201-205.
- Miloradovich, M. (2012). *Growing and using herbs and spices*. Courier Corporation.
- Mimica-Dukić N, Bozin B, Soković M, Mihajlović B, Matavulj M. Antimicrobial and antioxidant activities of three Mentha species essential oils. Planta Med. 2003 May;69(5):413-9. doi: 10.1055/s-2003-39704. PMID: 12802721.
- Mohammedi, H., Idjeri-Mecherara, S., Menaceur, F., Azine, K., & Hassani, A. (2018). Chemical compositions of extracted volatile oils of Ammodaucus leucotrichus L. fruit from different geographical regions of Algeria with evaluation of its toxicity, anti-inflammatory and antimicrobial activities. *Journal of Essential Oil Bearing Plants*, *21*(6), 1568-1584.
- MOHN, A., KAVAN, C., BOURCELOT, E., ZIMMERMANN, C. & PENFORNIS, A. 2012. Insulinothérapie fonctionnelle: un modèle d'approche éducative pour les patients ayant un diabète de type 1. Médecine des maladies Métaboliques, 6, 469-476.
- Moradi, S., Fazlali, A., & Hamedi, H. (2018). Microwave-assisted hydro-distillation of essential oil from rosemary: Comparison with traditional distillation. *Avicenna journal of medical biotechnology*, *10*(1), 22.
- Murti, Y., Jain, D., Semwal, B. C., Sıngh, S., Janmeda, P., & Bhaskar, P. (2023). Innovative methods for extraction of essential oils from medicinal plants. *International Journal of Secondary Metabolite*, *10*(2), 190-230.

- Murti, Y., Jain, D., Semwal, B. C., Sıngh, S., Janmeda, P., & Bhaskar, P. (2023). Innovative methods for extraction of essential oils from medicinal plants. *International Journal of Secondary Metabolite*, *10*(2), 190-230.
- Narendhirakannan RT, Subramanian S, Kandaswamy M. Biochemical evaluation of antidiabetogenic properties of some commonly used Indian plants on streptozotocin-induced diabetes in experimental rats. Clin Exp Pharmacol Physiol. 2006 Dec;33(12):1150-7. doi: 10.1111/j.1440-1681.2006.04507.x. PMID: 17184494.
- Nazare, J., Normand, S., Oste Triantafyllou, A, et al. (2008).Modulation of the postprandial phase by beta-glucan inoverweight subjects: effects on glucose and insulin kinetics. Molecular Nutrition and Food Research, 53: 361–369.
- Neghliz-Benabdelkader, H., Benabdelkader, T., Halladj, F., & Jullien, F. (2021). Characterization of the chemical diversity in essential oils from vegetative and reproductive organs of Ammodaucus leucotrichus subsp. leucotrichus Coss. & Dur. growing in Algeria. *Journal of Essential Oil Bearing Plants*, *24*(1), 75-85.
- Nouadri, T. 2011. L'α-amylase de Penicillium camemberti PL21 : Production, Purification, Caractérisation et Immobilisation. Diplôme de Doctorat, Université Mentouri, Constantine, p 19.
- Panda, M., Kumar, S., & Mahalik, G. (2020). An overview of medicinal plants of the family Rutaceae as a source of complementary therapeutics. *Journal of Biodiversity and conservation*, *3*(4), 13-14.
- Pandey, A., & Savita, R. (2017). Harvesting and post-harvest processing of medicinal plants: Problems and prospects. *The Pharma Innovation Journal*, *6*(12), 229-235.
- Perlemuter L et Collinde L'hortet G.(2002). Diabète et maladies métaboliques, Edition Masson, 3 édition Paris: 196- 197.
- Pia R.,et Bingbing W.,etYixian L.,et Weiping H .,2016.Pancreatic regulation of glucose homeostasis, 48(3):e219,DOI: 10.1038/emm.2016.6 :CC BY-NC-ND 4.0
- Pillot B , Soty M., Gautier-Stein A., Zitoun C. and Mithieux G.(2009). Protein feeding promotes redistribution of endogenous glucose production to the kidney and potentiates its suppression by insulin. Endocrinology. 150(2): 616-24.
- Porwal, O., Singh, S. K., Patel, D. K., Gupta, S., Tripathi, R., & Katekhaye, S. (2020). Cultivation, collection and processing of medicinal plants. *Bioactive Phytochemicals: Drug Discovery to Product Development*, *17*, 14-30.

- QUINTANILLA RODRIGUEZ, B. S. & MAHDY, H. 2019. Gestational Diabetes, StatPearls Publishing, Treasure Island (FL).
- Rasool Hassan, B. A. (2012). Medicinal plants (importance and uses). *Pharmaceut Anal Acta*, *3*(10), 2153-435.
- Rassem, H. H., Nour, A. H., & Yunus, R. M. (2016). Techniques for extraction of essential oils from plants: a review. *Australian Journal of Basic and Applied Sciences*, *10*(16), 117-127.
- Redouan, F. Z., Benítez, G., Picone, R. M., Crisafulli, A., Yebouk, C., Bouhbal, M., ... & Merzouki, A. (2020). Traditional medicinal knowledge of apiaceae at talassemtane national park (northern Morocco). *South African Journal of Botany*, *131*, 118-130.
- Riant E. (2009). Effets protecteurs des oestrogenes sur l'insulino-resistance et le diabete de type 2.Thèse de Doctorat en Médecine. Université Toulouse III - Paul Sabatier.P40.
- Romero-Jiménez M, Campos-Sánchez J, Analla M, Muñoz-Serrano A, Alonso-Moraga A. Genotoxicity and anti-genotoxicity of some traditional medicinal herbs. Mutat Res. 2005 Aug 1;585(1-2):147-55. doi: 10.1016/j.mrgentox.2005.05.004. PMID: 16005256.
- Rydberg, J. (Ed.). (2004). *Solvent extraction principles and practice, revised and expanded*. CRC press.
- Sandasi, M., Leonard, C. M., Van Vuuren, S. F., & Viljoen, A. M. (2011). Peppermint (Mentha piperita) inhibits microbial biofilms in vitro. *South African Journal of Botany*, *77*(1), 80-85.
- Safarov, J. E., & Khonboev, F. Z. (2016). Technology of convection drying of medicinal plants. *World science*, *1*(3 (7)), 41-42.
- Saju, F., & Sivaraman, C. M. (2021). Scope of herbal mucilage in pharmaceutical formulations. A review. *Herba Polonica*, *67*(1), 46-57.
- SAPRA, A., VAQAR, S. & BHANDARI, P. 2019. Diabetes Mellitus, StatPearls Publishing, Treasure Island (FL).
- Sarian MN, Ahmed QU, So'ad ZM, et al."Antioxidant and Antidiabetic Effects of Flavonoids: A Structure-Activity Relationship Based Study", BioMed Research International, vol. 2017, Article ID 8386065, 14 pages, doi.org/10.1155/2017/8386065.
- SCHEEN, A.-J. 2015. Antidiabétiques oraux dans le traitement du diabète de type 2: perspectives historique et médico-économique. Médecine des maladies Métaboliques, 9, 186-197.

