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Extraction, GC-MS Characterization, and In Vivo Evaluation of Anti-Thyroid Potential of Essential Oils from Medicinal Plants: In Silico Insights through ADMET Prediction, and Molecular Docking

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Steps would have faltered, and dreams would have faded, were it not for hearts that believed " in me when I doubted myself, and hands that reached out to lift me whenever I ".stumbled

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Dedication

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Imane

Abstract

The plant kingdom serves as an exceptional reservoir of bioactive compounds with antithyroid properties. This study aims to investigate the antithyroid effects of essential oils derived from *Cotula cinerea* and *Origanum majorana* L. through *in vivo*, and *in silico* approaches. Initially, the essential oils were obtained via hydrodistillation, and their chemical compositions were identified using gas chromatography–mass spectrometry (GC/MS) analysis, which revealed 31 compounds in Cotula cinerea and 44 in *Origanum majorana* L.

In vivo experiments were conducted on rats administered benzylthiouracil to induce thyroid dysfunction during the first phase of the study. After a 7-day treatment with 100 µL of essential oils from *C. cinerea* and *O. majorana L.*, the therapeutic efficacy was evaluated in comparison to control groups, including those treated with *levothyroxin.* The results demonstrated that *benzylthiouracil* induced alterations in hematological parameters (WBC, LYM, GRA), blood glucose levels, liver enzyme markers (AST, ALT), thyroid hormones (T3, T4, TSH), and renal function indicators (urea, creatinine, uric acid). Histopathological examinations revealed marked inflammation of the thyroid gland along with distinct tissue abnormalities, including severe follicular disruption and vascular dilation. However, the administration of essential oils significantly alleviated most of the side effects compared to *benzylthiouracil* In the computational domain, ADMET (absorption, distribution, metabolism, excretion, and toxicity) profiling was performed using the SwissADME platform to predict the physicochemical, pharmacokinetic, and toxicological properties of major compounds (>10%) found in each essential oil. The results highlighted favorable medicinal properties for all analyzed compounds.

During molecular docking studies, the major compounds (≥1%) from each essential oil were selected.

Keywords: essential oil, antithyroid activity, SwissADME, molecular docking, Levothyroxine, benzylthiouracil.

Résumé

Le règne végétal constitue une source exceptionnelle de composés bioactifs aux propriétés antithyroïdiennes. La présente étude vise à évaluer les effets antithyroïdiens des huiles essentielles extraites de *Cotula cinerea* et *Origanum majorana* L. in vivo, et in silico. Dans un premier temps, les huiles essentielles ont été obtenues par hydrodistillation, et leurs composants ont été caractérisés par chromatographie en phase gazeuse couplée à la spectrométrie de masse (GC/MS). L'analyse a révélé 31 composés pour *Cotula cinerea* et 44 pour *Origanum majorana* L.

Des expériences in vivo ont été menées sur des rats traités au *benzylthiouracile* pour induire un dysfonctionnement thyroïdien. Après une période de traitement de 7 jours avec 100 µL des huiles essentielles de *C. cinerea* et *O. majorana L.*, l'efficacité thérapeutique a été évaluée par comparaison avec des groupes témoins, y compris un groupe recevant la *Levothyroxine*. Les résultats ont montré que le *benzylthiouracile* entraînait des altérations des paramètres hématologiques (WBC, LYM, GRA), de la glycémie, des marqueurs hépatiques (AST, ALT), des hormones thyroïdiennes (T3, T4, TSH) ainsi que des fonctions rénales (urée, créatinine, acide urique). Les examens histologiques ont révélé une inflammation marquée de la thyroïde, accompagnée de déformations tissulaires évidentes, d'une désorganisation folliculaire sévère et d'une vasodilatation. Toutefois, l'administration des huiles essentielles a permis d'atténuer la majorité de ces effets indésirables, comparativement à la *benzylthiouracil*
Dans le domaine de la modélisation in silico, une analyse ADMET (absorption, distribution, métabolisme, excrétion et toxicité) a été réalisée à l'aide de la plateforme SwissADME pour prédire les propriétés physico-chimiques, pharmacocinétiques et toxicologiques des composés majoritaires (>10 %) présents dans chaque huile essentielle. Les résultats ont mis en évidence des profils médicinaux favorables pour l'ensemble des composés analysés.

Lors des études de docking moléculaire, les composés majeurs (≥1 %) ont été sélectionnés pour chaque huile.

Mots-clés : huile essentielle, activité antithyroïdienne, SwissADME, docking moléculaire, Levothyroxine, benzylthiouracile.

الملخص

تعمل مملكة النبات كمخزن استثنائي للمركبات النشطة بيولوجيًا ذات الخصائص المضادة لمرض اضطرابات الغدة الدرقية. تهدف هذه الدراسة إلى دراسة التأثيرات المضادة لمرضةاضطرابات الغدة الدرقية للزيوت العطرية المشتقة من Cotula cinerea و *Origanum majorana* L في كل من الجسم الحي ، وكذلك في الحاسوب. في البداية، تم الحصول على الزيوت العطرية من خلال التقطير المائي، وتم وصف مركباتها باستخدام تحليل كروماتوغرافيا الغاز-مطياف الكتلة (GC/MS)، حيث أنتجت Cotula cinerea 31 مركبًا وأنتج *Origanum majorana* L 44 مركبًا.

أُجريت تجارب حية باستخدام فئران أُعطيت benzylthiouracil لتحفيز الإصابة باضطرابات الغدة الدرقية خلال المرحلة الأولى من الدراسة. بعد فترة علاج لمدة7ايام بـ 100 ميكرولتر من الزيوت العطرية من كوتولا سينيريا وأوريجانوم ماجورانا إل، تم تقييم فعالية العلاج بالمقارنة مع مجموعات التحكم، بما في ذلك تلك التي تستخدم Levothyroxine. أظهرت النتائج أن benzylthiouracil تسبب في تغيير في المعايير الدموية. GRAWBC، LYM)، وسكر الدم، وعلامات إنزيمات الكبد (ALT، AST)، وهرمونات الغدة T3.T4.TCH، ووظائف الكلى (اليوريا، والكرياتينين، UA). كما كشفت الفحوصات النسيجية عن التهاب ملحوظ في الغدة الدرقية ,إلى تشوهات نسيجية واضحة. يتعطل البناء الجريبي بشدة، مع توسع الأوعية الدموية ومع ذلك، أدى إعطاء الزيوت العطرية إلى تحسين معظم الآثار الجانبية benzylthiouracil ، في مجال علوم الحاسوب، أُجري فحص لخصائص الامتصاص والتوزيع والاستقلاب والإفراز والسمية (ADMET) للتنبؤ بالخصائص الفيزيائية والكيميائية والصيدلانية والسمية لمركبات أساسية محددة موجودة في كل زيت (أكثر من01%). استخدم هذا التحليل خوادم SwissADME، حيث أشارت النتائج إلى خصائص طبية مواتية لجميع المركبات في عملية الإرساء.

الكلمات المفتاحية: زيت عطري، نشاط مضاد لمرض اضطرابات الغدة الدرقية ، swissADME، الإرساء, docking, Levothyroxine,benzylthiouracil,

ABREVIATIONS LIST

GΔ:	Binding free energy
A° :	Angstrom
Abs:	Absorbance
ADMET:	Absorption, Distribution, Metabolism, Excretion of drugs and Toxicity
:ALP	Alkaline phosphatase
ALT:	Aminotransferase
AST:	Aminotransferase
C° :	Degrees Celsius
Crea	Creatinine
DTA :	Ethylenediaminetetraacetic acid
EOs :	Essential Oils
EOCc :	Essential oils of Cotula cinerea
EOOm :	Essential oils of Origanum Majorana L
FBS:	Fasting blood sugar
g:	gramme
GC/MS :.	Gas Chromatography-Mass Spectrometry
GRA:	Granulocytes
GSH:	Glutathione
HGB:	Hemoglobin
IFD :	Induced Fit docking
K :	Constante de liaison
LYM:	Lymphocytes
mg :	Milligramme
min :	Minute
WHO:	World Health Organization
pH :	Potential hydrogen
PLT:	Platelets
RBC:	Erythrocytes
SIB:	Swiss Institute of Bioinformatics
TG:	Triglycerides
UA	Uric Acid
UV :	Ultra-violet
Vis :	Visible
WBC:	Leucocytes
µl :	Microlitre

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General Introduction

In recent years, plant-derived substances have garnered increasing attention due to their richness in molecules and bioactive compounds, which are essential for the development of new products used in the pharmaceutical, cosmetic, and food industries, particularly in therapeutic applications (Bekhaoua et al., 2019). Medicinal plants have been a focal point of human interest since ancient times, serving as a natural source of treatment either through traditional medicine or as purified active compounds. These plant-based compounds surpass synthetic drugs in terms of high therapeutic efficacy and minimal side effects (Farnsworth, 1986).

Algeria is distinguished by its diverse flora and a similarly rich ethnomedicinal tradition. Numerous plant-based formulations have been traditionally utilized in Algerian practice for the treatment of various ailments, including diabetes, with claims supporting their therapeutic effects (Khacheba et al., 2017).

Thyroid disorders are among the most common endocrine diseases worldwide, encompassing a range of conditions that affect the production and secretion of thyroid hormones, which play a vital role in regulating metabolism, growth, and the development of neural and muscular tissues (Brent, 2012). These disorders are mainly classified into hypothyroidism, which results from insufficient hormone secretion, and hyperthyroidism, where excessive amounts of hormones are produced, leading to accelerated metabolic processes and hormonal imbalance (Chaker et al., 2017; De Leo et al., 2016).

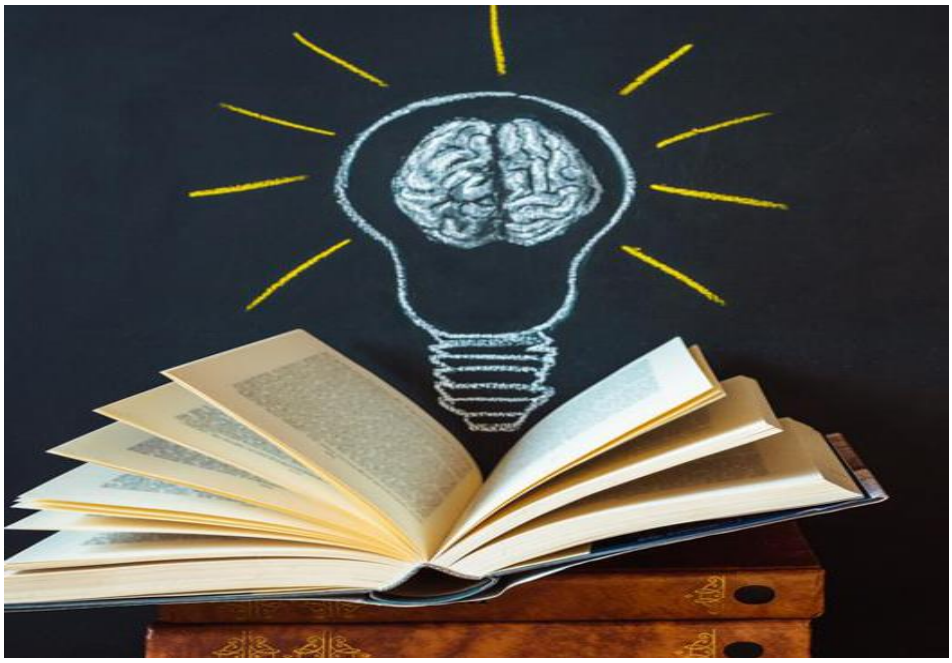
One of the most common causes of hypothyroidism is Hashimoto's thyroiditis, an autoimmune disease in which the immune system attacks and gradually destroys the thyroid gland (Chaker et al., 2017). In contrast, Graves' disease is the most frequent cause of hyperthyroidism, characterized by continuous stimulation of TSH receptors, resulting in excessive hormone production (De Leo et al., 2016). Epidemiological studies suggest that the prevalence of these disorders is increasing, especially among women, and is influenced by environmental, immunological, and genetic factors (Vanderpump, 2011).

Diagnosis is based on a thorough clinical assessment supported by laboratory tests measuring TSH, T3, and T4 levels, in addition to immunological assays and thyroid ultrasound imaging (Brent, 2012). Treatment varies depending on the disorder type, ranging from hormone replacement therapy (for hypothyroidism), to antithyroid drugs, radioactive iodine therapy, or surgery in cases of hyperthyroidism or suspicious nodules.

The present study focuses on the phytochemical valorization of two plant species native to the El Oued region of Algeria: *Cotula cinerea* and *Origanum majorana* L. Initially, essential oils were extracted from each plant using hydrodistillation (water distillation)

techniques. The chemical composition of the extracted oils was then identified through gas chromatography–mass spectrometry (GC-MS) analysis. Furthermore, both in vivo and in silico approaches were employed to evaluate the inhibitory effects of these essential oils on the enzymatic activity of the thyroïde.

FIRST PART: BIBLIOGRAPHICAL SYNTHESIS



Chapter one: Medicinal Plants

Introduction

Medicinal plants have long been recognized as one of the earliest and most enduring forms of therapeutic intervention used by humans. Throughout history, various civilizationsincluding the ancient Sumerians and Egyptians relied heavily on nature to treat illnesses by utilizing different parts of plants such as leaves, roots, and flowers (Benamara, 2022). This traditional knowledge laid the foundation for what would later become a vital component of modern pharmacology.

With the advancement of scientific research, active compounds within these plants have been identified, enabling the integration of traditional remedies into contemporary medicine. Numerous pharmaceutical drugs in use today are derived from plant-based substances; notable examples include aspirin, extracted from willow bark, and digoxin, sourced from the digitalis plant (World Health Organization [WHO], 2013).

Today, medicinal plants continue to play a crucial role in global health systems. According to the WHO, over 80% of the global population relies on traditional plant-based medicine for primary healthcare. This widespread use is attributed to the efficacy, accessibility, and cost-effectiveness of these natural remedies compared to synthetic pharmaceuticals (WHO, 2013).

2 . The studied plants:

2.1 .Cotula cinerea Plant:

2-1.1 . Cotula cinerea Definition:

Cotula cinerea, commonly known as Sheikh, is a distinctive annual herb characterized by its strong odor reminiscent of wormwood. Botanically, it features densely lobed leaves and sturdy, pale bristles. During the spring, it blooms with yellow, disc-shaped composite flowers. This species is well-adapted to desert environments and is especially abundant in Arab regions. Cotula cinerea is notable for its rich content of bioactive compounds, including flavonoids, terpenes, and volatile oils (Bohlmann et al., 1973; Mahran et al., 1976).

2-1.2. Vegetative classification of Cotula cinerea: (Halis Y,2007).

Scientific name: Cotula cinerea Del.

Synonymous: Brocchia cinerea (Del) Vis.

Common name: Sheikh, Rubaita, Sheha al-Bal

Table 1: Vegetative Classification of Cotula Cinerea (Quézel et Santa, 1963).

Division	Phanerogams (Spermatophytes)
Subdivision	Angiosperms
Class	Eudicots
Subclass	Asterids
Order	Asterales
Family	Asteraceae (Compositae)
Genus	Cotula
Species	Cotula cinerea Del., also known as Brocchia cinerea (Del.)

2-1.3. Vegetative description of Cotula cinerea:

Cotula cinerea is an annual, aromatic herb, growing between 10 to 20 cm in height. Its stems spread out and only stand upright at the tips. The leaves are woolly, light green, thick, and finely dissected. The tubular flowers start as brown buds and gradually turn yellow as they bloom. The plant flowers from March to May.((Chehma, 2006)).

This plant is widely found across the Sahara.especially in soils with a slight sandy texture(chehma.2006))

Figure 1. Cotula cinerea photograph (**Agrawal et ol,2011**)

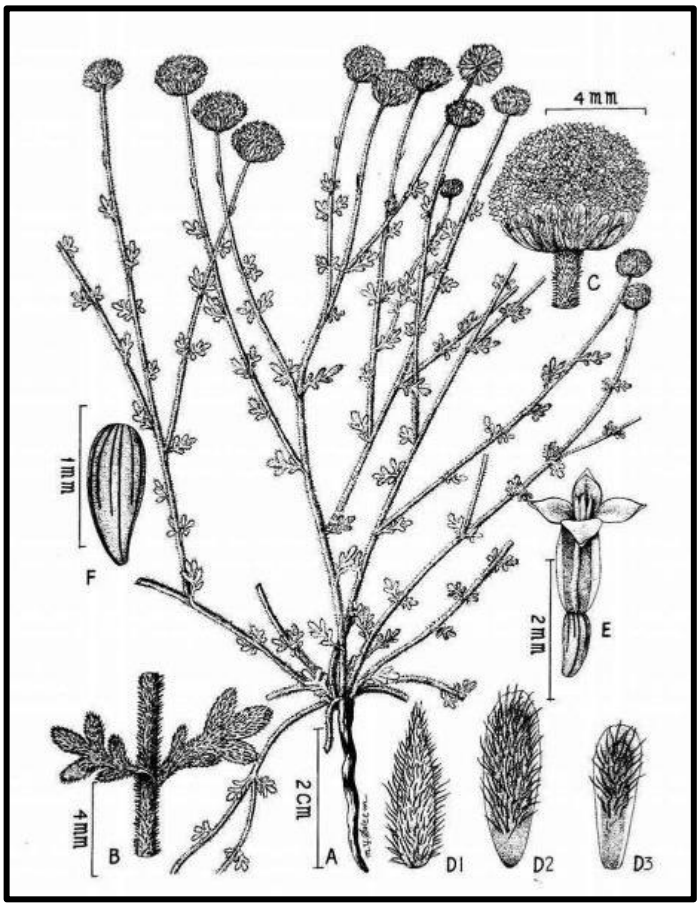


Figure 2. Diagram form of cotula cinerea(Ozenda P,1991).

2-1.4. Geographical Distribution of Cotula cinerea:

This plant is an endemic species of the Sahara. It primarily grows in small, slightly sandy depressions and is distributed throughout the northern and central regions of the Sahara Desert.(Djellouli, M. (2017)).

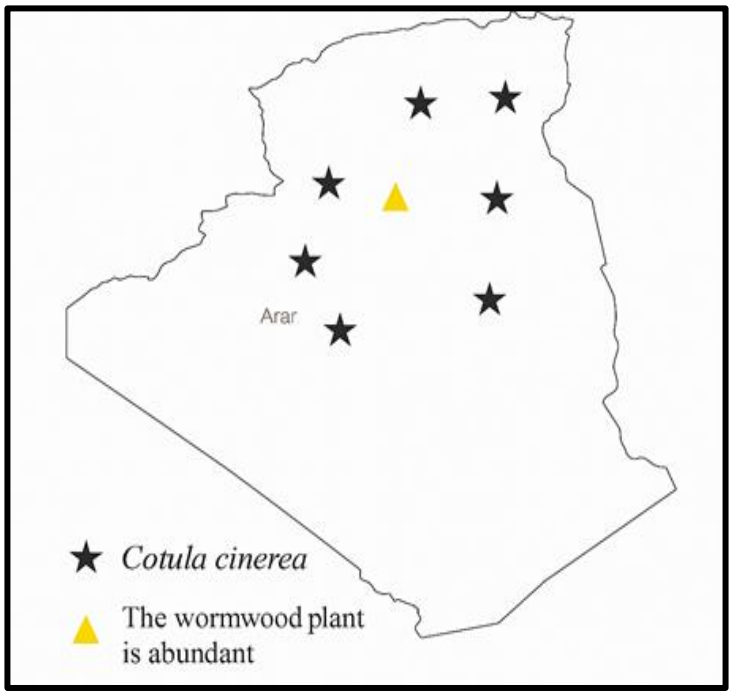


Figure 3. Distribution of Cotula cinerea in Algeria

2-1.5. Economic and Medicinal Uses of Cotula cinerea:

This species is widely used in traditional medicine for its various biological properties, including anti-inflammatory, analgesic, antiseptic, antibacterial, antipyretic, and larvicidal activities. It can be used in the treatment of stomach pain, fever, headaches, migraine, and cough.(Ghanmi, M., Ekhlil, B., Satrani, B., Alaoui, M. B., Rhafouri, R., Farah, A., Amusant, N., & Chaouch, A. (2016)).

- **General Use:** This plant is widely renowned for its notable aromatic properties.
- **Culinary Use:** It is primarily used to flavor soups, especially during the month of Ramadan.(Mahboub, N. (2018)).
- **Pharmacopoeia**: In traditional herbal medicine, it is consumed as an infusion to aid digestion.(Mahboub, N. (2018)).
- **Pastoral Interest**: From a pastoral perspective, it is mainly grazed by goats.(Mahboub, N. (2018)

2.2 . *Origanum Majorana* L Plant:

2.2.1. *Origanum Majorana* L Definition:

Marjoram (*Origanum majorana* L.), commonly referred to as Garden Oregano, is a herbaceous species from the Lamiaceae family, typically growing to a maximum height of 80 centimeters (Aiche-Iratni, G. (2016))

2.2.2. Vegetative Classification of *Origanum Majorana* L:

Scientific Name: *Origanum majorana* L.

Synonyms: Marjolaine.

Common Name: Al-Murdagush. (Baba Aissa,1991)

Table 2: Vegetative classification of *Origanum Majorana* L. (Gilles, 2007)

Division	Spermatophytes
Subdivision	Angiosperms
Class	Dicotyledons
Subclass	Gamopetalae
Order	Lamiales
Family	Lamiaceae
Genus	Origanum
Species	*Origanum Majorana* L

2.2.3.Vegetative Description of *Origanum Majorana* L:

Origanum majorana L., commonly known as marjoram or sweet marjoram, is a herbaceous plant belonging to the Lamiaceae family. It typically does not exceed 80 cm in height (Iratni, 2016). The plant features opposite, entire, grayish-green, oval leaves measuring between 1 and 2 cm in length. Its flowers are small, white or violet in color, and are arranged in dense axillary clusters accompanied by two spoon-shaped bracts (Lakhrissi, 2015).

