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THEME

Elevating Antioxidant Profiling of Medical Plant: Integration of Experimental Assays with Extensive in Silico Analyses for Comprehensive Evaluation

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Dedication

"And say, 'Do [as you will], for God will see your deeds, and [so, will] His Messenger, and the believers.' To those who conveyed the message and fulfilled the trust, to the Prophet of mercy and the light of the worlds, our Master Muhammad, peace be upon him.

To the spring that never ceases to give, to the one who wove my happiness with threads woven from her heart, to the meaning of love, tenderness, and dedication, to my angel in life and her smile, and the secret of existence, "My dear **mother".**

To the dignity and honor, to the one who taught me to give without expecting, to the one whose name I proudly bear, to the one who taught me to climb the ladder of life with wisdom, patience, and strength, "My dear **father**"

To the soul of our beloved departed, my brother **"Said"**, may God's mercy be upon him.

To those whose love runs through my veins and fills my heart with their memories, to "my dear **brothers** and **sisters"**

To those who shared our joy as we walked the path together towards success, to those who joined hands as we picked the flower of learning, to "my dear **Friends** and **Colleagues**".

To those who carried the noblest message in life, to those who paved the way for us to knowledge, "our honorable **Teachers**".

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We dedicate this humble work, hoping that it will be pleasing, accepted, and successful in the sight of the Almighty.

Souhila

Dedication

Thank God for love, thank you, and our gratitude for beginning and closing.

(And their last call is that praise belongs to God the Lord of the Worlds)

My studies are already over after years of tiredness and hardship for dreaming and science Today, I stand on my graduation threshold, picking the fruits of my labor, and raising my hat with pride. Thanks, God, for letting me complete this success and fulfilling my dream with all the love that I dedicate to the fruit of my success:

To whom my name was most beautiful, from my unbounded support, my first support in my career, my Sandy, my strength after God's honor and my pride, **My dear father**

who made heaven under her feet, embraced me with her heart before her, and made it easier for me to be hard to pray to those who were my joy and waxed my path to those who taught me patience for life **My Mather**

To those with whom I have gained strength and love... to those with whom I know the meaning of life... **My brothers** and **Sisters**

Until I wait these moments to be proud of my bond and my partner in life, to whom I see immortal in the midst of my heart and my chest to help me after God and my unflinching, firm rib (my precious husband, **Oussama**)

As a tribute and recognition, we extend our gratitude to the dedicated **Dr. Ilham Ben Amor**, who has strived so hard and who has helped us to help and guide us in the accomplishment of this humble work

Donía

Dedication

((And their last call to praise God the Lord of the World))

Praise be to Allah , and there is no way , nor is there any seal , nor is there any effort except Him

The journey was not short, nor should it be, nor was the dream near, nor was the road fraught with facilities, but I did it and I got it

Thank God for love and thanks and gratitude, thanks to which I am today looking at a long-awaited dream that has become a reality that I am proud of

Give me all the love of my graduate research:

To my strong self, who endured all the setbacks and continued despite the difficulties...

To whom God made heaven beneath its feet, to whom it was called the secret of my success, to which I had a light in my blackout**,"My Mother".**To the one who decorated my name with the most beautiful nicknames to the one who taught me that the world of struggle and its weapon of science and knowledge was **"My father"**

To those who said:(We'll pull your back with your brother)

My dear brother, **" Mohammed AlaEddine"**, is saved by God

To those I grew up with, and my twig grew among them, to those who were leaning on me, upon whom **"my sisters"**, God rest you, a firm side of me

To whom has she extended her hand tirelessly or bored with the time of my weakness, and gave me free of charge my first and eternal support and strength after God, and believed in me and in my abilities, my older sister**," KHAWLA**"...

You don't grow up in my eyes as much as your love grows in my heart

To sugar pieces and honey drops, my nephews kept you from all evil

Ikram

Abstract:

This work is part of a research programme in medicinal plants, where research was conducted on two types of plants: *cotula cinerea* and *Origanum Majorana L.* , the essential oil for which was derived and analysed by the gas chromatography linked to the mass spectrometer (GC/MS) to identify its components. The results showed the enrichment of plants under study with base oils, with a variation in the number and type of vehicles for the first type (*cotula cinerea*), which is growing particularly in the desert regions of the Arab region. The second type that is particularly developing around the Mediterranean regions is the origin of *Origanum Majorana L* with 42 known compounds and its main compound being trans thujone (33.3%).

Anti-oxidation effectiveness was also determined using the DPPH test, where it was found through the results that all the extracts had an anti-oxidant effectiveness, but compared to vitamin E, which was considered weak. By comparing the median concentration values of IC_{50}, the resulting compounds showed that vitamin E was the most powerful anti-oxidant activity.

Keywords: Bioefficiency (antioxidative) ,*Cotula cinerea*, *Origanum Majorana L,* IC_{50} , DPPH.

Résumé :

Ce travail fait partie d'un programme de recherche sur les plantes médicinales, où des recherches ont été menées sur deux types de plantes : cotula cinerea et Origanum Majorana L. dont l' huile de base a été dérivée et analysée par la chromatographie gazeuse liée au spectromètre de masse (GC/MS) pour identifier ses composants. Les résultats ont montré l' enrichissement des plantes étudiées avec des huiles de base, avec une variation du nombre et du type de véhicules pour le premier type (cotula cinerea), qui pousse en particulier dans les régions désertiques de la région arabe. Le deuxième type qui se développe particulièrement dans les régions méditerranéennes est l'origine de l' Origanum Majorana L avec 42 composés connus et son principal composé trans thujone (33,3%).

l' efficacité antioxydante a également été déterminée à l' aide de l'essai DPPH, où il a été constaté par les résultats que tous les extraits avaient un effet anti-oxydant, mais par rapport à la vitamine E, qui était considérée comme faible. En comparant les valeurs médiennes de concentration de IC50, les composés obtenus ont montré que la vitamine E était l' activité antioxydante la plus puissante.

Mots clés : Bioefficacité (effet antioxydant),Cotula cinerea,Origanum Majorana L,
IC50, DPPH.

ملخص

يندرج هذا العمل ضمن برنامج بحث في النباتات الطبية حيث أجريت الدراسة البحثية على نوعين من النباتات و هما (الشيحية و المردقوش) وتم استخلاص الزيت الأساسي لهما وتم تحليله بواسطة كروماتوغرافيا الغاز الموصول بمطياف الكتلة (GC/MS) للتعرف على مكوناته وأظهرت النتائج غنى النباتات قيد الدراسة بالزيوت الأساسية مع وجود تباين من حيث عدد ونوع المركبات بالنسبة للنوع الأول *(cotula cinerea)* الذي ينمو بشكل خاص في المناطق الصحراوية العربية و كان عدد مركبات 31 مركب مميزا بغناء مجموعة من الكيتونات ممثلا أساسا بالمركب trans thujone (51.86%) ، أما النوع الثاني النامي بشكل خاص حول مناطق البحر الأبيض المتوسط *(Origanum Majorana L)* ، كان عدد مركباته المعروفة 42 مركب والمركب الأساسي له هو trans thujone (33.3%) .

وكذلك تم تحديد الفعاليةالمضادةللأكسدة باستخدام اختبار DPPH حيث تبين من خلال النتائج أن لجميع المستخلصات فعاليةمضادةللأكسدة ولكنها مقارنه بفيتامين E اعتبرتضعيفة ومن خلال مقارنه قيم التركيز الوسطى IC_{50}تبين من خلال المركبات الناتجة أن فيتامين E هو الأكثر نشاط مضادةللأكسدة قوة.

الكلمات المفتاحية:الفعالية الحيوية (فعالية مضادة للأكسدة)، *Origanum MajoranaL*, *Cotula cinerea*, IC_{50}, DPPH.

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List of Abbreviations

A : Alpha

β : Beta

AE : Energy Difference

AG : Binding-free energy

Abs : Absorbance

ADMET : absorption, distribution, metabolism, excretion, and toxicity

C : Concentration

CCM : Thin Layer Chromatography

DMSO : Dimethylsulfoxide

E: energy

EDTA : Acide éthylène Diamine Tétra acétique

G : gramme

HPLC : High Performance Liquid Chromatography Chromatogram

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

K : Binding constant

KCI : Potassium chloride

Kg : Kilo gramme

KJ: Kilojoule

M : Mole

Mg : milligramme

pH : HydrogenPotential

T°: Temperature

UV : Ultraviolet

V : Volume

Vis : Visible

Summary

Chapter Two : Antioxidants

Second Part : Experimental

Chapter One : Materials And Methods

Chapter Two : Results And Discussion

GENERAL INTRODUCTION

In this context, we have studied the evaluation of antioxidants for two medical plants and have carried out extensive analyses in Silico to conduct a comprehensive assessment of the two plants growing in the city of El-Oued (south and east) of Algeria in order to promote scientific knowledge and to detect the medical uses and benefits of these plants.

Our choice has been made on the following plants (scarce alarm and almerdash plant) which have been selected because they grow extensively in many Algerian regions and have many medical benefits.

At first we looked at these plants thoroughly and then we extracted their essential oil in a hydro-distillation method, and the components of the essential oil derived from the plants were identified and the effectiveness of the estimated anti-oxidation oils was also defined.

We have divided our research into three sections, as follows:

Part One:

We did a thorough study of the plants studied in terms of description, uses and classification...etc., where through this door we gave a detailed idea of the medical plants studied.

Part Two:

We did chemical and physical studies of the base oil derived from the plants, where we explained the laboratory equipment, the tools used and the steps of the work for the extraction process.

Section III:

And it's the last door where the efficiency of the essential anti-oxidant oil has been estimated in theelectrochemical method.

FIRST PART:

THEORETICAL

CHAPTER ONE:

MEDICINAL PLANTS

I. Generalities about medicinal plants

1. Preface

The interest in studying medicinal plants and utilizing them in treating various diseases has increased significantly, as plants contain a vast number of medically active compounds that reflect their great therapeutic potential. It is known that some herbal remedies have greater therapeutic ability than manufactured drugs in treating certain diseases(Zulkipli*et al.*, 2015).

2. Historical overview of medicinal plants

Throughout the thousands of years humans have inhabited the Earth, they have experimented with the plants growing around them, testing their properties and conditions, often in search of food. However, through tasting these plants, they also learned that some cause illness while others can heal and relieve pain. God has endowed animals with instinctual properties to guide them to these plants without a guide or instruction, prompting humans to contemplate how to benefit from this instinct and these properties by accompanying animals and observing their eating and drinking whenever they needed medicine and food. In China, the first herbal medicine book appeared around 2700 BC, becoming the basis for all subsequent Chinese information written about plants, the most famous being the Great Herbal book (Giannenas *et al*., 2020).

In ancient Babylon, information about medicinal plants was recorded on stone and clay tablets, with over 250 plants listed, including cumin and turmeric. In Egypt, ancient writings, colored images on temple walls, and remnants of herbs found in tombs alongside mummified bodies indicate the use of these plants since 3000 BC. "Imhotep" is considered The first physician in the world used a lot of herbs such as myrrh, opium, aloe vera, and chaulmoogra in treating diseases. The whole world continued to treat their illnesses with the same ancient Pharaonic methods until the medical revolution occurred in the early 19th century (Giannenas *et al*., 2020).

Arabs before Islam did not have much knowledge about medicine and healing, as they relied on the advice of tribal elders for their treatment. They gained some knowledge from neighboring countries such as the Levant and Persia during their travels to these lands.

Like all societies in the world, Algeria has recently witnessed a great interest in herbal medicine. The culture of herbal treatment has spread among individuals of the society regardless of their gender, age, educational level, social, cultural, and economic background (Bahadur *et al*., 2007).

3. Definition

The medicinal plant is defined as a plant that contains in one or more of its organs (root / stem / leaf) one or more active chemical substances, regardless of the chemical nature of these substances and their concentration (low or high), they have the physiological ability to treat a specific disease or reduce the symptoms of this disease. The medicinal plant is also defined as anything of plant origin used medicinally and possessing therapeutic properties. Medicinal plants are commonly referred to as "herbs" in most scientific sources, where the correct concept of herbs is any plant valued for its medicinal and organic properties, distributed for its distinctive therapeutic or general medical properties. There must be medicinal plants known to be extremely toxic, as they are toxic and medicinal at the same time. Therefore, this plant is a remedy in small doses and lethal in large doses, so caution must be taken in determining doses when using some medicinal plants (Azmir*et al*., 2013; Petrovska, 2012).

4. Basis of classification of medicinal plants:

Classification methods for medicinal and aromatic plants have been divided into five categories, overlapping between agriculture, medicine, and pharmacy. Below are the classification methods in this field(Kan et al., 2017; Masarovičová *et al*., 2010).

4.1. Plant classification according to the effect of the active substance medically:

One plant may contain more than one active substance with different medical effects. For example, camphor leaves are used for containing essential oil, which is beneficial in treating nasal and throat inflammations as an antiseptic. It is also used in the production of popular expectorant medicines. Plants are classified in this regard as follows (Masarovičová*et al*., 2010; Vaou et al., 2022)

4.1.1. Plants that repel or kill worms

Thymus vulgaris (thyme), *Punicagranatum* (pomegranate), and *Citrullus colocynthis* (bitter apple) such as the fruits of colocynth which, when boiled, help expel thin worms from the intestines.

4.1.2. Plants used in cases of constipation and diarrhea:

They contain an astringent substance known as "tannin" used in the treatment of diarrhea. Plants like *Punicagranatum* (pomegranate) activate muscles in treating constipation, *Tamarindusindica* (tamarind), and *Cassia acutifoia* (senna) aid in the process of excretion.

4.1.3. Plants with antiseptic and antimicrobial effects:

Juniperuscommunis (juniper) whose oil was used by ancient Egyptians for embalming to resist microbes for centuries.

4.1.4. Plants with a heart-stimulating effect:

Plants containing "glycosides" like *Polygonum fagopyrum* (buckwheat) and *Allium cepa (onion)* whose leaves yield substances beneficial in treating some heart diseases.

4.1.5. Plants with analgesic and narcotic effects:

Plants containing "alkaloids" with roles in formulating pain-relieving drugs like *Papaver somniferum* (opium poppy) for pain relief and internal contractions.

4.1.6. Plants with Hormonal Effects:

There are some plants that have a feminine hormonal effect, while others have a masculine hormonal effect.

4.1.7. Plants with Effects on Kidney and Urinary Tract Treatment:

They assist in the expulsion of stones from the urinary system, and the therapeutic effect of the substance can benefit in a way like *Ocimumbasilicum*, such as basil, or in another way for animals and poultry, increasing production and improving its quality.

4.2. Chemical Classification of Plants:

This classification is based on the presence of active chemical substances in the plant. There may be more than one active chemical substance present in a single plant, necessitating classification based on the concentration of the most abundant substances in the plant, as follows (Tuyg'unovna, 2023):

4.2.1. Plants containing volatile aromatic oils

Such as *Cuminumcyninum L*, plants containing volatile oils like cumin.

4.2.2. Plants containing glycosides:

Rheum officinalis, with examples like rhubarb.

4.2.3. Plants containing tannins:

Lawsoniainermis, such as henna.

4.2.4. Plants containing resins:

Zingiber officinalis, like ginger.

4.2.5. Plants containing saponins:

Glycyrrhiza globra, such as licorice.

4.2.6. Plants containing carbohydrates:

Ceratonia siliqua and *carob Althea* officinalis are like marshmallow.

4.3. Classification based on the presence of the active ingredient in the plant:

The active ingredient may be concentrated in one plant organ or in more than one organ, and the division is as follows (Tsimogiannis& Oreopoulou, 2019):

4.3.1. Plants are used as a whole:

The active ingredient is concentrated in all parts of the plant without concentrating in one part at the expense of another part, like in the case of the plant Pelargonium.

4.3.2. Plants use their flowers or fruits:

Plants that concentrate their active substance in the flower petals, such as jasmine Jasminum officinalist.

4.3.3. Plants use their roots or steorchismascula:

Orchis mascula and *Glycyrrhiza globra* tubers are like salep.

4.3.4. Plants use their seeds:

Like black seed and cocoa *Theobroma cacao L*

4.3.5. Plants use their leaves:

Where the active ingredient is concentrated in the leaves, such as basil *Ocimumbasilcum*.

4.3.6. Plants use their fruits:

"Like cumin plant"*Cuminumcyminum L* and "Vanilla"*Vanillalanifolia*.

4.4. Classification in Botany

In this classification, plants are divided based on genetic traits, along with associated morphological, anatomical, and physiological characteristics. Degree of relatedness between plants and some specific features are displayed, and floral organs are considered the basis for classification and differentiation among plants. The classification units can be expressed in the following Figure (1) (Gledhill, 2008):

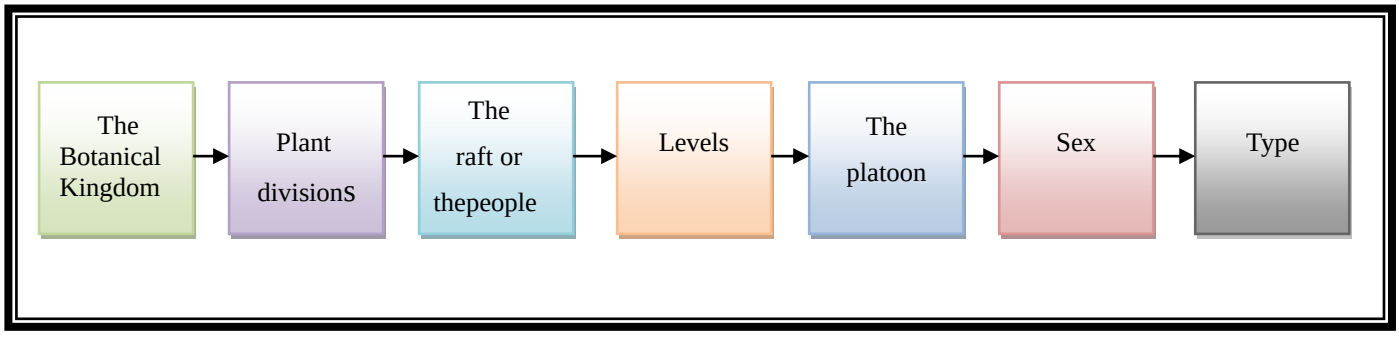


Figure 1: Classification Unit Diagram in Plant Science.

5. Source of Medicinal Plants:

Medical plants can be obtained from two sources, the first of which is wild plants where various types grow in valleys, plains, and forests, and this may be a sufficient source for some plants such as the Yonka plant, which grows wild in Central African countries. As for the second source of obtaining medical plants, it is through cultivation where pharmaceutical companies or investment institutions establish special farms to produce specific varieties or types needed by the local or international market in certain quantities (Wink, 2012).

6. The most important uses of medicinal and aromatic plants:

The medicinal and aromatic uses of plants are numerous, including:

- Preparation of some medications such as pain relievers for joint pain and rheumatoid arthritis, medications for high blood pressure, arterial stiffness, and disinfectants (Bajaj, 2012).

- Production of fixed oils where the seeds of some of these plants contain fixed oils used in the composition of some medical preparations.

- Preparation of foods for treating arterial sclerosis and angina such as sunflower seed oil, flaxseed, and castor oil.

- Preparation of cosmetic powders, hair creams, and soaps.

- Use in the manufacturing of fragrances and perfumes, including plants such as rose and jasmine (Bajaj, 2012).

- Manufacture of insecticides based on toxic substances found in medicinal and aromatic plants, whether for insects or fungi, including plants like henna and tobacco.

- Used as spices, seasonings, beverages, flavor enhancers, or fragrances.

- Sources for the production of aromatic and medicinal oils (Ghorbanpour*et al*., 2017; Lubbe & Verpoorte, 2011).