- SCHEEN, A.-J. 2015. Antidiabétiques oraux dans le traitement du diabète de type 2: perspectives historique et médico-économique. Médecine des maladies Métaboliques, 9, 186-197.
- Schomburg, D and Salzmann, M. 1991. Enzyme Hand book 4. Classe 3: Hydrolases Springer- Verlag (ed). Berlin Heidelberg. Germany. P : 1-12.
- secretion. Nature Reviews Molecular Cell Biology. févr 2021;22(2):142-58
- Shah, B. N. (2009). *Textbook of pharmacognosy and phytochemistry*. Elsevier India.
- Shankar, S., Segaran, G., Sundar, R. D. V., Settu, S., & Sathiavelu, M. (2019). Brassicaceae-A classical review on its pharmacological activities. *Int. J. Pharm. Sci. Rev. Res*, *55*(1), 107-113.
- Simon Nicolas.(2016). Modulation de l'homéostasie glucidique par transfert de microbiote intestinal chez la souris conventionnelle. Thèse de doctorat. Université Toulouse 3 Paul Sabatier (UT3 Paul Sabatier).P : 43 ,44.
- Singh, C. (1990). 2R-Hydroxymicromeric acid, a pentacyclic triterpene from Terminalia chebula. Phytochemistry, 29: 2348-2350.
- Singh, R. J., Lobeda, A., & Tucker, A. O. (2012). Medicinal plants—nature's pharmacy. Genetic resources, chromosome engineering, and crop improvement. Medicinal plants, 14-51.
- Small, J. David, and Rick Deitrich. "Environmental and equipment monitoring." The mouse in biomedical research. Academic Press, 2007. 409-436.
- Ssenku, J. E., Okurut, S. A., Namuli, A., Kudamba, A., Tugume, P., Matovu, P., ... & Walusansa, A. (2022). Medicinal plant use, conservation, and the associated traditional knowledge in rural communities in Eastern Uganda. Tropical Medicine and Health, 50(1), 39.
- Stumvoll M, Chintalapudi U., Perriello G., Welle S., Gutierrez O. and Gerich J. (1995).Uptake and release of glucose by the human kidney. Postabsorptive rates and responses to epinephrine. J Clin Invest. 96(5): 2528-33.
- Sun, Z., Wang, H., Wang, J., Zhou, L., & Yang, P. (2014). Chemical composition and anti-inflammatory, cytotoxic and antioxidant activities of essential oil from leaves of Mentha piperita grown in China. *PloS one*, *9*(12), e114767.
- Tariq, N. P. M. M., Ifham, S. M. R., & Ali, A. M. (2013). Data collection methods in research for medicinal plants of Javadhu hills, Tamilnadu, India. *International Journal of Current Microbiology and Applied Sciences*, *2*, 83-88.

- Teucher, E., Anton, R., and Lobstein, A. (2003). Plantes aromatiques : épices, aromates, condiments et huiles essentielles. Ed : Lavoisier.
- TIELMANS, A., LALOI-MICHELIN, M., COUPAYE, M., VIRALLY, M., MEAS, T. & GUILLAUSSEAU, P.-J. 2007. Traitement médicamenteux du diabète de type 2 (première partie). La Presse Médicale, 36, 269-278.
- Tormo, M. A., Gil-Exojo, I., de Tejada, A. R., & Campillo, J. E. (2004). Hypoglycaemic and anorexigenic activities of anα-amylase inhibitor from white kidney beans (Phaseolus vulgaris) in Wistar rats. British journal of nutrition, 92(05), 785-790.
- Vialettes B., Atlan C., Conte-D., Raccah D., Simonin G. (2006). Diabète sucré de type 1 et 2 de l'enfant et de l'adulte. Complications. Endocrinologie nutrition. Faculté de médecine de Marseille. P : 1-45.
- Voet, D. & Voet, J. G. (2005). Biochimie. Bruxelles. Editions de Boeck Université. 2ème édition. pp. 1600
- Williams BD.(2009). Can cow's milk increase your diabetic risk ?, Top external factor that can cause diabetes. www.ezinearticles.com. Mai. 2011.
- Xu, Z., Chang, L., Xu, Z., & Chang, L. (2017). *Asteraceae* (pp. 441-721). Springer Singapore.
- Yang, CY., Yen, YY., Hung, KC. et al. Inhibitory effects of pu-erh tea on alpha glucosidase and alpha amylase: a systemic review. Nutr. Diabetes 9, 23 (2019). https://doi.org/10.1038/s41387-019-0092-y
- Youmbai S., et Sahraoui A .,(2021). Etude de l'activité antidiabétique et amarrage moléculaire de quelques dérivés ferrocénylméthyl-bases nucléiques comme nouveaux inhibiteurs d'α-amylase. Université d'Echahid Hamma Lakhdar-El OUED.P:26.
- Yudharaj, P., Shankar, M., Sowjanya, R., Sireesha, B., Naik, E. A., & Priyadarshini, R. J. (2016). Importance and uses of medicinal plants–An overview. *Int. J. Preclin. Pharm. Res*, *7*(2), 67-73.
- Zhang, X., Caner, S., Kwan, E., Li, C., Brayer, G.D., Withers, S.G.(2016) .Starch binding sites on the Human pancreatic alpha amylase D300N variant complexed with an octaose substrate.DOI: 10.2210/pdb5TD4/pdb.
- Ziani BE, Rached W, Bachari K, Alves MJ, Calhelha RC, Barros L, Ferreira IC. Detailed chemical composition and functional properties of Ammodaucus leucotrichus cross. & Dur. And Moringa oleifera Lamarck. J Funct Foods. 2019;53:237–47.