This species closely resembles oregano and is characterized by its small leaves, typically ranging from 1 to 2 centimeters in length.(FURIA, T. E, 1971).

Unlike the leaves, the bracts are densely overlapping, gray-green in color, and positioned oppositely on a square-shaped stem alongside the calyces. Sweet marjoram produces tiny, tubular flowers about 0.3 cm long grouped in brush-like heads roughly 1.3 cm in length. These flowers can be hermaphroditic or female and typically feature two-lipped corollas, with the upper lip being entire or slightly toothed. The plant blooms from June to

September, displaying pale pink or white flowers with gray-green bracts in spiked inflorescences. Seed development occurs from August to September. The seeds are small, oval, and dark brown. Its roots are sub-cylindrical, displaying longitudinal coiling with transverse cracks, measuring 0.2 to 0.6 mm in diameter. The outer root surface is dark brown, while the interior is lighter brown and contains numerous elongated rootlets(Abdelhakim Bouyahya a et al. 2020).

Figure 4.*Origanum Majorana* L Photograph (Abdalla et al,2012)

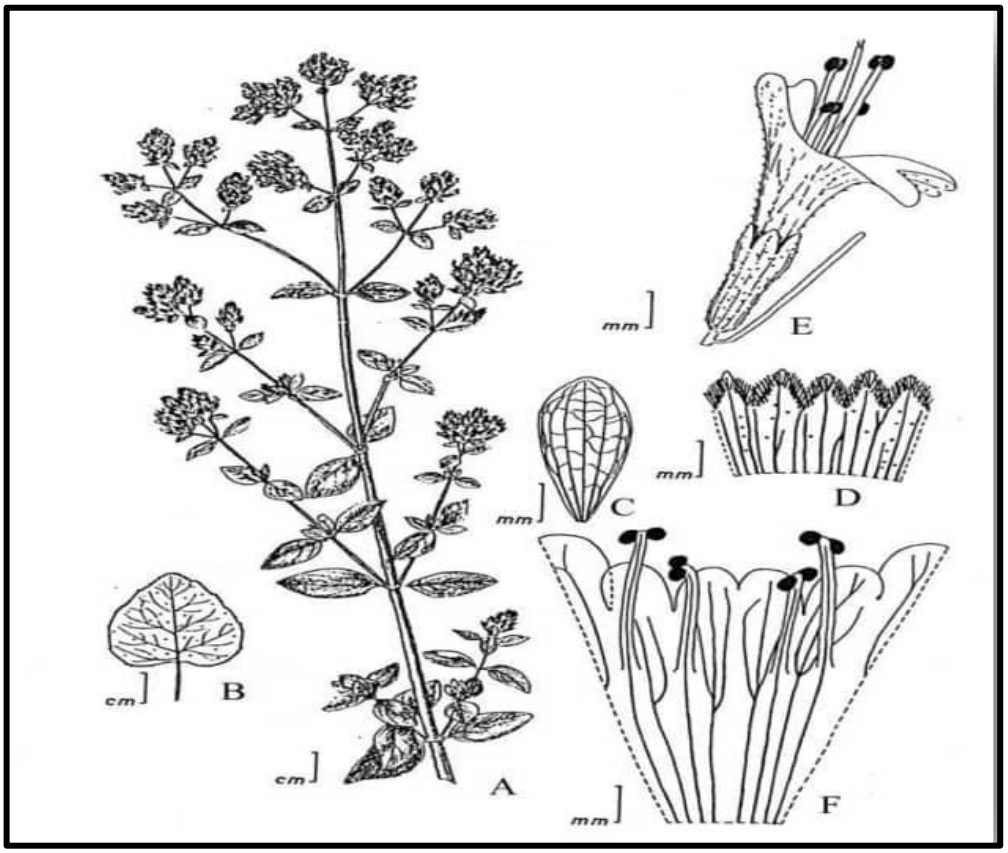


Figure 5.Diagram for *Origanum Majorana* L(Ietswaart,1980)

2.2.4. Geographical Distribution of *Origanum Majorana* L:

Currently found only in cultivation, *Origanum majorana* is believed to have originated in southwestern Asia, most likely in present-day Syria or Iran. It has been grown since ancient times across the Mediterranean basin, a region well-suited to the climate preferences of the Origanum genus. While it grows as a perennial in African regions, it is typically cultivated as an annual plant in Central European gardens due to climatic differences(Chevalier, 1938, Gérard et François., 2009, Ietswaart, 1980).

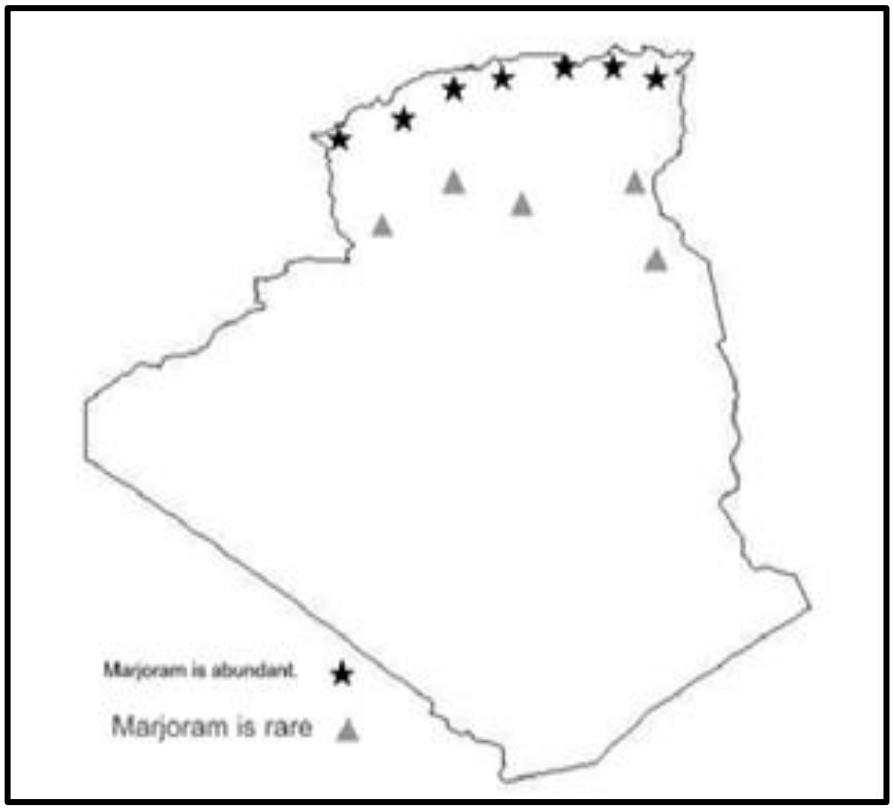


Figure 6. The spread of *Origanum majorana* L in Algeria

2.2.5. Economic and Medicinal Uses of *Origanum Majorana* L:

Origanum majorana L., commonly known as marjoram, has a long history of medicinal use dating back to Hippocrates, who employed it as an antiseptic. Today, it remains a popular natural remedy, particularly for treating respiratory ailments such as chest infections, coughs, and sore throats. In addition, it is used to relieve rheumatic pain, support cardiovascular and nervous system health, and manage conditions like epilepsy and insomnia. Marjoram is also valued for its benefits in skincare, as well as its ability to alleviate digestive issues such as flatulence and stomach discomfort 11.13 (Bremness L1994.Yazdanparast R and Shahriyary L 2008).

3. Essential Oils:

3.1. Generalities About Essential Oils:

3.1.1. Definition of Essential Oils:

Essential oils are generally composed of complex mixtures of volatile compounds naturally found in plants.(Bruneton, 1999)

Essential oils are highly concentrated aromatic oily liquids that contain complex mixtures of volatile substances composed of dozens of compounds. They are found in all parts of the plant bark, roots, leaves, flowers, and fruits and occur across all climatic regions of the world (Raul, 2005)

These volatile molecules have a low molecular weight, which gives plants such as peppermint, lemon, basil, and sage their characteristic aromas.(Peter B. Kaufman, Leland J Cseke, Sara Warber, Harry L. (Brielmann, James A Duke 1998)

3.1.2. Storage Sites for Essential Oils:

Essential oils are found in various botanical families and are widely distributed throughout the plant kingdom. They are present in significant amounts in approximately 2,000 species across 60 families. These essences are located in all living parts of the plant. Within a single plant, essential oils may be present in multiple organs, and their chemical composition can vary from one organ to another. These aromatic substances are produced by secretory glands located on almost all parts of the plant. (BOUCHIKHI TANI, Z.,2011)

All parts of aromatic plants, including all their vegetative organs, may contain essential oils.

Flowers such as orange blossom, rose, and lavender;

floral buds (e.g., clove) and bracts (e.g., ylang-ylang) are common sources.

Leaves of plants like eucalyptus, basil, mint, thyme, laurel, savory, sage, as well as pine and fir needles, are also rich in essential oils.

Underground organs include roots (e.g., vetiver, angelica) and rhizomes (e.g., ginger, calamus).

Fruits such as fennel, anise, and citrus peels, along with seeds like nutmeg, are important sources as well.

Additionally, essential oils can be extracted from wood and bark, including cinnamon, sandalwood, and rosewood.

These oils are stored in specialized cellular structures, such as essential oil cells, glandular trichomes (as in mint), and secretory canals.(Bruneton J., 2008)

3.1.3. Physical Properties of Essential Oils:

Essential oils are complex mixtures composed of a wide variety of molecules, mainly belonging to two chemical groups based on their biogenetic origin: terpenoid compounds (terpenes and their oxygenated derivatives) and aromatic compounds derived from phenylpropane (Boukhobza F et Goetz P.2014)

Other compounds from various chemical classes such as alcohols, aldehydes, ketones, and esters are typically present in trace amounts. All of these constituents are characterized by their low molecular weight.(Bakkali F, Averbeck S, Averbeck D and Idaomar M.2008)

1. Monoterpenes:

Monoterpenes (CH_{10}) can be classified into three main structural types: acyclic, monocyclic, and bicyclic. There is also a tricyclic monoterpene specifically known as tricyclene.

Each group contains oxygenated derivatives such as alcohols, aldehydes, ketones, oxides, and esters.

Monoterpenes are the primary constituents of a wide variety of essential oils obtained from flowers, leaves, or fruits.(P.Jose Teissiere ,1991)

2. Sesquiterpenes:

Sesquiterpenes are composed of 15 carbon atoms, equivalent to three isoprene units (P,Nivière, 1994) . They are mainly classified into two groups:

Linear sesquiterpenes, which are widely distributed throughout the plant kingdom (P. Ribereau et Gayon,1968)

Cyclic sesquiterpenes, among which monocyclic and bicyclic compounds are the most commonly found.(P. Ribereau et Gayon,1968)

3. Diterpenes:

Diterpenes are composed of four isoprene units (C_5H_8), resulting in a total of 20 carbon atoms. Their derivatives can be either cyclic or acyclic(P,Nivière, 1994) . Among the most important examples are vitamin A and certain aldehydes that play a key role in the visual mechanism. (P,Nivière, 1994)

4. Triterpenes:

Triterpenes are composed of six isoprene units (C_5H_8), amounting to a total of 30 carbon atoms. They include several groups of substances and numerous compounds of significant biological importance (P,Nivière, 1994).

For instance, squalene, extracted from shark liver, is an important triterpene that serves as a precursor to cholesterol.(P. Arnaud, 1997)

5. Tetraterpenes:

Tetraterpenes are composed of eight isoprene units (C_5H_8) (P,Nivière, 1994). resulting in a total of 40 carbon atoms. Among the most important tetraterpenes is carotene, which has a long aliphatic chain with eleven conjugated double bonds. This structure allows carotene to absorb light in the visible spectrum.

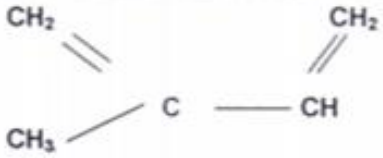
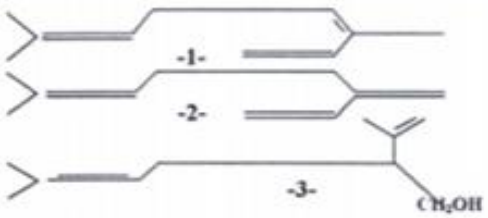
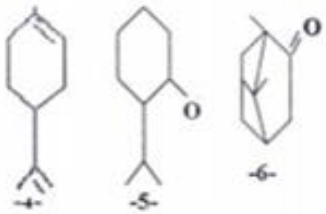
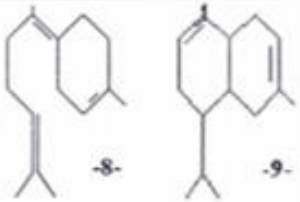
Tetraterpenes are highly colored substances that impart pigmentation to various plants. For instance, carotene is responsible for the orange color of carrots and autumn leaves.(P. Arnaud, 1997)

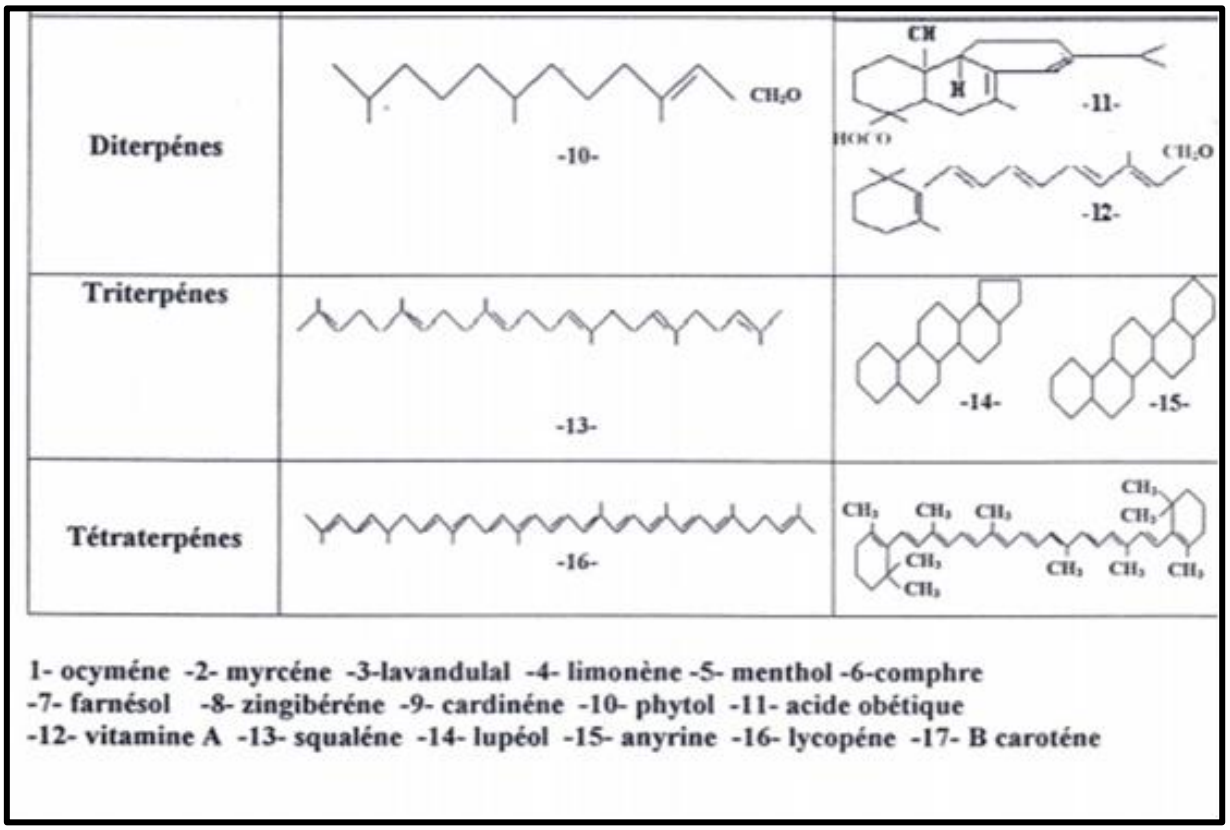
6. Les polyterpènes:

Butadiene can undergo polymerization to form long polyethylene chains, which make up elastomers materials that exhibit properties similar to rubber

(P. Arnaud, 1997)

Table 3: Different types of terrain in the vegetable region (Z.Messaouda et ol ,2003).

L`isoprène		
Terpènes	Acyclique	Cyclique
Monoterprénes		
Sesquiterpénes		



3.2. Methods of Essential Oil Extraction:

There are various techniques for extracting essential oils, and the selection of the most suitable method depends on several factors, including the nature of the plant material, the physicochemical properties of the oil to be extracted, the intended application of the extract, and the need to preserve its original aromatic profile. A range of extraction methods exists, each tailored to specific conditions and requirements (Bouzaa & Zid, 2022).

3.2.1. Hydro Distillation:

Hydro distillation is one of the oldest and simplest methods for extracting essential oils. It allows for the isolation of pure oils while achieving higher yields. The process involves immersing the plant material directly into a still filled with water, using a ratio of two to six times the volume of the plant material (Charie, 2019).

The mixture is then heated to the boiling point, causing various vapors to rise. These vapors are subsequently condensed on a cold surface, enabling the separation of the essential oil from the hydrosol due to their differing densities (Bruneton, 1993; Hellal, 2011). The steam and the volatile oil released from the plant form an immiscible mixture during this process (El-bahai et al., 2009).

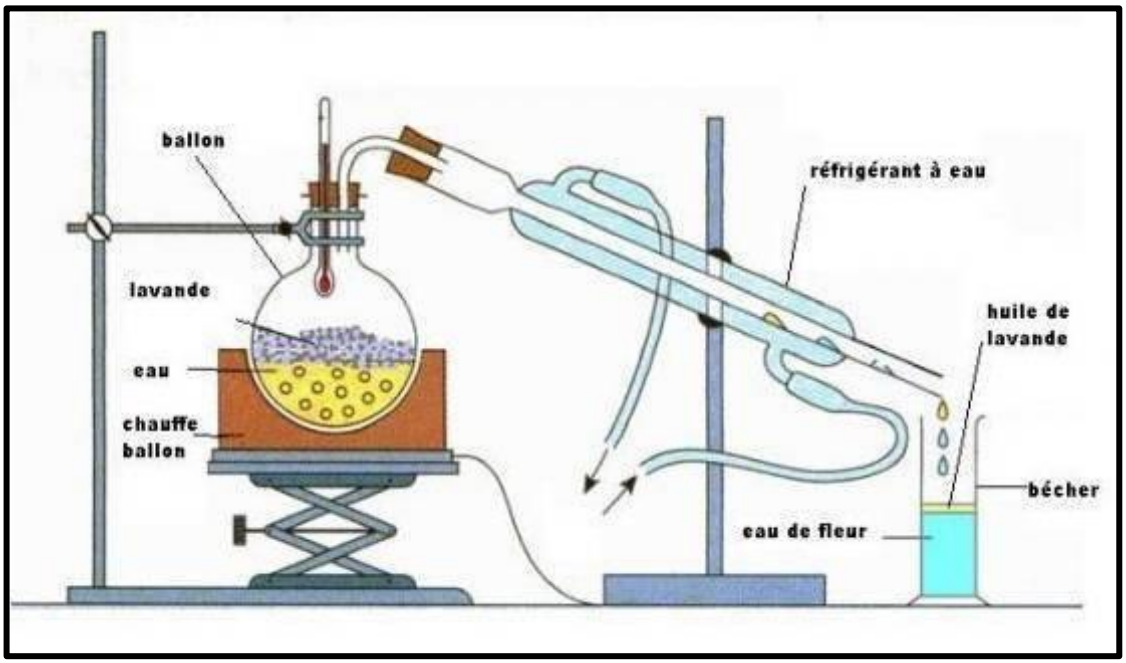


Figure 7: Diagram of a hydro-distillation assembly (El-bahai et al., 2009).

3.2.2. Cold Expression:

This specific extraction technique is exclusively applied to fruits belonging to the Rutaceae botanical family, such as lemon, orange, bergamot, and mandarin (Elhaib, 2011). It involves a simple mechanical process whereby the oil-containing sacs located in the peel or pericarp of the fruit are ruptured through abrasion to release their contents.

The essential oil is then separated from the fruit juice through a mechanical process known as decantation. This method is considered the simplest and the only one that does not alter the chemical composition of the obtained product. The oil extracted through this process is referred to as an "essence."

Essences obtained by cold expression retain their therapeutic properties significantly better than those extracted using other techniques. Unlike essential oils which are composed solely of volatile compounds essences also contain non-volatile substances such as flavonoids and steroids, enhancing their therapeutic potential (Lacoste, 2014).

3.2.3. Steam Distillation:

In this process, the plant material is not immersed in water; instead, it is exposed to a stream of steam without prior maceration. The steam, enriched with volatile compounds, is then condensed and separated through decantation . Steam is injected at the base of the distillation apparatus (Richard, 1992), allowing the extraction of essential oils in an efficient manner while preserving their sensitive constituents.