II. Studied plants:

1.Plant Cotula cinereai

1.1 Introducing the plant *Cotula cinerea*:

The plant *Cotula cinerea* The plant known as "Shihia" has a strong and pleasant smell resembling somewhat the smell of musk. ***Cotula cinerea*** is a herbaceous perennial plant with light green color and an average height of up to 30 cm. The plant is covered with dense white hairs. Its leaves are thick and divided at the upper part into two or three lobes. The branches end with composite flowers that resemble yellow discs. This plant grows in spring and blooms at the end of this season (Djellouli*et al*., 2015).

Cotula cinerea plant is found in the *Arabian Peninsula* and the Levant, but scattered. In highland areas and meadows close to agricultural areas, it thrives and may form dense communities. It is widespread in the southern part of the Earth in general and in the Arabian desert regions specifically.

This plant contains many active compounds, most notably flavonoids, terpenes, and volatile oils. These oils give Shihia its strong smell. Additionally, this plant has important biological activities due to the presence of these active compounds mentioned earlier (Lakhdar, 2018).

1.2 Vegetable Classification of *Cotula cinereai* : (Larbi BAM 2018).

Scientific name:*Cotula cinerea Del*

Synonym:*Broccchia cinerea* (Del) vis

Common name: Grease, rupees.

Table 1: Vegetable classification of *Cotula cinereal*.

Kingdom	**Plants**
Division	**Seed Cassettes**
Community	**Plague dialects**
Family	**Starry**
Type	**Cinrea**
Sex	***Cotula***

1.3 Vegetable Description of *Cotula cinerea*:

A small herb around me, lasting 10 to 40 cm, smells strong, distinctive, bleached, bleached, bleached, bleached, bleached at the top, broken up to 3 to 5 schizophrenics, tiny disc-shaped heads with a diameter of 6 to 10 centimeters, flowers are tubed, all of a four-year-old pressure, a canister-striped velvet, a budge at the beginning, a gold whistle at the opening, poor fruits are stripped and photographed in shape, as illustrated (Dendougui*et al*., 2012).

Figure 2: Photograph of the *Cotulacinerea.*

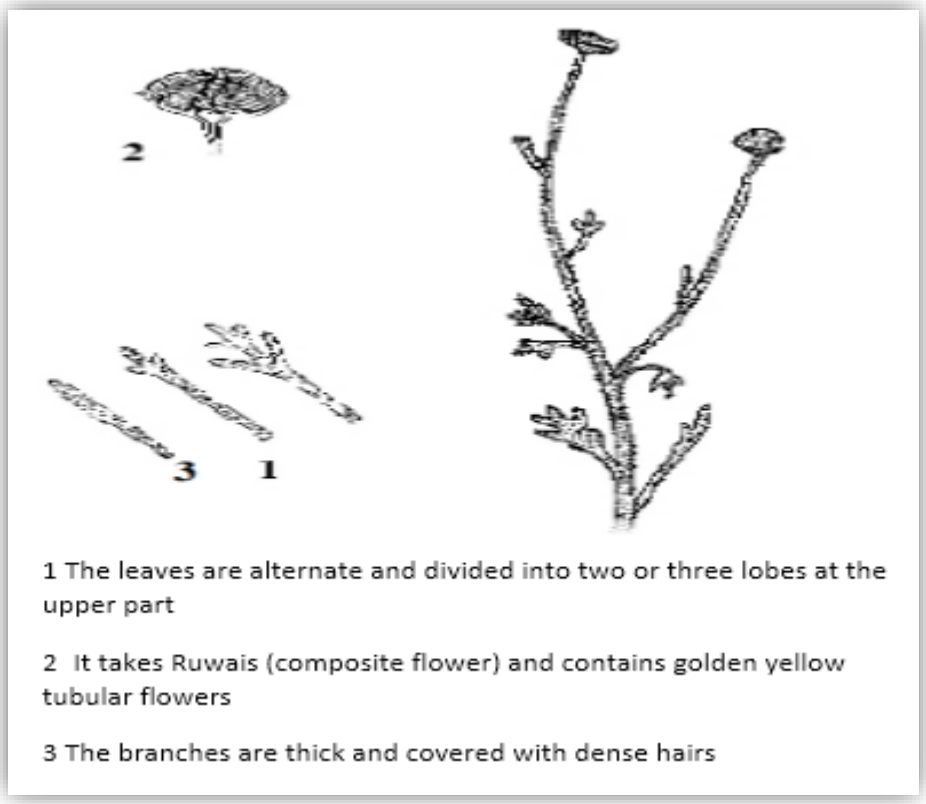


Figure 3: Schematic format for *Cotulacinerea*.

1.4. The geographical spread of *Cotula cinerea* :

*Cotula cinerea*is growing extensively in the southern part of the globe, spreading in the Sahara, in the Indian-Iranian deserts of Asia, as well as in the deserts of the *Arabian Peninsula*. In Algeria, it is growing in desert areas and semi-arid areas, especially in the south-eastern part of Algeria. And the (figure 4)shows the spread of Cotulacinerea in Algeria (Franke, 2005).

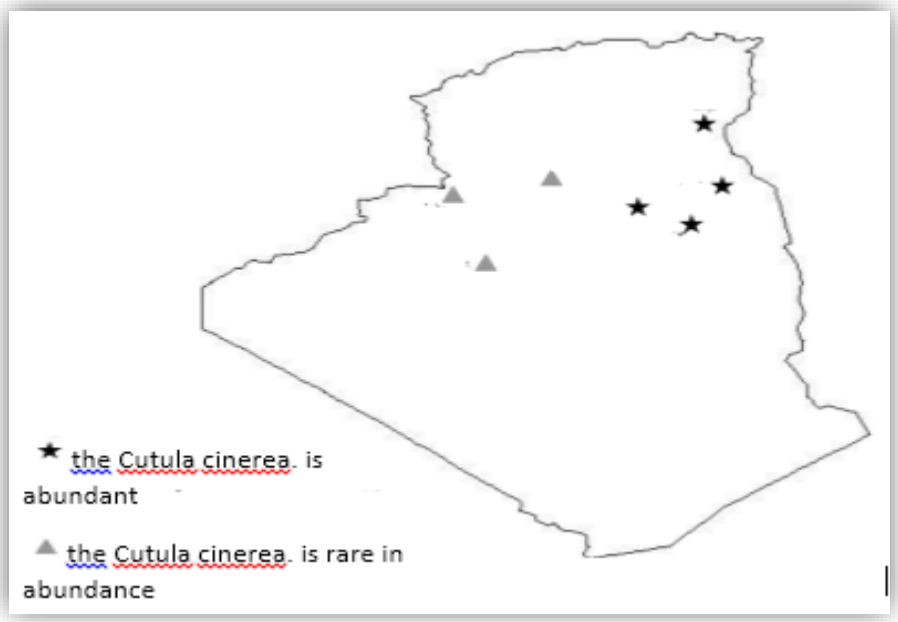


Figure4 : Prevalence of the *Cutulacinerea* plant in Algeria.

1.5 Economic and medical uses of *Cutula cinerea*:

Cotulacineren is used as a book, and is added as a tea and coffee muffin in some countries, and is used in popular medicine to treat abdominal abdominal pain, especially as an digestive aid, which is also used against people ' s air infections for their dilution properties of cough pain (Arbain*et al*., 2022; Mekhadmi *et al*., 2023).

It was noted that the paper extracts of the chip were characterized by anti-fungal activity (Zahari *et al*., 2014).

Flafonoid compounds derived from this plant also have an effect on a residential, anti-inflammatory and dehydration inhibitor. On the other hand, Kotulacinerea is used in some areas to treat stomach and abdominal pains (Buentzel*et al*., 2022).

2. A plant Origanum Majorana L:

2.1. Definition of the *Origanum Majorana L* plant:

The name Onegaman comes from the Greek words "oros" and means mountains and "gonos" and means sunshine, and thus became known for the beauty and abundance of mountains in the mountain areas of the Mediterranean basin (Pezzani*et al*., 2017).

Origanum Majorana L, formerly known as MajoranahortensisMoench, is a herb of age with large morphological and chemical diversity and forty-nine locally divided classifications distributed around the Mediterranean Sea (Vasudeva, 2015). Grass sex, commonly known as sweet parody, native home in Anatolia (Turkey) and Cyprus and naturalized in parts of the

Mediterranean region, especially Egypt was initially used as a purified agent by pirate earrings. It's a good home therapy for bra infection, cough and throat infection (Vasudeva, 2015).

2.2. Plant classification of *Origanum Majorana L:* (Larbi BAM 2018).

Scientific name: *Origanum Majorana*

A synonym: Marjolaine

Common name: Mercenary.

Table2: Plant Classification of *Origanum Majorana L*.

Kingdom	**Plants**
Division	**Flower Plants**
Community	**Plague dialects**
Level	**No Mileage**
Family	**Oral**
Type	***Majorana L***
Sex	***Origanum***

2.3. Vegetation of*Origanum Majorana L*: It is a half-lived tree that grows every year sensitive to the cold, an aromatic tree up to 30-60 centimeters high, with square red trunks with multiple branches spreading to form a pile. Legs are straight, weak, hairy, green round with red stains (Paudel *et al*., 2022). The leaves are soft, simple, spicy and oval to an oval rectangle, gray green linked to the opposite of each other on a square leg are very soft because there are so many hairs of 0.5-1.5 cm wide, 0.2-0.8 cm wide, with a piercing top, a full edge, a corresponding but straight base, and retinal veins. It has tiny white or pale pink tube flowers with gray green brackets blooming in the squirrel as mid to late summer (Juan to September). It's less than 0.3 cm long and it's about 13 cm long. Flowers are female in nature accurate seeds, dark oval and brown color that mature from August to September. She's got a subcylinder. Roots are taller with occasional incisions: 0.6 - 0.2 mm in the outer diameter of the dark brown root while the light brown color is internal with many long roots and root scars are also present. The cracks are long, symbiotic and fibrotic with a scent of perfume and unsolved (Hajlaoui *et al*., 2016).

Figure5: Photograph of *Origanum Majorana L.*

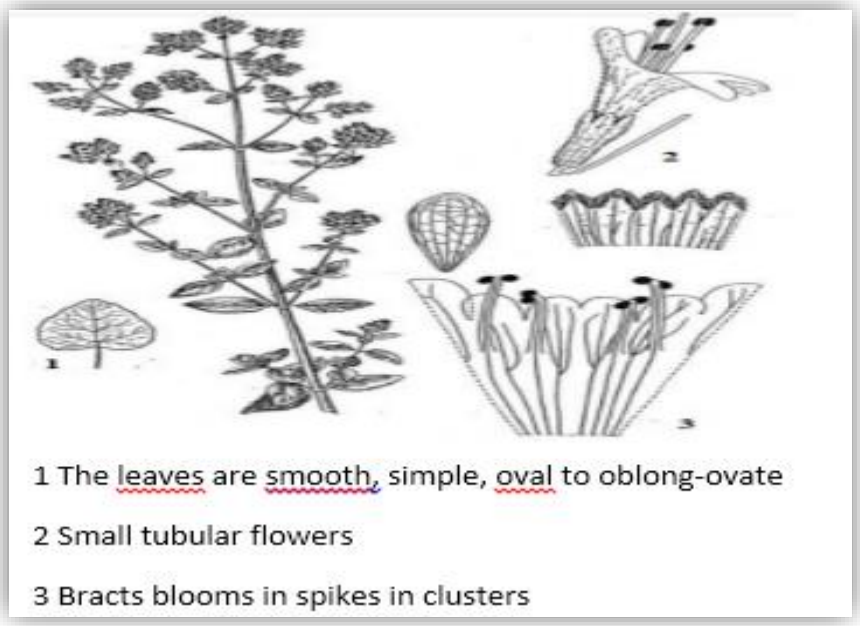


Figure 6: A schematic format *for Origanum Majorana L*

2.4. Geographical spread of *Origanum Majorana L*:

It is home to Cyprus and Turkey and has spread to Mediterranean countries (e.g. Lebanon), Iran, North America, the Arab island, and India. It grows on sunny slopes in meadows, fields and stonelands in dry weather. It is widely cultivated in the southern regions of Saudi Arabia (Tahmasebi *et al*., 2016).

The Figure (7) shows the spread of the Origanum Majorana L plant in Algeria.

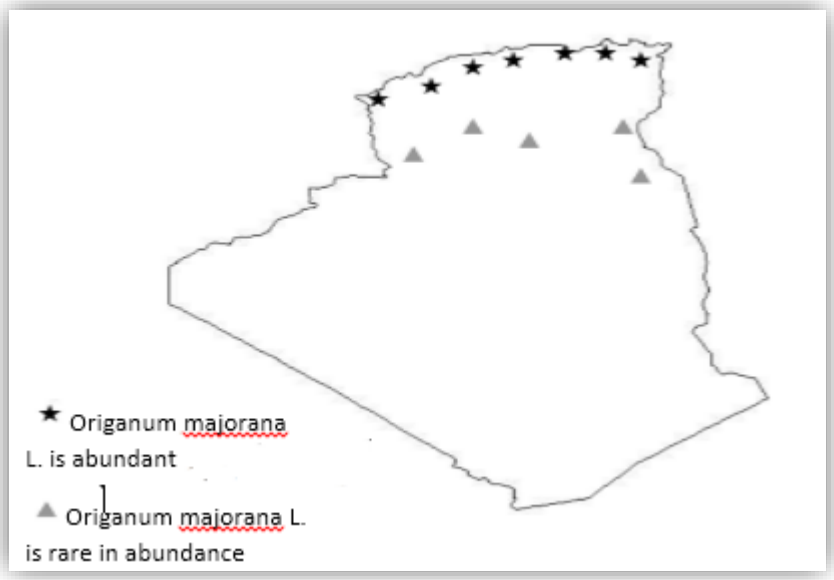


Figure 7:*OriganumMajoranaL* in Algeria.

2.5. Economic and medical uses of *Origanum Majorana L*:

Good home therapy for chest infections, cough, throat infections, romantic pain, neurological disorders, cardiovascular diseases, epilepsy, abdominal swelling, skin care and stomach disorders (Vasudeva, 2015).

It is widely used in the food industry as cables with meat and vegetables. It is advised not to take it during pregnancy, as it is an active uterus alarm.

In paper traditions it is used to treat sugar, insomnia, asthma and panic its pilot oil is used to provide flavor for various foods, especially soup, gravy, meat, fish, pained foods, bottles of wine, and beer (Vasudeva, 2015).

III. General about essential oils:

1. Definition of essential oils:

Essential oils are defined as oils that evaporate or volatilize without decay, and this distinguishes them from those fixed oils that do not volatilize. If exposed to evaporation or heating, they decompose and condensate (Gołębiowski*et al*., 2018).

Essential oils are the mixture of aromatic compounds and a pilot with a plant source, resulting from the metabolic transformation of aromatic plants as secondary receivers (Aqeel *et al*., 2023). We get them by dragging with water vapor or an afternoon on the cold of lemon crusts, which are complex and volatile, and may be liquid or half liquid, transparent or colorful.

Flying oils (les huilesessentiels) have the advantage of smell and are used as bait and flavorimproveers, such as calamitous oils and cauliflower, aromatic oils are concentrated in the green and unhorticultural total as in the cafeteria, flowers as in the narciss and jasmine, and sometimes in the tulips.

Flying oil is defined as a compound or material with a rather complex composition, which contains elements of a plane found in plants, which are elements with a volatile oil smell (volatile), uncolourable or inflammable (Jaunatres), which easily damage in the air a glue problem, and which naturally is liquid and is at surrounding normal temperatures, and which does not possess the grease and ointmentary properties (Oncteneux) of oils at the time they are touched (Hudaib*et al*., 2015; Radulescu *et al*., 2010) .

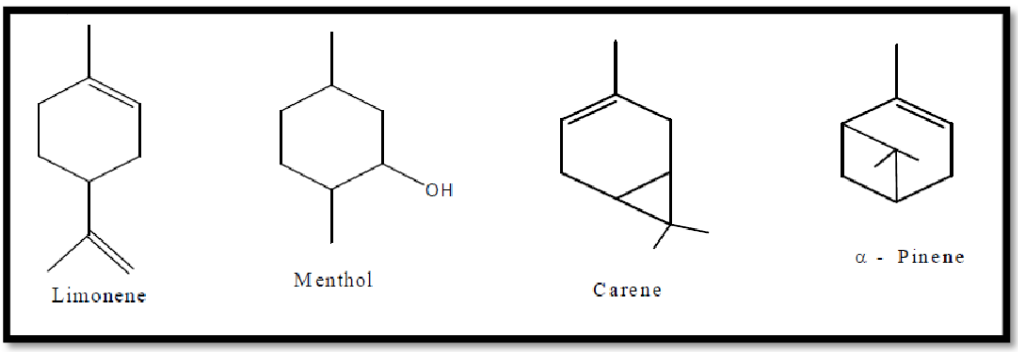


Figure 8: Structure of some essential oils (Sell, 2020).

2. Location of essential oils in vegetation:

In a plant, essential oils can be stored in various organs, such as flowers, leaves, bark, wood, roots, rhymes, fruit or seeds (De Castro *et al*., 1999; Sharifi-Rad *et al*., 2017).

The manufacture and assembly of essential oils is usually associated with the existence of a specialized fabric structure, Figure(9), the basic oils are manufactured in the Euphrates Cytoplasm and then clustered into gland cells covered by kyotkel, the shape and number of euphoria structures differ from one vegetarian family to another, and even from the other type, several categories of euphoria can be found in the same species (Morsy, 2017; Sell, 2020).

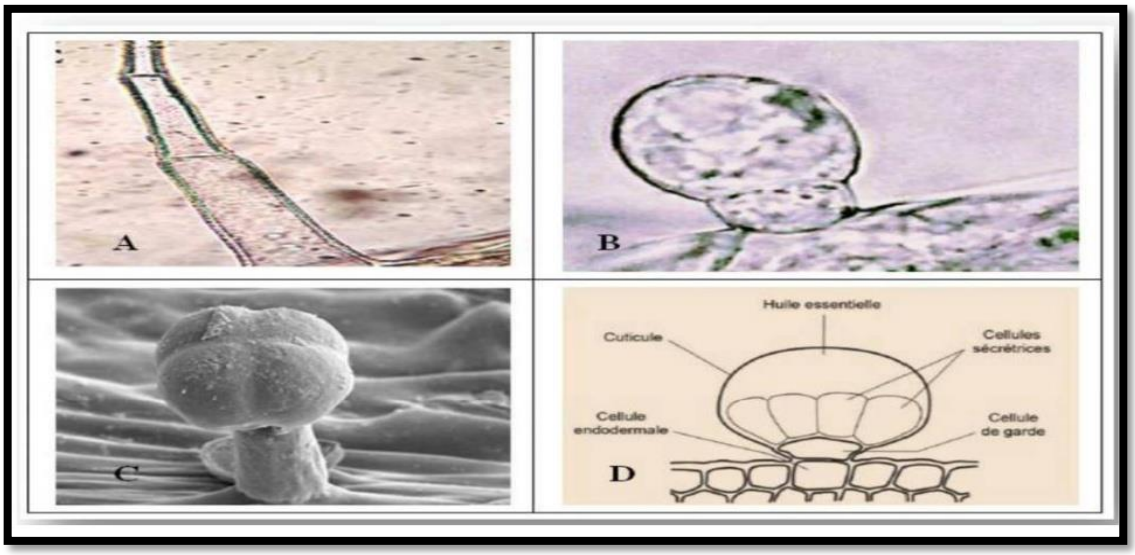


Figure9: Different types of infrastructure responsible for the formation of essential oils.

(A): Mentha pulegiumPoilsécréteur

(B): Mentha pulegium mint trichome glandulaire

(C): Lippiascaberrima for trichome glandulaire

(D): Thymus vulgaris for trichome glandulaire

3. Installation of essential oils:

All of the essential oils are complex mixtures of several compounds, but they consist essentially of two parts, one hydrocarbon, which is the main part of perfumed oil, the other is oxygen compounds, which belong to an organic group of acids, alcohol, esters, aldhydrates, kentons and ethers, and to a small extent these components may also contain sulfur or nitrogen compounds (Moghaddam & Mehdizadeh, 2017; Sadgrove *et al*., 2022).