References Experimental

- Adefegha, S. A., & Oboh, G. (2012). In vitro inhibition activity of polyphenol-rich extracts from Syzygium aromaticum (L.) Merr. & Perry (Clove) buds against carbohydrate hydrolyzing enzymes linked to type 2 diabetes and Fe2+-induced lipid peroxidation in rat pancreas. Asian Pacific Journal of Tropical Biomedicine, 2(10), 774–781. https://doi.org/10.1016/S2221-1691(12)60228-7
- Adefegha, S. A., Olasehinde, T. A., & Oboh, G. (2017). Essential oil composition, antioxidant, antidiabetic and antihypertensive properties of two Afromomum species. Journal of Oleo Science, 66(1), 51–63.
- Adesokan, A., Oyewole, O.,Turay, B. (2010). Kidney and Liver Function Parameters in Alloxan-Induced Diabetic Rats Treated with Aloe Barbadensis Juice Extract. Sierra Leone Journal of Biomedical Research, 1(1). doi:10.4314/sljbr.v1i1.52431
- Afnor, Ø. (1982). Recueil de normes françaises des produits dérivés des fruits et légumes jus de fruits. AFNOR, 325.
- Ahmad, A., Khan, A., Samber, N., & Manzoor, N. (2014). Antimicrobial activity of Mentha piperita essential oil in combination with silver ions. Synergy, 1(2), 92–98.
- Akbar, S., Das, S., Iqubal, A., & Ahmed, B. (2022). Synthesis, biological evaluation and molecular dynamics studies of oxadiazine derivatives as potential anti-hepatotoxic agents. Journal of Biomolecular Structure and Dynamics, 40(20), 9974–9991. https://doi.org/10.1080/07391102.2021.1938233
- Alajtal, A. I., Sherami, F. E., & Elbagermi, M. A. (2018). Acid, peroxide, ester and saponification values for some vegetable oils before and after frying. AASCIT Journal of Materials, 4(2), 43–47.
- Alexopoulos, A.-S., Qamar, A., Hutchins, K., Crowley, M. J., Batch, B. C., Guyton, J. R. (2019). Triglycerides: Emerging Targets in Diabetes Care? Review of Moderate Hypertriglyceridemia in Diabetes. Current Diabetes Reports, 19(4). doi:10.1007/s11892-019-1136-3
- Anigboro, A. A., Avwioroko, O. J., Ohwokevwo, O. A., & Nzor, J. N. (2018). Phytochemical constituents, antidiabetic and ameliorative effects of Polyalthia longifiola leaf extract in alloxan-induced diabetic rats. Journal of Applied Sciences and Environmental Management, 22(6), 993. https://doi.org/10.4314/jasem.v22i6.25
- Anigboro, A. A., Avwioroko, O. J., Ohwokevwo, O. A., Pessu, B., & Tonukari, N. J. (2021). Phytochemical profile, antioxidant, α-amylase inhibition, binding interaction and

docking studies of Justicia carnea bioactive compounds with α-amylase. Biophysical Chemistry, 269, 106529. https://doi.org/10.1016/j.bpc.2020.106529

- Atti-Santos, A. C., Rossato, M., Pauletti, G. F., Rota, L. D., Rech, J. C., Pansera, M. R., Agostini, F., Serafini, L. A., & Moyna, P. (2005). Physico-chemical evaluation of Rosmarinus officinalis L. essential oils. Brazilian Archives of Biology and Technology, 48, 1035–1039.
- Avwioroko, O. J., Oyetunde, T. T., Atanu, F. O., Otuechere, C. A., Anigboro, A. A., Dairo, O. F., Ejoh, A. S., Ajibade, S. O., & Omorogie, M. O. (2020). Exploring the binding interactions of structurally diverse dichalcogenoimidodiphosphinate ligands with α-amylase: Spectroscopic approach coupled with molecular docking. Biochemistry and Biophysics Reports, 24, 100837. https://doi.org/10.1016/j.bbrep.2020.100837
- Bacanlı, M.; Anlar, H.G.; Aydın, S.; Çal, T.; Arı, N.; Ündeğer Bucurgat, Ü.; Başaran, A.A.; Başaran, N. (2017). D-limonene ameliorates diabetes and its complications in streptozotocin-induced diabetic rats. Food Chem. Toxicol. Int. J. Publ. Br. Ind. Biol. Res. Assoc. 110, 434–442.
- Banerjee, P., Eckert, A. O., Schrey, A. K., & Preissner, R. (2018). ProTox-II: A webserver for the prediction of toxicity of chemicals. Nucleic Acids Research, 46(W1), W257–W263. https://doi.org/10.1093/nar/gky318
- Baser, K. H. C., Özek, T., & Kirimer, N. (1993). The essential oil of Laser trilobum fruit of Turkish origin. Journal of Essential Oil Research, 5(4), 365–369.
- Benesi, H. A., & Hildebrand, J. H. (1949). A Spectrophotometric Investigation of the Interaction of Iodine with Aromatic Hydrocarbons. Journal of the American Chemical Society, 71(8), 2703–2707. https://doi.org/10.1021/ja01176a030
- Biswa, M. S., Varsha, T., Abhishek, T., & Bimal, K. B. (2023). Green Chemistry using Essential Oils as Synthons. Journal of Indian Chemical Society, Mar2023, 1–24. https://doi.org/10.5281/zenodo.7841465
- Bowers, K. J., Chow, E., Xu, H., Dror, R. O., Eastwood, M. P., Gregersen, B. A., Klepeis, J. L., Kolossvary, I., Moraes, M. A., & Sacerdoti, F. D. (2006). Scalable algorithms for molecular dynamics simulations on commodity clusters. Proceedings of the 2006 ACM/IEEE Conference on Supercomputing, 84-es.
- Brenk, R., Schipani, A., James, D., Krasowski, A., Gilbert, I. H., Frearson, J., & Wyatt, P. G. (2008). Lessons learnt from assembling screening libraries for drug discovery for neglected diseases. ChemMedChem, 3(3), 435–444.