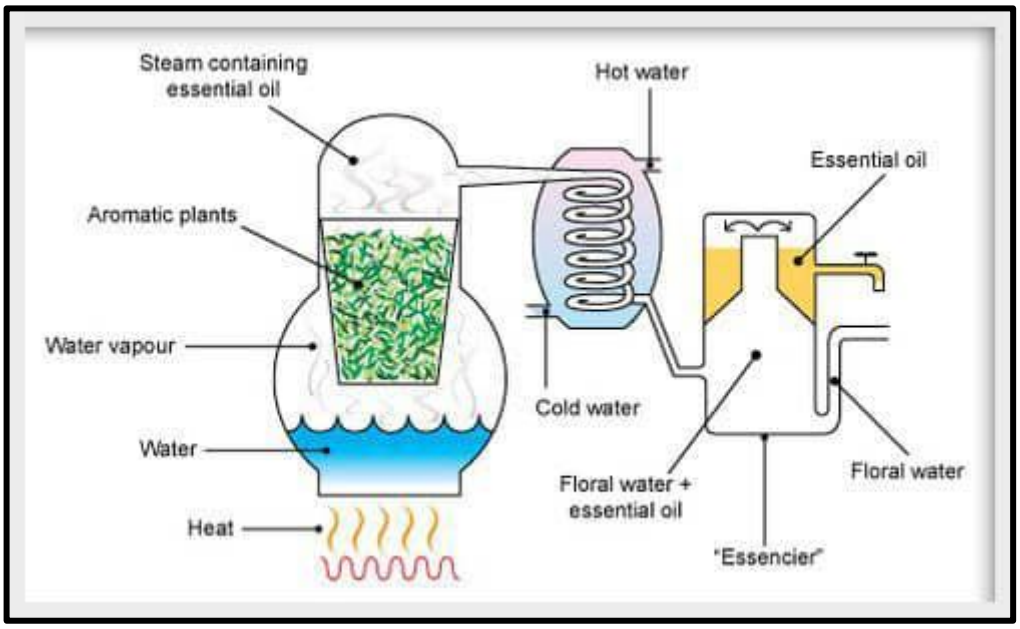


Figure 8: Steam drive method (Richard, 1992)

3.2.4. Solvent Extraction:

This extraction technique is based on the solubility of aromatic essences in most organic solvents. After the extraction phase, the solvent is removed through low-pressure distillation, resulting in a fragrant, paste-like substance. The essential oil is then isolated from this mixture using alcohol, yielding a pure essential oil (Bruneton, 1999).

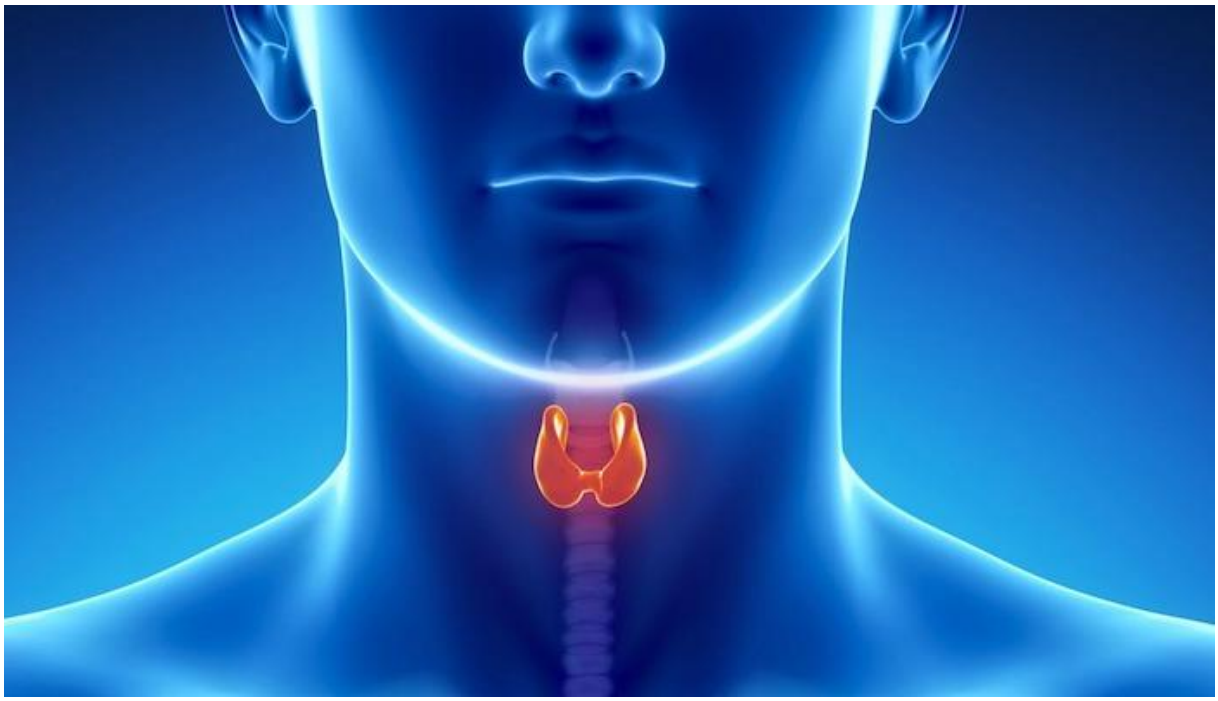
The solvents employed in this method can include commonly used substances in organic chemistry such as hexane and petroleum ether, as well as fats, oils, and even gases in some cases (Richard, 1992).

3.2.5. Microwave Extraction:

This innovative process is based on the principle of traditional hydro distillation, where a microwave oven is used to heat a part of the hydro distillation setup. The plant material is placed with water inside a flask within the microwave oven. The cooling system and the part dedicated to the recovery of the essences are located outside the oven, allowing for efficient extraction of aromatic compounds while maintaining precise control over temperature and the extraction process (Chemat & Lucchesi, 2005).

CHAPTER TWO:

ANTITHYROÏDE ACTIVITY



1-Introduction:

thyroid gland is an endocrine organ located at the base of the neck. Its name is derived from the Greek word thyreoeides, meaning "shield-shaped." This gland produces hormones that are transported through the bloodstream tovarious parts of the body, functioning as chemical messengers that transmit

instructions to distant organs. The thyroid is unique among the body's glands in that it stores its secretory products within its own cells. The hormones secreted by the thyroid are essential for growth, the regulation of metabolism, and the proper functioning of nearly all tissues and organs in the human body. (BRAUNSTEIN, 2022)

2- Anatomy and morphology of the glande :

The thyroid gland is a single, midline structure situated in the anteroinferior portion of the neck, aligned with the second and third tracheal rings, to which it is anchored via the Gruber ligament (Figure 09) (LECLERE et al., 2001). It comprises two asymmetrical lateral lobes joined by a central isthmus, from which a pyramidal lobe—also referred to as Lalouette's lobe may occasionally emerge as a superior projection, slightly deviated to the left.

Viewed anteriorly, the thyroid generally resembles an "H" or butterfly in shape (RYNDAK-SWIERCZ, 2010).

Typically, four parathyroid glands are present small clusters of cells located on the posterior aspect of the thyroid gland. Each gland weighs around 40 mg

and can be embedded within, behind, or beneath the thyroid tissue. These glands produce parathyroid hormone (PTH), which plays a key role in maintaining calcium homeostasis in the bloodstream and tissues (XING et al., 2021

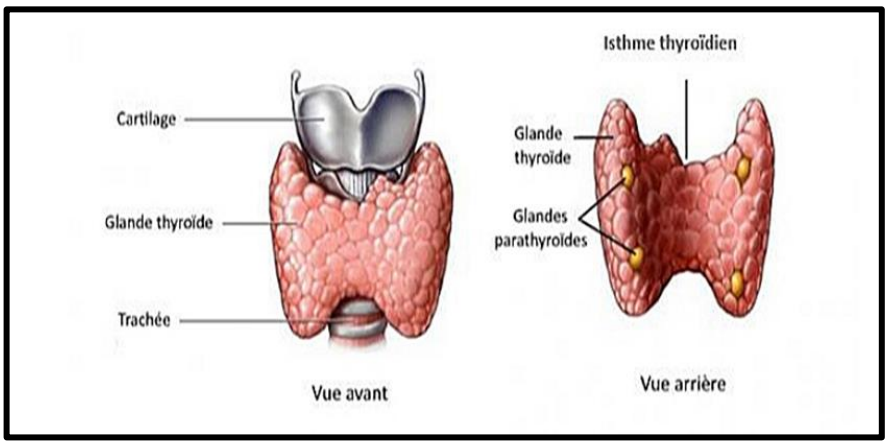


Figure 09: localization and morphology of the thyroid gland (MARRET;2019)

The thyroid gland has a height ranging from 6 to 8 cm at the lobes and from1 to 2 cm at the isthmus. Its weight varies between 20 and 30 grams.Physiologically, it tends to be larger in women than in men, undergoes enlargement during pregnancy, and diminishes in size with aging [Chevrel JP, Fontaine C. (1996).]. An anatomical representation of the thyroid gland is shown in Figure 10

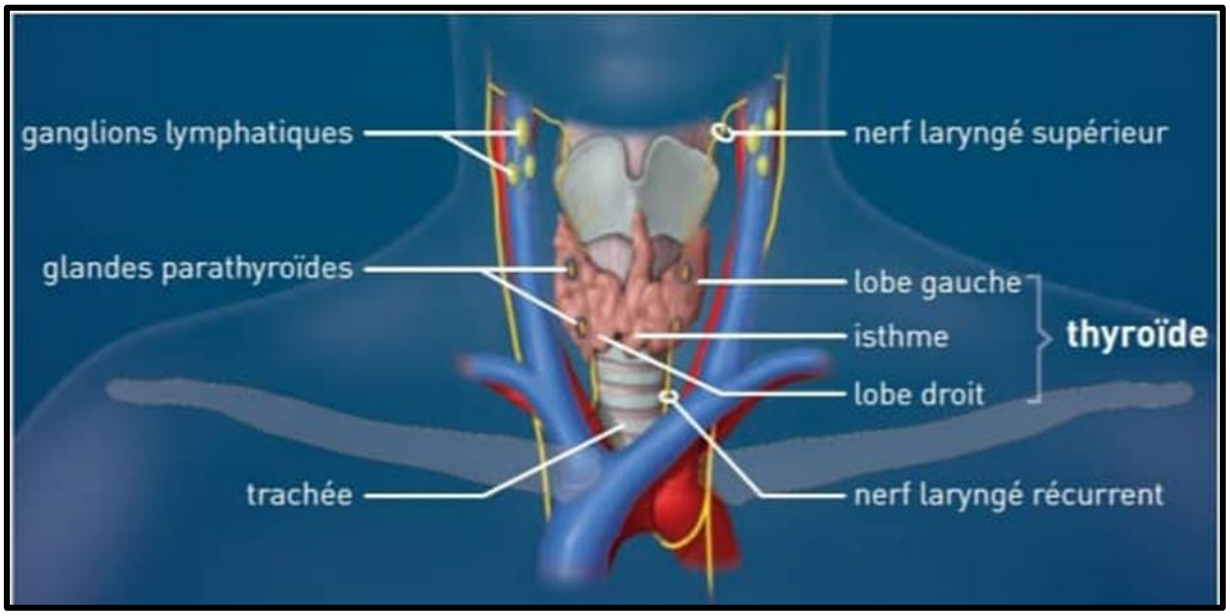


Figure 10 : présentation of the thyroïde [Institut national du cancer. Juillet 2013.].

3.Thyroid hormones:

The thyroid gland primarily secretes two iodinated polypeptide hormones: triiodothyronine (T3) and tetraiodothyronine (thyroxine or T4). These hormones act as key regulators of the activity of nearly all organs, are essential for normal growth and development, and exert multiple effects on metabolic processes (Pérez-Martin, 2007; Bernard et al., 2015).

4-Structure of Thyroid Hormones:

Thyroid hormones (TH) share a common organic structure centered on the thyronine nucleus, which is derived from the amino acid tyrosine. This structure consists of two phenolic rings connected by a diphenyl ether bridge. Both major hormones, T4 (3,5',3',5-tetraiodothyronine) and T3 (3,5,3'-triiodothyronine), have two iodine atoms attached to the inner ring of the tyrosine molecule. They differ in the number and position of iodine atoms on the outer phenyl ring: T4 carries two iodine atoms on the outer ring, whereas T3 has only one.

The removal of an iodine atom from the inner ring of T4 results in the formation of reverse T3 (rT3), which is biologically inactive (Figure 11) (LACOUR and BELON, 2015).

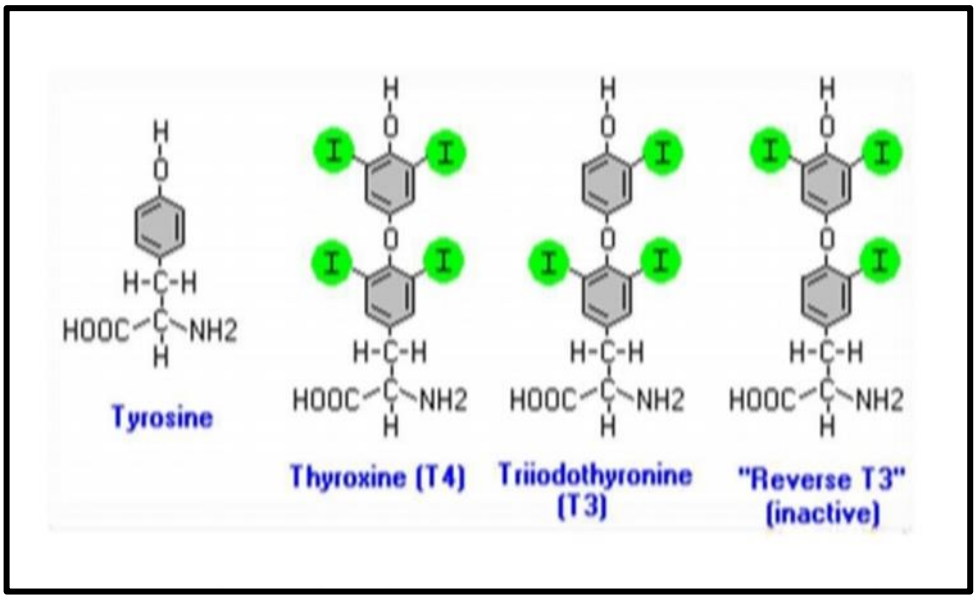


Figure 11: Structure of tyrosine and thyroïdiennes hormones

(TAGHRIED et al., 2021)

5-Histology of the thyroid gland:

The histological structure of the thyroid gland is characterized by its functional units known as "thyroid follicles" or vesicles. These spherical formations are composed of a single layer of cuboidal epithelial cells resting on a basal membrane. A central colloid material is enclosed within the follicles, serving as a storage site for hormones (T3 and T4), thyroglobulin, and transport proteins, as depicted in Figure12.(Omri M. (2011))

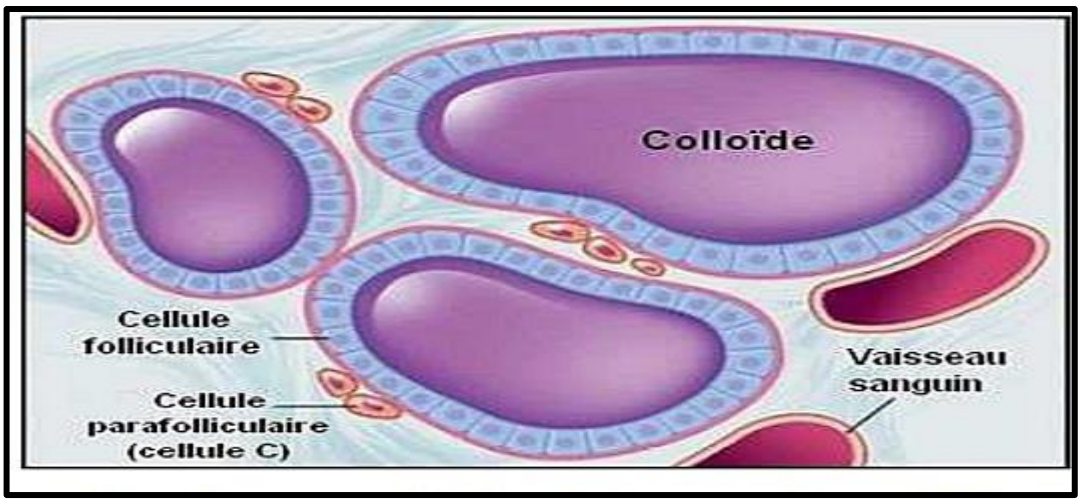


Figure 12. structure of the thyroïd (source : quizlet.com) [Hazard J, Perlemuter L. (2000).]

Each thyroid follicle is surrounded by a very thin basal lamina, reinforced by reticulin fibers, and is embedded within a dense capillary network. The structural characteristics of the follicles vary depending on several factors:

Their anatomical location within the gland (peripheral follicles are generally larger than . .central follicles)

Their functional activity status (inactive or resting follicles are large and lined with low . cuboidal epithelium, whereas active follicles are smaller and exhibit tall columnar epithelium)]Belarbi AN. (2018).[

5.1. Follicular Cells (Thyrocytes)

The follicular cells, or thyrocytes, represent the principal component of the follicular epithelium of the thyroid gland. They form a simple epithelial layer resting on a fine layer of connective tissue. The apical pole of these cells features microvilli extending into the colloid, while their basal pole is in direct contact with the vascular network. The rough endoplasmic reticulum and Golgi apparatus are particularly well-developed due to their role in the synthesis of thyroid hormones. Follicular cells are interconnected by tight junctions located ,] exclusively at the apical side, establishing a sealed follicular lumenHazard J, Perlemuter L. (2000))(Leclère, J. Orgiazzi, J. Rousset BJ. Schlienger L. Wémeau JL. (2001))

5.2. C Cells or Parafollicular Cells

C cells, also known as parafollicular cells, are pale cells significantly less numerous than thyrocytes (representing less than 0.1% of the thyroid parenchyma). Not involved in the primary thyroid hormonal activity, they do not contact the colloid but instead lie adjacent to the capillaries, predominantly in the central regions of the lateral lobes. These cells are located between the basal lamina and the follicular epithelium, exhibit a globular shape, and secrete calcitonin, a hormone with hypocalcemic action. Serum calcitonin levels serve as a specific marker for medullary thyroid carcinoma (Belarbi AN. (2018))

5.3. Colloid

The colloid is a yellowish, paste-like mass whose abundance varies according to the functional activity of the gland. It fills the follicular lumen and serves as a reservoir for thyroid hormones (Omri M. (2011)). Approximately 70% of the colloid is composed of thyroglobulin, with its polysaccharide fraction accounting for positivity in periodic acid-Schiff (PAS) staining. It also contains iodinated and non-iodinated proteins detectable by PAS staining (Belarbi AN. (2011))

5.4. Interstitial Cells

Interstitial cells are clear cytoplasm cells located between thyroid follicles. They are found either isolated or grouped in small clusters and correspond to Weber's interstitial cells, also referred to as Wolfler's clusters. Their exact significance remains under debate (Belarbi AN. (2018).)

6-Thyroid hormones Mécanisme:

Thyroid hormones primarily exert their mechanism of action at the nuclear level by binding to specific receptors within target cells. Triiodothyronine (T3) demonstrates a significantly higher affinity for these receptors compared to tetraiodothyronine (T4), thereby establishing T3 as the metabolically active form. The conversion of T4 into T3 predominantly occurs within target cells and depends on the availability of deiodinase enzymes, whose expression varies according to cell type and expected functional response.

The cellular response to thyroid hormone stimulation involves two distinct phases:

The early response: characterized by the binding of T3 to nuclear receptors (TRα and TRβ), leading to the activation of specific genes and the production of primary proteins. TRα receptors predominantly activate genes involved in cardiac function, whereas TRβ receptors stimulate genes associated with metabolic regulation.

The delayed (or secondary) response: consists of the stimulation or inhibition of the synthesis of additional proteins mediated by the primary proteins produced during the early response phase.

It is thus evident that thyroid hormones can exert prolonged effects even after their elimination from the circulation, owing to the continued action of the induced proteins. Moreover, T3 and T4 can bind to low-affinity cytoplasmic receptors, serving to maintain a local reservoir of hormones near their sites of action. Additionally, direct interactions between thyroid hormones and various cytoplasmic organelles, such as mitochondria, have been reported (Grammer D K. (1995))(Harriet M, Syme BSc, BvetMed, PhD, MRCVS. (2007)

7-The regulation of thyroid hormone:

The hypothalamus, a region of the central nervous system, initiates a cascade of hormonal reactions regulating thyroid function. It contains multiple nuclei capable of secreting thyrotropin-releasing hormone (TRH, also known as protirelin) in response to specific stimuli. TRH, a tripeptide prohormone, is released toward the anterior pituitary (adenohypophysis), where it binds to specific receptors, activating adenylyl cyclase and

leading to phosphorylation of protein kinases, thereby stimulating the secretion of thyroid-stimulating hormone (TSH) by pituitary thyrotropic cells.

TSH, a dimeric glycoprotein, enters the systemic circulation and exerts its effects via a G protein-coupled receptor (TSHr), activating the adenylyl cyclase-cAMP system and likely also the phospholipase and protein kinase A pathways at the basal pole of thyrocytes. This stimulation triggers the various stages of thyroid hormone biosynthesis, from iodine uptake to the secretion of T3 and T4.

In turn, thyroid hormones exert a negative feedback effect by inhibiting the synthesis of TRH and TSH, specifically targeting the follicular cells. Additionally, an intra-thyroidal autoregulatory mechanism known as the Wolff-Chaikoff effect transiently inhibits thyroid hormone production by blocking iodine organification when blood iodide levels are elevated.Furthermore, various endogenous factors including stress, insomnia, age, sex, pregnancy, and metabolic disorders can influence thyroid function (Ryndak-Swiercz, 2022; Shahid et al., 2023).