3.1. Trophies:

They are natural products found in different parts of plants and represent the large proportion of by-products of metabolic metabolism that have the distinctive scent of many plants. The name of turbines has derived from vehicles that could be separated from turbine oil , and most turbines are volatile oils that are separated from plants such as basic oil separation routes, and the turbines play great importance especially in our daily lives, such as vitamin A, which is one of the turbines whose decrease in the body leads to a weakening of human sight . Studies carried out by Owlach at Göttingen University, Germany, in 1877 showed that the variety of turbines was due to the number of zombie units and Table (**3**) showing the types of turbines.

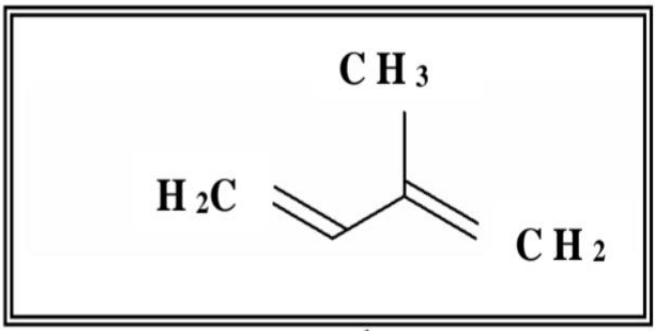


Figure10: Isoburn Unit.

3.1.1. Monotrophies:

Moitrates are among the most common species in the plant kingdom, consisting of two Isoprene units divided into monocyclic and its most important lemonene and a-Phyllandrene compounds (Vinckenet *al*., 2007).

And the two rings and the most important of them: α-binene and Sabinene β-Pinene.

The two-ring monotropanes are in large preparation and are divided by carbon structure of hydrocarbons into four groups (Oyman, 2017):

✓Thujine Group

✓Karan Group Carene

✓Pinene Group

✓Kamphene Group

Togan and Karan derivatives are found in rice wood oil, and the most important source of the pine group is crude turpentine oil, which can be obtained from pine bark.

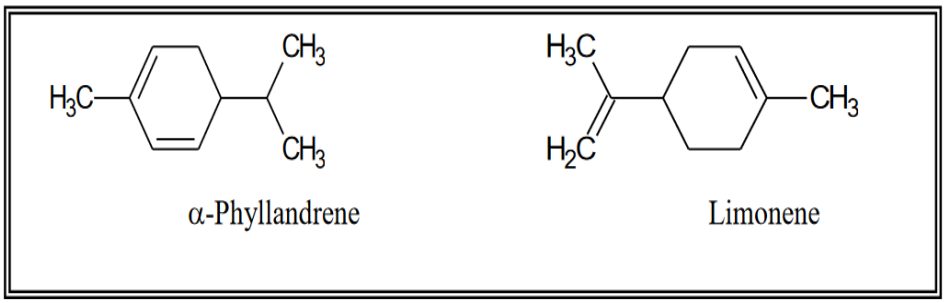


Figure 11: Monocyclic monotrophies.

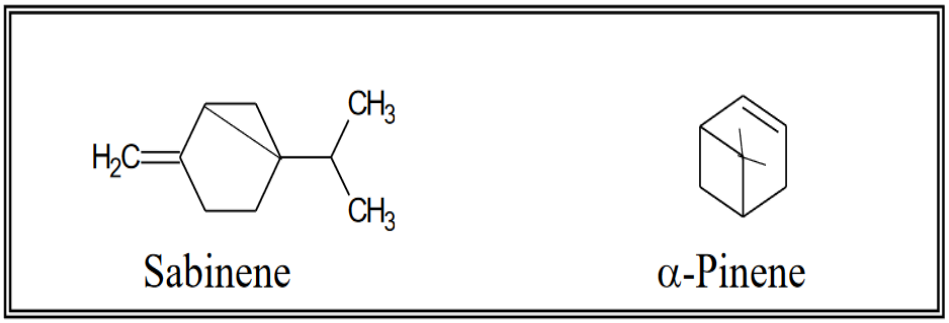


Figure 12: Monotrophine binary ring.

3.1.2. Bilateral (digen) turbines:

These turbines contain 20 carbon atoms, corresponding to four Isobrin units, for example, Vitol Phytol and vitamin A.

3.1.3. Triple turbines:

More than 2,500 molecules of three Isoprene units, each containing 15 carbon atoms. It is usually divided into two categories according to its carbon structure. One example is the type I turbine known as the Cadden. The second type of turbine is known as the Célineen, which is found in celery oil.

3.1.4. Triple turbines:

One example is the compound known as Escoline, which is considered to be the cholesterol makeup.

3.1.5. Quadrant turbines:

Each of them consists of eight Isoprene units, which are also called the Cartoins, and it has been possible to separate three carotins from the islands: α-carene and δ-carene β-carene.

3.1.6. Multiple turbines:

One of its most important examples is natural rubber, which is found in nature in the form of an emulsive brown, obtained from rubber trees found in cascading areas, and its null form around bilateral ties with the formation of cis (Sarkar & Bhowmick, 2018).

Table (3): Types of turbines.

Type of turbine	Number of carbon atoms	Number of Isopren units
Monotheistic	10	2
Raising half a trio	15	3
Tuppering's a couple	20	4
Triple turbine	30	6
Tetra-turbation	40	8
Polytechnic	Over 40	More then 8

4. Aromatic oil extraction methods:

Essential oils are derived by a number of methods, the most important of which are: water distillation, water vapor distillation, cold pressure extraction and organic pesticide extraction (Stratakos& Koidis, 2016).

4.1.Distillation

Aromatic oils are extracted in most plants in this way, and this process is done by vaporizing essential oil using heat, and can therefore be separated from other plant components. Oil is then intensified by reducing temperature whenever high-quality oil, natural and chemical specifications can be obtained and by distilling two methods (Aziz *et al*., 2018; Directions)

4.1.1 .Hydrodestination method:

Plants are flooded in the water in a lounge or metal bath, and they are heated either directly by fire or heated in a water bath so as to prevent the burning of parts of the plant touching the walls. This is especially the method for plants that bear boiling and partially dried and contain a high proportion of oil, such as seeds and crusts (Aziz *et al*., 2018; Directions).

4.1.2. Entrainement à la vapeur vapor:

Plants are placed in retina receptacles in such a way that the water sailors can intersect and extract from volatile oils, carry them into condensation pipes and easily break off from water, preferably breaking the plant material into small parts so that it can be infused by water vapor and collect the largest amount of volatile oil. This method can be used with all plants that contain volatile oils and bear high temperatures (Aziz *et al.*, 2018; Directions).

5 .Basic oil uses:

These natural products are of great importance to many sectors such as *(Bolouriet* al., *2022)*:

5.1 Pharmacist:

It can be used as follows:

You're depressing someone's oral medication.

For her physiological effects.

5.2. In industry:

5.2.1. Perfume and beauty industry

Many perfumes are natural, and some essential oils form the basis of many of them: roses and jasmine (de Sousa *et al*., 2023).

5.2.2. Nutrition

Essential oils are used like lemon oil, mints are too much to give flavor to food (jus de fruits, pâtisserie) (de Sousa *et al*., 2023).

CHAPTER TWO: ANTIOXIDANTS

I. Antioxidants:

1. Public about antioxidants:

Antioxidants prevent the formation or prevention of the effect of oxygen and active nitrogen that originates within the body and that causes damage to nuclear acids, fats, proteins and other biological particles. Antioxidants are classified as a substance that has the ability to inhibit or reduce free radicals. Thus, few antioxidants molecules as some enzymes are insufficient to prevent such damage completely. The removal of free radicals by antioxidants appears important to human health. However, we cannot live without free roots.

The body uses free roots to destroy germs, and it is also used to produce energy. The problem is that most people are exposed to excess (plus) amounts of free roots, but factors that increase our exposure to free roots or increase the production of our bodies can be avoided by using antioxidant-rich foods such as vegetables and fruits, and through the above, some basic concepts have to be taken, especially free roots and antioxidants.

2. Definition of antioxidants:

Antioxidants are a set of substances that protect cells from damage caused by unstable molecules known as free radicals, which can be defined in the biological system as a substance that is a low congener relative to what oxidable materials were and prevents their oxidation. Antioxidation is naturally found in most vegetables, fruits and medicinal herbs, which are considered as hydrogen grants or free root receptors and therefore their primary role is to break the chain of radical reactions resulting from oxidation (Lobo *et al*., 2010).

3. Anti-oxidant types:

There are types of antioxidants (Yadav *et al*., 2016):

3.1. Initial oxidation inhibitors:

It removes oxygen and nitrogen cracks after they are formed and even, giving them electron, and converts them into a fixed image of the oxidative capacity, or prevents their formation (Yadav *et al*., 2016).

3.2. Secondary oxidation inhibitors:

They are the mechanisms that stop the meta-oxidation of fat after it begins with oxygen or nitrogen cracks, so they are called super-grease oxidation chains, and are considered to be extremely important, as their failure leads to cell death or the mutation of their genetic content (Yadav *et al*., 2016).

3.3.Triple oxidizers:

They are complex mechanisms of a wide range of enzymes, working to repair damage caused by free roots after past systems have failed to prevent it, such as nuclear acid repair enzymes (Yadav *et al*., 2016).

4. Classification of antioxidants:

Antioxidants are classified from source to natural and manufactured (Fliege*et al*., 2021):

4.1. Natural antioxidants:

In a physiological state, the concentration of roots is like..˙O˙ and OOH˙ and OH˙It is controlled by cells that use many anti-oxidant strategies and consume considerable energy to monitor the level of oxygen reactions, using internal natural defensive devices (e.g. enzymes catalases, Pyroxydasessuperoxydedismutases) Anti-oxidation factors derived from food (external sources) suchas *vitaminCVitaminQ* and*vitaminE*. Antioxidants form a trap of free roots where free electrons are acquired and converted into fixed compounds, and on this basis it can be said that antioxidants are internal or external materials that can prevent the modification or repair of damage caused by free radicals (Fliege*et al*., 2021; Mamta *et al*., 2014).

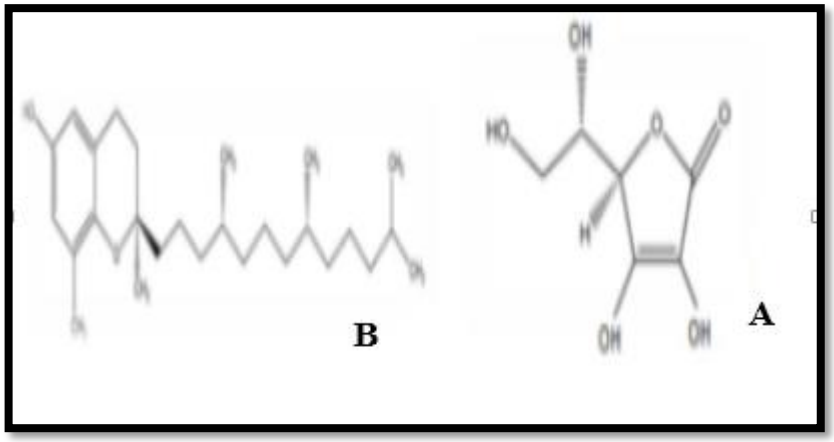


Figure 13: Chemical composition of some natural antioxidants (Rasheed & Azeez, 2019).

A- scorbick acid C6H8O6.

B-Vitamin$EC_{29}H_{50}O_2$ - D-alpha-tocopherol E

4.2.Manufactured anti-oxidants:

Antioxidants manufactured as an essential element must be added to canned foods to minimize their damage to the speed of oxidation From her BHT , BHA ,TBHQ , PG

These compounds are widely used in the food industry, because they're cost-effective compared to antibodies. Natural oxidation is as untoxic as it is (Fliegeret *al*., 2021).

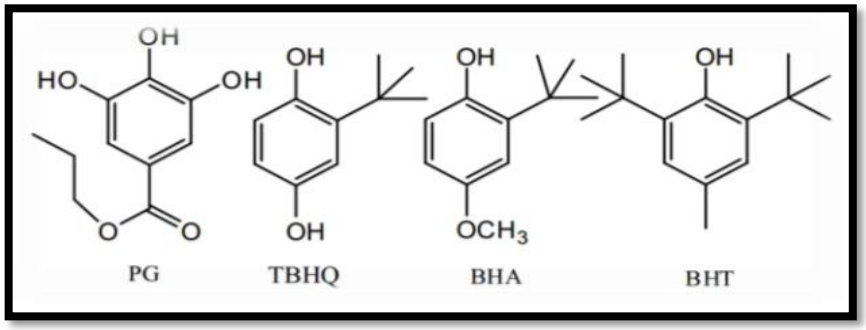


Figure 14: Antioxidants used in the food industry.

PG: Propyl Gallate

TBHQ: Tertiobutylhydroxyquinone

BHA: 3-tertiobutyl-4-hydroxyanisole

BHT: 2,6-ditertiobutyle-4-hydroxytoluéne

II. Free Root:

1. Definition of free roots :

Free radicals are defined as chemical units (atoms or molecules) that possess one or more free electrons in their outer orbit, making them unstable, and react quickly to other compounds, trying to capture what they lack from electrons to chemical stability. Free radicals usually attack the nearest constant molecule to which they need to take their electrons, in which case the attack molecules, which we have lost, turn into free radicals seeking stability, starting a series of reactions that exacerbates the membrane of the living cell and its components,And until the molecule of the AND (Martemucci et al., 2022).

2. Type of free root :

Free roots in terms of stability are divided into two types (Fitter, 2002):

2.1. Active roots (unstable):

Free roots are unstable in normal conditions, and this type includes elemental atoms such as hydrogen, nitrogen, chlorine and fluorine And so on. These roots are estimated to be in microscopic again or even less: CH HO NO :Roots with generally weak molecular weight, such as picoseconds (10-12 seconds). Notices the interactions of these roots and diagnoses their interaction with modern spectral methods (Fitter, 2002).

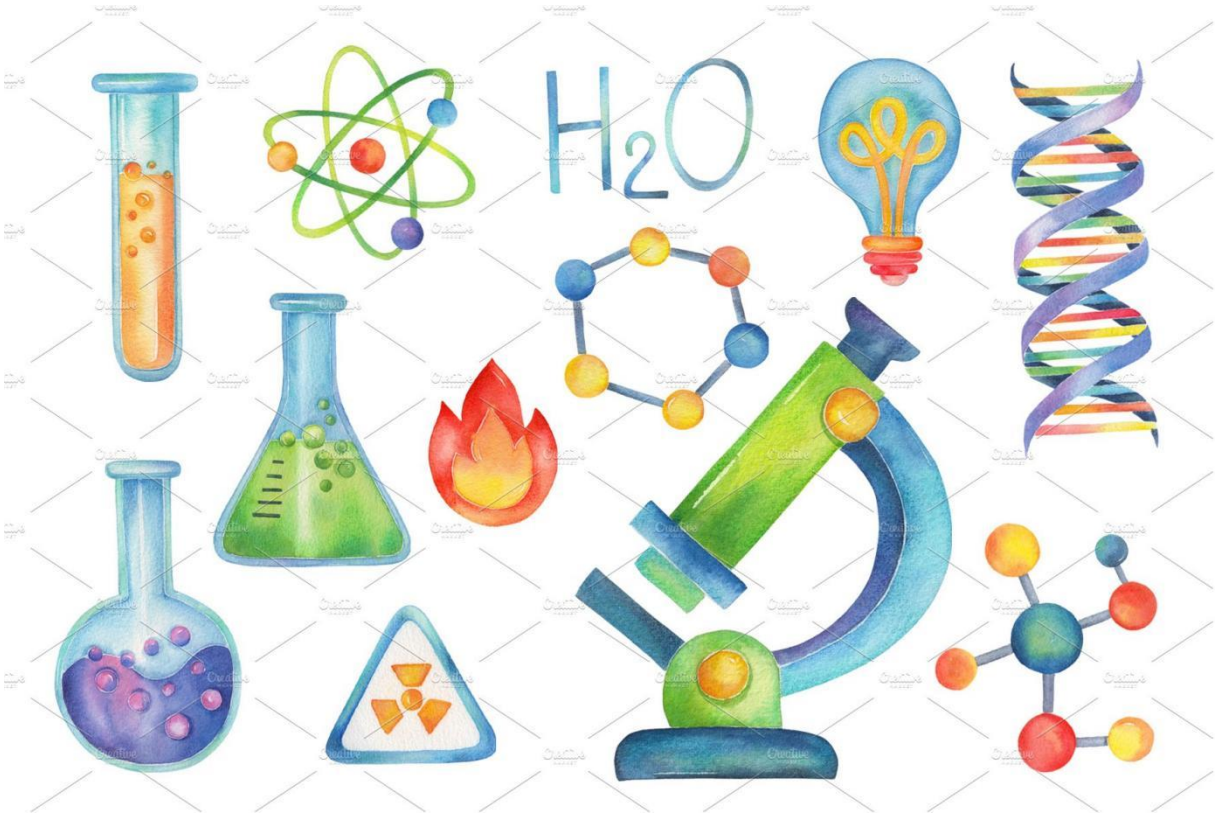
2.2 Stable (sustained) roots:

Triphenylmethyl Secondary metabolite products and antioxidants are roots that are estimated to be two seconds, minutes, hours, even days, like root It's a black purple and its •DPPH) diphenyl1-1-picrylhydrazyl Yellow-colored and stable at room temperature by hours and root2,2- It's a solid, solutionable and stable substance for several days Most of the free, amphibious roots with the resonant structures are often stable, and the stability of this type of root is due to the fact that the free electron is not concentrated, that is, moving from one location to another along the root structure, like the electron is not concentrated at the root of (DPPH•)2,2-diphenyl1-1-picrylhydrazyl . In addition, there are also free fat roots such as unsaturated fat, and free toxic roots such as chemical carcinogens (Kochar*et al*., 2024).

SECOND PART:

EXPERIMENTAL

CHAPTER ONE: MATERIALS AND METHODS



1. Introduction:

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

2. Plant Material:

2.1. Cotulacinerea

The *Cotulacinerea* plant was harvested at various time intervals ranging from December 2023 to January 2024 in a desert forest area in southeastern Algeria, specifically in Hassi Khalifa region in El Oued province. This region is characterized by the following specifications:

- Geographic Coordinates: Longitude 48°10' East, Latitude 23°09' North.
- Elevation above Sea Level: 44 meters.
- Distance from Sea Level: 290 kilometres.
- Bioclimatic Characterization: Desert.

2.2. Origanum Majorana L:

The *Origanum Majorana L* plant was harvested at different time intervals extending from March 2023 to April 2023 in a desert forest area in southeastern Algeria, specifically in Akfadou region in El Oued province. This region is characterized by the following specifications:

- Geographic Coordinates: Longitude 67°6' East, Latitude 33° North.
- Elevation above Sea Level: 58 meters.
- Distance from Sea Level: 300 kilometres.
- Bioclimatic Characterization: Desert.

3. Chemicals and reagents:

Acetonitrile (ACN) (HPLC-grade from Sigma-Aldrich) was utilized as a solvent in the experimental procedures. Additionally, 2,2-diphenyl-1-picrylhydrazyl (DPPH), a stable free radical commonly employed in antioxidant assays, served as the reagent for assessing

antioxidant activity. Tetrabutylammonium tetrafluoroborate (Bu_4NBF_4) (electrochemical grade, 99% purity, Sigma-Aldrich) was employed as the supporting electrolyte, maintaining a concentration of 0.1M. All other reagents utilized were of analytical grade.