https://doi.org/10.1002/cmdc.200700139

- Bungau SG, Vesa CM, Bustea C, Purza AL, Tit DM, Brisc MC, Radu AF. (2023). Antioxidant and Hypoglycemic Potential of Essential Oils in Diabetes Mellitus and Its Complications. Internatinal Journal Moleculaires Sciences. 24(22):16501. doi: 10.3390/ijms242216501. PMID: 38003691; PMCID: PMC10671358.
- Cheng, T., Zhao, Y., Li, X., Lin, F., Xu, Y., Zhang, X., Li, Y., Wang, R., & Lai, L. (2007). Computation of octanol-water partition coefficients by guiding an additive model with knowledge. Journal of Chemical Information and Modeling, 47(6), 2140–2148. https://doi.org/10.1021/ci700257y
- Chiu, S. Y. C., Dobberstein, R. H., Fong, H. H. S., & Farnsworth, N. R. (1982). Oxoaporphine alkaloids from Siparuna gilgiana. Journal of Natural Products, 45(2), 229–230.
- Daina, A., Michielin, O., & Zoete, V. (2014). ILOGP: A simple, robust, and efficient description of n-octanol/water partition coefficient for drug design using the GB/SA approach. Journal of Chemical Information and Modeling, 54(12), 3284–3301. https://doi.org/10.1021/ci500467k
- Daina, A., Michielin, O., & Zoete, V. (2017). SwissADME: A free web tool to evaluate pharmacokinetics, drug-likeness and medicinal chemistry friendliness of small molecules. Scientific Reports, 7(1), 1–13. https://doi.org/10.1038/srep42717
- Deveci, E., CAYAN, G. T. E. L., & Duru, M. E. (2020). In vitro antidiabetic activity of seven medicinal plants naturally growing in Turkey. European Journal of Biology, 79(1), 23–28.
- Dey, D., Paul, P. K., Azad, S. Al, Mazid, M. F. Al, Khan, A. M., Sharif, M. A., & Rahman, M. H. (2021). Molecular optimization, docking, and dynamic simulation profiling of selective aromatic phytochemical ligands in blocking the SARS-CoV-2 S protein attachment to ACE2 receptor: an in silico approach of targeted drug designing. Journal of Advanced Veterinary and Animal Research, 8(1), 24–35. https://doi.org/10.5455/javar.2021.h481
- Drwal, M. N., Banerjee, P., Dunkel, M., Wettig, M. R., & Preissner, R. (2014). ProTox: A web server for the in silico prediction of rodent oral toxicity. Nucleic Acids Research, 42(W1), W53–W58. https://doi.org/10.1093/nar/gku401
- Dumitrică, T., & James, R. D. (2007). Objective molecular dynamics. Journal of the Mechanics and Physics of Solids, 55(10), 2206–2236.

https://doi.org/10.1016/j.jmps.2007.03.001

- Friesner, R. A., Murphy, R. B., Repasky, M. P., Frye, L. L., Greenwood, J. R., Halgren, T. A., Sanschagrin, P. C., & Mainz, D. T. (2006). Extra precision glide: Docking and scoring incorporating a model of hydrophobic enclosure for protein− ligand complexes. Journal of Medicinal Chemistry, 49(21), 6177–6196.
- Furman, B.L. (2017). Diabetogenic Agents. Reference Module in Biomedical Sciences, Elsevier, ISBN 9780128012383, https://doi.org/10.1016/B978-0-12-801238-3.98060-7.
- Gargouri, M., Magné, C., El Feki, A. (2016). Hyperglycemia, oxidative stress, liver damage and dysfunction in alloxan-induced diabetic rat are prevented by Spirulina supplementation. Nutrition Research, 36(11), 1255–1268. doi:10.1016/j.nutres.2016.09.011
- Gavahian, M., Farahnaky, A., Farhoosh, R., Javidnia, K., & Shahidi, F. (2015). Extraction of essential oils from Mentha piperita using advanced techniques: Microwave versus ohmic assisted hydrodistillation. Food and Bioproducts Processing, 94, 50–58.
- Ghose, A. K., Viswanadhan, V. N., & Wendoloski, J. J. (1998). Prediction of hydrophobic (lipophilic) properties of small organic molecules using fragmental methods: An analysis of ALOGP and CLOGP methods. Journal of Physical Chemistry A, 102(21), 3762–3772. https://doi.org/10.1021/jp980230o
- Gil, M. I., Tomás-Barberán, F. A., Hess-Pierce, B., & Kader, A. A. (2002). Antioxidant capacities, phenolic compounds, carotenoids, and vitamin C contents of nectarine, peach, and plum cultivars from California. Journal of Agricultural and Food Chemistry, 50(17), 4976–4982. https://doi.org/10.1021/jf020136b
- Gorla, U. S., Gsn, K. R., Kulandaivelu, U., Alavala, R. R., Das, S., & Joseph, A. (2021). Bioflavonoids as potential target inhibitors in covid-19: An in silico analysis. Journal of Research in Pharmacy, 25(6), 982–997. https://doi.org/10.29228/jrp.94
- Guinaudeau, H., Leboeuf, M., & Cave, A. (1975). Aporphine alkaloids.
- Halder, D., Das, S., Joseph, A., & Jeyaprakash, R. S. (2023). Molecular docking and dynamics approach to in silico drug repurposing for inflammatory bowels disease by targeting TNF alpha. Journal of Biomolecular Structure and Dynamics, 41(8), 3462–3475. https://doi.org/10.1080/07391102.2022.2050948
- Hatmal, M. M., & Taha, M. O. (2017). Simulated annealing molecular dynamics and ligand-receptor contacts analysis for pharmacophore modeling. Future Medicinal Chemistry, 9(11), 1141–1159. https://doi.org/10.4155/fmc-2017-0061