8-Dysfonctionnement de la thyroïde :

8.1. Hyperthyroidism:

Hyperthyroidism, also referred to as thyrotoxicosis, is characterized by the excessive secretion of thyroid hormones [FAUCHER J., LACHAINE R., 2012.], most commonly associated with thyroid tumors (Lacroix L et al .(2004))Clinical manifestations include elevated body temperature, profuse sweating, weight loss, irritability, hypertension , (FAUCHER J., LACHAINE R., 2012)increased basal metabolic rate, tachycardia, nervousness, restlessness, and a general inability to relax (MARIEBE M., 2008.)

The main causes of hyperthyroidism include (FAUCHER J., LACHAINE R., 2012.)

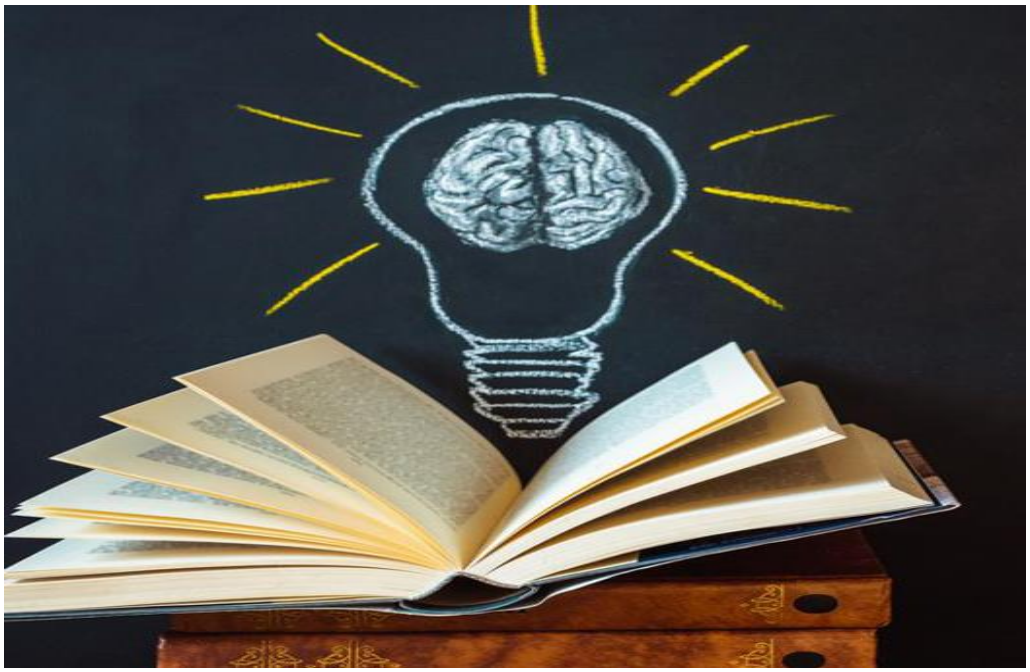
- Diffuse toxic goiter (Graves' disease);
- Toxic adenoma;
- Toxic multinodular goiter (Plummer’s disease);
- Painful subacute thyroiditis;
- Silent thyroiditis, including lymphocytic and postpartum variants;
- Iodine-induced hyperthyroidism (e.g., associated with amiodarone therapy);
- Excessive ingestion of thyroid hormones (Baskin HJ et al 2002)

8.2. Hypothyroidism:

Hypothyroidism results from insufficient production of thyroid hormones by the thyroid gland. In the United States, the most frequent cause of primary hypothyroidism is chronic autoimmune thyroiditis (Hashimoto's disease). Other causes include surgical removal of the thyroid gland, external irradiation, defects in iodine organification, replacement of thyroid tissue by tumors (e.g., lymphoma), and certain medications such as lithium or interferon (Baskin HJ et al 2002)

SECOND PART:

EXPERIMENTAL



CHAPTER ONE: MATERIALS AND METHODS

Introduction

This research was collaboratively conducted by two specialized laboratories: the Valorisation and Technology of Saharian Resources Laboratory (VTRS), affiliated with the Faculty of Exact Sciences, Department of Chemistry at the University of El Oued, and the Centre de Recherche Scientifique et Technique en Analyses Physico-Chimiques (CRAPC) in Ouargla, Algeria. The VTRS laboratory was responsible for the extraction of essential oils, performing both in vivo and in vitro biological assays, as well as conducting in silico studies. Concurrently, the CRAPC laboratory undertook the gas chromatography-mass spectrometry (GC/MS) analysis of the extracted essential oils. This collaborative framework ensured comprehensive and precise experimental procedures, leveraging the combined expertise and technical resources of both institutions.

Plant Material

2-1- Cotula cinerea:

Cotula cinerea specimens were collected over multiple intervals between December 2024 and January 2025 from a desert forest environment located in southeastern Algeria, specifically in the Hassi Khalifa region of El Oued Province. The ecological and geographical characteristics of the collection site are as follows:

- Geographic Coordinates: 48°10' East longitude, 23°09' North latitude.
- Elevation: 44 meters above sea level.
- Distance from the coast: approximately 290 kilometers.
- Bioclimatic Classification: Desert climate.

2-2- *Origanum majorana* L:

Origanum majorana L. samples were harvested during different periods spanning from March 2024 to April 2025 in a desert forest area in southeastern Algeria, specifically in the Akfadou region of El Oued Province. The collection site is characterized by the following parameters:

- Geographic Coordinates: 67°6' East longitude, 33°00' North latitude.
- Elevation: 58 meters above sea level.
- Distance from the coast: approximately 300 kilometers.
- Bioclimatic Classification: Desert climate.

3-Materials and Methods:

3-1- Essential Oil Extraction :

3-1-1- Apparatus :

Essential oil extraction was performed using specialized apparatus to ensure accuracy and reproducibility. The equipment employed included:

- Adventurer – Pro AV53 precision balance.
- Heating flask.
- Refrigerant
- Clevenger-type apparatus
- Separating funnel.
- Rotary evaporator.

The Clevenger apparatus, an essential component of the extraction system, is illustrated in Figure 13 (Biswa et al., 2023).

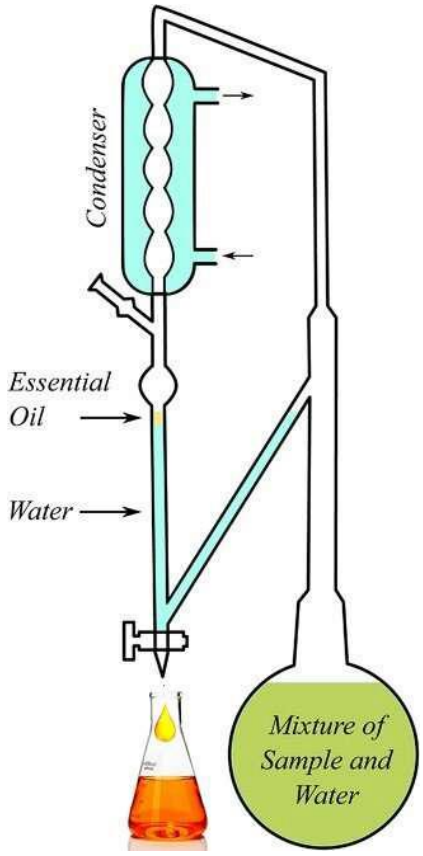


Figure 13.Schematic representation of the Clevenger apparatus used in the essential oil extraction process (Biswa et al., 2023).

3-1-2- Procedure:

The extraction procedure was carried out meticulously to maximize the yield and preserve the chemical integrity of the essential oils. The steps undertaken were as follows:

- The plant samples were carefully cleaned to eliminate surface impurities.
- Precisely 100 g of the plant material were weighed using the Adventurer – Pro AV53 sensitive balance.
- The samples were washed with water to avoid charring and subsequently placed into a heating flask.
- Small pieces of a boiling regulator were added to promote uniform boiling.
- The mixture was subjected to direct heating at 100°C for three and a half hours.
- Steam distillation was conducted, followed by the separation of the aqueous and oily phases through liquid-liquid extraction using diethyl ether.
- The organic (oily) phase was dried over anhydrous sodium sulfate to remove residual moisture.
- The dried oil was filtered through filter paper to eliminate any remaining diethyl ether particles.
- The filtered oil phase was concentrated by evaporating the remaining organic solvent using a rotary evaporator.
- The resulting essential oil was stored in small amber glass bottles and refrigerated at 5°C to preserve its quality.

3-2- Yield of Essential Oil Extraction:

Two distinct methodologies are commonly utilized to determine the yield of essential oil extraction, each offering a specific perspective on process efficiency.

3-2-1- Volumetric Yield – Mass-based Method (Naima et al., 2019)

This method assesses the mass of the plant material used for extraction in relation to the volume of essential oil obtained. The yield is calculated according to Equation 1:

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

Where: REO: Essential oil yield, m0: Mass of the utilized plant sample, VEO: Volume of the extracted essential oil.

3-2-2- Mass-based Yield – Mass-based Method (Larbi et al., 2018)

Alternatively, this method defines the yield as the ratio of the mass of the extracted essential oil to the mass of the plant material used. The yield is computed using Equation 2:

$$d = \frac{m_{EO}}{m_{H_2O}}$$

Both methodologies were systematically applied to determine the essential oil yields of all examined plant specimens.

3-3- Characterization of Essential Oils:

The characterization of the essential oils consisted of two primary aspects: evaluation of physicochemical properties and analysis via Gas Chromatography–Mass Spectrometry (GC/MS).

3-3-1- Physicochemical Properties:

This section examines the fundamental physicochemical parameters of essential oils, including relative density, acidity index, ester index, and refractive index. These properties provide valuable insights into the chemical composition and environmental interactions of the oils (Atti-Santos et al., 2005).

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

The relative density was determined at 20°C. A 1 mL aliquot of essential oil was weighed, and the same procedure was performed for distilled water. The relative density was calculated using Equation 3 (Valarezo et al., 2015):

$$d = \frac{m_{EO}}{m_{H_2O}}$$

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

❖ **Acidity Index (AFNOR NF T75-111 Standard)**

The acidity index, representing the concentration of free acids in 1 g of essential oil, was determined by titration with 0.5 N potassium hydroxide (KOH) solution (Sahoo et al., 2007).

❖ **The procedure involved:**

- Mixing 0.5 mL of essential oil with 2–3 drops of phenolphthalein indicator in a small vessel.

- Titrating the mixture with 0.5 N KOH solution until the appearance of a faint pink color.

❖ **The acidity index was calculated using Equation 4:**

$$I_a = \frac{56.11 \times V \times C}{m}$$

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 ester index was determined to quantify the esters hydrolyzed into free acids within the essential oil (Alajtal et al., 2018). The procedure was as follows:

- 0.5 mL of essential oil was placed into a small vessel.
- 1 mL of 0.5 N KOH solution was added.
- The mixture was heated in a gas-evacuated water bath for a specified duration to facilitate hydrolysis.
- After cooling, 0.5 mL of distilled water and 3 drops of phenolphthalein indicator were added.
- Titration was performed with 0.5 N hydrochloric acid (HCl) until a color change indicated neutralization.
- The volume of HCl required to neutralize the excess KOH was recorded.

❖ **The ester index was then calculated using Equation 5:**

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

Where: V0 (ml): Volume of hydrochloric acid (HCl) without essential oil, V1 (ml): Volume of hydrochloric acid (HCl) in the presence of essential oil, Ia: Acid value, Ie: Ester value and m: Mass of the essential oil sample.

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

The refractive index of the essential oil is defined as the ratio of the sine of the angle of incidence to the sine of the angle of refraction of a light ray of a specific wavelength as it passes from air into the essential oil at a constant temperature (Singh, 2002).

The refractive index was measured directly using a refractometer at a reference temperature of 20°C.

3-3-2- Gas Chromatography–Mass Spectrometry (GC/MS) Analysis:

The GC/MS technique is the most widely utilized method for the analysis of essential oils, allowing for the simultaneous separation, identification, and quantification of their constituents.

❖ **Principle**

The GC/MS technique is based on the differential affinities of the sample's constituents towards two phases: a stationary phase and a mobile phase.

The stationary phase consists of a silicone-based liquid that coats an inert, granular solid material within a typically coiled steel or glass column measuring 1 to 3 meters in length and 2 to 4 millimeters in internal diameter.

The mobile phase comprises an inert carrier gas such as nitrogen, helium, or argon.

Compounds are separated based on their interactions with these two phases, leading to differences in retention times, which are then used for their identification and quantification.

The column is maintained at elevated temperatures within a controlled furnace environment. Under the influence of heat, the constituents of the essential oil vaporize, thereby allowing their separation based on differences in volatility. The separation principle relies on the disparity in the partition coefficients of volatile compounds between the stationary liquid phase and the mobile gas phase.

As each compound elutes from the column, it is detected by a detection system that generates a signal, resulting in a chromatogram with peaks corresponding to individual constituents.

Gas chromatography is coupled with mass spectrometry (GC-MS), a powerful analytical combination that enables the identification of compounds. This coupling relies on computerized comparison between the mass spectrum of an unknown compound and spectra stored in reference libraries, allowing precise compound identification.

❖ **Apparatus**

The chemical constituents of the essential oils were identified using a gas chromatographic system (HP 5890 Series II) equipped with an HP-5 MS capillary column (30 m length, 0.25 mm internal diameter, 0.25 μm film thickness), coupled to a mass spectrometer (HP-MSD 5972).

Nitrogen (N_2) was employed as the carrier gas for the analyses. Spectra were recorded at an ionization energy of 70 eV. The identification of individual constituents was achieved by

comparing the obtained spectra with those present in the WILEY275 spectral library (Chiu et al., 1982; Guinaudeau et al., 1975; Kiryakov, 1968; Shamma, 1972).

❖ **Procedure**

The carrier gas (N_2) was introduced at a constant flow rate of 1 mL/min. An aliquot of 1 µL of essential oil was injected in split mode. The injector and detector temperatures were set at 250°C and 320°C, respectively. The oven temperature was programmed as follows:

- Initially held at 60°C for 8 minutes
- Increased gradually to 250°C at a rate of 2°C/min and maintained isothermally at 250°C for 15 minutes,
- Subsequently increased to 300°C at a rate of 10°C/min.

3-4- Invivo antithyroid activities:

3-4-1- Animal Treatment:

The study was conducted in vivo using 25 adult male Albino Wistar rats, with an average weight of 223.8 ± 25.5 g, obtained from the Pasteur Institute in Algiers. The rats were acclimatized to the animal housing conditions for a period of 15 days, maintained at a temperature of 25°C, relative humidity of 62%, and a 12-hour light/dark photoperiod. They were treated in accordance with the guidelines specified in the Guide for the Care and Use of Experimental Animals (Albus, 2012). Polypropylene cages measuring 50 x 35 x 20 cm were used, with five rats per cage, allowing free access to both water and food. The cages were lined with sawdust and cleaned daily throughout the experiment. The rats were provided with standard food as described by(Southon et al.1984), and tap water was available ad libitum throughout the study.

3-4-2- Experimental Groups:

❖ **Group 01 (Cont - Control):**

All individuals in this group were fed exclusively with a standard diet and were provided with regular drinking water without any additives throughout the experimental period.

❖ **Group 02 (BTU):**

All individuals in this group were fed exclusively with a standard diet and received drinking water supplemented with Benzylthiouracil (BTU) at a concentration of 2 mg/kg for a duration of 21 days.

❖ **Group 03 (BTU + CC):**

All individuals in this group were fed exclusively with a standard diet and received drinking water supplemented with Benzylthiouracil (BTU) at a concentration of 2 mg/kg for 21 days. Subsequently, they were administered cotula cinerea. chamaeleon essential oil at a dose of 100 µL/rat/day via oral gavage for 7 consecutive days.

❖ **Group 04 (BTU + OM):**

All individuals in this group were fed exclusively with a standard diet and received drinking water supplemented with Benzylthiouracil (BTU) at a concentration of 2 mg/kg for 21 days. Subsequently, they were administered *Origanum majorana* essential oil at a dose of 100 µL/rat/day via oral gavage for 7 consecutive days.

❖ **Group 05 (BTU + Levo):**

All individuals in this group were fed exclusively with a standard diet and received drinking water supplemented with Benzylthiouracil (BTU) at a concentration of 2 mg/kg for 21 days.

Afterwards, they received drinking water containing Levothyroxine sodium at a concentration of 1.5 mg/kg for 7 consecutive days.

3-4-3- Sacrifice of Rats and Collection of Blood and Organs:

Following a fasting period of 16 hours, the rats were anesthetized using chloroform (94%) and subsequently sacrificed. Blood samples were collected immediately at the time of sacrifice: into EDTA-containing tubes for hematological analyses, and into dry tubes for biochemical analyses.

After dissection, the thyroid were carefully excised, rinsed first with distilled water and subsequently with 0.9% NaCl solution. A portion of each organ was used for the preparation of for histological examination.

3-5- Methods of Blood Analysis:

3-5-1- Hematological Parameter Assay:

Hematological parameters including white blood cells (WBC), lymphocytes (LYM), granulocytes (GRA), red blood cells (RBC), hemoglobin concentration (HGB), mean corpuscular volume (MCV), and platelets (PLT) were assessed using the Coulter principle.

Measurements were performed with a Medonic automated hematology analyzer designed for Complete Blood Count (CBC) analysis.

3-5-2- Biochemical Parameter Assay:

❖ **Blood Glucose Determination:**

Plasma beta-D-glucose was quantified through enzymatic oxidation by glucose oxidase, resulting in the formation of D-glucono-1,5-lactone and hydrogen peroxide. The lactone subsequently undergoes slow hydrolysis to D-gluconic acid.

The generated hydrogen peroxide is catalytically decomposed by peroxidase into water and nascent oxygen. The liberated oxygen reacts with a chromogenic oxygen acceptor, resulting in the formation of a colored compound. The intensity of the color is directly proportional to the glucose concentration and is measured colorimetrically (Trinder, 1969).

The reaction mechanisms are summarized as follows:

$$Glucose + Glucose\ Oxidase + O_2 \rightarrow Gluconic\ Acid + H_2O_2$$

$$H_2O_2 + Peroxidase + Oxygen\ Acceptor\ (Colorless) \rightarrow H_2O + Oxidized\ Acceptor\ (Colored)$$

❖ **Thyroid Hormones Assay:**

Serum concentrations of thyroid hormones including free triiodothyronine (FT3), free thyroxine (FT4), and ultra-sensitive thyroid-stimulating hormone (TSH) were determined using the Cobas e411 analyzer. This fully automated immunoassay system operates based on the electrochemiluminescence (ECL) detection method (Marwaha et al., 2008.

❖ **C-Reactive Protein (CRP) Assay:**

Serum C-reactive protein (CRP) levels were measured by the turbidimetric method using the COBAS INTEGRA 400 analyzer. This method employs a highly efficient, monospecific antibody-based equilibrium turbidimetric immunoassay, where polyethylene glycol-6000 accelerates and enhances the immunoprecipitation reaction, and Tween-20 surfactant minimizes and stabilizes sample blank values (Otsuji et al., 1982).

❖ **Aspartate Transaminase (AST) Assay:**

Aspartate aminotransferase (AST) activity was determined using an enzymatic kinetic method, with readings performed spectrophotometrically. AST, also known as glutamate oxaloacetate transaminase (GOT), catalyzes the transfer of an amino group from aspartate to α-ketoglutarate, producing oxaloacetate and glutamate. Subsequently, malate dehydrogenase (MDH) and NADH reduce oxaloacetate to malate. The decrease in NADH concentration,

which correlates with AST activity, was monitored by measuring absorbance at 340 nm (Schumann et al., 2010).

The reactions are as follows:

$$Aspartate + 2-Oxoglutarate \rightarrow Oxaloacetate + Glutamate$$

$$Oxaloacetate + NADH + H^{+} \rightarrow Malate + NAD^{+}$$

❖ **Alanine Transaminase (ALT) Assay:**

Alanine aminotransferase (ALT) activity was assessed using an enzymatic kinetic method with spectrophotometric measurement. ALT, also referred to as glutamate-pyruvate transaminase (GPT), catalyzes the transfer of an amino group from L-alanine to α-ketoglutarate, resulting in the production of pyruvate and L-glutamate. Subsequently, pyruvate is reduced to lactate-by-lactate dehydrogenase (LDH) using NADH as a cofactor. The decrease in NADH concentration, which is proportional to the ALT activity, was monitored by measuring absorbance at 340 nm (Schumann et al., 2010).

The reactions are as follows:

$$Alanine + 2-Oxoglutarate \rightarrow Pyruvate + Glutamate$$

$$Pyruvate + NADH + H^{+} \rightarrow Lactate + NAD^{+}$$

❖ **Urea Assay:**

The concentration of urea in serum was determined based on the enzymatic hydrolysis of urea by urease, resulting in the production of ammonia (NH_4^+) and carbon dioxide (CO_2). The ammonia formed is subsequently incorporated into α-ketoglutarate by the action of glutamate dehydrogenase (GLDH), accompanied by the oxidation of NADH to NAD^+. The reduction in NADH concentration, which is directly proportional to the urea concentration, was monitored spectrophotometrically (Kaplan, 1984).

The reactions are as follows:

$$Urea + H_2O + 2H^{+} \rightarrow (NH_4^{+})_2 + CO_2$$

$$NH_4^{+} + \alpha-Ketoglutarate + NADH \rightarrow L-Glutamate + H_2O + NAD$$

❖ **Creatinine Assay:**

Creatinine concentration was measured based on its reaction with sodium picrate under alkaline conditions, leading to the formation of a red-colored complex. The measurement interval was carefully selected to minimize interference from other serum constituents. The

intensity of the resulting color is directly proportional to the creatinine concentration in the sample (Peake and Whiting, 2006).