4. Materials and Methods:

4.1. Essential Oils Extraction:

4.1.1. Apparatus:

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

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

The Clevenger apparatus, a key component in the extraction process, is depicted in Figure 15

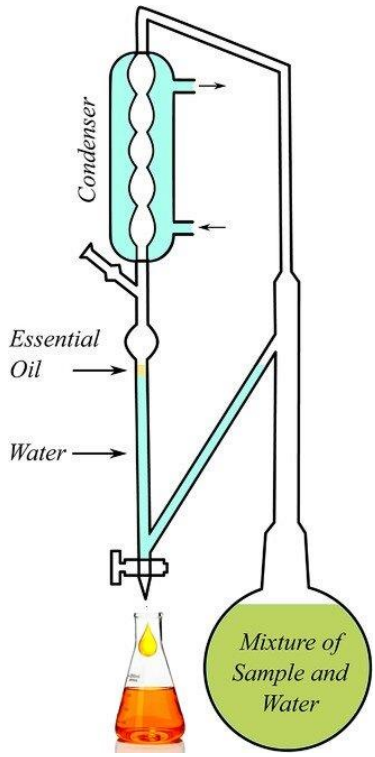


Figure 35. Schematic representation of the Clevenger apparatus used in the essential oil extraction process (Biswa *et al.*, 2023)

4.1.2. Procedure:

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

- ✓ Cleaning of the plant sample to remove any impurities.
- ✓ Weighing the plant sample to a quantity of 100 g using a sensitive balance.
- ✓ Washing the plant sample with water to prevent burning, followed by placement in a heating flask.
- ✓ Addition of small pieces of boiling regulator to the flask.
- ✓ Direct heating of the mixture at a temperature of 100 degrees Celsius for three and a half hours.
- ✓ Conducting the steam distillation process and subsequent separation of oil and water phases using liquid-liquid separation with diethyl ether.
- ✓ Drying of the organic oily phase with anhydrous sodium sulphate.
- ✓ Filtration of the dried oil phase through filter paper to remove diethyl ether particles.
- ✓ Evaporation of the filtered oil phase using a rotary evaporator to remove any remaining organic solvent.
- ✓ Storage of the obtained essential oil in small brown bottles and refrigeration at a temperature of 5°C.

4.2. Yield of Essential Oil Extraction:

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

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

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

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

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

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

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

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

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

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

4.3. Characterization of Essential Oils:

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

4.3.1. Physicochemical Properties:

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

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

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

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

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

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

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

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

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

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

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

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

- Begin by placing 0.5 mL of the essential oil into a small vessel.
- Add 1 mL of 0.5 N potassium hydroxide (KOH) solution to the vessel to initiate the titration process.
- Place the mixture in a gas-evacuated water bath for a specified duration to facilitate reaction.
- After cooling, introduce 0.5 mL of distilled water and add 3 drops of phenolphthalein indicator to the mixture.
- Titrate the excess potassium hydroxide (KOH) using 0.5 N hydrochloric acid (HCl) until a colour change is observed, indicating neutralization.
- Quantify the volume of hydrochloric acid (HCl) required to neutralize the excess potassium hydroxide (KOH).
- Calculate the ester content using the following Equation 5:

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

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

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

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

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

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

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

➢ **Principle:**

The principle is founded on the varying affinities of compounds within the mixture towards two phases: a stationary phase and a mobile phase. This technique relies on the distribution of

constituents between a stationary phase and a gas phase. The stationary phase comprises a silicone-based liquid that permeates an inert and granular solid material, housed within a typically coiled steel or glass column measuring 1 to 3 meters in length and 2 to 4 millimetres in diameter. The mobile phase consists of an inert carrier gas such as nitrogen, helium, or argon.

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

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

- **Apparatus:**

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

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

- **Procedure:**

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

4.4. In VitroDPPH inhibition assays:

4.4.1. Cyclic Voltammetric Measurements:

- ✓ **Apparatus:**

The apparatus used for voltammetric measurements is a Potentiostat/Galvanostat Model PGZ301 (Radiometer Analytical SAS) connected to a three-electrode electrochemical cell,

- A glassy carbon electrode with a diameter of 5.2 mm^2.
- A mercury/mercurous chloride/saturated KCl reference electrode.

- A platinum auxiliary electrode with a diameter of 3 mm².

All of this is controlled by a Pentium IV microcomputer (CPU 4.0 GHz and RAM 2 GB) equipped with VoltaMaster4 software, version 7.08.

✓ **Reagents:**

- Acetonitrile solution
- Electrolyte support (Tetra-n-butylammonium tetrafluoroborate, 99%)
- Nitrogen (Gas)
- 2,2-diphenyl-1-picrylhydrazyl

✓ **Procedure:**

The reaction between the essential oils and the DPPH takes place in the electrochemical cell. The reaction medium is an acetonitrile solution.

Before each measurement, the working electrode is polished using p4000 abrasive paper. After polishing, the electrode is washed with ultra-pure water and dried with air. The surface of the electrode thus appears mirror-like. Then, the electrochemical cell is equipped with the reference electrode and the auxiliary electrode, as well as the working electrode. The cell is filled with 10 ml of a solution consisting of ACN solution containing the essential oils under study. Nitrogen gas is bubbled through the solution for 10 to 15 minutes to remove oxygen from the cell. A variable potential is applied at a fixed scan rate and at a temperature of 28 ± 2°C. The obtained voltammogram is analysed to access the interaction parameters between the studied essential oil and the DPPH.

The ability of the test sample to scavenge the DPPH radicals (% Inhibition of DPPH) was calculated usingEquation 6(Lanez *et al.*, 2023; Lanez *et al.*, 2019),

$$\% \, DPPH \, radical \, scavenging \, activity = \frac{i_0 - i}{i_0} \times 100 \quad (6)$$

where i_0 and i are the anodic peak current densitiesof the DPPH radical in the absence andpresence of *Cotulacinerea*and*Origanum Majorana L*essential oils, respectively

4.4.2. Spectroscopic Measurements:

✓ **Apparatus:**

UV-Vis measurements were performed using a UV-Vis spectrometer (Shimadzu 1800) and a quartz cell with a volumetric capacity of 5 ml. Data acquisition was carried out with a Pentium IV microcomputer (CPU 4.0 GHz and RAM 2 GB) equipped with UV probe software version 2.34 (Shimadzu). The data is processed using OriginLab 9.0 software.

✓ **Reagents:**

- Acetonitrile solution

- 2,2-diphenyl-1-picrylhydrazyl

✓ **Procedure:**

The electronic spectra of DPPH in the acetonitrile solution were obtained in the absence and presence of increasing concentrations of *Cotula cinerea* and *Origanum Majorana L* essential oils.

The ability of the test sample to scavenge the DPPH radicals (% Inhibition of DPPH) was calculated using Equation 7 (Filote *et al.*, 2022),

$$\%\ DPPH\ radical\ scavenging\ activity = \frac{A_0 - A}{A_0} \times 100 \quad (7)$$

where A_0 and A are the absorbances of the DPPH radical in the absence andpresence of *Cotulacinerea*and*Origanum Majorana L*essential oils, respectively.

4.5. In-Silico analysis:

4.5.1. Software

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

4.5.2. ADMET and drug-likeness evaluation:

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

4.5.3. Docking setup:

➢ **Ligands preparation:**

The three-dimensional configurations of the major compounds isolated from *Cotulacinerea* and *Origanum Majorana L* essential oilswere obtained from the National Library of Medicine

(NCBI) database (Kim *et al.*, 2016), accessible through the NCBI website (https://pubchem.ncbi.nlm.nih.gov/).

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

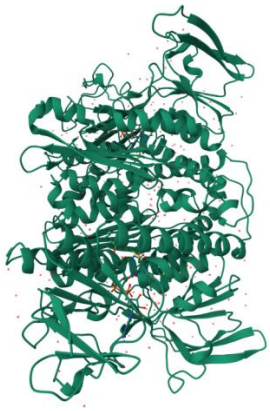
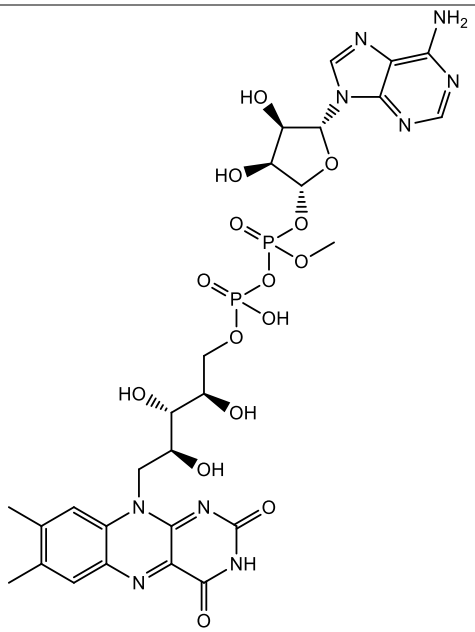
- **Receptor Preparation:**

The crystallographic data for Human pancreatic alpha-amylase (Table 4) (PDB ID: 1BWC) (Gallwitz *et al.*, 1999)was retrieved from the Protein Data Bank (http://www.rcsb.org) (Rose *et al.*, 2017), adhering to specific parameters such as a resolution of 1.35Å and R value-free of 0.211. Processing of the protein structure was executed through the "protein preparation Workflow" module within the Schrödinger suite (Madhavi Sastry *et al.*, 2013), involving consecutive stages of import and processing, review and modification, and refinement.

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

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

Table 4. Target receptor information chosen for docking studies

Human glutathione reductase	Detaille's	
	PDB ID	1BWC
	Mutation	No
	Resolution (Å)	2.10
	R-Value Free	0.230
	R-Value Work	0.180
	R-Value Observed	0.180
	Organism	Homo sapiens
	Expression System	Escherichia coli
	Space Groupe	C22 2_1
	Sequence Length	478
Co-Crystallized Ligand	**Detaille's**	
	Name	FLAVIN-ADENINE DINUCLEOTIDE
	Formula	$C_{27}H_{33}N_9O_{15}P_2$

- **Molecular Docking:**

Computational simulations, including Induced Fit Docking, molecular dynamics studies, and MM-GBSA calculations, were conducted employing the Glide module, Induced Fit Docking module, Prime module, and the Desmond module within the Maestro version 11.7 user interface of the Schrödinger suite (Small-Molecule Drug Discovery Suite 2021-4, Schrödinger, LLC, New York, NY, 2021) (Schrödinger, 2015). The simulations were executed on a DELL Intel(R) Core(TM) i9-13900HX CPU @ 2.20 GHz processor, equipped with 32,0 GB RAM, and operated on a 64-bit Linux Ubuntu 18,04.1 LTS operating system.

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

- **Induced Fit Docking (IFD):**

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

- **Molecular Dynamics Simulation (MDS):**

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

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

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

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

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

OPLS4 force field, stands as a methodologically rigorous approach for the comprehensive evaluation of molecular interactions and binding strengths (Gorla *et al.*, 2021).

CHAPTER TWO: RESULTS AND DISCUSSION

1. Introduction:

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

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

2. Extraction Yield:

Figure 16 illustrates the yields of essential oils obtained through hydrodistillation of the aerial parts of *Cotulacinerea*and *Origanum Majorana L*. variety of Eloued.

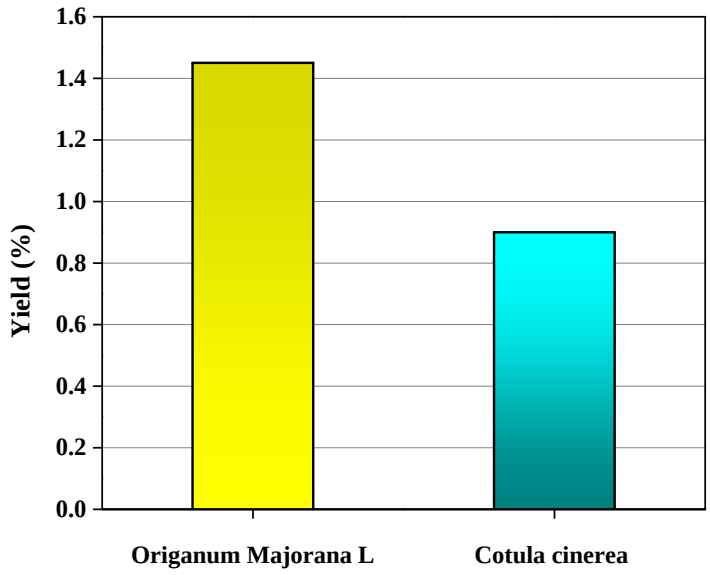


Figure 16. The yields of extracted essential oils

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, findings reported by (Alimi *et al.*, 2022) indicate lower yields of essential oils extracted by hydrodistillation from *Origanum Majorana L* and *Cotula cinerea* at ambient temperature.

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

Furthermore, significantly higher yields were achieved by (Vera *et al.*, 1999) during the hydrodistillation of *Origanum Majorana L*, Indian variety.

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

3. Chemical composition of essential oils:

3.1. Organoleptic characteristics:

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

Table 5. The organoleptic characteristics of essential oils

Plant	Smell	Aspect	Colour
Cotulacinerea	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 meticulously determined according to the standards of the French Association for Standardization (AFNOR) using established methodologies to measure relative density, refractive index, acidity index, and ester index, as depicted in the following Table 6.

Table 6. The physicochemical properties of essential oils

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

By comparing these results to those obtained by (Naima *et al.*, 2019) (density 0.953, refractive index 1.474, acidity index 4.93 and ester index 45.96) and (Alimi *et al.*, 2022) (density: 0.834 ± 0.02, 0.835 ± 0.02, refractive index, at 25°C 1.4596 ± 0.03, 1.4622 ± 0.04), and considering that the refractive index was measured at a temperature of 23°C, we can conclude that these values are in accordance with the standards described by AFNOR (Afnor, 1982).

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

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

Figure 17 and Figure 18 depict the chromatograms illustrating the essential oil compositions of *Cotulacinerea* and *Origanum Majorana L*, respectively, as obtained through gas chromatography-mass spectrometry (GC/MS) analysis:

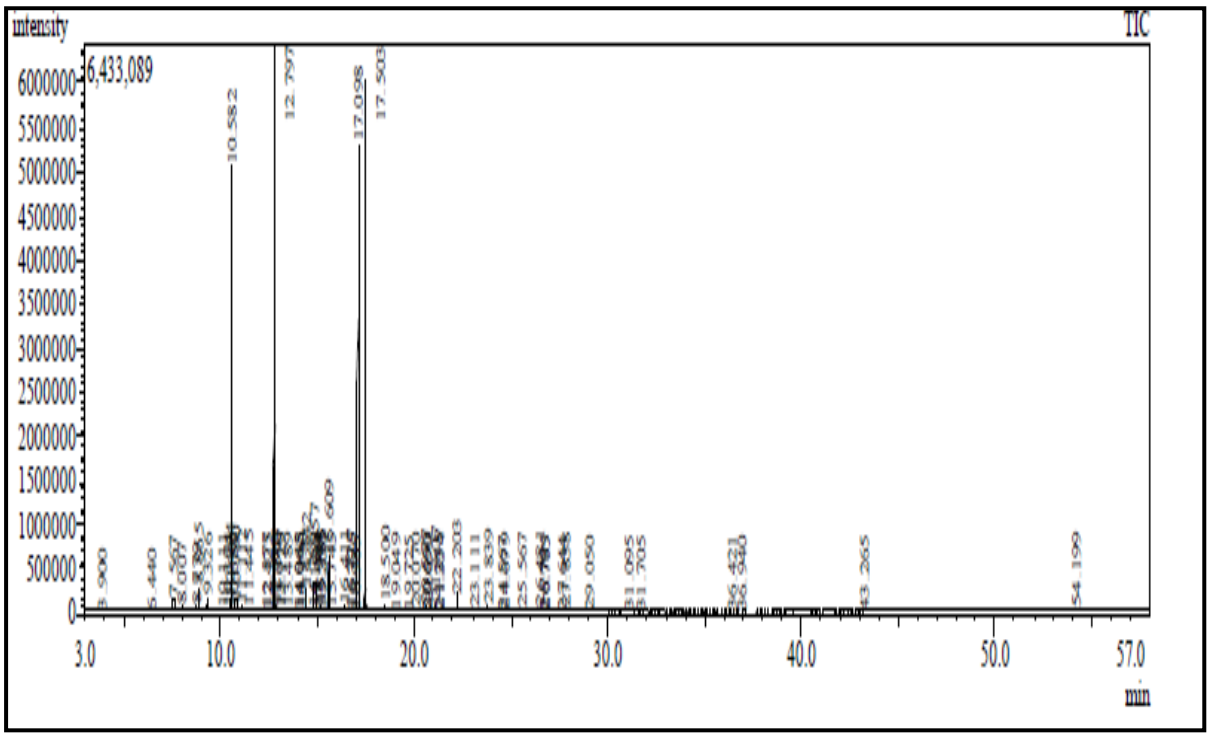


Figure 17. Chromatogram of the essential oil of *Cotulacinerea* plant obtained by GC/MS

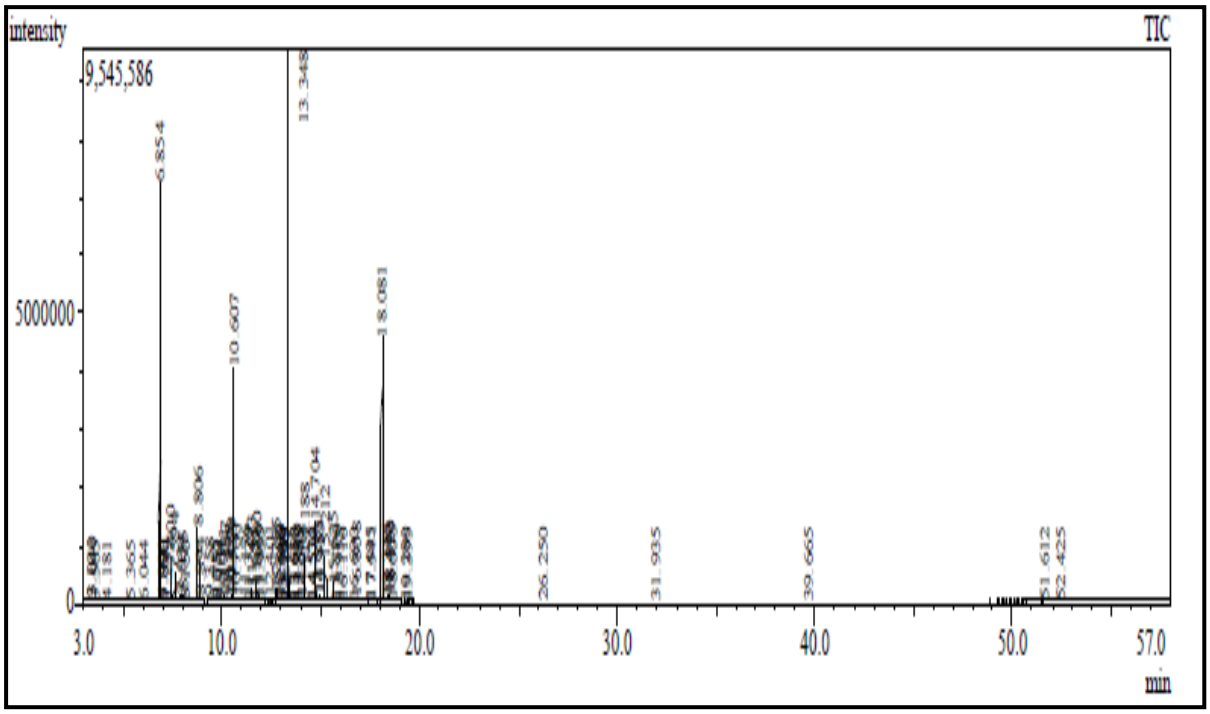


Figure 18. Chromatogram of the essential oil of *Origanum Majorana L* plant obtained by GC/MS

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 *Cotulacinerea* and *Origanummajorana L*, have been identified and collated as follows

3.3.1. Cotula cinerea:

The hydrodistillation extraction of *Cotulacinerea* yielded a dark yellow oil with a yield of 0.91%. Gas chromatography-mass spectrometry (GC/MS) analysis identified 31 compounds, collectively constituting 95.64% of the oil's composition (as presented in Table 7). Predominantly, oxygenated monoterpenes accounted for 68.16%, followed by hydrocarbon monoterpenes at 23.27%, while the percentage of oxygenated sesquiterpenes was minimal at 0.15%. The remaining 4.06% comprised various other compounds.