- Hay, M., Thomas, D. W., Craighead, J. L., Economides, C., & Rosenthal, J. (2014). Clinical development success rates for investigational drugs. Nature Biotechnology, 32(1), 40–51. https://doi.org/10.1038/nbt.2786
- Horai, H., Arita, M., Kanaya, S., Nihei, Y., Ikeda, T., Suwa, K., Ojima, Y., Tanaka, K., Tanaka, S., & Aoshima, K. (2010). MassBank: a public repository for sharing mass spectral data for life sciences. Journal of Mass Spectrometry, 45(7), 703–714.
- Hossain, M. A., Al-Hdhrami, S. S., Weli, A. M., Al-Riyami, Q., & Al-Sabahi, J. N. (2014). Isolation, fractionation and identification of chemical constituents from the leaves crude extracts of Mentha piperita L grown in Sultanate of Oman. Asian Pacific Journal of Tropical Biomedicine, 4, S368–S372.
- Ibrahim, F. A., Usman, L. A., Akolade, J. O., Idowu, O. A., Abdulazeez, A. T., & Amuzat, A. O. (2019). Antidiabetic potentials of Citrus aurantifolia leaf essential oil. Drug Research, 69(04), 201–206.
- Ikewuchi, C. C., Ikewuchi, J. C., Ifeanacho, M. O. (2017). Restoration of plasma markers of liver and kidney functions/integrity in alloxan-induced diabetic rabbits by aqueous extract of Pleurotus tuberregium sclerotia. Biomedicine & Pharmacotherapy, 95, 1809–1814. doi:10.1016/j.biopha.2017.09.075
- Kakouros N, Rade JJ, Kourliouros A, Resar JR. (2011). Platelet function in patients with diabetes mellitus: from a theoretical to a practical perspective. International Journal of Endocrinology. ;2011:742719. doi: 10.1155/2011/742719. Epub 2011 Aug 21. PMID: 21869886; PMCID: PMC3159301.
- Ke, J.; Zhu, C.; Zhang, Y.; Zhang, W. (2020). Anti-Arrhythmic Effects of Linalool via Cx43 Expression in a Rat Model of Myocardial Infarction. Front. Pharmacol. 11, 926.
- Khelil, C. K. M., Amrouche, B., soufiane Benyoucef, A., Kara, K., & Chouder, A. (2020). New Intelligent Fault Diagnosis (IFD) approach for grid-connected photovoltaic systems. Energy, 211, 118591.
- Kim, S., Thiessen, P. A., Bolton, E. E., Chen, J., Fu, G., Gindulyte, A., Han, L., He, J., He, S., Shoemaker, B. A., Wang, J., Yu, B., Zhang, J., & Bryant, S. H. (2016). PubChem substance and compound databases. Nucleic Acids Research, 44(D1), D1202–D1213. https://doi.org/10.1093/nar/gkv951
- Kiryakov, H. G. (1968). Structure of dehydroglaucine: a new aporphine alkaloid.
- Klopman, G., Li, J. Y., Wang, S., & Dimayuga, M. (1993). Computer automated log P calculations based on an extended group contribution approach. Journal of Chemical

Information and Computer Sciences, 33(4), 752–781.