3-5-3- Histopathological Study:

For histological analysis, fragments of organs from all experimental groups were promptly collected post-sacrifice to prevent autolysis, which begins shortly after the death of the animal. After rinsing the tissues first with distilled water and then with 0.9% NaCl solution, the samples were immediately immersed in a fixative solution of 10% formaldehyde (Leclerc-Mercier et al., 2016).

❖ **The histological preparation involved several steps:**

The tissue fragments were placed into specialized cassettes with perforated walls to facilitate the passage of liquids.

Dehydration of the samples was performed using an automatic tissue processor, allowing a gradual transfer through ethanol baths of increasing concentrations (70%, 95%, and 100%).

Following dehydration, the samples were cleared and infiltrated with liquid paraffin.

The paraffin-impregnated tissues were subsequently embedded by enclosing them in paraffin blocks using embedding stations. These stations maintained the paraffin in a liquid state with a heating system and utilized a refrigerated metal plate for rapid solidification of the paraffin blocks containing the tissues.

Thin sections, approximately 5 μm in thickness, were prepared using specialized instruments known as microtomes. The obtained sections were carefully placed onto microscope slides, spread evenly, and affixed by immersion in heated gelatinous water.

For staining, the Hematoxylin-Eosin (H&E) technique was employed (Elmalti et al., 2007). The reagents required for this process included acid alcohol (prepared by mixing 100 mL of 70% ethyl alcohol with 50 mL of hydrochloric acid), ammoniacal water (prepared from 100 mL of distilled water and 2 mL of ammonia), and an eosin solution (100 mL of 3% aqueous eosin solution, 125 mL of 95% ethyl alcohol, 375 mL of distilled water, and 2 drops of acetic acid).

❖ **The staining procedure involved the following steps:**

Deparaffinization and Hydration: Slides were first deparaffinized and rehydrated using tap water, followed by rinsing with distilled water.

Nuclear Staining: Slides were immersed in Harris Hematoxylin for 15 minutes to stain basophilic structures (nuclei) purplish-blue.

Differentiation: After hematoxylin staining, the slides were dipped briefly (1–2 times) in acid alcohol for differentiation, then rinsed in tap water, with microscopic verification of the differentiation.

Bluing: Slides were immersed in ammoniacal water to intensify the nuclear staining.

Cytoplasmic Staining: The slides were subsequently immersed in eosin for 15 seconds to 2 minutes to stain acidophilic structures (cytoplasm) pink.

Each staining step was separated by thorough rinsing with tap water.

Microscopic Examination: After drying, the stained preparations were observed under an optical microscope and photographed using a digital camera (Bremond-Gignac et al., 2004).

3-5-4- In-Silico Analysis:

- **Software:**

Computational simulations, including Induced Fit Docking, , and MM-GBSA calculations, were performed using the Glide module, Induced Fit Docking module, Prime module, and 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.

- **ADMET and Drug-Likeness Evaluation:**

Drug candidates are expected to possess favorable ADMET properties and ideally be non-toxic. Consequently, the major compounds identified from the essential oils extract were evaluated for their ADME profile, which includes physicochemical properties, lipophilicity, water solubility, pharmacokinetics, drug-like characteristics, medicinal chemistry, and various other parameters. This evaluation was carried out using the SwissADME module provided by the Swiss Institute of Bioinformatics (SIB) webserver (Daina et al., 2017; Riyadi et al., 2021) [https://www.sib.swiss]. Additionally, the toxicity of the compounds was predicted using the ProTox webserver (Banerjee et al., 2018) [https://comptox.charite.de/protox3/].

❖ **Docking Setup:**

- **Ligand Preparation**: The three-dimensional structures of the major compounds isolated from *Cotula cinerea* and *Origanum majorana* L essential oils were obtained from the National Library of Medicine (NCBI) database (Kim et al., 2016), which is accessible through the NCBI website [https://pubchem.ncbi.nlm.nih.gov/].
- **Ligand Preparation:** Ligand preparation involved an energy optimization process to derive the most energetically favorable conformations for each compound. This was achieved using the LigPrep module within the Schrödinger Suite (Schrödinger, 2024). The optimization procedure ensured the compounds, including Montbretin A (a co-crystallized ligand), reached their lowest energy states. Ionization states were determined at a , as computed by the Epik Classic module, while maintaining specified chirality and generating relevant tautomeric forms. Additionally, partial atomic charges were calculated using the Optimized Potentials for Liquid Simulations (OPLS4) force field (Lu et al., 2021).
- **Receptor Preparation:** Crystallographic data for Human thyroid (PDB ID: 3GWS) (Williams et al., 2015) was retrieved from the Protein Data Bank (http://www.rcsb.org) (Rose et al., 2017), with specific parameters such as a resolution of 2.20 Å and an R value-free of 0.233. The protein structure was processed using the Protein Preparation Work flow module in the Schrödinger suite (Madhavi Sastry et al., 2013), which involved multiple stages of import, processing, review, modification, and refinement.

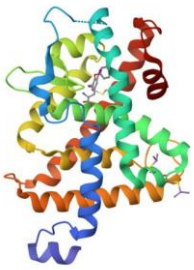
The initial stage employed the Prime tool to address missing residues and side chains, maintaining a using PROPKA. Subsequent steps included optimization and assignment of hydrogen bonds, as well as the removal of water molecules beyond 8 Å. Restraint minimization was performed using the OPLS4 force field, achieving a low-energy state for the protein (Lu et al., 2021). This stage of protein preparation signifies an energy optimization methodology, ensuring that the protein is in its energetically favorable state for subsequent in-silico studies.

The Receptor Grid Generation panel was then utilized to create a grid encompassing the active site of the protein, defined by the co-crystallized ligand Montbretin A. Default parameters were maintained, with the grid center generated at coordinates

X=4.24 Y=18.96

Z=29.85

Table 04 . Target receptor information chosen for docking studies

	
Parameter	Information
Target receptor	thyroide
PDB ID	3GWS
Mutation	NO
Resolution (Å)	2.20
R-Value Free	0.233
R-Value Work	0.191
R-Value Observed	0.193
Organism	HOMO SAPIENS
Expression System	Escherichia Coli
Space Groupe	P 31 21
Sequence Length	458

❖ **Molecular Docking:**

Molecular docking simulations, including Induced Fit Docking (IFD), molecular dynamics (MD) studies, and MM-GBSA calculations, were conducted using the Glide module, Induced Fit Docking module, Prime module, and 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).

All computational experiments were performed on a DELL Intel(R) Core (TM) i9-13900HX CPU @ 2.20 GHz processor, equipped with 32.0 GB RAM, and operating on a 64-bit Linux Ubuntu 18.04.1 LTS 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 were docked into the predefined active site of the receptor using the Glide module in Standard Precision (SP) mode (Friesner et al., 2006), optimizing the ligand binding poses based on scoring functions that consider both steric and electrostatic interactions.

CHAPTER TWO: RESULTS AND DISCUSSION

Introduction:

This chapter presents the findings related to the extraction yield, chemical characterization, and in-depth investigation of the anti-thyroïde properties of two essential oils obtained from the aerial parts of Cotula cinerea and *Origanum majorana* L.. The primary objective of this study was to evaluate the potential anti-thyroïde efficacy of these essential oils, highlighting their promise as candidates in the development of novel pharmaceutical agents.

The results provide a detailed analysis of the chemical composition of the oils, which facilitated their application in in-silico studies. Advanced computational techniques.

2. Extraction Yield:

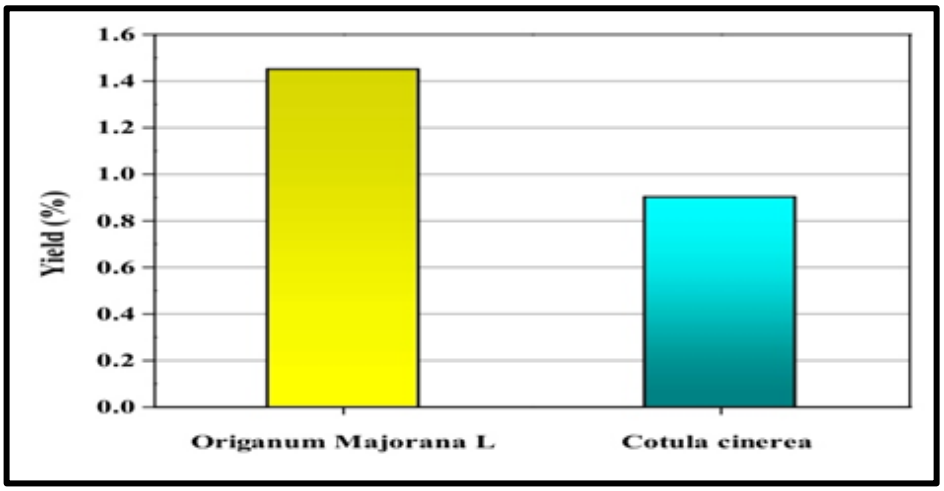


Figure 17 illustrates the yields of essential oils obtained through hydrodistillation of the aerial

parts of Cotula cinerea and *Origanum Majorana* L. variety of Eloued.

We observe that *Origanum Majorana* L exhibits a higher essential oil yield than Cotula cinerea (1.45% and 0.90% respectively). However, the difference between the two yields is non significant.

Nevertheless, the findings reported by Alimi et al. (2022) demonstrated lower yields of essential oils extracted via hydrodistillation from *Origanum majorana* L. and Cotula cinerea under ambient temperature conditions.

In contrast, results consistent with those obtained in the present study were reported by Naima et al. (2019), who employed the same extraction method on the same plant species.

Additionally, Vera and Chane-Ming (1999) reported significantly higher yields from the hydrodistillation of the Indian variety of *Origanum majorana* L..

Such variations in extraction yield can be attributed to multiple influencing factors, either related to the plant itself (species, variety, chemical composition, etc.) or to the experimental conditions (extraction technique, duration, temperature, 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 05. The organoleptic characteristics of essential oils

Plant	Smell	Aspect	Colour
Cotula cinerea	A distinctive aroma	liquid at room temperature	Dark-yellow
Origanum Majorana L	A pleasant fragrance	liquid at room temperature	Light-yellow

3.2. Physicochemical Properties:

The physicochemical properties were rigorously assessed in accordance with the standards set by the French Association for Standardization (AFNOR), employing validated methodologies to determine relative density, refractive index, acid value, and ester value, as presented in Table 6.

Table 06.The physicochemical properties of essential oils

Plant	Relative Density	Refractive Index	Acidity Index	Ester Index
Cotula cinerea	0.958	1.4720	5.01	43.72
Origanum Majorana L	0.908	1.4512	4.39	41.23

By comparing these findings with those reported by Naima et al. (2019) who recorded a density of 0.953, a refractive index of 1.474, an acid value of 4.93, and an ester value of 45.96 and by Alimi et al. (2022), who reported densities of 0.834 ± 0.02 and 0.835 ± 0.02 and refractive indices at 25°C of 1.4596 ± 0.03 and 1.4622 ± 0.04, it can be observed that the values obtained in the present study, with the refractive index measured at 23°C, are consistent with the standards established by AFNOR (Afnor, 1982).

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

The analysis centered on identifying the constituents of essential oils extracted from two selected plant species using gas chromatography–mass spectrometry (GC/MS). Mass spectra associated with each chromatographic peak were compared against reference spectra from scientific literature and the Wiley mass spectral database (Horai et al., 2010). Retention indices were used to confirm the identity of the compounds. Additionally, the use of an identical non-polar HP5 stationary phase in the gas chromatography column ensured consistency in peak retention and elution order for the compounds under analysis.

Figures 15 and 16 present the GC/MS chromatograms illustrating the essential oil profiles of Cotula cinerea and *Origanum majorana* L., respectively.

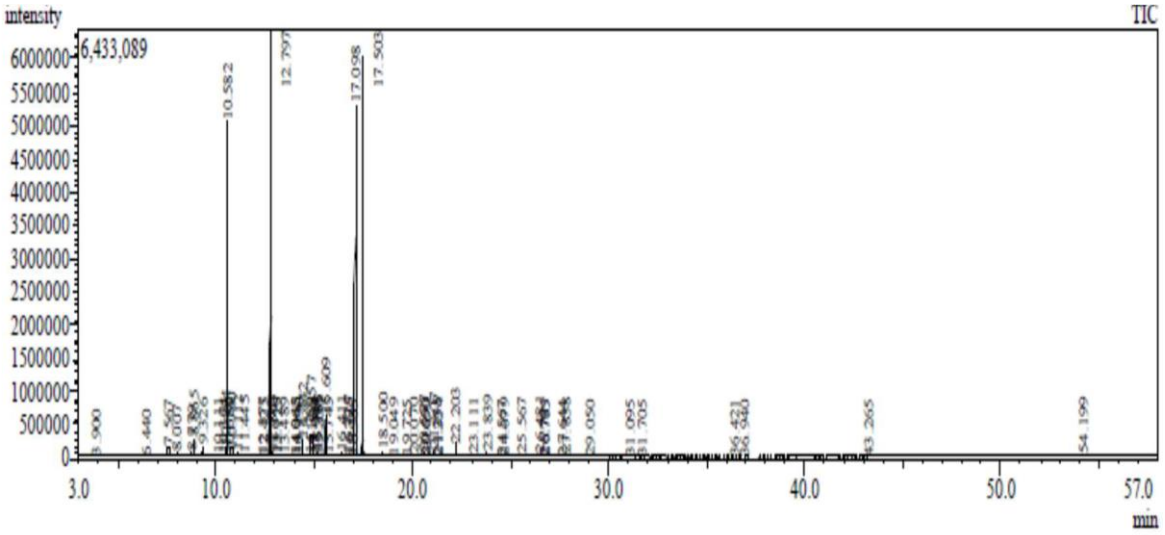


Figure15. Chromatogram of the essential oil of Cotula cinerea plant obtained by GC/MS

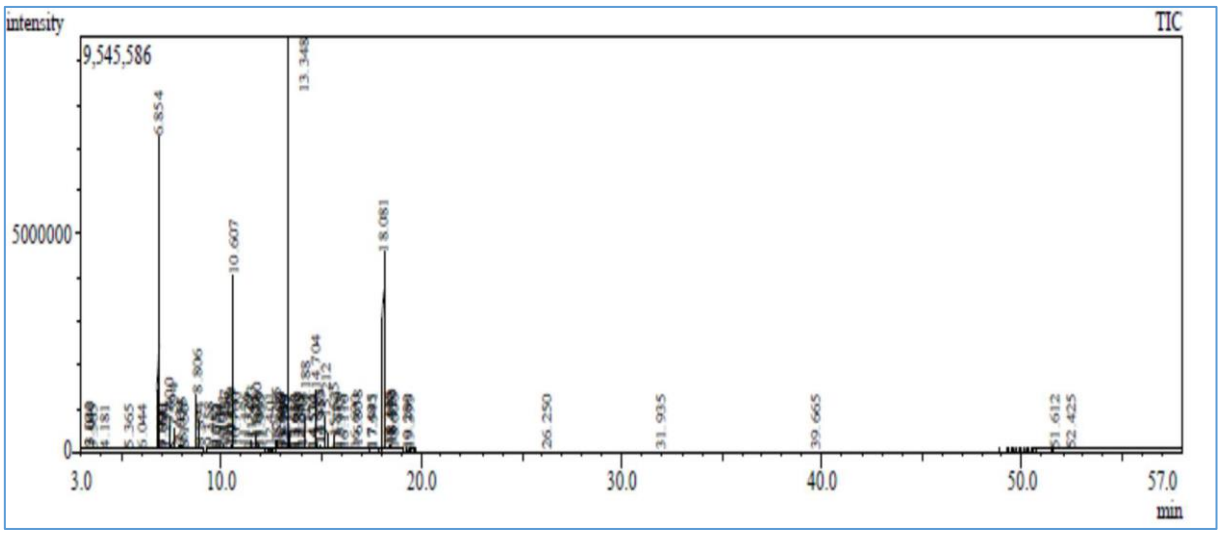


Figure 16. Chromatogram of the essential oil of *Origanum Majorana* L plant obtained by GC/MS

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 Cotula cinerea and *Origanum majorana* L, have been identified and collated as follows

3.3.1. Cotula cinerea:

The hydrodistillation of Cotula cinerea resulted in the extraction of a dark yellow essential oil with a yield of 0.91%. Gas chromatography–mass spectrometry (GC/MS) analysis revealed the presence of 31 distinct compounds, collectively representing 95.64% of the total oil composition (as detailed in Table 7). The oil was primarily composed of oxygenated monoterpenes (68.16%), followed by hydrocarbon monoterpenes (23.27%). Oxygenated sesquiterpenes were present in a much lower proportion (0.15%), while the remaining 4.06% consisted of various other minor constituents.

Table 07. Essential oil constituents of Cotula cinerea identified by GC/MS

No	Compounds	IR_{Exp}	IR_{Ref}	(%)
01	**Santolina triene**	**908**	**914**	**10.6**
02	Alpha-thujene	931	935	0.88
03	**Alpha-pinene**	**939**	**943**	**2.02**
04	camphene	953	956	0.85
05	**Sabinene**	**976**	**976**	**6.17**
06	Beta- pinene	980	981	0.58
07	Dehydro-1.8-cineole	991	988	0.64
08	Myrcene	991	993	0.07
09	Meta mentha-1(7)-8dien	999	997	0.06

10	Alpha-terpinene	1018	1017	0.83
11	Ortho cymene	1022	1020	0.43
12	Para cymene	1026	1029	0.6
13	**1.8-cineole**	**1033**	**1033**	**5.34**
14	**Delta-terpinene**	**1062**	**1057**	**1.57**
15	Cis sabinene hydrate	1068	1069	0.46
16	Terpinolene	1088	1088	0.36
17	Cis thujone	1102	1100	0.52
18	**Trans thujone**	**1114**	**1117**	**51.86**
19	**Camphor**	**1143**	**1140**	**2.63**
20	**Beta-terpineol**	**1163**	**1160**	**1.39**
21	**Terpin-4-ol**	**1177**	**1178**	**1.73**
22	Alpha-terpineol	1189	1190	0.58
23	Myrtenol	1194	1195	0.13
24	Neo iso dihydro carveol	1226	1224	0.53
25	Carvotanacetone	1246	1247	0.9
26	**Cis verbenyl acetate**	**1262**	**1264**	**5.07**
27	Iso pulegol acetate	1273	1274	0.06
28	Para cymen-7-ol	1287	1285	0.08
29	Neryl acetate	1365	1367	--
30	Cis jasmone	1394	1390	0.15
31	Germacrene D	1480	1481	0.06
Total		**95.64**		

Our analysis revealed ten compounds with concentrations exceeding 1% (Table 7), with trans-thujone being the dominant constituent at 51.86%, followed by santolina triene at 10.69% and sabinene at 6.17%. Additionally, 1,8-cineole accounted for 5.34%, while α-pinene, terpin-4-ol, β-terpineol, and camphor were present in lower proportions 2.02%, 1.73%, 1.39%, and 2.63%, respectively.

A comparison with previous studies highlighted certain similarities, particularly regarding the percentage of santolina triene, which was reported at 11.67% in the study by Ekhlil et al. (2016). However, differences were observed in the major constituent: iso-thujanol was the principal compound in their findings, comprising 47.38%. Conversely, the results of El Bouzidi et al. (2011) closely aligned with ours, especially for trans-thujone (41.4%), 1,8-

cineole (8.2%), and santolina triene (7.2%), while camphor was reported at a higher percentage (5.5%). In contrast, Fournier et al. (1989) identified camphor as the major component, comprising 50% of the oil.

For a visual representation of the major constituents (those exceeding 1%) and their respective structures, see Figure 20, which illustrates the chemical structures and IUPAC names of the predominant compounds identified in the essential oil of Cotula cinerea.

3.3.2. *Origanum Majorana* L:

The essential oil of *Origanum majorana* L. was extracted via hydrodistillation, yielding a notable 1.5%. Gas chromatography–mass spectrometry (GC/MS) analysis enabled the identification of 42 compounds, accounting for 98.84% of the total oil composition (as shown in Table 8). The oil was predominantly composed of oxygenated monoterpenes (over 57%), followed by hydrocarbon monoterpenes at 25.14%.

Of particular interest is the high concentration of trans-thujone, which constituted 33.3% of the oil an observation not commonly reported in prior literature. For example, in the study by Vera and Chane-Ming (1999), terpinen-4-ol was identified as the principal component at 38.4%, a compound completely absents in the present analysis. Likewise, santolina triene emerged as the second most abundant constituent in this study at 16.40%, yet it has not been commonly reported in existing studies.

Despite these discrepancies, some consistency was observed concerning sabinene, which was detected at 3.12% in the current study, compared to 15% in Vera and Chane-Ming's (1999) study and 17% in that of Baser et al. (1993). Similarly, Sellami et al. (2009) reported sabinene at 2.14%.

Other notable constituents identified included α-thujene (1.44%), α-pinene (1.19%), and β-pinene oxide (4.42%).