Table 7. Essential oil constituents of *Cotulacinerea* 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 jasmine	1394	1390	0.15

31	Germacrene D	1480	1481	0.06
	Total		**95.64**	

Our analysis unveiled ten compounds with concentrations surpassing 1% (Table 7), with trans-thujone emerging as the predominant compound at 51.86%, followed by santolina triene at 10.69%, and sabinene at 6.17%. Additionally, 1,8-cineole constituted 5.34%, while four compounds α-pinene, terpin-4-ol, β-terpineol, and camphor appeared in lesser proportions at 2.02%, 1.73%, 1.39%, and 2.63%, respectively.

Comparison with other studies revealed some congruence, particularly with the percentage of santolina triene in Ekhlil et al.'s (Ekhlil *et al.*, 2016) investigation (11.67%). However, disparities arose in the primary compound, with iso-thujanol predominating at 47.38% in their study. Conversely, El bouzidi et al.'s (Bouzidi *et al.*, 2011) findings aligned closely with ours, particularly regarding trans-thujone (41.4%), 1,8-cineole (8.2%), and santolina triene (7.2%), while reporting camphor at 5.5%. Notably, Fournier et al.'s (Fournier *et al.*, 1989) study diverged, identifying camphor as the principal compound at 50%.

For a visual representation of the top major compounds (more than 1%) and their structures, refer to Figure 19, which depicts the chemical structures and IUPAC names of the predominant constituents identified in the essential oil of *Cotula cinerea*.

3.3.2. Origanum Majorana L:

The hydrodistillation extraction method was employed to obtain the essential oil from *Origanum Majorana L*., yielding a noteworthy 1.5% output. Analysis revealed the identification of 98.84% of the constituents, comprising a total of 42 compounds (as presented in Table 8). Oxygenated monoterpenes dominated the composition, constituting over 57%, followed by hydrocarbon monoterpenes at 25.14%.

Of particular interest is the prominent presence of trans-Thujone, constituting 33.3% of the essential oil, a compound conspicuously absents in the majority of prior studies. For instance, J. Chane et al.'s (Vera *et al.*, 1999) investigation reported Terpinen-4-ol as the primary compound at 38.4%, a constituent entirely absents in our study. Similarly, Santolina triene emerged as the second major compound in our analysis at 16.40%, a finding not corroborated in extant literature.

Despite these disparities, concordance exists with previous studies regarding the occurrence of Sabinene, which manifested at 3.12% in our study, compared to 15% in J. Chane et al.'s (Vera *et al.*, 1999)study and 17% in K.H.C Baser et al.'s study (Baser *et al.*, 1993). Additionally, Sellami et al (Sellami *et al.*, 2009) documented Sabinene at 2.14%. Supplementary compounds identified include α-Thujene (1.44%), α-Pinene (1.19%), and β-Pinene oxide (4.42%).

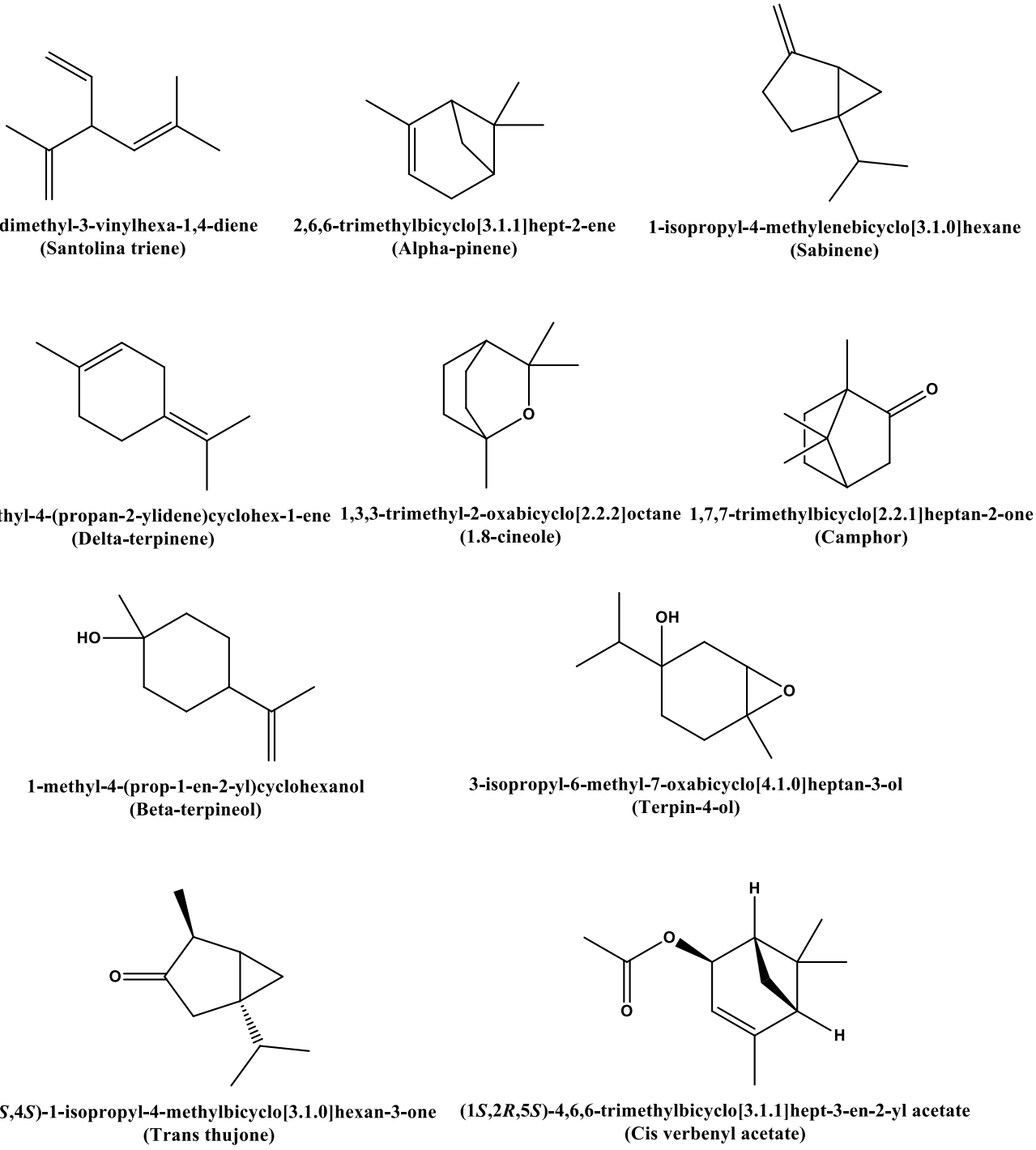
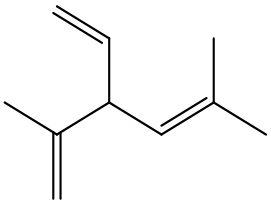


Figure 19. Chemical structures and IUPAC names of the top major compounds identified in the essential oil of *Cotula cinerea*

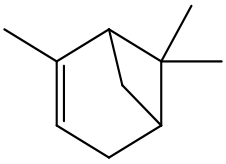
In summary, the essential oil derived from *Origanum Majorana L.* exhibits distinctive chemical compositions in Eloued region compared to oils from other geographical locales, characterized notably by the prevalence of trans-Thujone and Santolina triene as major constituents. This variance likely stems from multifactorial influences such as soil quality, climatic conditions, and harvest timing. For an illustrative overview of the major compounds identified, Figure 20, displaying the structures and IUPAC nomenclature of these constituents.

Table 8. Essential oil constituents of *Origanum Majorana L* identified by GC/MS

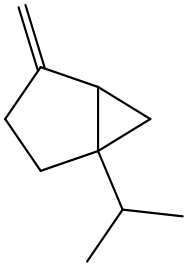
No	Compounds	IR_{Exp}	IR_{Ref}	(%)
01	Pentanol	759	762	0.12
02	Cis-2-Penten-1-ol	762	765	0.03
03	Hexanal	801	801	0.02
04	(Z)-Salvene	844	847	0.02
05	Isopentyl acetate	869	869	0.02
06	**Santolina triene**	**898**	**906**	**16.42**
07	Tricyclene	912	921	0.02
08	**Alpha-Thujene**	**918**	**924**	**1.44**
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**	



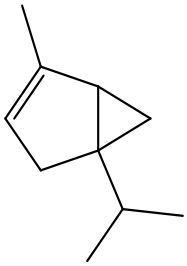
2,5-dimethyl-3-vinylhexa-1,4-diene
(Santolina triene)



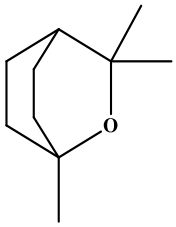
2,6,6-trimethylbicyclo[3.1.1]hept-2-ene
(Alpha-pinene)



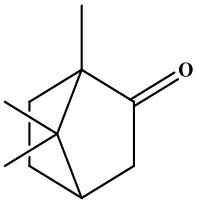
1-isopropyl-4-methylenebicyclo[3.1.0]hexane
(Sabinene)



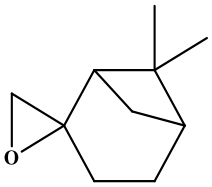
5-isopropyl-2-methylbicyclo[3.1.0]hex-2-ene
(Alpha-Thujene)



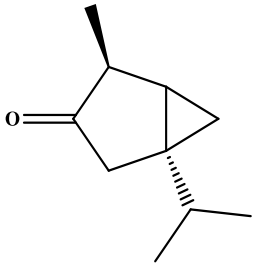
1,3,3-trimethyl-2-oxabicyclo[2.2.2]octane
(1.8-cineole)



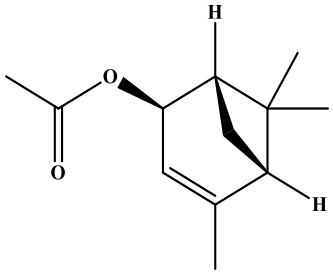
1,7,7-trimethylbicyclo[2.2.1]heptan-2-one
(Camphor)



6,6-dimethylspiro[bicyclo[3.1.1]heptane-2,2'-oxirane]
(Beta pinene oxide)



(1*S*,4*S*)-1-isopropyl-4-methylbicyclo[3.1.0]hexan-3-one
(Trans thujone)



(1*S*,2*R*,5*S*)-4,6,6-trimethylbicyclo[3.1.1]hept-3-en-2-yl acetate
(Cis verbenyl acetate)

Figure 20. Chemical structures and IUPAC names of the top major compounds identified in the essential oil of *Origanum Majorana L*

4. *In Vitro* Antioxidant Activity:

4.1. Cyclic Voltametry interaction study:

4.1.1.2,2-diphenyl-1-picrylhydrazylscavenging activity (IC_{50}):

The electrochemical behavior of the DPPH radical in acetonitrile, accompanied by 0.1M Bu_4NBF_4 as a supporting electrolyte, within a potential window of 0.0–1.2V at a glassy carbon electrode, is elucidated in Figure 21. Within this setup, the free DPPH redox couple manifests two oxidation peaks at 0.394 and 0.848V, as well as two reduction peaks at 0.312 and 0.766V. Figure 21 further illustrates the impact of varying concentrations of *Cotulacinerea* and *Origanum Majorana L*essential oils, introduced into a solution of DPPH in acetonitrile, on the oxidation peak current density of the DPPH couple.

The addition of different concentrations of *Cotula cinerea* and *Origanum Majorana L*essential oilsin acetonitrileto 300 µM acetonitrile solution of DPPH causes a remarkable decrease in the peakcurrent density, Figure 21. The significant decrease in peak current density can be attributedto the diminution in freeDPPHradical concentration as indicated by the equilibriumof theelectron transfer between DPPH radical and the studied essential oils, Equation 8.

$$DPPH^{\bullet} + EO \text{€} \quad DPPH + EO^{\bullet} \quad (8)$$

Since the peak current density is directly proportional to the concentration of $DPPH^{\bullet}$(Brand-Williams *et al.*, 1995)the $DPPH^{\bullet}$concentration is decreased upon reaction with *Cotulacinerea* and *Origanum Majorana L*essential oils.

The ability of the studied essential oils to quench DPPH radicals(% Inhibition of DPPH) was calculated using the previously cited Equation 6(Rebiai *et al.*, 2014).

The effective concentration (IC_{50}) values were obtainedfrom the linear regression of the percentages of DPPH radical scavenging activity against differentcompound concentrations, Figure 22.

The equations obtained from the linear calibration graph in the studied concentrationrange for *Cotulacinerea* and *Origanum Majorana L*essential oils and α-tocopherol used as a positive control aresummarized in Table 9 (where y represents the value of the anodic peak current density of DPPH radical and x, the value of samples concentration, expressed in mg/mL).

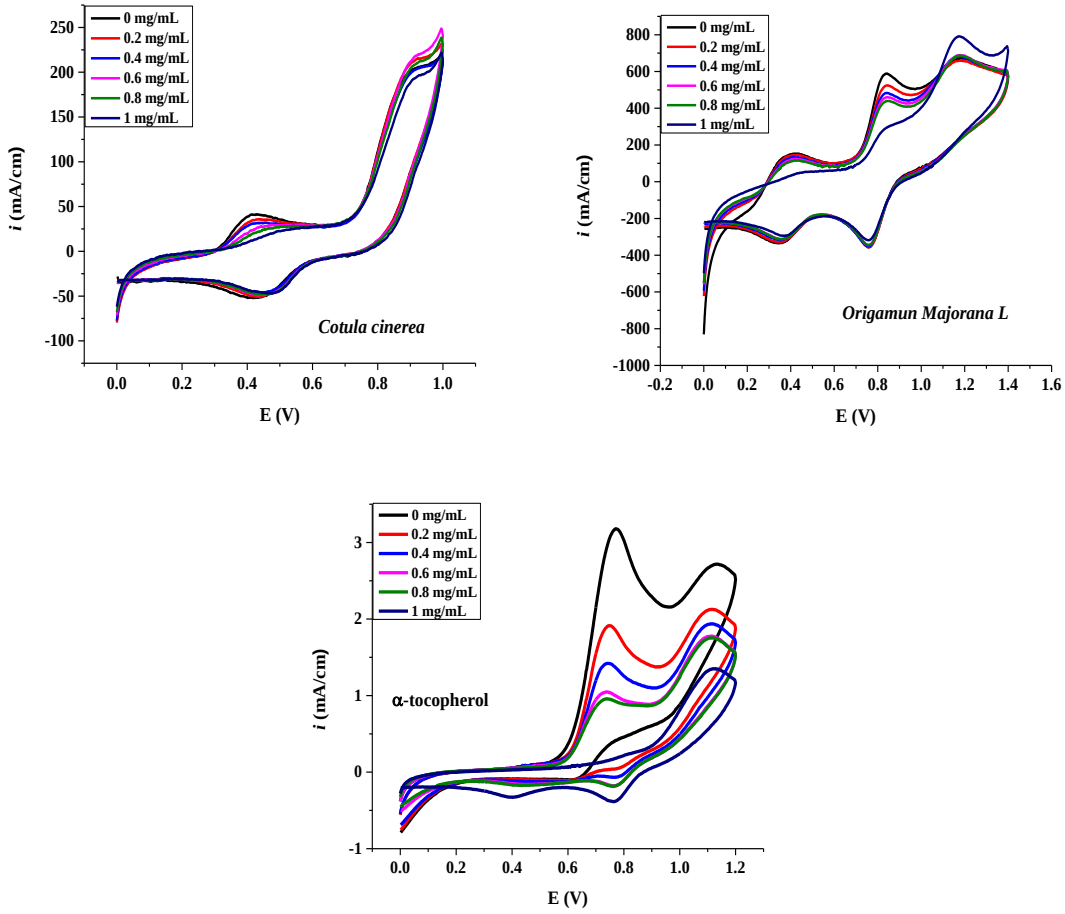


Figure 21. Cyclic voltammograms of 300 µM acetonitrile solution of DPPH in the absence and presenceof increasing concentration of *Cotulacinerea* and *Origanum Majorana L* essential oils and alpha-tocopherol, scan rate 100 mV/s, T = 28° C.

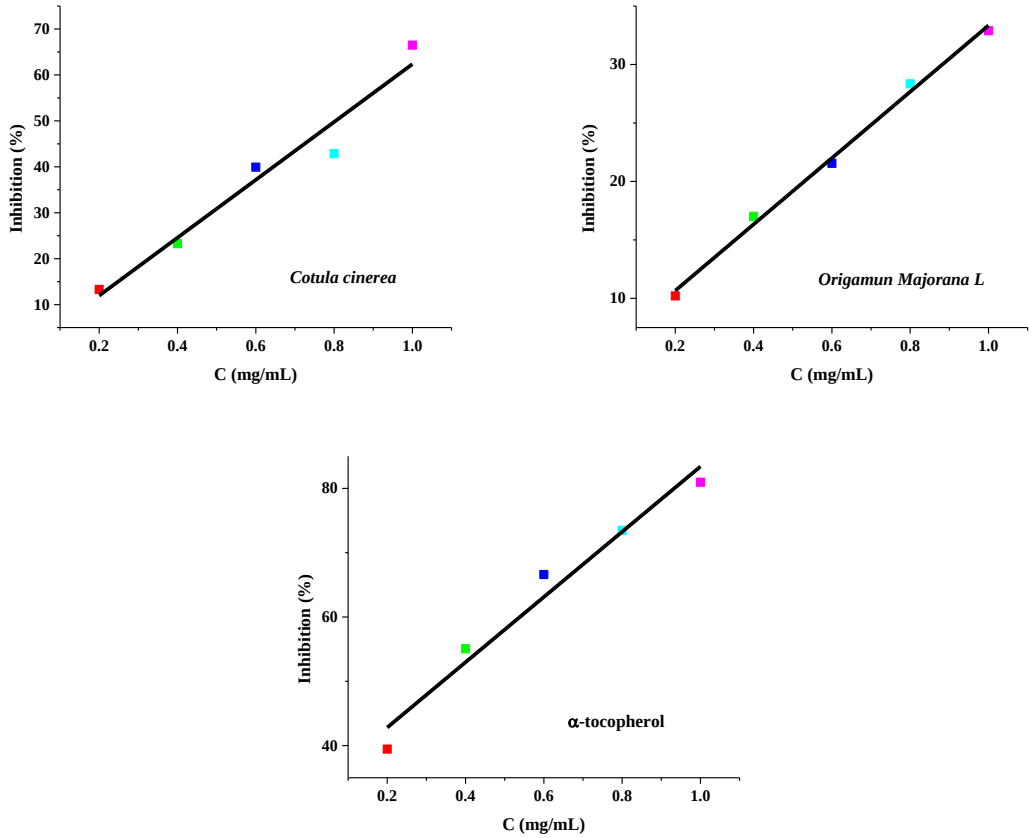


Figure 22. Plot of scavenging activities $[(i_0-i)/i_0]\times100$ versus sample concentrations for *Cotula cinerea* and *Origanum Majorana L* essential oils and alpha-tocopherol.

Table 9. Antioxidant efficiency values IC_{50} for *Cotula cinerea* and *Origanum Majorana L* essential oilsand the standardα-tocopherol obtained using cyclic voltammetry assays.

Entry	Equation	R^2	IC_{50} (mg/ml)
Cotulacinerea	y = 62.982x -0.623	0.939	0.804
Origanum Majorana L	y = 28.347x + 4.991	0.993	1.588
alpha-tocopherol	y = 50.717x + 32.67	0.957	0.341

Table 9 presents the IC_{50} values, indicative of antioxidant efficiency, for *Cotula cinerea* and *Origanum Majorana L* essential oils, as well as the standard α-tocopherol, derived from cyclic voltammetry assays. These IC_{50} values represent the concentration of the respective compounds required to scavenge 50% of the DPPH radicals present in the assay solution.

