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Memoir

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اهداء

"بسم الله خالقي وميسر اموري وعصمة امري لك كل الحمد والامتنان

من قال انا لها " نالها "

لم تكن الرحلة قصيرة ولا ينبغي لها ان تكون لم يكن الحلم قريبا ولا الطريق محفوفا بالتسهيلات لكني فعلتها ونلتها.

الى من كلله الله بالهيبة والوقار.. الى من احمل اسمه بكل فخر.. الى من حصد الاشواك عن دربي ليمهد لي طريق العلم بعد فضل الله ما انا فيه يعود الى- ابي- الرجل الذي سعى طوال حياته لكي نكون أفضل منه

– ابي الغالي –

الى ملاكي في الحياة الى معنى الحب والى معنى الحنان والتفاني.. الى بسمة الحياة وسر الوجود الى من كان دعاؤها سر نجاحي وحنانها بلسم جراحي التي كانت لي الام والاخت والصديقة داعمي الأول ووجهتي التي استمد منها القوة.

–امي الحبيبة –

الى مصدر قوتي الداعمين الساندين ارضي الصلبة وجداري المتين الى من وهنو على نجاتي.. ويذكروني بمدى قوتي واستطاعتي اللذين لا يحبطوني ويؤمنوا بشجاعتي مهما ضعفت وارتخيت واقفين خلفي.. مثل ظلا مهما كثرة تخبطاتي من بذلوا جهدا في مساعدتي وكانوا عونا وسندا الى الشموع التي تنير طريقي

-اخواني واخواتي – امينة– مخطارية–زهرة– منال -

ولا أنسي رفقاء الروح واللذين شاركوني خطوات هذا الطريق الى من هونوا تعب الطريق الى من شجعوني على المثابرة لإتمام مسيرتي الى رفقاء السنين ممتنة لكم كلكم باسمه

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الى سندي ومسندي صديقتي واختي ...الى من شاركتني خطوات هذا الطريق بكل عقباته ونجاحاته.. خيرة ايامي وصفوتها زميلتي في هذا العمل غاليتي

-ايمان-

لله الشكر كله ان وفقني لهذه اللحظة فالحمد لله رب العالمين والصلاة والسلام على نبيه الكريم

اهداء

"بسم الله خالقي وميسر اموري وعصمة امري لك كل الحمد والامتنان "

من قال انا لها " نالها "

لم تكن الرحلة قصيرة ولا ينبغي لها ان تكون لم يكن الحلم قريبا ولا الطريق محفوفا بالتسهيلات لكني فعلتها ونلتها.

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الملخص

يركز عملنا على استخلاص المركبات العلاجية من النباتات، وصياغة العلاجات القائمة على المستخلصات النباتية وتقييم إمكاناتها العلاجية. تم تفصيل طرق الاستخلاص وتحضير المستخلص، بالإضافة إلى الاختبارات المختبرية والحية لتقييم النشاط المضاد للأكسدة والمضاد للالتهابات والقدرة على تعزيز نمو الشعر في الفئران. أظهرت النتائج أن مستخلص المثنان يُظهر نشاطًا كبيرًا مضادًا للأكسدة، بينما يُظهر مستخلص البابونج نشاطًا أقل وضوحًا مضادًا للالتهابات. تم اختبار التركيبات الجالينية التي تم تحضيرها على الفئران، وأظهرت تأثيرات إيجابية على الالتهاب .

الكلمات المفتاحية : تجارب على الجسم الحي، نشاط مضاد للجراثيم ,مستخلصات المثنان والبابونج.

Résumé

Notre travail concentre sur l'extraction de composés thérapeutiques à partir de plantes, la formulation de remèdes à base d'extraits de plantes et l'évaluation de leur potentiel thérapeutique. Des méthodes d'extraction et de préparation d'extraits sont détaillées, ainsi que des tests in vitro et in vivo pour évaluer l'activité antioxydante, anti-inflammatoire et la capacité de favoriser la croissance des poils chez les rats. Les résultats montrent que l'extrait de Thymelaea présente une activité antioxydante significative, tandis que l'extrait de Chamomile montre une activité anti-inflammatoire moins prononcée. Les formulations galéniques préparées ont été testées sur des rats, démontrant des effets positifs sur l'inflammation.