- Korb, O., Olsson, T. S. G., Bowden, S. J., Hall, R. J., Verdonk, M. L., Liebeschuetz, J. W., & Cole, J. C. (2012). Potential and limitations of ensemble docking. Journal of Chemical Information and Modeling, 52(5), 1262–1274.
- Kumar V, Mahdi F, Chander R. (2010). Hypolipidemic and antioxidant activity of Anthocephalus indicus(Kadam) root extract. Indian Journal of Biochemistry and Biophysics. 47(2):104–109.
- Laraoui, H., Lanez, E., Zegheb, N., Adaika, A., Lanez, T., & Benkhaled, M. (2023). Anti-Diabetic Activity of Flavonol Glucosides From Fumana montana Pomel: In vitro Analysis, In Silico Docking, ADMET Prediction, and Molecular Dynamics Simulations. ChemistrySelect, 8(8), e202204512. https://doi.org/10.1002/slct.202204512
- Larbi, B. A. M., Naima, B., Elsharkawy, E. R., & Neghmouche, N. S. (2018). Phytochemical characterization, in-vitro cytotoxic and antibacterial activity of Cotula cinerea (Delile) Vis essential oil. Journal of Natural Remedies, 107–112.
- Liu, X., Testa, B., & Fahr, A. (2011). Lipophilicity and its relationship with passive drug permeation. Pharmaceutical Research, 28(5), 962–977. https://doi.org/10.1007/s11095-010-0303-7
- Louail, Z., Kameli, A., Benabdelkader, T., Bouti, K., Hamza, K., & Krimat, S. (2016). Antimicrobial and antioxidant activity of essential oil of Ammodaucus leucotrichus Coss. & Dur. seeds. J. Mater. Environ. Sci, 7(7), 2328–2334.
- Lu, C., Wu, C., Ghoreishi, D., Chen, W., Wang, L., Damm, W., Ross, G. A., Dahlgren, M. K., Russell, E., & Von Bargen, C. D. (2021). OPLS4: Improving force field accuracy on challenging regimes of chemical space. Journal of Chemical Theory and Computation, 17(7), 4291–4300.
- Madhavi Sastry, G., Adzhigirey, M., Day, T., Annabhimoju, R., & Sherman, W. (2013). Protein and ligand preparation: parameters, protocols, and influence on virtual screening enrichments. Journal of Computer-Aided Molecular Design, 27, 221–234.
- Mahmoud, A.M., Ahmed, O.M., Ashour, M.B. and Abdel-Moneim, A. (2013) Upregulation of PPARγ Mediates the Antidiabetic Effects of Flavonoids in High Fat Diet Fed-Streptozocin Induced Type 2 Diabetic Rats. International Journal of Bioassays, 2, 756-761.
- Malekaneh, M., & Zarban, A. (2011). Antidiabetic activity of water extract of B-100 (a herbal formulation) in streptozotocin-induced diabetic rats. Clinical Biochemistry, 44(13),

S348. https://doi.org/10.1016/j.clinbiochem.2011.08.869

- Manssouri, M., Znini, M., & Majidi, L. (2020). Studies on the antioxidant activity of essential oil and various extracts of Ammodaucus leucotrichus Coss. & Dur. Fruits from Morocco. Journal of Taibah University for Science, 14(1), 124–130.
- Mao, X.-M., He, K., Shi, J.-C. (2014). Safety and efficacy of acarbose in the treatment of diabetes in Chinese patients. Therapeutics and Clinical Risk Management, 505. doi:10.2147/tcrm.s50362
- Meylan, W. M., & Howard, P. H. (2000). Estimating log P with atom/fragments and water solubility with log P. Perspectives in Drug Discovery and Design, 19(1), 67–84. https://doi.org/10.1023/A:1008715521862
- Mimica-Dukić, N., Božin, B., Soković, M., Mihajlović, B., & Matavulj, M. (2003). Antimicrobial and antioxidant activities of three Mentha species essential oils. Planta Medica, 69(05), 413–419.
- More, T.; Kulkarni, B.; Nalawade, M.; Arvindekar, A. (2014). Antidiabetic activity of linalool and limonene in streptozotocin-induced diabetic rat: A combinatorial therapy approach. Int. J. Pharm. Pharm. Sci., 6, 159–163.
- Moriguchi, I., Hirano, H., & Nakagome, I. (1994). Comparison of Reliability of log P Values for Drugs Calculated by Several Methods. Chemical and Pharmaceutical Bulletin, 42(4), 976–978. https://doi.org/10.1248/cpb.42.976
- Moriguchi, I., Hirono, S., Liu, Q., Nakagome, Izum., & Matsushita, Y. (1992). Simple Method of Calculating Octanol/Water Partition Coefficient. Chemical and Pharmaceutical Bulletin, 40(1), 127–130. https://doi.org/10.1248/cpb.40.127
- Moulay, A., Brahim, S., Satrani, B., Ghanmi, M., Aafi, A., Amusant, N., El Antry, S., & Chaouch, A. (2014). Bioactivity and chemical quality of Ammodaucus leucotrichus ssp. Leucotrichus Coss. & Durieu essential oils from Morocco.
- Naima, B., Abdelkrim, R., Ouarda, B., Salah, N. N., & Larbi, B. A. M. (2019). Chemical composition, antimicrobial, antioxidant and anticancer activities of essential oil from Ammodaucus leucotrichus Cosson & Durieu (Apiaceae) growing in South Algeria. Bulletin of the Chemical Society of Ethiopia, 33(3), 541–549.
- Narendhirakannan RT, Subramanian S, Kandaswamy M. (2006) Biochemical evaluation of antidiabetogenic properties of some commonly used Indian plants on streptozotocin-induced diabetes in experimental rats. Clinical and Experimental Pharmacology and Physiology. 33(12):1150–1157.