The equations provided alongside each entry in the table depict the linear regression models utilized to calculate the IC_{50} values. These equations are of the form y = mx + b, where 'y' represents the response variable (in this case, the reduction in DPPH radical concentration), 'x' represents the independent variable (the concentration of the antioxidant compound), 'm'

represents the slope of the regression line (indicative of the efficiency of the antioxidant), and 'b' represents the y-intercept. The coefficient of determination (R^2) associated with each equation indicates the goodness of fit of the regression model to the experimental data(Muselík *et al.*, 2007). A higher R^2 value (closer to 1) signifies a better fit of the model to the data, implying greater reliability in the derived IC_{50} value(Zou *et al.*, 2004).

The obtained IC_{50} values for *Cotula cinerea*, *Origanum Majorana L*, and α-tocopherol are 0.804 mg/ml, 1.588 mg/ml, and 0.341 mg/ml, respectively. These values reflect the potent antioxidant activities exhibited by these compounds, with lower IC_{50} values indicating higher antioxidant efficiency(Hsiang Wei Wang *et al.*, 2021).

Comparing the IC_{50} values among the tested compounds, it is evident that α-tocopherol demonstrates the most potent antioxidant activity, as reflected by its lowest IC_{50} value of 0.341 mg/ml. *Cotula cinerea* follows with an IC_{50} value of 0.804 mg/ml, while *Origanum Majorana L* exhibits relatively lower antioxidant efficiency with an IC_{50} value of 1.588 mg/ml.

Overall, the data presented in Table 9 underscore the significant antioxidant potential of the tested essential oils and α-tocopherol, as evidenced by their respective IC_{50} values. These findings highlight the importance of these compounds in mitigating oxidative stress-related damage and emphasize their potential utility in various biomedical and therapeutic applications.

4.1.2. Electrochemical DPPH radical interaction study:

In the presence of *Cotulacinerea* and *Origanum Majorana L* the anodic peak potentials of DPPH were shifted to more negative potentials. This shift was associated with a significant decrease in anodic peak current density. The significant drop in anodic peak current density can be assigned to the decrease in *Cotulacinerea* and *Origanum Majorana L* concentrations due to the formation of slowly diffusing EO-DPPH$^{\cdot}$complexes.

Typical CV behavior of DPPH in ACN/0.1M Bu_4NBF_4 in the potential window of 0.0 to -1.4 V at a glassy carbon electrode in the absence and presence of a solution of increasing concentration of essential oils in the same solvent as it shown in Figure 21. The decrease in the anodic peak current density, caused by the addition of *Cotula cinerea*or *Origanum Majorana L*, can be explained by the reaction of DPPH with the studied essential oils(Lanez *et al.*, 2018). This decrease can be used for the calculation of the binding constant, whereas the shift in peak potential values can be exploited for the determination of the mode of interaction (Lanez *et al.*, 2023; Kedadra *et al.*, 2022). Figure 21 further indicates that in the presence of *Cotulacinerea*and*Origanum Majorana L* the anodic peak potentials of all compounds were shifted to more negative potentials. This shift was associated with a significant decrease in anodic peak current density. The significant drop in anodic peak current density can be assigned

to the decrease in *Cotula cinereaOriganum Majorana L* concentrations due to the formation of slowly diffusing complexes.

- **Binding constant:**

The addition of different concentrations of *Cotula cinerea Origanum Majorana L* essential oils to a solution of ACNcontain 300 µM of DPPH provokes remarkable decrease in the peak current density, Figure 21. The substantial diminution in peak current density can be attributed to the decrease in free DPPH radical concentration due to the formation of DPPH-EO product.

The gradual decrease in peak current density by increasing *Cotulacinerea*or*Origanum Majorana L*essential oils concentrations can be exploited to calculate the binding constant by applying the following Equation 9(Boutarfaia *et al.*, 2019),

$$\log\frac{1}{C}=\log K_b+\log\frac{ip}{ip_0-ip} \quad (9)$$

where C is the essential oils concentration (mg/mL); $K_b$represents the binding constant (M^{-1}); ip_0 and ipdenote the anodic peak current density of the free and bound DPPH compound, respectively ($\mu A/cm^2$).

The plot of log $1/C$ versus $\log 1/1-(i/i_0)$ gave a straight line (Figure 23) with a 'y' intercept equal to the logarithm of binding constant K_b.

- **Binding free energy:**

The binding free energy change was calculated using the following Equation 10 (Atkins *et al.*, 2010)

$$\Delta G=-RT\ln K_b \quad (10)$$

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

Binding parameters of the studied essential oils are listed in Table 10.

Table 10. Binding constants and binding free energies values for EOs and α-tocopherol with DPPH from CV data

Adduct	Equation	R^2	K (M^{-1})	-ΔG ($KJ.mol^{-1}$)
***Cotulacinerea*_DPPH**	y = 0.631x + 2.636	0.905	433.17	15.05
Origanum Majorana L_DPPH	y = 1.101x + 2.197	0.991	157.73	12.55
α-tocopherol_DPPH	y = 0.866x + 3.138	0.979	1374.13	17.91

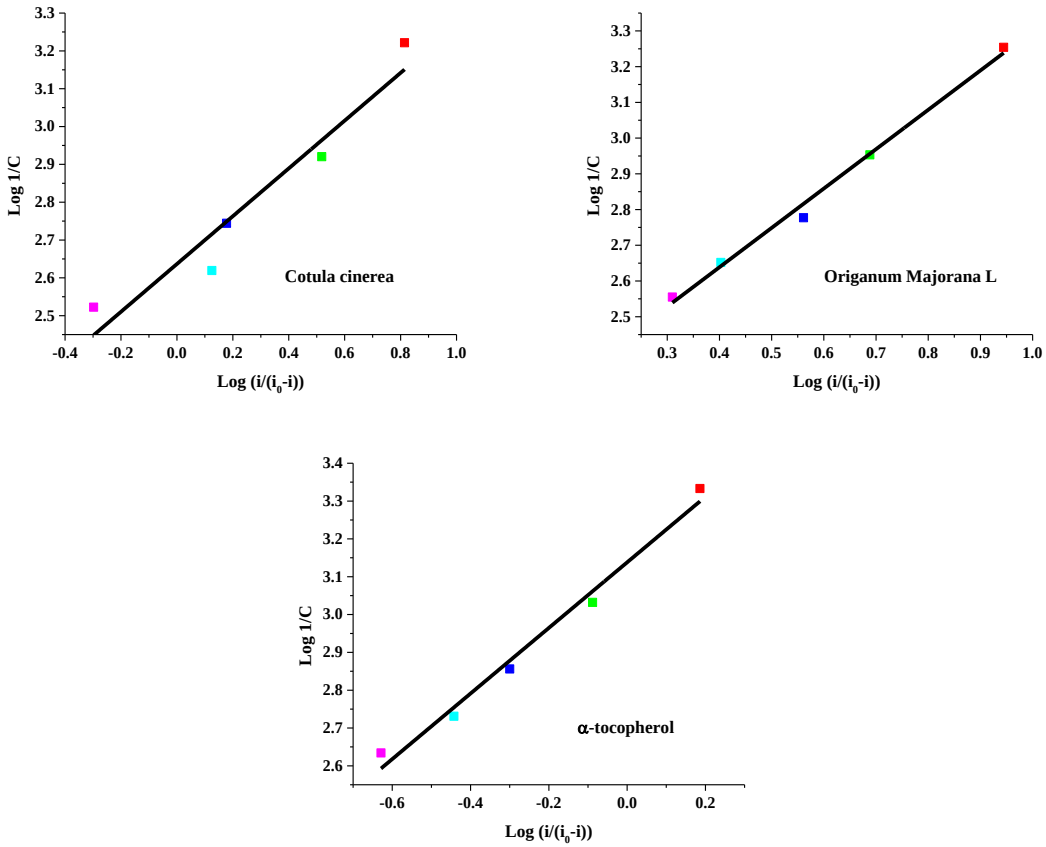


Figure 23. Plots of $\boldsymbol{log\left(1/1-(i_p/i_{p_0})\right)}$ versus $\boldsymbol{log\,1/C}$ used to calculate the binding constants of studied essential oil with DPPH.

The Table 10 delineates the binding constants and binding free energy values derived from cyclic voltammetry (CV) data, representing the interactions between essential oils (EOs) from *Cotula cinerea* and *Origanum Majorana L*, as well as alpha-tocopherol, with DPPH free radicals.Each adduct is associated with a linear regression equation depicting the relationship between the concentration of the antioxidant compound and the corresponding response observed in the CV data. The coefficient of determination (R^2) values, ranging from 0.905 to 0.991, affirm the robustness of the linear regression models, signifying a satisfactory fit of the experimental data(Milardović *et al.*, 2006).

The binding constant (K) delineates the equilibrium constant governing the formation of the adduct between the antioxidant compound and DPPH radicals, thereby quantifying the strength of their interaction.Notably, alpha-tocopherol exhibits the highest binding constant (1374.13 M^{-1}), indicative of its pronounced affinity towards DPPH radicals. Conversely, *Cotula cinerea* and *Origanum Majorana L* essential oils manifest comparatively lower binding constants (433.17 M^{-1} and 157.73 M^{-1}, respectively), albeit still demonstrating significant interactions with DPPH radicals.

The binding free energy (ΔG) elucidates the thermodynamic favorability of the interaction between the antioxidant compound and DPPH radicals, with more negative values denoting heightened spontaneity and favorability of the binding process(Laraoui *et al.*, 2023).

Alpha-tocopherol emerges with the most negative free binding energy (-17.91 kJ·mol⁻¹), underscoring the thermodynamically favorable nature of its interaction with DPPH radicals. *Cotulacinerea* and *Origanum Majorana L* essential oils also exhibit negative free binding energies, indicative of favorable interactions, albeit marginally less pronounced than alpha-tocopherol.

4.2. Electronic Spectroscopy interaction study:

4.2.1. 2,2-diphenyl-1-picrylhydrazyl scavenging activity (IC50):

The independence of absorbance upon addition of gradually concentration of *Cotulacinerea* and *Origanum Majorana L* essential oils to DPPH is presented in Figure 24, the figure clearly shows the decrease of the absorbance following the addition of the studied compounds.

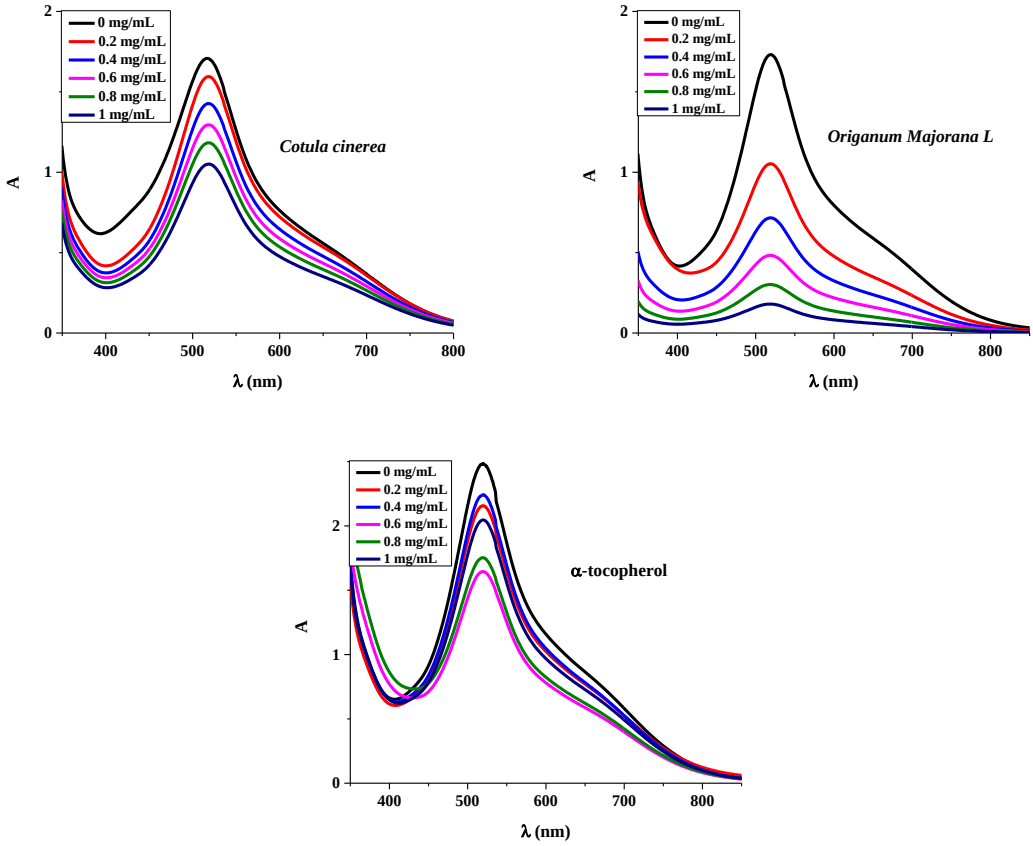


Figure 24. UV-visible absorption spectra of DPPH in presence of increasing concentrations of *Cotula cinerea*, *Origanum Majorana L* essential oilsand α-tocopherol in Acetonitrile at 298K

The Figure 24 shows a series of absorption spectra of the DPPH radical at different concentrations of *Cotulacinerea*, *Origanum Majorana L* essential oils, and α-tocopherol.

Observing drop in absorbance peaks and changes in peak intensity can indicate the extent of interaction between the DPPH radical and the compounds being tested.

The absorbance results obtained reveal a proportional correlation between absorbance and increasing concentrations of the compounds studied, suggesting an inhibitory activity of DPPH.

The ability of the studied essential oils to quench DPPH radicals(% Inhibition of DPPH) was calculated using the previously cited Equation 7(Rebiai *et al.*, 2014).

The effective concentration (IC_{50}) values were obtainedfrom the linear regression of the percentages of DPPH radical scavenging activity against differentcompound concentrations, Figure 25.

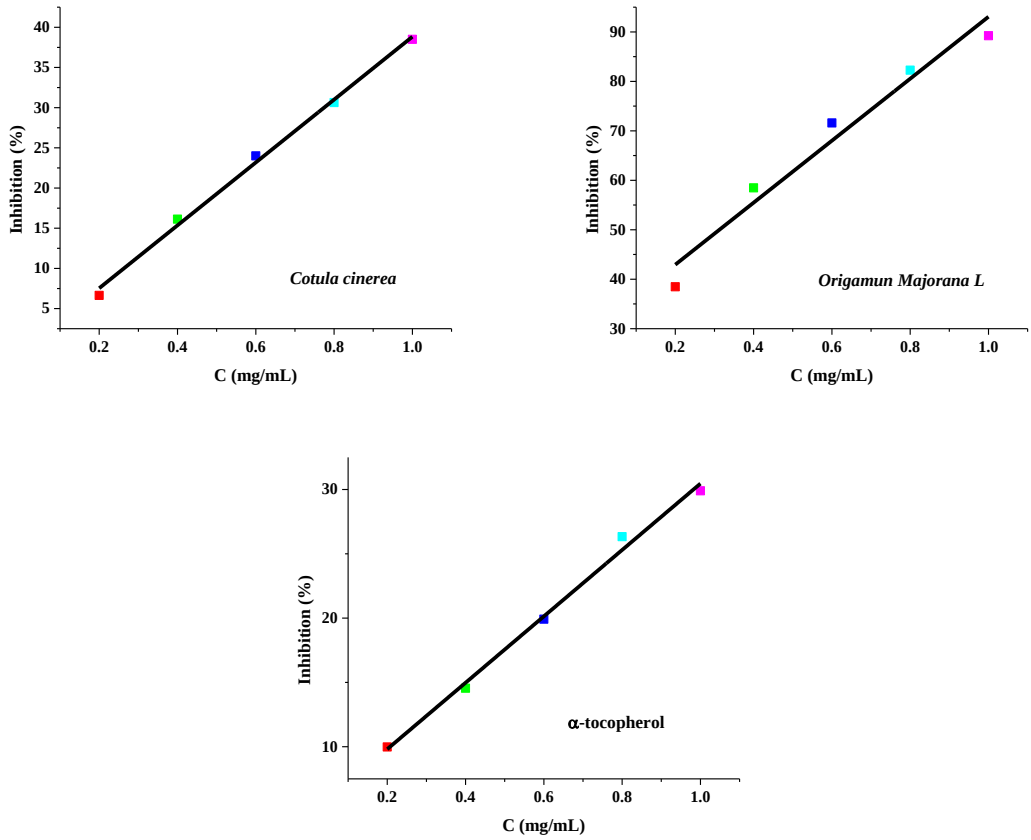


Figure 25. Linear regression of the inhibition of DPPH activity by the essential oils.

The equations obtained from the linear calibration graph in the studied concentrationrange for *Cotulacinerea* and *Origanum Majorana L*essential oils and α-tocopherol used as a positive control aresummarized in Table 11 (where y represents the value of the absorbance ofDPPH radical and x, the value of samples concentration, expressed in mg/mL).

Table 11. Antioxidant efficiency values IC_{50} for *Cotula cinerea* and *Origanum Majorana L* essential oilsand the standardα-tocopherol obtained using electronic spectroscopic assays.

Entry	Equation	R^2	IC_{50} (mg/ml)
Cotulacinerea	y = 39.108x - 0.285	0.994	1.2857
Origanum Majorana L	y = 62.644x + 30.42	0.951	0.3125
alpha-tocopherol	y = 25.811x + 4.649	0.991	1.7569

The Table 11 presents the antioxidant efficiency values, specifically the IC_{50} values, for *Cotulacinerea* and *Origanum Majorana L* essential oils, as well as the standard α-tocopherol, obtained through electronic spectroscopic assays.The R^2 value indicates the goodness of fit of the regression model to the data points. Higher R^2 values, closer to 1, suggest better agreement between the experimental and predicted values(Pisoschi *et al.*, 2009). All three compounds demonstrate relatively high R^2 values (*Cotulacinerea*: 0.994, *Origanum Majorana L*: 0.951, α-tocopherol: 0.991), suggesting robust relationships between concentration and antioxidant efficiency.

The IC_{50} values represent the concentration of each compound required to scavenge 50% of the DPPH radicals in the assay. Lower IC_{50} values indicate higher antioxidant efficiency, as it implies that a lower concentration of the compound is needed to achieve the desired antioxidant effect(Anigboro *et al.*, 2021).In this context, *Origanum Majorana L* essential oil exhibits the lowest IC_{50} value (0.3125 mg/ml), indicating superior antioxidant potency compared to *Cotulacinerea* essential oil (1.2857 mg/ml) and α-tocopherol (1.7569 mg/ml).Compounds with lower IC50 values, such as *Origanum Majorana L* essential oil, may offer enhanced protection against oxidative stress-related ailments. Understanding their antioxidant potency can inform the development of antioxidant-rich products and therapies aimed at combating various diseases associated with oxidative damage.

4.2.2. Electronic spectroscopicDPPH radical interaction study:

- **Binding constant:**

The change in absorbance values by increasing *Cotulacinerea* and *Origanum Majorana L* essential oils concentration was used to evaluate the intrinsic binding constant employing the following Equation 11, (Khennoufa *et al.*, 2021; Lanez *et al.*, 2018; Lanez *et al.*, 2023).

$$\frac{A_0}{A-A_0} = \frac{\varepsilon}{\varepsilon_0-\varepsilon} + \frac{\varepsilon}{\varepsilon_0-\varepsilon}\frac{1}{K_b \times C} \quad (11)$$

where C is the *Cotulacinerea* and *Origanum Majorana L* essential oils concentration, K_b is the binding constant, $A_0$and A are respectively the absorbance of DPPH in the absence and presence of essential oils, and ε_G and ε_{H-G} are their extinction coefficient respectively.