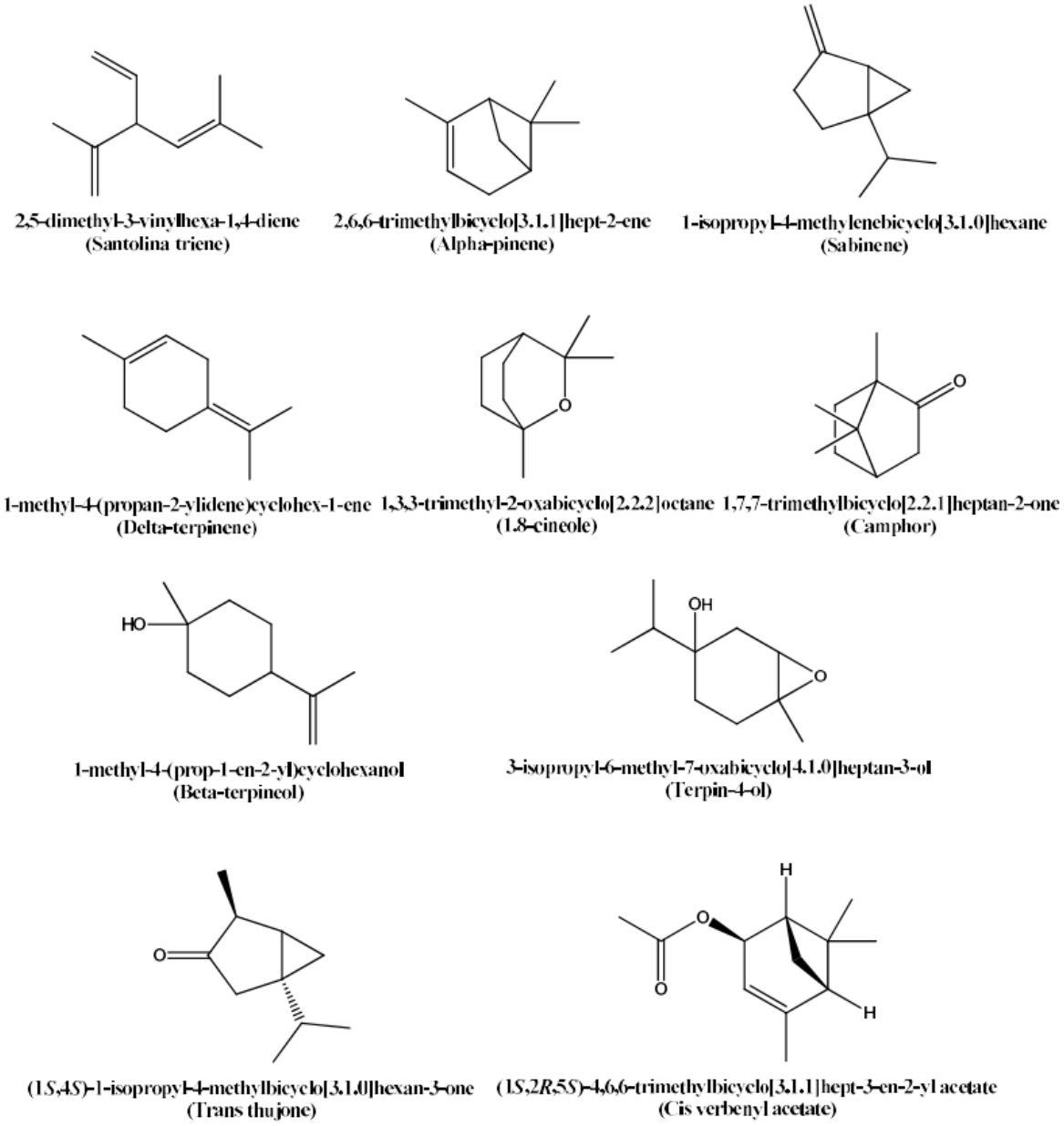


Figure 17. Chemical structures and IUPAC names of the top major compounds identified in the essential oil of Cotula cinerea

In summary, the essential oil derived from *Origanum Majorana* L. exhibits distinctive chemical compositions in Eloued region compared to oils from other geographical locales, characterized notably by the prevalence of trans-Thujone and Santolina triene as major constituents. This variance likely stems from multifactorial influences such as soil quality, climatic conditions, and harvest timing. For an illustrative overview of the major compounds identified, Figure 17. displaying the structures and IUPAC nomenclature of these constituents.

Table 08.Essential oil constituents of *Origanum Majorana* L identified by GC/MS

No	**Compounds**	**IRExp**	**IRRef**	**(%)**
01	Pentanol	759	762	0.12
02	Cis-2-Penten-1-ol	762	765	0.03
03	Hexanal	801	801	0.02
04	(Z)-Salvene	844	847	0.02
05	Isopentyl acetate	869	869	0.02
06	**Santolina triene**	**898**	**906**	**16.42**
07	Tricyclene	912	921	0.02
08	**Alpha-Thujene**	**918**	**924**	**1.44**
09	**Alpha-Pinene**	**925**	**932**	**1.19**
10	Camphene	941	946	0.43
11	**Sabinene**	**969**	**969**	**3.12**
12	Beta-Pinene	972	974	0.22
13	Beta-Myrcene	989	988	0.14
14	Alpha-Phellandrene	1003	1002	0.03
15	Propanoic acid, 2-methyl-, 3-methylbutyl ester	1012	1007	0.02
16	Alpha-Terpinene	1016	1014	0.36
17	Para cymene	1024	1020	0.20
18	Ortho Cymene	1026	1022	0.45
19	Sylvestrene	1028	1025	0.20
20	**1,8-Cineole**	**1031**	**1026**	**10.71**
21	Trans-decahydroNaphthalene	1054	1053	0.03
22	Terpinene	1059	1054	0.76
23	Cis Sabinene hydrate	1068	1065	0.89
24	Para-Mentha-2'4(8)-diene	1088	1085	0.11
25	Trans-Sabinene hydrate	1100	1198	0.86
26	Isopentyl-2-methylbutanoate	1104	1100	0.14
27	cis-Thujone	1107	1101	0.24
28	**trans-Thujone**	**1119**	**1112**	**33.30**
29	iso-3-Thujanol	1136	1134	0.04
30	**Camphor**	**1146**	**1141**	**2.85**
31	**Beta pinene oxide**	**1163**	**1154**	**4.42**

32	3-Thujanol	1168	1164	0.48
33	Alpha Terpineol	1192	1186	0.88
34	Dihydrocarveol	1197	1192	0.06
35	Safranal	1203	1197	0.01
36	Cis-3-Hexenyl 2-methyl butanoate	1233	1229	0.25
37	trans-Myrtanol	1257	1258	0.04
38	2-(1E)-propenyl Phenol	1258	1264	0.09
39	cis Verbenyl acetate	1277	1280	15.05
40	neoiso-3Thujanol acetate	1287	1281	0.09
41	Isobornyl acetate	1290	1283	0.21
42	Lavandulyl acetate	1293	1288	0.32
Total		95.64		

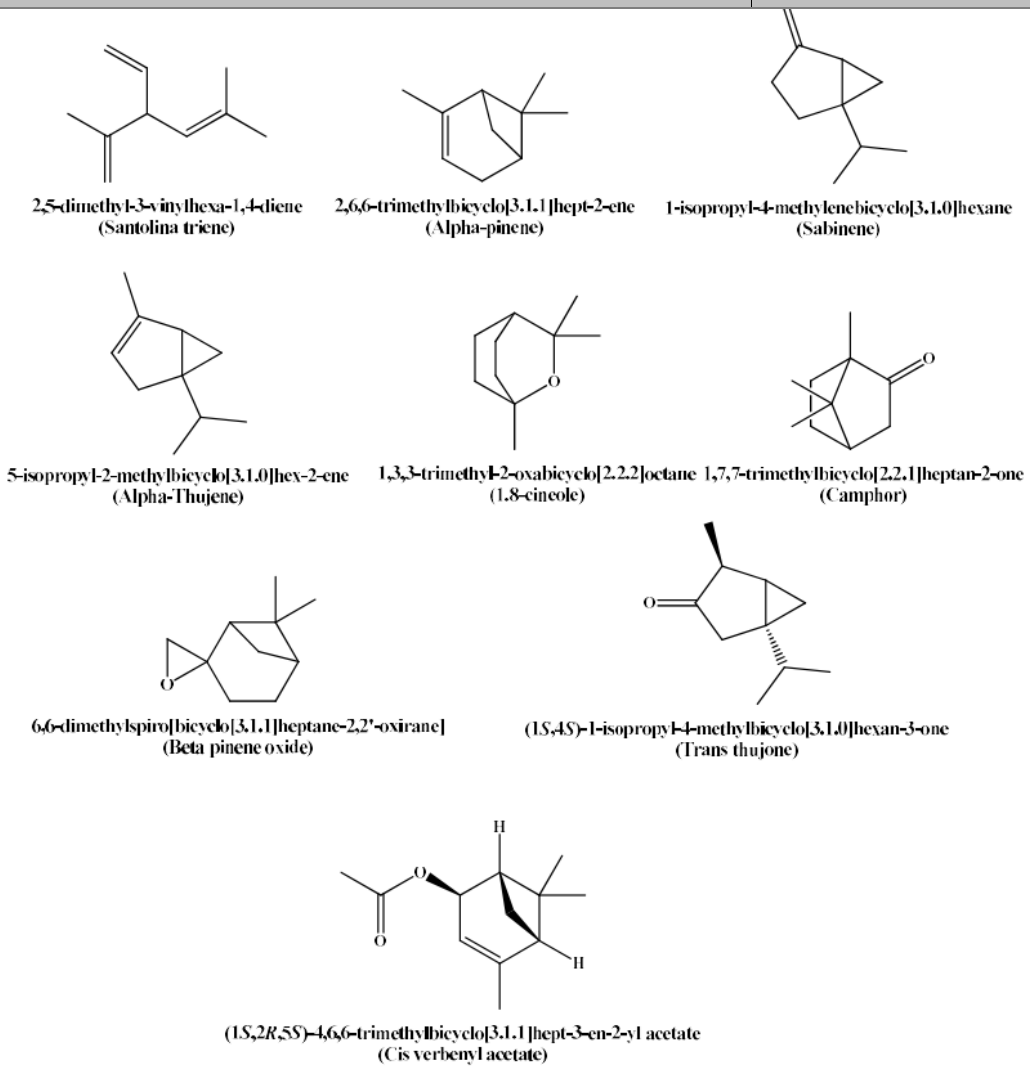


Figure18 . Chemical structures and IUPAC names of the top major compounds identified in the essential oil of *Origanum Majorana* L

4. In vivo antithyroïde activities:

4.1.Weight Gain Index :

Table 09 displays the weight gain index of the various experimental groups before and after treatment. The control group exhibited stable body weight, with values of 6.2 ± 0.1 before treatment and 6.48 ± 0.67 after treatment. In contrast, exposure to benzylthiouracil (BTU) led to a significant elevation in the weight gain index, increasing to 23.39 ± 1.84 before treatment and further to 26.69 ± 0.02 after treatment ($p < 0.05$ vs. control), indicating a marked weight gain induced by BTU administration.

The treatment with *Cotula cinerea* (BTU + CC) and *Origanum majorana L.* (BTU + OM) resulted in non-significant changes in the pre-treatment weight gain index compared to the BTU group, with values of 24.34 ± 1.35 and 24.06 ± 2.06, respectively. However, post-treatment values in both groups showed a significant reduction in weight gain index compared to the BTU group (6.66 ± 0.68 for CC and 6.16 ± 0.48 for OM; $p < 0.05$), indicating the potential ameliorative effects of both herbal treatments. On the other hand, the treatment with Levothyroxine (BTU + Levo) resulted in a pre-treatment weight gain index of 25.25 ± 0.89, which was not significantly different from the BTU group. However, the post-treatment value remained elevated at 21.16 ± 1.69, showing no significant reduction compared to BTU alone, suggesting a limited effect of levofloxacin in mitigating BTU-induced weight gain.

Table 09:Weight Gain Index of different experimental groups

	Weight Gain Index	
	Before treatment	After treatment
Control	6.2±0.1	6.48±0.67
BTU	23.39±1.84[a]	26.69±0.02 [a]
BTU + CC	24.34±1.35[b NS]	6.66±0.68 [NS*]
BTU + OM	24.06±2.06[a NS]	6.16±0.48 [NS *]
BTU + Levo	25.25±0.89 [b NS]	21.16±1.69 [a NS]

NS: Non-significant differences; Comparison with the control group: $p < 0.05$ (a), $p < 0.01$ (b), $p < 0.001$ (c); Comparison with BTU group: $p < 0.05$ (*), $p < 0.01$ (**), $p < 0.001$ (***)

4.2. Hematological parameters

Table 10 outlines the effects of various treatments on hematological parameters in rats subjected to benzylthiouracil (BTU)-induced toxicity. In the control group, all values remained within normal physiological ranges. However, BTU exposure significantly disrupted hematological balance. White blood cell count (WBC) increased significantly to $4.23 \pm 0.18 \times 10^9$/L ($p < 0.01$ vs. control), along with a significant rise in lymphocytes (LYM)

($3.1 \pm 0.08 \times 10^9$/L; $p < 0.05$). Conversely, BTU induced a significant reduction in hemoglobin (HGB) (9.4 ± 0.21 g/dL; $p < 0.05$), red blood cells (RBC) ($4.9 \pm 0.14 \times 10^{12}$/L; $p < 0.05$), and platelets (PLT) increased sharply to $676 \pm 11.5 \times 10^9$/L ($p < 0.001$), indicating anemia and inflammation. Granulocytes (GRA) were reduced, though the change was not statistically significant.

The treatment with *Cotula cinerea* attenuated BTU-induced hematological alterations. WBC and LYM values decreased compared to BTU, although not significantly. Notably, HGB and RBC levels significantly improved to 15.4 ± 0.12 g/dL and $7.17 \pm 0.11 \times 10^{12}$/L, respectively ($p < 0.01$ vs. BTU), reflecting recovery from anemia. PLT count also declined to $456.3 \pm 44 \times 10^9$/L ($p < 0.05$ vs. BTU), indicating an anti-inflammatory effect. GRA levels increased, though without statistical significance.

Treatment with *Origanum majorana* led to a further elevation in WBC and LYM (5.1 ± 0.05 and $3.6 \pm 0.26 \times 10^9$/L, respectively; $p < 0.01$ vs. control), suggesting a potential immune-stimulatory response. HGB (15.26 ± 0.76 g/dL) and RBC ($6.77 \pm 0.57 \times 10^{12}$/L) significantly improved compared to BTU ($p < 0.05$), indicating effective recovery from anemia. However, PLT remained markedly elevated ($800.67 \pm 5.4 \times 10^9$/L; $p < 0.01$ vs. control and $p < 0.05$ vs. BTU), possibly reflecting sustained immune activation.

Levothyroxine administration also helped restore hematological parameters. WBC and LYM levels remained elevated (5.7 ± 0.65 and $3.46 \pm 0.63 \times 10^9$/L), while HGB ($15.13 \pm 0.2$ g/dL; $p < 0.01$) and RBC ($6.1 \pm 0.44 \times 10^{12}$/L) significantly increased compared to BTU. GRA levels (0.84 ± 0.34) and PLT ($751 \pm 18 \times 10^9$/L) also increased, indicating partial hematological recovery with a persistent inflammatory signature.

Table 10 : Plasma concentration of hematological parameters of different experimental groups

	WBC($\times 10^9$/L)	LYM($\times 10^9$/L)	GRA($\times 10^9$/L)	HGB (g/dL)	RBC($\times 10^{12}$/L)	PLT ($\times 10^9$/L)
Control	1.46±0.29	1.4±0.26	1.1±0.4	14.46±0.78	7.8±0.47	516±9.23
BTU	4.23±0.18 ^{b}	3.1±0.08^{a}	0.16±0.08 ^{NS}	9.4±0.21^{a}	4.9±0.14^{a}	676±11.5 ^{c}
BTU + CC	2.63±0.31 ^{NS}	2.23±0.12^{NS*}	1.7±0.49^{NS}	15.4±0.12^{NS**}	7.17±0.11^{NS**}	456.3±44 $^{NS *}$
BTU + OM	5.1±0.05 $^{b\,NS}$	3.6±0.26 $^{b\,NS}$	1.45±0.55 ^{NS}	15.26±0.76^{NS*}	6.77±0.57 $^{a\,NS}$	800.67±5.4 ^{b*}
BTU + Levo	5.7±0.65 $^{a\,NS}$	3.46±0.63 ^{NS}	0.84±0.34 ^{NS}	15.13±0.2 ^{NS**}	6.1±0.44 ^{NS}	751±18 $^{b\,NS}$

NS: Non-significant differences; Comparison with the control group: $p < 0.05$ (a), $p < 0.01$ (b), $p < 0.001$ (c); Comparison with BTU group: $p < 0.05$ (*), $p < 0.01$ (**), $p < 0.001$ (***)

4.3. Glycemia, liver and kidney's function parameters

Administration of benzylthiouracil (BTU) led to significant disruptions in glucose metabolism, liver function, and renal parameters. Compared to the control group, BTU significantly elevated fasting blood sugar (FBS) levels (0.93 ± 0.01 g/L; $p < 0.05$), indicating hyperglycemia. Hepatic enzymes were markedly increased, with aspartate aminotransferase (AST) rising to 226.06 ± 8.84 U/L ($p < 0.05$) and alanine aminotransferase (ALT) to 116 ± 2.63 U/L ($p < 0.01$), reflecting liver injury. Moreover, BTU treatment significantly increased blood urea (0.66 ± 0.011 g/L; $p < 0.01$) and creatinine (CT) levels (5.56 ± 0.033 mg/L; $p < 0.05$), suggesting impaired renal function.

The treatment with *Cotula cinerea* (BTU + CC) demonstrated a significant improvement in glycemic control, reducing FBS to 0.76 ± 0.01 g/L ($p < 0.05$ vs. BTU). Liver function was also restored, with AST and ALT activities significantly decreased to 111.1 ± 18.7 U/L and 71.56 ± 2.17 U/L, respectively ($p < 0.01$ vs. BTU), approaching control values. Urea levels normalized to 0.43 ± 0.015 g/L ($p < 0.01$), and CT levels dropped to 4.63 ± 0.27 mg/L, indicating a notable renoprotective effect.

Similarly, *Origanum majorana* (BTU + OM) showed effective restoration of metabolic and organ function. FBS was reduced to 0.64 ± 0.05 g/L, which was not significantly different from the control, suggesting near-complete normalization of glycemia. Both AST (99.6 ± 0.31 U/L) and ALT (76.11 ± 1.15 U/L) were significantly lowered ($p < 0.01$ vs. BTU), and renal markers showed improvement, with urea at 0.47 ± 0.03 g/L ($p < 0.05$ vs. BTU) and CT at 4.86 ± 0.38 mg/L.

Levothyroxine treatment (BTU + Levo) also demonstrated strong protective effects. FBS significantly decreased to 0.67 ± 0.02 g/L ($p < 0.01$ vs. BTU), indicating improved glycemic regulation. Liver enzymes were reduced to 104.3 ± 7.5 U/L ($p < 0.001$) for AST and 77.0 ± 8.5 U/L for ALT, confirming attenuation of hepatocellular injury. Urea returned to control levels (0.42 ± 0.011 g/L; $p < 0.01$), while CT was stabilized at 5.1 ± 0.05 mg/L, suggesting effective renal protection.

Table 11: Glycemia, liver and kidney's function parameters of different experimental groups

	FBS (g/L)	AST (U/L)	ALT (U/L)	Urea (g/L)	CT (mg/L)
Control	0.58±0.04	126.13±6.48	79.23±2.9	0.43±0.017	5.06±0.066
BTU	0.93 ± 0.01^{a}	226.06 ± 8.84^{a}	116 ± 2.63^{b}	0.66 ± 0.011^{b}	5.56 ± 0.033^{a}
BTU + CC	$0.76 \pm 0.01^{a*}$	$111.1 \pm 18.7^{NS**}$	$71.56 \pm 2.17^{NS**}$	$0.43 \pm 0.015^{NS**}$	4.63 ± 0.27^{NS}
BTU + OM	0.64 ± 0.05^{NS}	$99.6 \pm 0.31^{NS**}$	$76.11 \pm 1.15^{NS**}$	$0.47 \pm 0.03^{NS*}$	4.86 ± 0.38^{NS}
BTU + Levo	$0.67 \pm 0.02^{NS**}$	$104.3 \pm 7.5^{NS***}$	77.0 ± 8.5^{NS}	$0.42 \pm 0.011^{NS**}$	$5.1 \pm 0.05^{NS**}$

NS: Non-significant differences; Comparison with the control group: $p < 0.05$ (a), $p < 0.01$ (b), $p < 0.001$ (c); Comparison with BTU group: $p < 0.05$ (*), $p < 0.01$ (**), $p < 0.001$ (***)

4.4. Thyroid function parameters

Benzylthiouracil (BTU) administration induced significant alterations in thyroid hormone levels, confirming its antithyroid effect. In BTU-treated rats, serum levels of thyroxine (T4) and triiodothyronine (T3) were significantly reduced to 16.2 ± 0.1 pmol/L ($p < 0.05$) and 3.9 ± 0.4 pmol/L ($p < 0.05$), respectively, compared to the control group (T4: 19.5 ± 0.28 pmol/L; T3: 8.65 ± 0.32 pmol/L). Conversely, thyroid-stimulating hormone (TSH) levels were significantly elevated in the BTU group (0.37 ± 0.01 µUI/L; $p < 0.01$), suggesting a negative feedback response to hypothyroidism.

The treatment with *Cotula cinerea* (BTU + CC) showed promising effects in restoring thyroid function. Although T4 and T3 levels were not significantly different from the control (23.6 ± 3.09 pmol/L and 8.38 ± 0.8 pmol/L, respectively), TSH levels were significantly decreased compared to the BTU group (0.09 ± 0.005 µUI/L; $p < 0.01$), indicating a reversal of the hypothyroid state.