- Peana, A.T.; D'Aquila, P.S.; Panin, F.; Serra, G.; Pippia, P.; Moretti, M.D. (2002). Anti-inflammatory activity of linalool and linalyl acetate constituents of essential oils. Phytomed. Int. J. Phytother. Phytopharm. 9, 721–726.
- Ranjith D, & Viswanath S. (2019). In silico antidiabetic activity of bioactive compounds in Ipomoea mauritiana Jacq. ~ 5 ~ The Pharma Innovation Journal, 8(10), 5–11. http://www.thepharmajournal.com
- Ranjith, D., & Ravikumar, C. (2019). SwissADME predictions of pharmacokinetics and drug-likeness properties of small molecules present in Ipomoea mauritiana Jacq. Journal of Pharmacognosy and Phytochemistry, 8(5), 2063–2073.
- Riyadi, P. H., Romadhon, Sari, I. D., Kurniasih, R. A., Agustini, T. W., Swastawati, F., Herawati, V. E., & Tanod, W. A. (2021). SwissADME predictions of pharmacokinetics and drug-likeness properties of small molecules present in Spirulina platensis. IOP Conference Series: Earth and Environmental Science, 890(1), 2063–2073. https://doi.org/10.1088/1755-1315/890/1/012021
- Roos, K., Wu, C., Damm, W., Reboul, M., Stevenson, J. M., Lu, C., Dahlgren, M. K., Mondal, S., Chen, W., Wang, L., Abel, R., Friesner, R. A., & Harder, E. D. (2019). OPLS3e: Extending Force Field Coverage for Drug-Like Small Molecules. Journal of Chemical Theory and Computation, 15(3), 1863–1874. https://doi.org/10.1021/acs.jctc.8b01026
- Rose, P. W., Prlić, A., Altunkaya, A., Bi, C., Bradley, A. R., Christie, C. H., Di Costanzo, L., Duarte, J. M., Dutta, S., Feng, Z., Green, R. K., Goodsell, D. S., Hudson, B., Kalro, T., Lowe, R., Peisach, E., Randle, C., Rose, A. S., Shao, C., … Burley, S. K. (2017). The RCSB protein data bank: Integrative view of protein, gene and 3D structural information. Nucleic Acids Research, 45(D1), D271–D281.
- Sahin Basak, S., & Candan, F. (2010). Chemical composition and in vitro antioxidant and antidiabetic activities of Eucalyptus camaldulensis Dehnh. essential oil. Journal of the Iranian Chemical Society, 7, 216–226.
- Sahoo, P. K., Das, L. M., Babu, M. K. G., & Naik, S. N. (2007). Biodiesel development from high acid value polanga seed oil and performance evaluation in a CI engine. Fuel, 86(3), 448–454.
- Samber, N., Khan, A., Varma, A., & Manzoor, N. (2015). Synergistic anti-candidal activity and mode of action of Mentha piperita essential oil and its major components. Pharmaceutical Biology, 53(10), 1496–1504.

- Schreiner, W., Karch, R., Knapp, B., & Ilieva, N. (2012). Relaxation estimation of RMSD in molecular dynamics immunosimulations. Computational and Mathematical Methods in Medicine, 2012. https://doi.org/10.1155/2012/173521
- Schrödinger. (2015). Small-Molecule Drug Discovery Suite 2015-3: Schrödinger Suite 2015-3 Induced Fit Docking protocol; Glide version 6.8; Prime version 4.1. Glide Version, 6.
- Schrödinger. (2024). Schrödinger Release 2024-1: LigPrep, Schrödinger, LLC.
- Shamma, M. (1972). The Isoquinoline Alkaloids, New York and London. Academic Press, 81, 335–341.
- Singh, S. (2002). Refractive index measurement and its applications. Physica Scripta, 65(2), 167.
- Sliwoski, G., Kothiwale, S., Meiler, J., & Lowe, E. W. (2014). Computational methods in drug discovery. Pharmacological Reviews, 66(1), 334–395.
- Smaoui, S., Hsouna, A. Ben, Lahmar, A., Ennouri, K., Mtibaa-Chakchouk, A., Sellem, I., Najah, S., Bouaziz, M., & Mellouli, L. (2016). Bio-preservative effect of the essential oil of the endemic Mentha piperita used alone and in combination with BacTN635 in stored minced beef meat. Meat Science, 117, 196–204.
- Sonboli, A., Gholipour, A., & Yousefzadi, M. (2012). Antibacterial activity of the essential oil and main components of two Dracocephalum species from Iran. Natural Product Research, 26(22), 2121–2125.
- Tu, T., Rendleman, C. A., Borhani, D. W., Dror, R. O., Gullingsrud, J., Jensen, M. O., Klepeis, J. L., Maragakis, P., Miller, P., Stafford, K. A., & Shaw, D. E. (2008). A scalable parallel framework for analyzing terascale molecular dynamics simulation trajectories. 2008 SC - International Conference for High Performance Computing, Networking, Storage and Analysis, SC 2008, 1–12. https://doi.org/10.1109/SC.2008.5214715
- Valarezo, E., Rosales, J., Morocho, V., Cartuche, L., Guaya, D., Ojeda-Riascos, S., Armijos, C., & González, S. (2015). Chemical composition and biological activity of the essential oil of Baccharis obtusifolia Kunth from Loja, Ecuador. Journal of Essential Oil Research, 27(3), 212–216.
- Volpato GT, Vieira FL, Damasceno DC, Câmara FLA, Di Stasi LC. (2007). Lemonica IP. Effects of leaves of Polymnia sonchifolia (yacon) aqueous extract in diabetic ratsEfeito do extrato aquoso de folhas de Polymnia sonchifolia (yacon) em ratas diabéticas. Revista Brasileira de Plantas Medicinais. 9(2):88–93.

- Wang, B. L., Pan, D. Q., Kou, S. B., Lin, Z. Y., & Shi, J. H. (2020). Exploring the binding interaction between bovine serum albumin and perindopril as well as influence of metal ions using multi-spectroscopic, molecular docking and DFT calculation. Chemical Physics, 530, 110641. https://doi.org/10.1016/j.chemphys.2019.110641
- Wang, E., Fu, W., Jiang, D., Sun, H., Wang, J., Zhang, X., Weng, G., Liu, H., Tao, P., & Hou, T. (2021). VAD-MM/GBSA: a variable atomic dielectric MM/GBSA model for improved accuracy in protein–ligand binding free energy calculations. Journal of Chemical Information and Modeling, 61(6), 2844–2856.
- Wang, R., Fu, Y., & Lai, L. (1997). A new atom-additive method for calculating partition coefficients. Journal of Chemical Information and Computer Sciences, 37(3), 615–621.
- Wang, S. Y., & Chen, C.-T. (2010). Effect of allyl isothiocyanate on antioxidant enzyme activities, flavonoids and post-harvest fruit quality of blueberries (Vaccinium corymbosum L., cv. Duke). Food Chemistry, 122(4), 1153–1158.
- Williams, L. K., Zhang, X., Caner, S., Tysoe, C., Nguyen, N. T., Wicki, J., Williams, D. E., Coleman, J., McNeill, J. H., Yuen, V., Andersen, R. J., Withers, S. G., & Brayer, G. D. (2015). The amylase inhibitor montbretin A reveals a new glycosidase inhibition motif. Nature Chemical Biology, 11(9), 691–696. https://doi.org/10.1038/nchembio.1865
- Yang, Y., Yao, K., Repasky, M. P., Leswing, K., Abel, R., Shoichet, B. K., & Jerome, S. V. (2021). Efficient exploration of chemical space with docking and deep learning. Journal of Chemical Theory and Computation, 17(11), 7106–7119.