The constant K_b is obtained from the intercept to slope ratio of the plot of $A_0/(A-A_0)$ versus$1/C$, Figure 26.

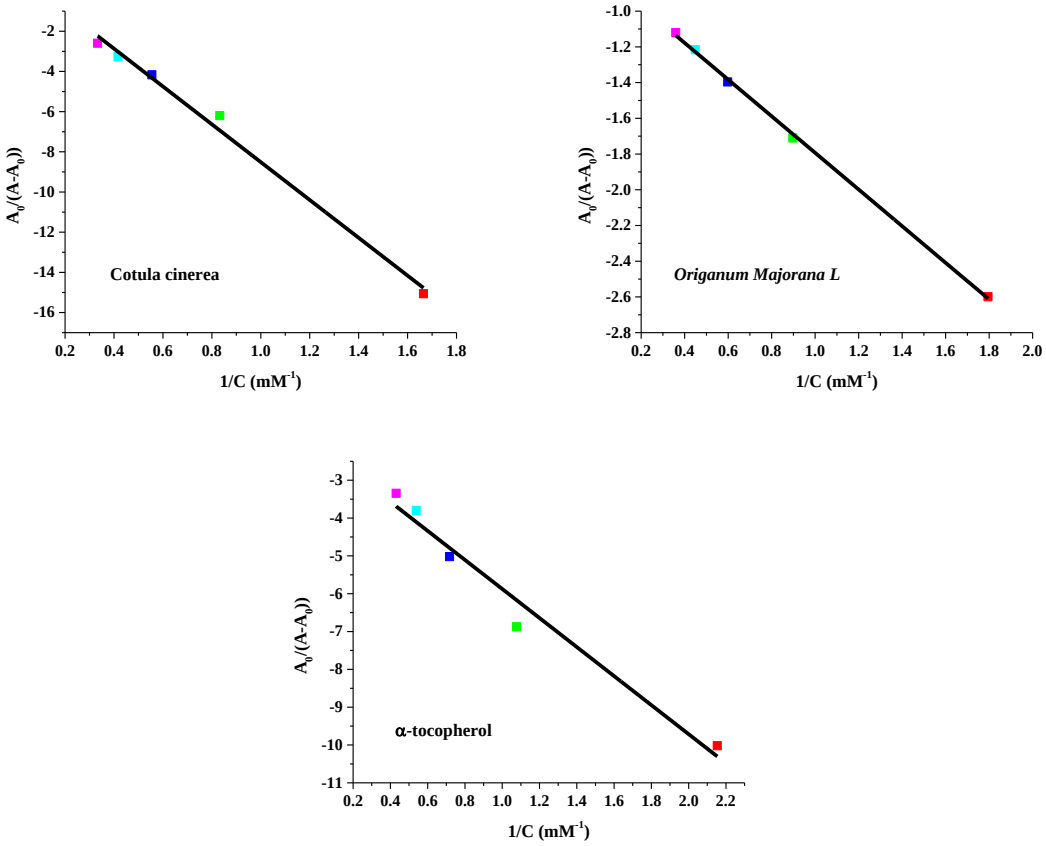


Figure 26. Plots of $\boldsymbol{A_0/(A-A_0)}$ versus $\boldsymbol{1/C}$ used to calculate the binding constants of *Cotulacinerea* and *Origanum Majorana L* essential oilswith DPPH.

- **Binding free energy:**

The binding free energy change was calculated using the following Equation 12(W., 1998)

$$\Delta G = -RT \ln K_b \quad (12)$$

where ΔG is the binding free energy in KJ.mol^{-1}, R is the gas constant, 8.32 J.mol^{-1}K^{-1} and T is the absolute temperature, 298K. Binding parameters are listed in Table 12.

Table 12. Binding constant and binding free energy values for *Cotulacinerea* and *Origanum Majorana L* essential oilswith DPPH from UV data.

Adduct	Equation	R^2	K (M^{-1})	-ΔG (KJ.mol^{-1})
Cotulacinerea_DPPH	y = -9.413x - 0.899	0.989	95.56	11.31
OriganumMajorana_DPPH	y = -1.025x -0.767	0.998	748.26	16.41
α-tocopherol_DPPH	y = -3.838x -2.035	0.962	530.31	15.55

The binding constant (K) represents the strength of interaction between the compounds (essential oils or α-tocopherol) and DPPH(Lanez *et al.*, 2016). Higher K values indicate stronger binding affinity between the compound and DPPH(Adisa *et al.*, 2011). *Origanum Majorana L*

essential oil exhibits the highest binding constant (748.26 M^{-1}), followed by α-tocopherol (530.31 M^{-1}), and *Cotulacinerea* essential oil (95.56 M^{-1}). This suggests that *Origanum Majorana L* essential oil has the strongest binding affinity for DPPH among the compounds tested.

The binding free energy (ΔG) represents the thermodynamic driving force for the formation of the compound-DPPH adduct(Toan *et al.*, 2022). More negative ΔG values indicate a more favorable binding interaction(Mouada *et al.*, 2019). *Origanum Majorana L* essential oil also demonstrates the most negative ΔG value (-16.41 $KJ.mol^{-1}$), followed by α-tocopherol (-15.55 $KJ.mol^{-1}$), and *Cotulacinerea* essential oil (-11.31 $KJ.mol^{-1}$). This further supports the notion that *Origanum Majorana L* essential oil has the strongest binding interaction with DPPH.

5. In-Silico analysis:

5.1. ADMET and drug-likeness prediction:

Modern drug discovery involves assessment of competence of the dynamic molecules and their strength to reach target site in bioactive form, which involves cellular, animal and human clinical trials which are highly priced and encumbered with risks (Ranjith D *et al.*, 2019; Ranjith *et al.*, 2019). Presently computer aided drug development encouraged the estimate of absorption, distribution, metabolism and excretion of drugs (ADME), they postulate anticipatory and dependable data very quickly and compliment for experimental approaches (Ranjith *et al.*, 2019; Sliwoski *et al.*, 2014). It has been determined that the initial appraisal of ADME properties in the discovery period diminishes remarkably the fraction of pharmacokinetics related failures in the clinical phase (Ranjith *et al.*, 2019; Hay *et al.*, 2014).

In the present study we evaluated the ADME properties of the major compounds (more than 10%) present in both essential oils using SwissADME web tool. A total of 4 potent phytoconstituents were analysed to study general characteristics (Table 13), Physicochemical properties (Table 14), lipophilicity and water solubility characteristics (Table 15&16), pharmacokinetic parameters (Table 17), drug likeness rule and bioavailability score (Table 18) and medicinal chemistry properties (Table 19), respectively. General characteristics of the studied compounds revealed all the compounds having molecular weight less than 500 Da, which is a good prime property to be called as drug likeness of the small molecules.

Table 73. 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 84. 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 ($Å^2$)	0.00	17.07	9.23	26.30

Table 95. 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 106. 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 117. 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 128. 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

Table 139. 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

Lipophilicity property of the compounds portrays an important role for molecular discovery activities in multifarious domains. The quantitative descriptor of the lipophilicity is the partition coefficient P of a given molecule between *n*-octanol and water system (Daina *et al.*, 2014). Because of its amphiphilic nature, *n*-octanol is considered a good mimic of phospholipid membrane characteristics (Liu *et al.*, 2011). Multifarious algorithms are accessible to compute log Po/w, which rely on factual methodologies. The classic log P predictors branched in to two division, first ones split molecular structures into molecular fragments includes fragmental approach e.g. KLOGP (Klopman *et al.*, 1993), KOWWNIN (Meylan *et al.*, 2000) or atomicapproach e.g. ALOGP (Ghose *et al.*, 1998; Wang *et al.*, 1997), XLOGP (Moriguchi *et al.*, 1994; Cheng *et al.*, 2007). The second division gathers the topological methods in which, the molecules description is related to its topology being as count or flags for specific atoms, groups or structural properties e.g. MLOGP (Moriguchi *et al.*, 1992; Brenk *et al.*, 2008), the prediction attained by manifold linear regression trained on large molecular data sets. The SILICOS-IT is a hybrid technique which combines both molecular fragments and topological parameters, which confide on 27 fragments and 7 topological descriptors, it was disciplined on 23,455 molecules with experimental *n*-octanol/water partition values (Daina *et al.*, 2014). The version three of the XLOGP atomic model is established on a system of 87 fragments and two corrective factors. If the input structures are similar to a reference compound, the fragments differentiating them are treated and the corresponding log P contributions added to the reference structure log P value (Cheng *et al.*, 2007). Lipophilicity estimated as consensus Log P(Mouada *et al.*, 2019), which is the average value of all Log P evaluated with various lipophilicity criteria, determined acarbose as most lipophilic whereas santolina triene as least lipophilic and water solubility of the small molecules ranged from highly water soluble to water soluble.

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

The pharmacokinetics and drug likeness performed using SwissADME showed a low level of GI absorption and BBB permeant with santolina triene and cis-Verbenyl acetate while a high absorption detected with trans-Thujone and 1,8-Cineole. All the compounds present in the essential oils are not the substrates for P-gp (Table 15), so they are not susceptible to the efflux mechanism performed by this transporter which is used by many tumours cell lines as a drug-resistance mechanism(Ranjith *et al.*, 2019)

All of the small molecules returned as non-inhibitors for inactivation for CYP isoenzymes. The skin permeability coefficient (Log Kp), a multiple linear regression, the more negative the log Kp (with Kp in cm/s), the less skin permeant is the molecule. Among the phytoconstituents,

trans-Thujone (-5.62) is the least permeant andsantolina triene (-4.13) is highly permeant respectively. This SwissADME section gives access to five different rule-based filters, with diverse ranges of properties inside of which the molecule is defined as drug-like. The Lipinski (Pfizer) filter is the pioneer rule-of-five implemented and with the Ghose (Amgen), Veber (GSK), Egan (Pharmacia) and Muegge (Bayer) methods. Multiple estimations allow consensus views or selection of methods best fitting the end-user's specific needs in terms of chemical space or project-related demands. Any violation of any rule described here appears explicitly in the output panel. All the four compounds followed the filtered rule invoked in the Swiss ADME; the violation shown by the molecules are minimal.

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

In silico toxicity study aims to help in optimizing compounds regarding their toxicity proprieties. The study could offer an important improvement to the awareness of the full perspective of virtual screening for the identification of target compounds with negligible or no toxicity, which may open a path for the selection of novel nontoxic phytoconstituents present in *Cotula cinerea* and *Origanum Majorana L* essential oilswith high antidiabetic activity. In silico toxicity study of the chosen compounds was performed using the ProTox-II web server (Drwal *et al.*, 2014). It aims to predict hepatotoxicity (Dili), carcinogenicity (Carcino), immunotoxicity (Immuno), mutagenicity (Mutagen), cytotoxicity (Cyto), median lethal dose (LD50), and toxicity class (TC).

According to in silico toxicity profiles presented in Table 20, the toxicity class of all the phytoconstituents was detected to be equal to 5 except the trans-Thujone which predicted to be 4. Santolina triene, trans-Thujone**,** 1,8-Cineole and cis-Verbenyl acetatewere predicted to be nontoxic except in immunotoxicity for the last wrote compound.

Table 20. In silico toxicity profiles of the studied compounds.

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 interaction of all the major compounds presented in *Cotula cinerea* and *Origanum Majorana L* essential oils compounds with human glutathione reductase was further investigated by carrying out a molecular docking analysis of the binding interaction between human glutathione reductase with compounds: 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 **(**the major phytochemicals present in the two plants with yield > 1) using Maestro version 11.7 user interface of the Schrödinger suite (Small-Molecule Drug Discovery Suite 2021-4, Schrödinger, LLC, New York, NY, 2021) (Schrödinger, 2015).

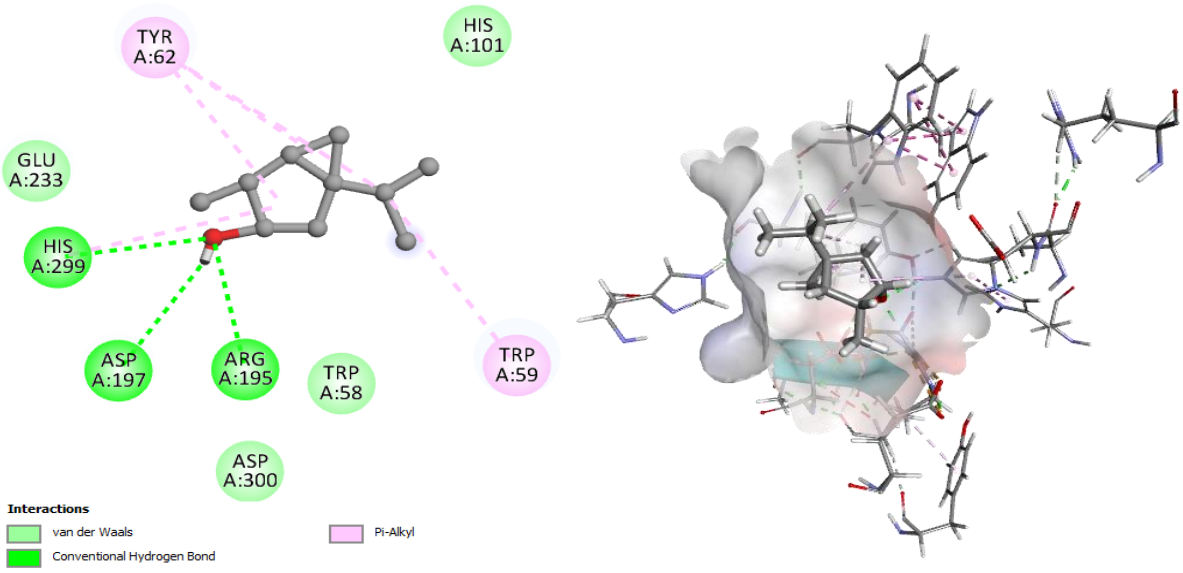
Each compound was docked with Human glutathione reductase (GR) to understand their interactions at the enzyme's active site. Of all the major compounds present in *Cotulacinerea* and *Origanum Majorana L* essential oils, the binding affinities of them for glutathione reductase were comparable (Table 19). This result indicates that the plant is a rich source of compounds which, either individually or synergistically, resulted in the decrease of human glutathione reductase activity as observed in the *in vitro* experimental study. Also, the interactions were spontaneous as observed from the negative Gibb's free energy values (Avwioroko *et al.*, 2020).

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

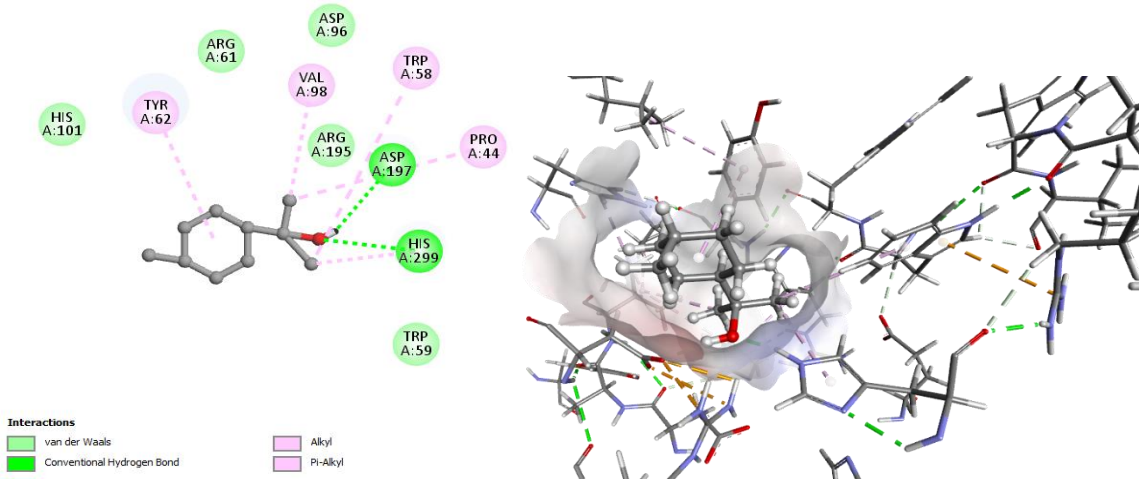
Table 21. Docking score of the studied compounds.

Compound	Rigid Docking Score (Kcal/mol)	IFD scores (Kcal/mol)
trans thujone	-5.569	-6.679
alpha-Terpineol	-5.052	-6.162
beta-Terpineol	-5.033	-6.143
cis Verbenyl acetate	-4.944	-6.054
Camphor	-4.505	-5.615
Sabinene	-4.365	-5.475
gamma terpinene	-4.185	-5.295
alpha-Pinene	-3.884	-4.994
1,8-Cineole	-3.706	-4.816
Santolina triene	-2.986	-4.096
alpha-Thujene	-4.41	-5.522
Beta Pinene oxide	-4.115	-5.225

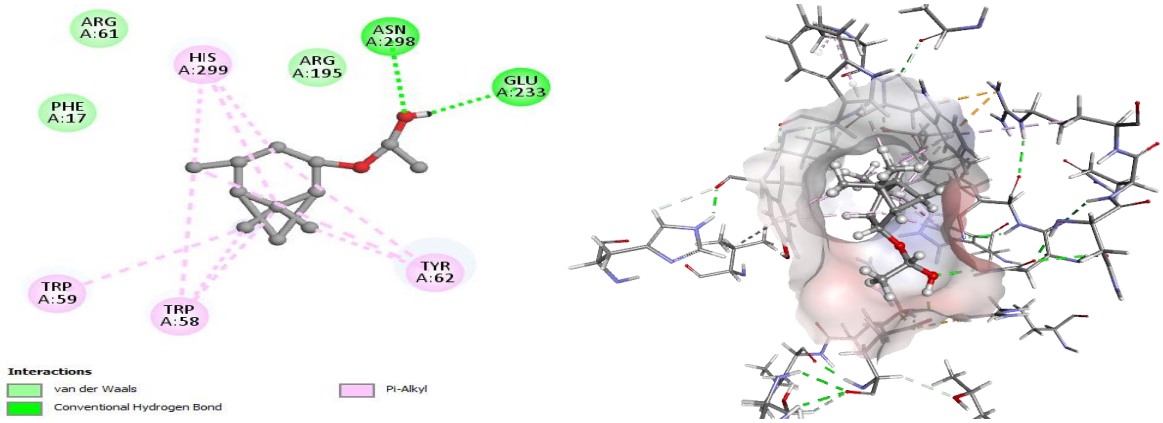
The results in Figure 23 revealed that the best two compounds in each plant interacted with a similar set of amino acid residues at the active site of the protein. It is noticed in their interaction with the enzyme's key catalytic residues (ASP-197, GLU-233 and ASP-300). compoundtrans thujoneshowed interaction with the three key amino acids involved in catalysis at GR active site, whereas alpha-Terpineol showed interaction with two of the catalytic triads (GLU-197 and ASP-197). It is quite surprising to note that cis Verbenyl acetateshow interaction with only one of the three key glutathione reductase catalytic residues even though it had the second highest binding affinity to glutathione reductase compared to the other compounds. This perhaps denotes that the inhibitory effect contributed by cis Verbenyl acetateto the overall inhibitory effect of the other compounds may be that of non-competitive inhibition whereas that of other compounds includingtrans thujone(the compound with the best binding affinity) may be that of competitive mode. Overall, the *in-silico* analysis of the ligand protein interaction of these compounds with amino acid residues present in the glutathione reductase active site domain (such as TRP-58, TRP-59, TYR-62, GLN-63, ASP-197, GLU-233, ASP-300 etc.) gave credence to the inhibitory effect of the compounds reported in this study. Furthermore, it also confirmed the possibility of the binding interaction between glutathione reductase and the phytoconstituents of *Cotulacinerea* and *Origanum Majorana L* essential oilsinvestigated and observed using spectroscopic methods reported. The best ligand-binding poses in the catalytic domain of HPA obtained after docking as well as the amino acid residues involved in the interaction are as illustrated in Figure 23.