Similarly, *Origanum majorana* (BTU + OM) effectively improved thyroid hormone profiles. T4 increased to 21.4 ± 1.34 pmol/L and T3 to 7.2 ± 0.02 pmol/L ($p < 0.05$ vs. BTU), while TSH significantly decreased to 0.07 ± 0.006 µUI/L ($p < 0.01$ vs. BTU), suggesting a marked restoration of thyroid axis homeostasis.

Treatment with levothyroxine (BTU + Levo) effectively normalized all thyroid function parameters. T4 levels returned to control values (19.5 ± 0.28 pmol/L; $p < 0.01$ vs. BTU), and TSH significantly declined to 0.08 ± 0.006 µUI/L ($p < 0.001$ vs. BTU), further confirming the efficacy of this standard hormone replacement therapy. Although T3 levels (6.41 ± 1.12 pmol/L) remained slightly below normal, the values were not statistically different from the control group.

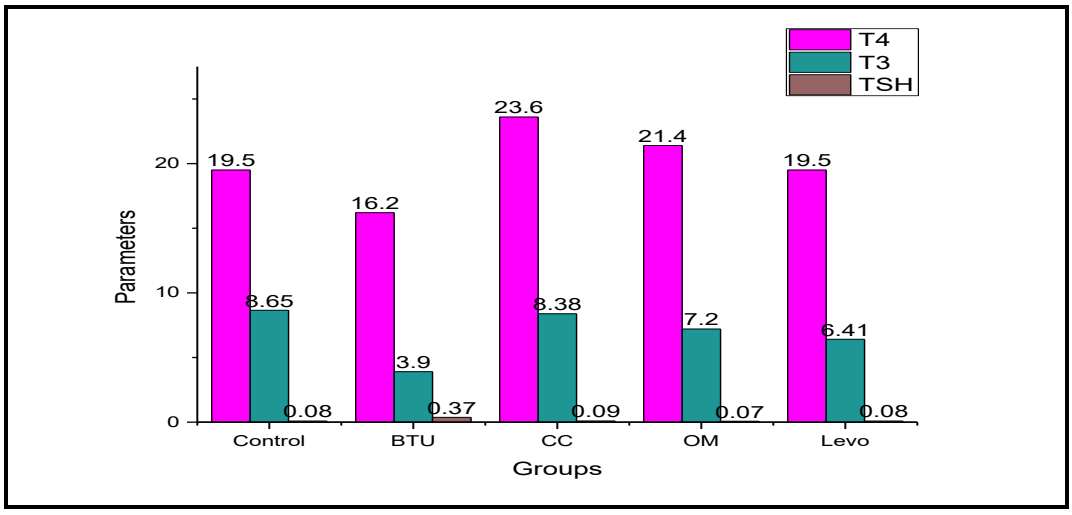


Figure 19: Thyroid function parameters of different experimental groups

4.5. Histopathological studies

The histological images stained with hematoxylin and eosin (H&E) further illustrates the structural alterations in thyroid tissue induced by benzylthiouracil (BTU), along with the comparative effects of additional therapeutic agents.

- **Control (C):** Normal thyroid follicles (V) are lined by cuboidal follicular cells (FC), with uniform colloid-filled lumens and minimal interstitial cellularity, indicating healthy and functional thyroid tissue.
- **BTU Group:** BTU administration leads to pronounced histological abnormalities. The follicular architecture is severely disrupted, with dilated blood vessels (BV), interstitial congestion, increased inflammatory infiltration, and reduced colloid density within follicles. This reflects a hypothyroid state induced by BTU's antithyroid effects.
- **Levothyroxine (BTU + Levo):** Treatment with levothyroxine shows improved follicular morphology compared to the BTU group. There is partial restoration of colloid content and a more organized follicular lining, demonstrating levothyroxine's therapeutic effect in restoring thyroid hormone balance.
- ***Cotula cinerea* (BTU + CC):** This group exhibits moderate structural improvement. Follicles are more regularly shaped, and colloid reaccumulation is observed. The number of inflammatory cells is reduced, suggesting anti-hypothyroidism and anti-inflammatory activity of the *Cotula cinerea* treatment.
- ***Origanum Majorana L* (BTU + OM):** Histological improvements are evident with more prominent colloid reaccumulation and preserved follicular integrity. The epithelium is largely intact, and interstitial inflammation appears reduced. This indicates a protective effect likely due to *Origanum Majorana* L's anti-hypothyroidism and anti-inflammatory activity.

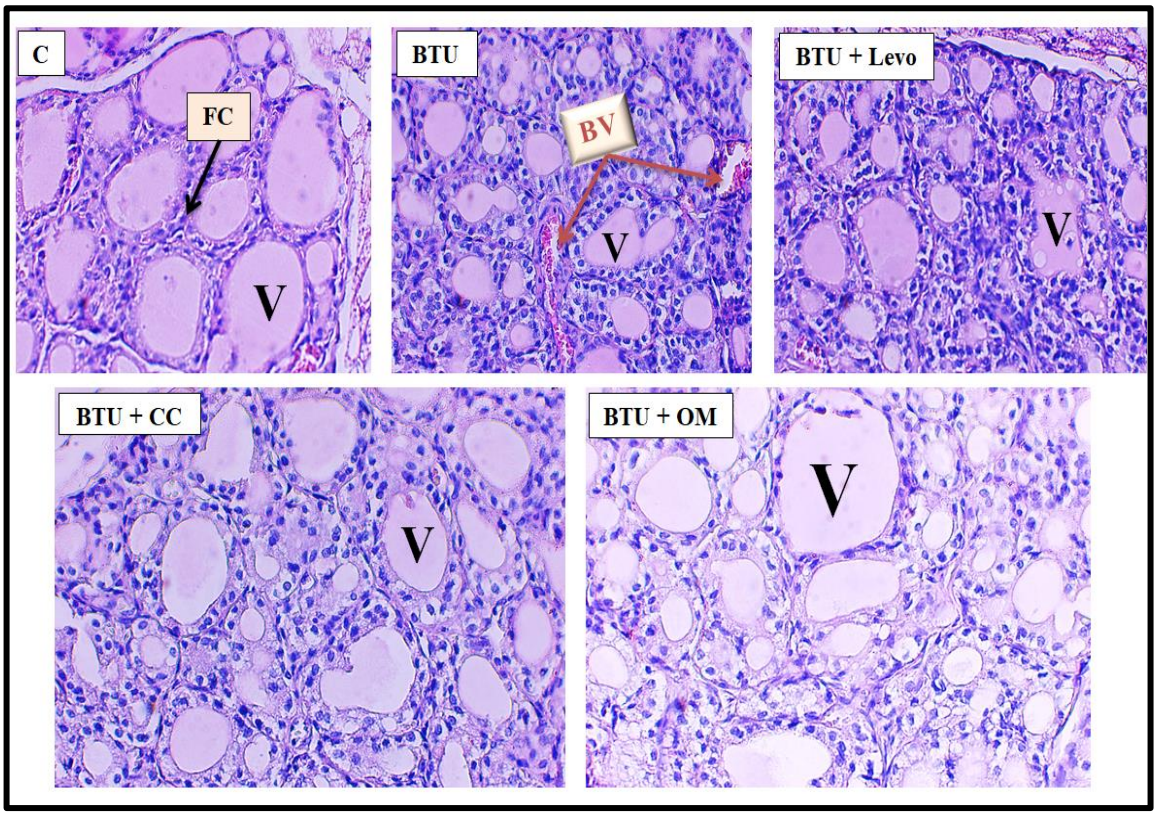


Figure 20: Microscopic observation of thyroid gland histological sections from different experimental groups,

(C) Control group, (BTU) Benzylthiouracil group, (BTU+Levo) Group treated with levothyroxine, (BTU+CC) Group treated with cotula cinerea, and (BTU+OM) Group treated with *Origanum Majorana* L , (V) Indicates thyroid follicles, (FC) Indicate follicular cells, (BV) Indicate blood vessel, Magnification ×40.

The data clearly demonstrate that BTU administration results in a significant- increase in WGI, supporting the hypothesis that thyroid suppression leads to metabolic slowdown and subsequent weight gain. These findings are consistent with previous reports that link antithyroid agents with metabolic disturbances (Alam et al., 2020.)

On the other hand, co-treatment with the plant extracts Cotula cinerea and *Origanum majorana* L. did not significantly alter WGI prior to treatment compared to BTU alone. However, both groups showed a substantial reduction in WGI post-treatment, nearly returning to control levels. This suggests that these herbal treatments might exert corrective effects on BTU-induced weight gain, possibly through their bioactive compounds such as alpha-pinene and trans-thujone known for modulating metabolism and inflammation (Ben Salem et al., 2021.)

In contrast, Levothyroxine treatment failed to reverse the effects of BTU on weight, as WGI remained elevated after treatment, indicating a limited therapeutic effect in this context. These findings are aligned with prior clinical observations suggesting that Levothyroxine

does not consistently promote weight loss and may even contribute to BMI increase under certain conditions (Ito et al., 2012.)

Table 10 and the associated findings illustrate the profound hematological -disturbances induced by benzylthiouracil (BTU) exposure, and the varying efficacy of treatments using *Cotula cinerea*, *Origanum majorana*, and Levothyroxine in mitigating these alterations.

BTU administration led to a significant increase in total white blood cell count and lymphocyte levels , indicating a pro-inflammatory or immune-stimulatory response. Simultaneously, BTU exposure caused a marked reduction in hemoglobin concentration , red blood cell count , and a significant elevation in platelet count , suggesting an anemic state likely associated with bone marrow suppression and a reactive thrombocytosis—a pattern previously reported in thyroid dysfunction models (Kumar et al., 2015; DOI: 10.1016/j.toxlet.2015.06.009).

Treatment with Cotula cinerea resulted in a partial normalization of the hematological profile. Although reductions in WBC and LYM were not statistically significant, HGB and RBC levels significantly increased, suggesting effective hematopoietic restoration. Moreover, the decline in PLT highlights potential anti-inflammatory properties, possibly mediated by polyphenolic compounds with known immunomodulatory effects (Ben Salem et al., 2021; Journal of Ethnopharmacology).

Conversely, *Origanum majorana* treatment yielded an unexpected increase in WBC and LYM , both significantly higher than control values ($p < 0.01$), which could reflect an immune-stimulating mechanism or a sustained inflammatory response. Nonetheless, it effectively ameliorated anemia, as evidenced by improved HGB and RBC . The persistent elevation in PLT raises concerns about ongoing systemic inflammation or platelet hyperreactivity (Rafieian-Kopaei et al., 2014; DOI: 10.1016/j.jep.2013.12.036).

Levothyroxine-treated animals also demonstrated partial hematological recovery. HGB and RBC values significantly improved , indicating erythropoietic activity. However, elevated WBC and PLT levels suggest incomplete resolution of inflammation. The relatively modest increase in granulocytes further supports this observation. These findings align with clinical reports on levothyroxine, which indicate variability in its impact on immune and hematopoietic systems depending on dosage and disease context (Ito et al., 2012; PubMed).

The administration of benzylthiouracil (BTU) resulted in significant metabolic -disturbances, notably hyperglycemia, hepatic injury, and renal dysfunction. The observed elevation in fasting blood glucose (FBS) levels indicates impaired glucose metabolism, potentially due to BTU-induced hypothyroidism affecting carbohydrate metabolism. This

aligns with findings that thyroid dysfunction can disrupt glucose homeostasis by altering hepatic glucose production and renal glucose reabsorption (Teixeira et al., 2016)

Elevated hepatic enzymes, aspartate aminotransferase (AST) and alanine aminotransferase (ALT), suggest hepatocellular damage. Such increases are consistent with studies indicating that antithyroid agents like BTU can induce oxidative stress, leading to liver injury (Kamel et al., 2017). Additionally, the significant rise in blood urea and creatinine levels points to compromised renal function, corroborating reports that hypothyroidism can adversely affect renal physiology (Naderi et al., 2020)

Treatment with Cotula cinerea (BTU + CC) demonstrated a notable improvement in glycemic control, reducing FBS to . Liver function markers AST and ALT were significantly decreased, indicating hepatoprotective effects. Renal parameters also improved, with urea and creatinine levels approaching normal values, suggesting nephroprotective properties.

Similarly, *Origanum majorana* (BTU + OM) treatment effectively restored metabolic and organ functions. FBS levels were reduced to 0.64 ± 0.05 g/L, not significantly different from the control group, indicating near-complete normalization of glycemia. AST and ALT activities were significantly lowered, and renal markers showed improvement, reflecting both hepatoprotective and nephroprotective effects. These findings are supported by studies highlighting the antioxidant and anti-inflammatory properties of O. majorana, which contribute to its protective roles in liver and kidney functions (El-Beshbishy et al., 2015).

Levothyroxine treatment (BTU + Levo) also exhibited protective effects. FBS significantly decreased to , indicating improved glycemic regulation. Liver enzymes were reduced, and renal function markers were stabilized, suggesting effective restoration of organ functions. This is consistent with research demonstrating that thyroid hormone replacement can ameliorate metabolic disturbances and organ dysfunctions associated with hypothyroidism (Teixeira et al., 2016)

The results of the study demonstrated that administration of Benzylthiouracil (BTU) led to significant physiological alterations in thyroid hormone levels. Notably, serum thyroxine (T4) and triiodothyronine (T3) levels decreased markedly , while thyroid-stimulating hormone (TSH) levels increased significantly (0.37 ± 0.01 µUI/L). These findings confirm BTU's antithyroid action through inhibition of thyroid hormone biosynthesis, consistent with previous reports indicating that thiouracil derivatives suppress iodine oxidation and its binding to tyrosine residues in thyroid follicular cells, leading to a hypothyroid state (Hood et al., 1999)

Conversely, co-administration of Cotula cinerea with BTU showed a restorative effect on thyroid function. Serum T4 and T3 levels nearly returned to normal values , and TSH levels decreased markedly . Although direct studies on Cotula cinerea's effects on the thyroid are limited, preliminary evidence suggests its phytochemical constituents especially alpha-pinene and trans-thujone may exert antioxidant and anti-inflammatory effects that support thyroid function.

Similarly, treatment with *Origanum majorana* significantly improved thyroid hormone profiles, with increased levels of T4 and T3 , and a notable reduction in TSH . These outcomes align with previous studies demonstrating the antioxidant and anti-inflammatory properties of *O. majorana*, attributed to its active components such as carvacrol and thymol (Gheitasi et al., 2021; Kakouri et al., 2022)

Treatment with levothyroxine (BTU + Levo), a standard therapy for hypothyroidism, effectively normalized serum T4 and TSH levels . T3 levels also approached the reference range without significant statistical deviation, reinforcing levothyroxine's therapeutic efficacy in correcting BTU-induced thyroid hormone imbalances (Hood et al., 1999)

Histological examination using hematoxylin and eosin (H&E) staining revealed a pronounced impact of benzylthiouracil (BTU) on thyroid tissue architecture, supporting its efficacy as an experimental model for inducing hypothyroidism in animal subjects. The observed pathological alterations included disrupted follicular architecture, dilated blood vessels, interstitial congestion, significant inflammatory cell infiltration, and marked depletion of colloid content within follicular lumens. These features are consistent with BTU's known mechanism of action, which involves inhibition of thyroid peroxidase activity and subsequent suppression of thyroid hormone biosynthesis (Braverman & Cooper, 2021).

In contrast, treatment with levothyroxine demonstrated partial histological restoration, characterized by more organized follicular structures and moderate reaccumulation of colloid, reflecting the therapeutic effect of exogenous T4 in reestablishing hormonal and functional homeostasis of the thyroid gland.

Administration of Cotula cinerea extract resulted in moderate improvement in thyroid histoarchitecture. The follicles appeared more regularly shaped, colloid deposition was partially restored, and inflammatory cell infiltration was reduced. These outcomes suggest potential anti-inflammatory and thyroid-protective properties of Cotula cinerea, aligning with previous findings that highlight its antioxidant and immunomodulatory capabilities (Bousta et al., 2017).

On the other hand, treatment with *Origanum majorana* L. exhibited more substantial histological recovery. Follicular integrity was largely preserved, colloid content was prominently restored, and inflammatory infiltration was markedly reduced. These findings indicate a strong protective and possibly therapeutic effect, likely attributed to the plant's rich phenolic content with well-documented antioxidant and anti-inflammatory activities (Sivropoulou et al., 1996).

Collectively, these results suggest that both plant-based treatments may offer promising supportive or alternative strategies for mitigating structural thyroid damage associated with hypothyroidism, particularly in cases where conventional hormone replacement therapies are contraindicated or insufficient.-

5. In-Silico analysis:

5.1. ADMET and drug-likeness prediction:

Modern drug discovery involves the evaluation of dynamic molecules for their efficiency and ability to reach the target site in their bioactive form. This process requires cellular, animal, and human clinical trials, which are costly, complex, and carry significant risks (Ranjith & Ravikumar, 2019; Ranjith D & Viswanath S, 2019). Currently, computer-aided drug development has facilitated the prediction of drug absorption, distribution, metabolism, and excretion (ADME), offering reliable and rapid data that complement experimental approaches (Ranjith & Ravikumar, 2019; Sliwoski et al., 2014). Early assessment of ADME properties during the discovery phase has been shown to significantly reduce the rate of pharmacokinetics-related failures in clinical stages (Hay et al., 2014; Ranjith & Ravikumar, 2019).

In the present study, the ADME properties of major compounds (greater than 10%) found in the essential oils were evaluated using the SwissADME web tool. Four active phytoconstituents were analyzed to investigate their general characteristics . physicochemical properties , lipophilicity and water solubility pharmacokinetic parameters (Table 12), drug-likeness and bioavailability score , and medicinal chemistry properties . The six studied compounds were compared to the reference drug thyroïde. The general characteristics revealed that all compounds had molecular weights below 500 Da, which is a key indicator of drug-likeness in small molecules. Moreover, these compounds possessed lower molecular weights than thyroïde

Table 12. General characteristics of the phytoconstituents of essential oils

SI. No	Compounds	Molecular formula	Canonical SMILES	Molecular weight (g/mol)
1	Santolina triene	$C_{10}H_{16}$	CC(=CC(C=C)C(=C)C)C	136.23
2	trans-Thujone	$C_{10}H_{16}O$	CC1C2CC2(CC1=O)C(C)C	152.53
3	1,8-Cineole	$C_{10}H_{18}O$	CC1(C2CCC(O1)(CC2)C)C	154.25
4	cis-Verbenyl acetate	$C_{12}H_{18}O_2$	CC1=CC(C2CC1C2(C)C)OC(=O)C	194.27

Table 13. Physicochemical properties of the phytoconstituents of essential oils :

Properties	Santolina triene	trans-Thujone	1,8-Cineole	cis-Verbenyl acetate
Num. heavy atoms	10	11	11	14
Num. arom. heavy atoms	0	0	0	0
Fraction Csp3	0.40	0.90	1.00	0.75
Num. rotatable bonds	3	1	0	2
Num. H-bond acceptors	0	1	1	2
Num. H-bond donors	0	0	0	0
Molar refractivity	48.76	45.90	47.12	56.12
TPSA (Å²)	0.00	17.07	9.23	26.30

Table 14. Lipophilicity characteristics of the phytoconstituents of essential oils

Properties	Santolina triene	trans-Thujone	1,8-Cineole	cis-Verbenyl acetate
iLOGP	2.90	2.27	2.58	2.53
XLOGP3	4.22	2.27	2.74	3.73
WLOGP	3.33	2.26	2.74	2.54
MLOGP	3.56	2.30	2.45	2.65
SILICOS-IT	2.88	2.63	2.86	2.26
Consensus Log Po/w	3.38	2.35	2.67	2.74

Table 15. 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	
Santolina triene	-3.15	9.75e-2	7.16e-4	S	-3.93	1.60e-2	1.17e-4	S	-2.04	1.24e-0	9.10e-3	S
trans-Thujone	-2.15	1.08e-0	7.11e-3	S	-2.27	8.27e-1	5.43e-3	S	-2.15	1.08e-0	7.10e-3	S

1,8-Cineole	-2.52	4.63e-1	3.00e-3	S	-2.59	3.98e-1	2.58e-3	S	-2.45	5.45e-1	3.53e-3	S
cis-Verbenyl acetate	-3.26	1.06e-1	5.47e-4	S	-3.97	2.06e-2	1.06e-4	S	-2.11	1.51e-0	7.78e-3	S

Table 16. Pharmacokinetics parameters of the phytoconstituents of essential oils

Proprieties	**Santolina triene**	**trans-Thujone**	**1,8-Cineole**	**cis-Verbenyl acetate**
GI absorption	Low	High	High	Low
BBB permeant	Yes	Yes	Yes	Yes
P-gp substrate	No	No	No	No
CYP1A2 inhibitor	No	No	No	No
CYP2C19 inhibitor	No	No	No	No
CYP2C9 inhibitor	No	No	No	Yes
CYP2D6 inhibitor	No	No	No	No
CYP3A4 inhibitor	No	No	No	No
Log Kp (Skin Permeation) (cm/s)	-4.13	-5.62	-5.30	-4.84

Table 17. Druglikeness rule and bioavailability score of the phytoconstituents of essential oils

Proprieties	Santolina triene	trans-Thujone	1,8-Cineole	cis-Verbenyl acetate
Lipinski	Yes; 0 violations	Yes; 0 violations	Yes; 0 violations	Yes; 0 violations
Ghose	No; 1 violation: MW<160	No; 1 violation: MW<160	No; 1 violation: MW<160	Yes
Veber	Yes	Yes	Yes	Yes
Egan	Yes	Yes	Yes	Yes
Muegge	No; 2 violations: MW<200, XLOGP3>3.5	No; 2 violations: MW<200, Heteroatoms<2	No; 2 violations: MW<200, Heteroatoms<2	No; 1 violation: MW<200,
Bioavailability score	0.55	0.55	0.55	0.55

Table18. Medicinal Chemistry properties of the Phytoconstituents of essential oils

Proprieties	Santolina triene	trans-Thujone	1,8-Cineole	cis-Verbenyl acetate
PAINS	0 alert	0 alert	0 alert	0 alert
Brenk	1 alert: isolated_alkene	0 alert	0 alert	1 alert: isolated_alkene
Leadlikeness	No; 2 violations: MW<250, XLOGP3>3.5	No; 1 violation: MW<250	No; 1 violation: MW<250	No; 2 violations: MW<250, XLOGP3>3.5
Synthetic accessibility	3.22	2.79	3.65	4.50

The lipophilicity property of compounds plays a crucial role in molecular discovery across various scientific domains. It is quantitatively represented by the partition coefficient (P) of a molecule between an n-octanol and water system (Daina et al., 2014). Due to its amphiphilic characteristics, n-octanol serves as a suitable analog for phospholipid membrane properties (Liu et al., 2011). Numerous algorithms are available for calculating log Po/w, based on diverse methodologies. Classic log P prediction methods are classified into two main categories. The first includes fragment-based models, such as KLOGP (Klopman et al., 1993) and KOWWIN (Meylan & Howard, 2000), or atomic-based models, such as ALOGP (Ghose et al., 1998; R. Wang et al., 1997) and XLOGP (Cheng et al., 2007; Moriguchi et al., 1994). The second category comprises topological approaches, which describe molecules using their structural topology as counts or indicators of specific atoms, groups, or features—for instance, MLOGP (Brenk et al., 2008; Moriguchi et al., 1992). These predictions are typically derived from multilinear regression models trained on extensive molecular datasets.