Annexes

Annexe:

Figure 1: Plant Essential Oil Extraction by Clevenger

Figure 2: The cages of the different batches of rats

Figure 3: The stages of nursing and treating mice

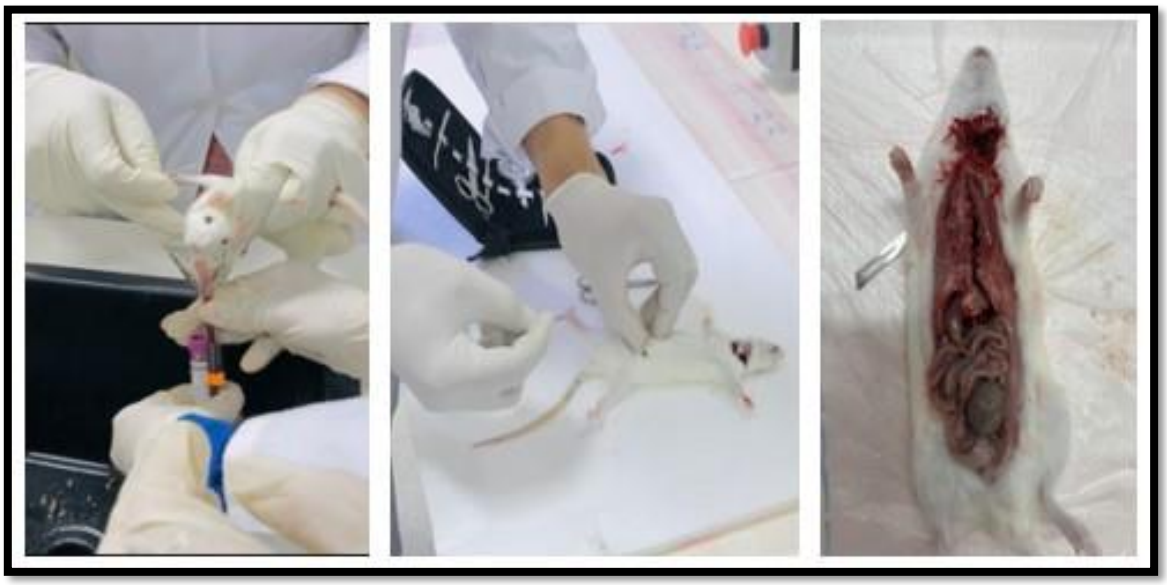


Figure 4: Slaughtering and dissecting mice

Figure 5: Steps and tools used when preparing histological cuts

Figure 6: Preparing samples for oxidative stress test

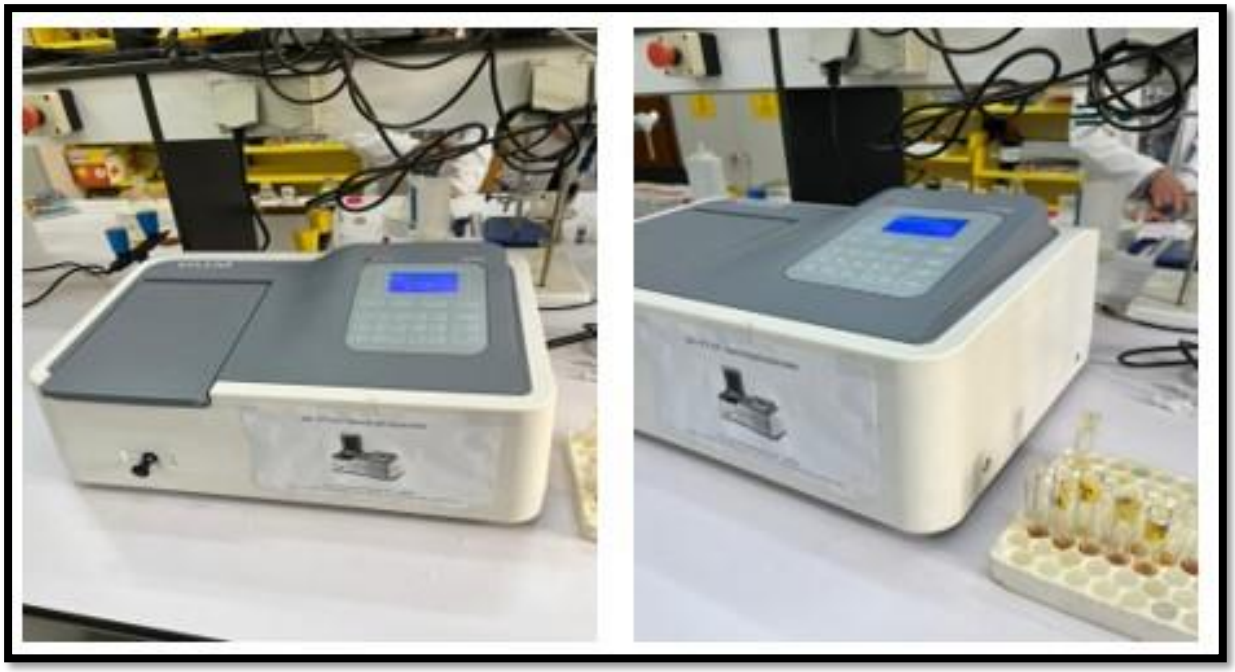


Figure 6: Transmission spectrophotometry