Docking complex and interaction plot for compound trans- thujone with GR



Docking complex and interaction plot for compound alpha-Terpineolwith GR



Docking complex and interaction plot for compound cis Verbenyl acetatewith GR

Figure 27. Molecular interactions of studied compounds & Acarbose with humane pancreatic α-amylase.

5.3. Molecular Dynamics Simulation:

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

The Root Mean Square Deviation (RMSD) values for the complex consistently fall within the acceptable range of 0 – 2 Å. Examination of the analysed complex revealed no significant conformational alterations; however, an initial minor deviation was discerned in the vicinity of 8 – 20 nanoseconds, and a subtle drift was observed approximately between 45 – 55 nanoseconds. Beyond these periods, the complex demonstrated marked stability throughout the entirety of the simulation process, with no notable occurrence of major conformational changes. (Figure 24).

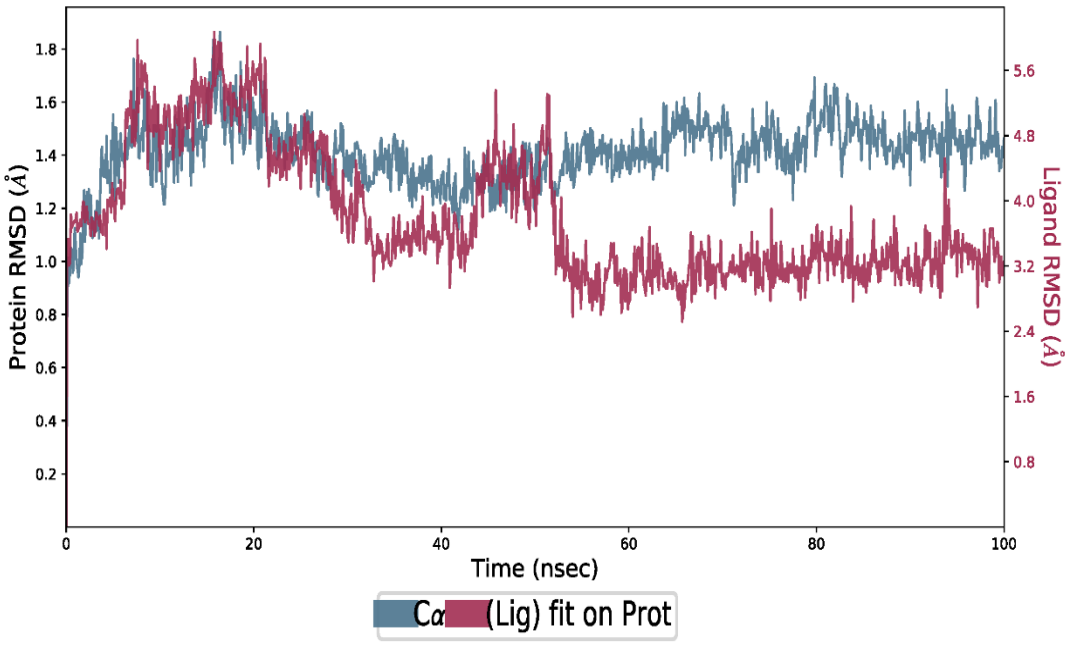


Figure 28. RMSD plot of trans- thujone and glutathione reductase complex.

The Root Mean Square Fluctuation (RMSF) analysis provides insights into local variations along the protein chain, facilitating the identification of residues contributing to structural

fluctuations within the complex(Dey *et al.*, 2021). In the examined complex, the observed fluctuation variation remained below 3 Å. Major fluctuations (2.20-2.63 and 1.81-2.96Å) were observed in the C-terminal and loopregions (223-225 and 363-366), respectively, which are positioned awayfrom the binding pocket of glutathione reductase. Except for the loop regions, the RMSFvalues of most residues are less than 1.5 Å, indicating that theresidue conformation is relatively stable during the simulation.A graphical representation of the RMSF plot depicting the specific ligand interactions with amino acid residues in the protein is presented in Figure 25.

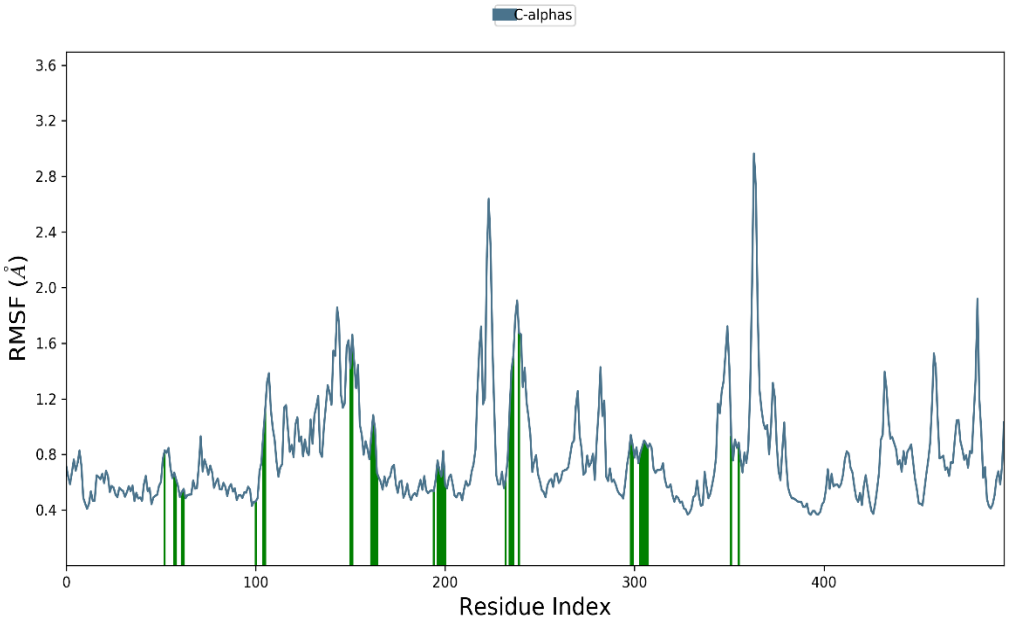


Figure 29. RMSF plot of trans- thujone and glutathione reductase complex.

Within the investigated complex, notable hydrogen bond interactions were observed involvingHIS299, ASP197, and TRP58. Additionally, water-mediated hydrogen bond interactions were formed, encompassing the same residues, as visually depicted in Figure 26.

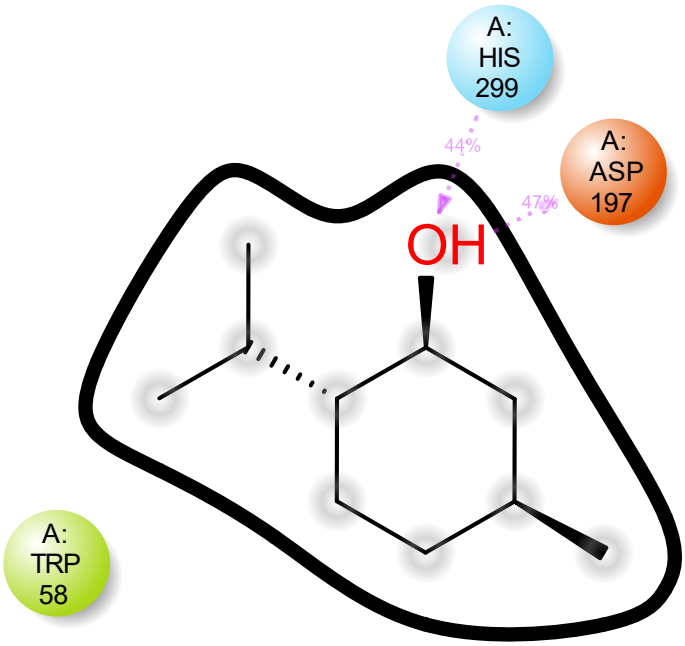


Figure 30. Interaction diagram of protein-ligand after MDS.

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

In the context of the investigated complex, the interaction values for the residues GLU240, HIS201, ASP197, GLN63, GLU233, and THR163 are recorded as 1.25, 1, 1.75, 1, 0.60, and 1.5, respectively. These values signify varying degrees of sustained interactions between the ligand and corresponding amino acid residues. The graphical representation of the protein-ligand contact histograms for the complex is depicted in Figure 27 for visual elucidation.

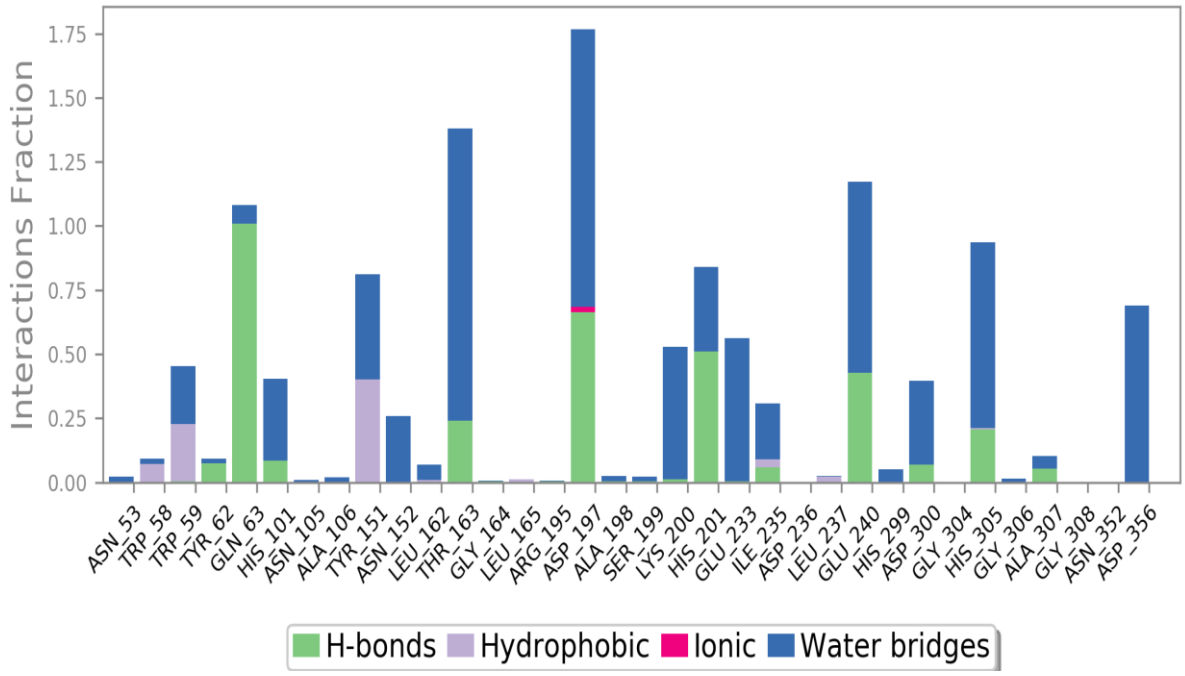


Figure 31. Histogram of protein-ligand complex.

To comprehensively investigate the interactions between the ligand and human glutathione reductase receptor, two supplementary panels were introduced by Schrödinger post Molecular Dynamics Simulation (MDS), as depicted in Figure 28. The upper panel provides insights into the total number of specific contacts established by the protein with the ligand throughout the trajectory. Conversely, the lower panel delineates the residues engaged in interactions with the ligand in each trajectory frame.

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

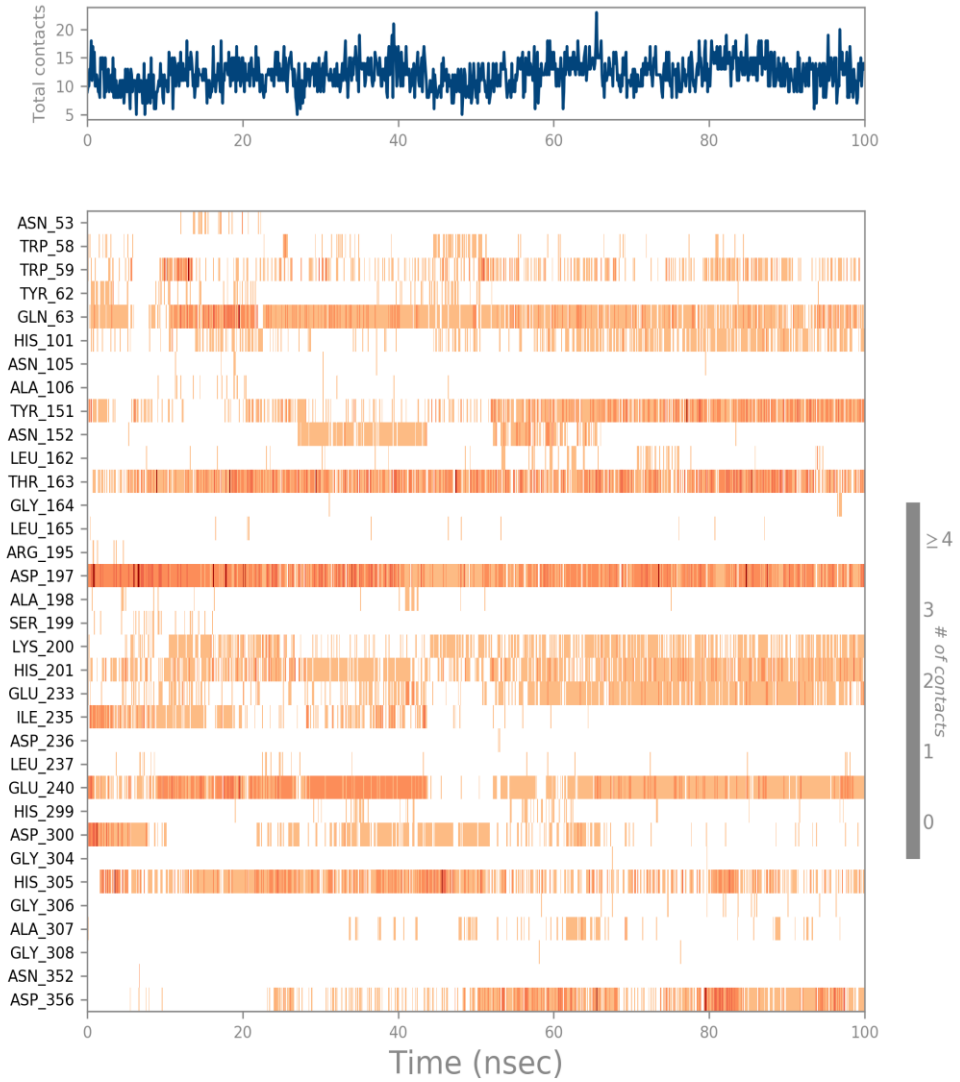


Figure 32. Details of the protein ligand contact.

6.4. Free Energy (MM-GBSA) Calculation:

To evaluate the binding efficacy of the preeminent compound, trans- thujone, with theglutathione reductase protein, the Prime-MM-GBSA method was applied to compute the binding free energies. The establishment of stable complexes is evidenced by favourable binding interactions between the compound and the protein. The determination of binding free energy entails the consideration of various energy components, encompassing van der Waals (vdW), lipophilic (lipo), Generalized Born electrostatic solvation (Solv GB), Coulomb, and hydrogen-bonding (hbond) energies. The discrete contributions of these energy components to the overall binding free energy of the trans- thujone-alpha-amylase complex are delineated in Table 20.

Table 22. Energy components of the studied complex.

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

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

CONCLUSION

Conclusion:

ELOUED area is flourishing with many plant species that are frequently used for medical and therapeutic purposes. At present, despite the development, interest in medicinal plants has increased over time, and most people tend to use it as a substitute for preparations of chemical origin. Basic oils have occupied their place and are of great importance in many areas (such as medicine, chemistry, cosmetics and perfume), making them increasingly interesting. Through this platform, it has become a commitment to follow up on this growing interest in medicinal plants and to deepen the most important aspects of medical science with a view to finding an alternative and completely eliminating chemicals and the portfolio used in its food industry.

On this subject, we highlighted two plants,***cotula cinerea*** *and* ***OriganumMajorana L***. The results of our study are as follows:

At the beginning, we extracted base oil for the plants, so we noticed that the yield rate for the base oil in the marijuana plant is (1.45%)higher than that of the ***cotula cinerea***plan (%0 .90) It has also been noted that there is a great similarity in the natural properties of the essential oils of the plants, both of which appear to be yellow. We have calculated the physicochemical properties of the essential oils and the tail on what is dependent on A.F.N.O.R., both the relative density, acidity, the aster presumption and the refrigeration index. For the qualitative and quantitative analysis of the underlying oils derived from both plants by the gas chromatography associated with the mass spectrometer (GC/MS) and gave the following results:

cotula cinerea plant gave 31 compounds, most of which were ketonate, where the analysis revealed 10 compounds at a concentration greater than 1%, with two dominant compounds appearing:

trans thujone at 51.86% and 10.69% for the santalinatriène.

As for the ***Origanum Majorana L*** plant, about 42 compounds have been identified, most of which are also kitonets. One of its dominant compounds is

trans thujone (33.3%) and (10,40%) of the santalinatriène.

As for the oxidation effectiveness of the essential oils of the plants, it was done in two ways: we found that there was oil efficiency, but it was considered to be weak compared to the efficacy

of vitamin E, where its path of inhibition with DPPH was the IC_{50} values for the gravity plant and the mesh 0.804mg/mlu 1.588 mg/ml respectively.

And the voltage method didn't differ from the DPPH method where we found the same order of oil in the anti-oxidant capacity.

By comparing IC_{50} values between tested compounds, it is clear that alpha-tocofirol (Vitamin E) shows the most robust antioxidant activity.

Expectations:

We look forward in the future to a deeper and more comprehensive study of these plants:
- Use other advanced methods in the extraction process, such as CO_2 and microwind. Using other techniques in chemical analysis, such as... GC - FTR, LC - MS, RM)
- Deepening biological study in anti-microbial and bacterial capacity.

-Retrieval ofmetal compounds and flafonics for these plants
- Study of the anti-corrosive potential of the extracts of these oils

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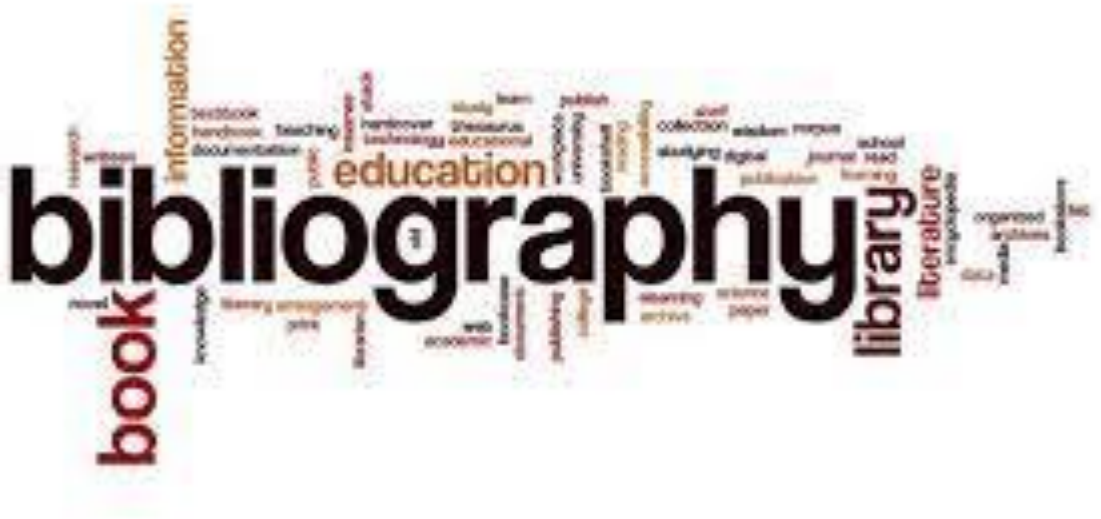
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