SILICOS-IT represents a hybrid approach that combines molecular fragments and topological parameters, utilizing 27 fragment descriptors and 7 topological descriptors, trained on 23,455 molecules with experimentally determined n-octanol/water partition values (Daina et al., 2014). The third version of the XLOGP atomic model is based on 87 molecular fragments and two correction factors. When input structures resemble a reference compound, the distinguishing fragments are analyzed and their log P contributions are added to that of the reference compound (Cheng et al., 2007). Lipophilicity, estimated as consensus Log P an average of log P values computed using different prediction models identified thyroïde as the most lipophilic compound, while santolina triene exhibited the lowest lipophilicity. The water solubility of the compounds ranged from highly water-soluble to moderately water-soluble.

SwissADME also evaluates the pharmacokinetic potential of compounds, indicating with a “Yes” or “No” whether a molecule is likely to be a substrate or non-substrate of P-

glycoprotein (P-gp), or an inhibitor or non-inhibitor of major cytochrome P450 isoenzymes (CYP1A2, CYP2C9, CYP2C19, CYP2D6, and CYP3A4). The pharmacokinetic and drug-likeness analysis showed low gastrointestinal absorption and blood-brain barrier permeability for santolina triene and cis-verbenyl acetate, whereas high absorption was observed for trans-thujone and 1,8-cineole. Notably, all the compounds present in the essential oils were found to be non-substrates of P-gp, in contrast to thyroïde (Table 16), indicating they are not subject to the efflux mechanisms mediated by this transporter, which is commonly employed by cancer

All evaluated small molecules were found to be non-inhibitors of cytochrome P450 (CYP) isoenzymes. The skin permeability coefficient (Log Kp), derived from multiple linear regression, indicates that the more negative the Log Kp (measured in cm/s), the lower the compound's ability to penetrate the skin. Among the phytochemicals analyzed, trans-Thujone exhibited the lowest skin permeability (Log Kp = -5.62), while santolina triene demonstrated the highest permeability (Log Kp = -4.13).

The SwissADME platform utilizes five different rule-based filters to assess the drug-likeness of molecules, including the Lipinski (Pfizer), Ghose (Amgen), Veber (GSK), Egan (Pharmacia), and Muegge (Bayer) rules. These filters cover a wide range of molecular properties, enabling tailored assessments depending on project requirements or chemical space. Any violations of these rules are clearly highlighted in the output. All four studied compounds adhered to these guidelines, with minimal violations noted.

SwissADME also reported zero PAINS (Pan Assay Interference Compounds) alerts for the analyzed compounds. According to Brenk's criteria, which emphasizes smaller and less hydrophobic compounds excluding those with mutagenic or reactive groups like nitro, sulfate, phosphate, 2-halopyridine, and thiol groups only one alert was raised per compound. Nonetheless, none of the compounds met the criteria for lead-likeness due to their molecular weights.

The in-silico toxicity assessment was performed using the ProTox-II web tool (Drwal et al., 2014), designed to predict various toxicity parameters including hepatotoxicity (DILI), carcinogenicity, immunotoxicity, mutagenicity, cytotoxicity, median lethal dose (LD50), and overall toxicity class. This computational approach aims to facilitate the selection and optimization of compounds with minimal or no toxicity, thus contributing to the identification of safe, bioactive phytochemicals from Cotula cinerea and *Origanum majorana* L. essential oils with potential antithyroid properties.

According to the toxicity profiles generated (Table 19), all phytoconstituents were classified as toxicity class 5, except trans-Thujone, which was predicted as class 4. The compounds santolina triene, trans-Thujone, 1,8-Cineole, and cis-Verbenyl acetate were considered non-toxic across most evaluated categories, with the exception of immunotoxicity observed in the latter

Table19. In silico toxicity profiles of the studied componds

Molecule	Dili	Carcino	Immuno	Mutagen	Cyto	LD_{50} ($mg.Kg^{-1}$)	TC
Santolina triene	Inactive	Active	Inactive	Inactive	Inactive	2610	5
trans-Thujone	Inactive	Inactive	Inactive	Inactive	Inactive	500	4
1,8-Cineole	Inactive	Inactive	Inactive	Inactive	Inactive	2480	5
cis-Verbenyl acetate	Inactive	Inactive	Active	Inactive	Inactive	2600	5

5.2. Molecular Docking Study:

In the current study, the binding interactions of the major compounds identified in Cotula cinerea and *Origanum majorana* L. essential oils with thyroïde were further explored through molecular docking analysis. The compounds included trans-Thujone, alpha-Terpineol, beta-Terpineol, cis-Verbenyl acetate, Camphor, Sabinene, gamma-Terpinene, alpha-Pinene, 1,8-Cineole, Santolina triene, alpha-Thujene, and beta-Pinene oxide—each selected for their significant presence (yield > 1%) in the two plant extracts. Molecular docking was performed using the Maestro version 11.7 interface within the Schrödinger Small-Molecule Drug Discovery Suite (version 2021-4; Schrödinger, LLC, New York, NY) (Schrödinger, 2015).

Each phytochemical was docked with human thyroïde to assess their binding behavior within the enzyme's active site. The results revealed that several compounds exhibited binding affinities comparable to standard reference ligands (Rigid Doking score Table 20). These findings suggest that the essential oils of both plants contain constituents capable either individually or synergistically of significantly reducing thyroïde activity, as also observed in corresponding experimental inhibition studies. Furthermore, the negative values of Gibb's free energy indicated that the interactions were thermodynamically spontaneous (Avwioroko et al., 2020).

Insights obtained from structural and enzymatic studies of thyroïde highlighted the presence of three key amino acid residues-ILE276, PHE269, and PHE272-at the enzyme's active site, which play critical roles in its catalytic mechanism. ILE276 functions as the catalytic nucleophile during substrate cleavage, PHE269 acts as the acid-base catalyst, and PHE272 helps orient the substrate for optimal catalysis (Anigboro et al., 2021).

Table 20.Docking score of the studied compounds in plants Cotula cinerea

Compound	Rigid Docking Score (Kcal/mol)
1.8cineole	-6.12
Alpha-pinene	-6.29
Beta-terpineol	-6.11
Camphor	**-6.88**
Cis verbenyl acetate	-6.86
Delta-terpinene	-5.87
Sabinene	-5.65
Santolina triene	-5.05
Terpin-4-ol	-6.24
Trans thujone	-5.84

Table 21. Docking score of the studied compounds in plants *Origanum Majorana* L

Compound	Rigid Docking Score (Kcal/mol)
Trans thujone	-5.84
Santolina triene	-5.23
Sabinene	-5.62
Cis verbenyl acetate	-6.77
Camphor	**-6.88**
Beta pinene oxide	-6.2
Alpha-Thujene	-5.4
Alpha-pinene	-6.29
1.8Cineole	-6.12

The results shown in Figure (21) revealed that the best compound in both plants interacted with a similar set of amino acid residues at the active site of the protein, just as the

standard inhibitor of the thyroïde enzyme did. The compound camphor showed interaction with the amino acid His435, which is involved in catalysis at the active site of thyroïde, for both plants.

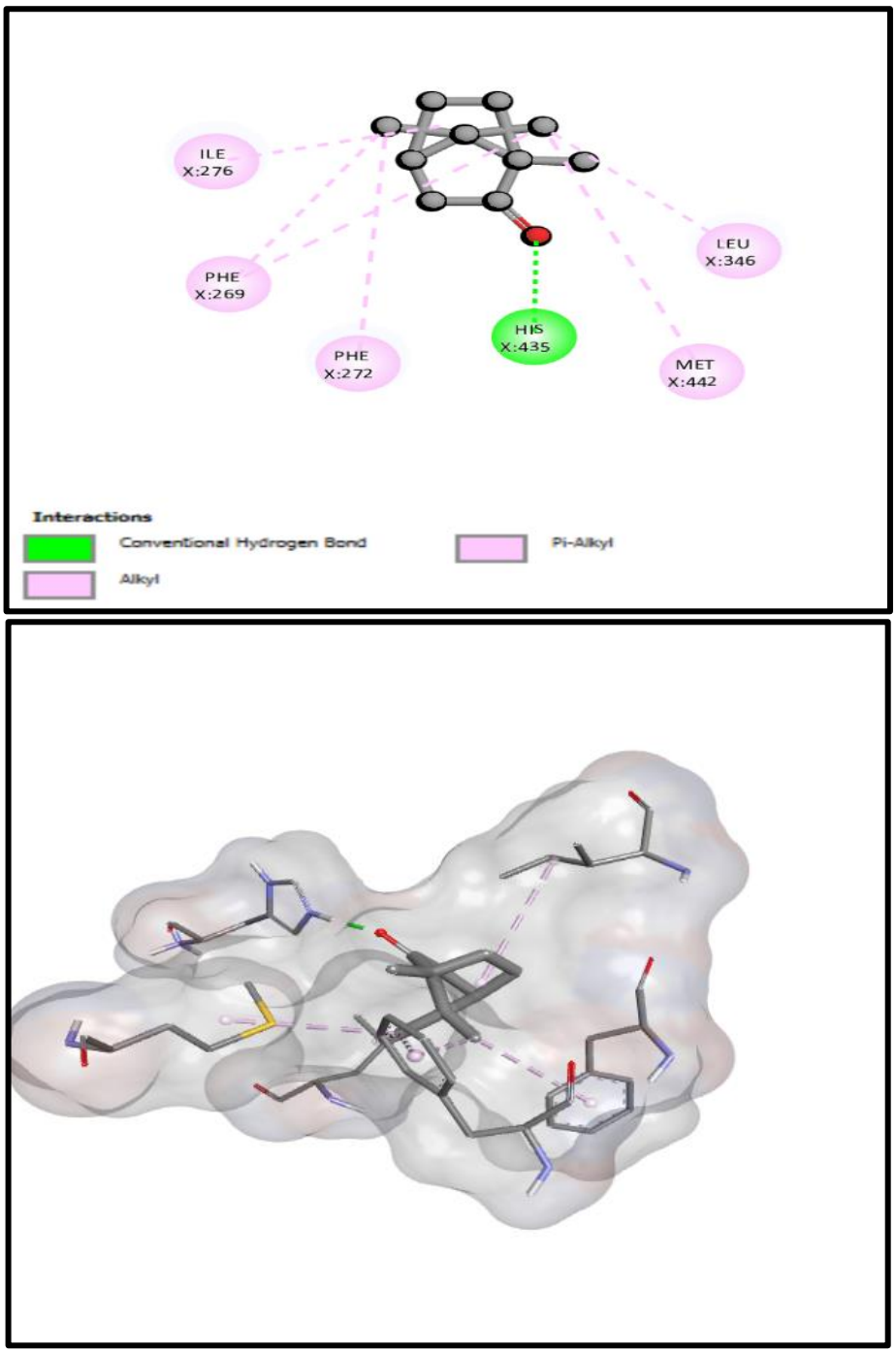


Figure21 . Molecular interactions of studied compounds

Overall, the in-silico analysis of the ligand-protein interaction with the amino acid residues present in the active domain of the thyroïde enzyme supports the inhibitory effect of the studied compounds in this research. It also confirms the possibility of a binding

interaction between thyroïde and the phytoconstituents of Cotula cinerea and *Origanum majorana* L. essential oils, as verified using the spectroscopic methods described. The optimal ligand-binding poses in the catalytic domain of thyroïde, along with the amino acid residues involved in the interaction

Docking complex and interaction plot for compound Camphore. with thyroïde

Conclusion

In recent years, phytotherapy has experienced significant advancement, driven by increasing public interest in natural remedies and the desire to minimize the adverse effects commonly associated with synthetic pharmaceuticals. Within this framework, the present study focused on two medicinal plants-*Cotula cinerea* and *Origanum majorana* L.-from which essential oils were extracted and chemically characterized.

Both in vivo and in silico investigations were undertaken to evaluate the inhibitory potential of these essential oils on the thyroid gland, a central organ in metabolic and physiological regulation. Experimental findings in animal models demonstrated that the essential oils from *Cotula cinerea* and *Origanum majorana* L. exert a modulatory effect on thyroid function, indicating their possible therapeutic utility in the management of thyroid disorders.

Furthermore, in silico analyses including SwissADME profiling, molecular docking, — validated the pharmacological potential of the bioactive constituents within the oils, revealing favorable pharmacokinetic and toxicological profiles.

Collectively, this study underscores the relevance of local medicinal flora in promoting endocrine health and highlights their promise as natural adjuncts in thyroid disorder management. Nonetheless, these findings are preliminary and necessitate further comprehensive studies to fully elucidate their therapeutic potential.

❖ **Future Perspectives**

The outcomes of this investigation pave the way for expanded research into the therapeutic applications of plant-derived essential oils, particularly in the domain of endocrine dysfunctions such as thyroid disorders. Future research should aim to explore additional pharmacological properties of these oils, including antimicrobial, antioxidant, anti-inflammatory, and anticancer activities.

Advanced analytical techniques such as Gas Chromatography-Fourier Transform Infrared Spectroscopy (GC-FTIR), Liquid Chromatography-Mass Spectrometry (LC-MS), and Nuclear Magnetic Resonance (NMR) could provide deeper insights into the structural and functional profiles of the active constituents.

Moreover, extending in vivo experimentation to diverse biological models could further elucidate the efficacy, safety, and mechanisms of action of these compounds.

Investigating potential synergistic interactions among oil components may also enhance their pharmacological potency and therapeutic index.

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Appendix

Figure 22 : Rat cages (Original photo, 2025).

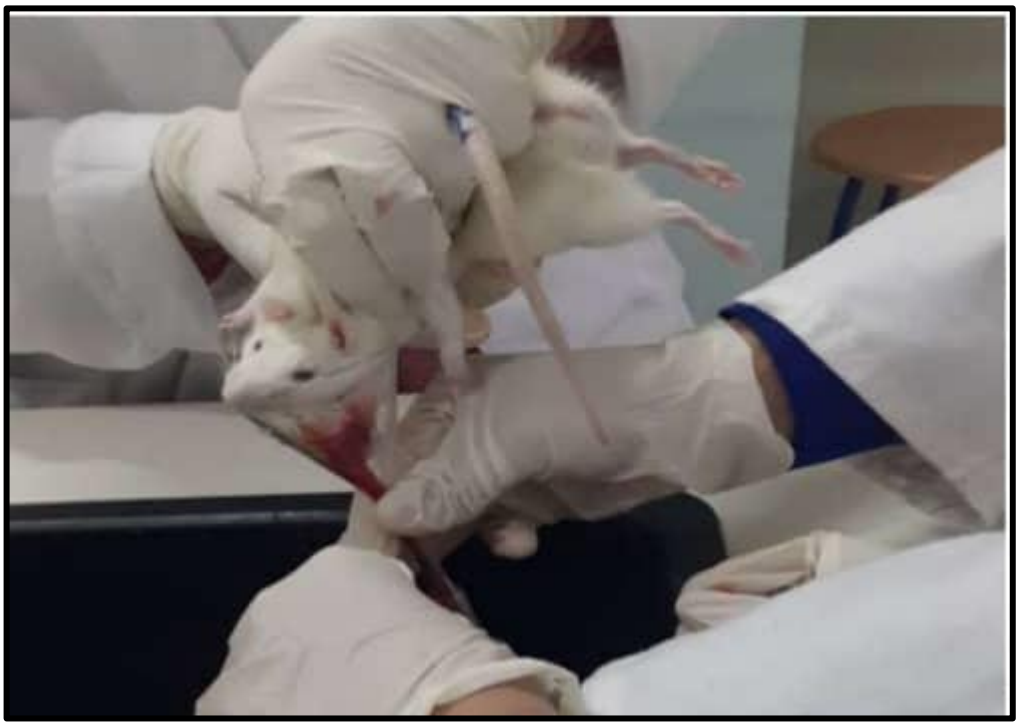

Figure 23 . Sacrifice, blood drawing (Original photo, 2025)

Figure 24. Dissection and removal of organs (Original photo, 2025).

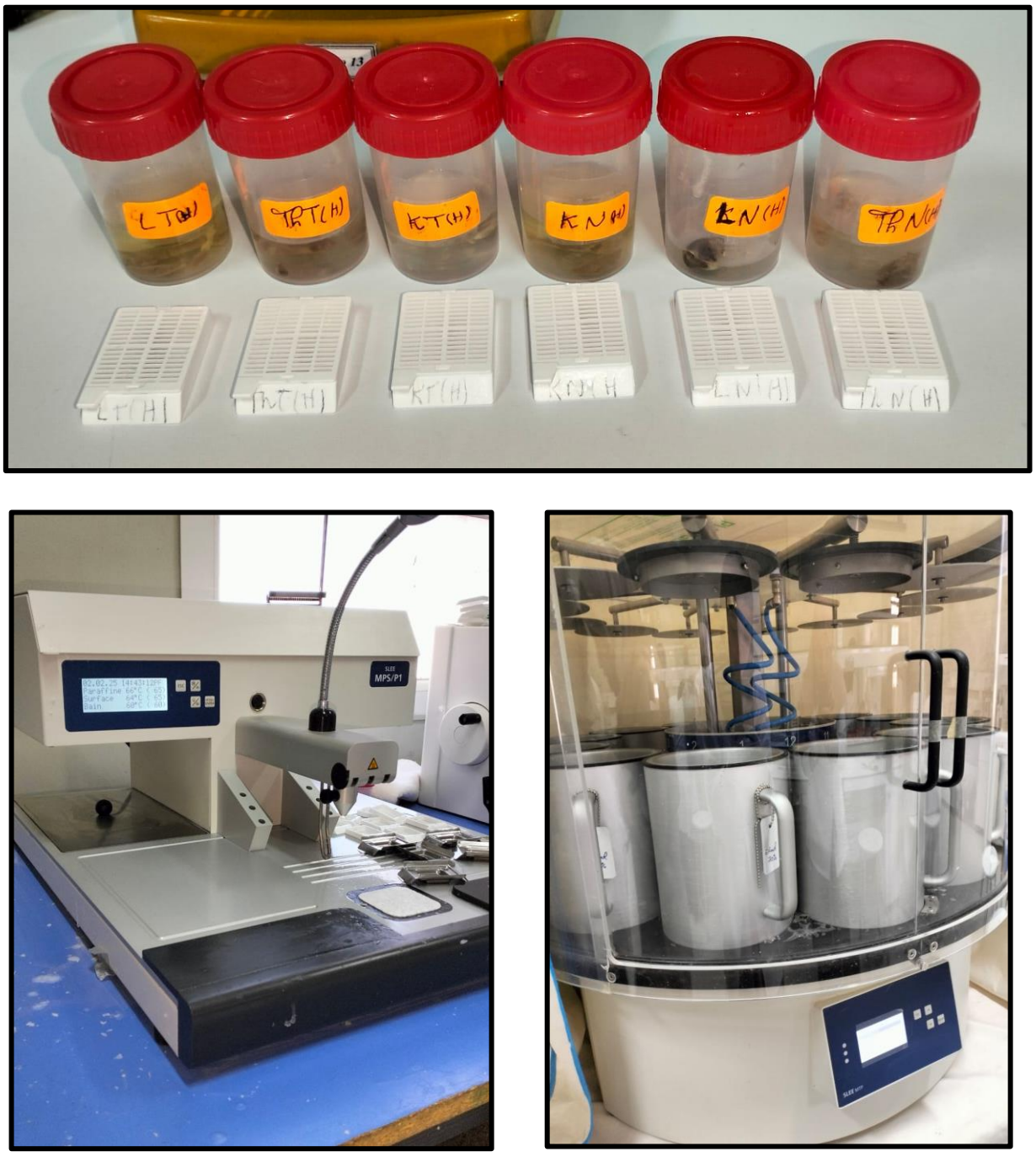

Figure 25:Steps and tools used when preparing histological cuts (Original photo, 2025)