Mots-clés : Méthane et extraits de camomille, expériences in vivo, activité antibactérienne.

Abstract

Our work focuses on the extraction of therapeutic compounds from plants, the formulation of plant extract-based remedies and the evaluation of their therapeutic potential. Extraction and extract preparation methods are detailed, as well as in vitro and in vivo tests to assess antioxidant and anti-inflammatory activity and the ability to promote hair growth in rats. The results show that Thymelaea extract exhibits significant antioxidant activity, while Chamomile extract shows less pronounced anti-inflammatory activity. The galenic formulations prepared were tested on rats, demonstrating positive effects on inflammation .

Keywords: Methane and chamomile extracts, in vivo experiments, antibacterial activity

List of abbreviations

Abs	Absorption.
ATCC	American Type Culture Collection.
C	Concentration of the solution in mole/L .
°C	Celcious degree .
Cam	Matricaria Chamomile .
Candida	*Candida albicans.*
Cur	Curcuma.
cm^3	Centimeter square.
DPPH	2,2-diphenyl-1-picrylhydrazyl.
DMSO	Di Methyl Sulfoxyde.
E.coli	*Escherichia coli.*
g	Gram.
I max	Maximal Intensity.
I_0	Initial Intensity.
l	Length of quartz cell (1 cm).
L1	Cream of Curcuma 1 %.
L1.0	Blank of L1.
L2	Cream of curcuma 1% + zinc oxyde.
L2.0	Blank of L2.
L3	Cream of Chamomile 1 %.
L3.0	Blank of L3.
L4	Cream of Chamomile 1.5% .
L4.0	Blank of L4.
L5	Cream of curcumin 2%+1% Chamomile.
L5.0	Blank of L5.
L6	Cream of curcumin 1%+2% Chamomile.
L6.0	Blanc of L6.
m	Mass of dry extract .
MIC	Minimal Inhibitory Concentration .
mg	Milligram .
min	Minute .
ml	Mililiter .
nm	Nano miter .
pH	Hydrogen potential.
Pseudo	*Pseudomonas aeruginosa.*
rfm	Rote for minute.
Staph	*Staphylococcus aureus.*
TC	Tannin Content.
TFC	Total Flavonoid Content.
TPC	Total Polyphenols Content.
UV	Ultra- Violet.
V	Extract volume.
VF	Volume of residues solution in the flacon following each precipitation.
Vf	Volume of solution after dilution.
Vp	Taken Volume.
ε	the specific absorption coefficient.
µL	Micro liter.

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Summary

General Introduction

Plants have been used for medical purposes for almost 60,000 years. There is evidence that medicinal plants have been cultivated for at least 2,500 years in Greece and Central Asia, and for about 5,000 years in Egypt, China, and India. The use of plants as medicine was first empirical but has progressively developed into a science supported by data. The earliest known written records are from about 5,000 years ago **[1]**.

Despite their well-known therapeutic benefits, medicinal plants can contain hundreds or even thousands of active compounds that are frequently difficult, if not impossible, to detect. While certain ingredients have specific purposes, others have broader applications **[2]**.

Herbal medicine has many advantages over contemporary medicine, reflecting the past's reliance on plants for healing. As antibiotics become less effective against emerging bacteria and viruses, plant-based remedies are becoming more and more popular. As worries about drug side effects increase, herbal medicine is seeing a resurgence. This is especially true when it comes to managing chronic diseases. Ten to twenty percent of hospital admissions are related to adverse reactions to chemical medications **[3-5]**.

The aim of this work is to demonstrate the efficacity of some plants Matrecaria Chamomile and Turmeric in the pharmaceutical domain, the essential oils of these plants were sought after for this purpose, however no extraction has taken place for these products. To achieve our goal, water-based extracts have been prepared, formulated to galenic forms and tested to evaluate their effectiveness.

This memory will be summarized in three parts:

- The first chapter presents a bibliographic study on medicinal plants and their composition.
- The second chapter describes the methodology used in the exaction, characterization of extracted products, preparation of galenic forms and effectiveness's tests.
- The obtained results and their interpretation are presented in the third chapter.

A global summary succinct the main findings is presented to close.

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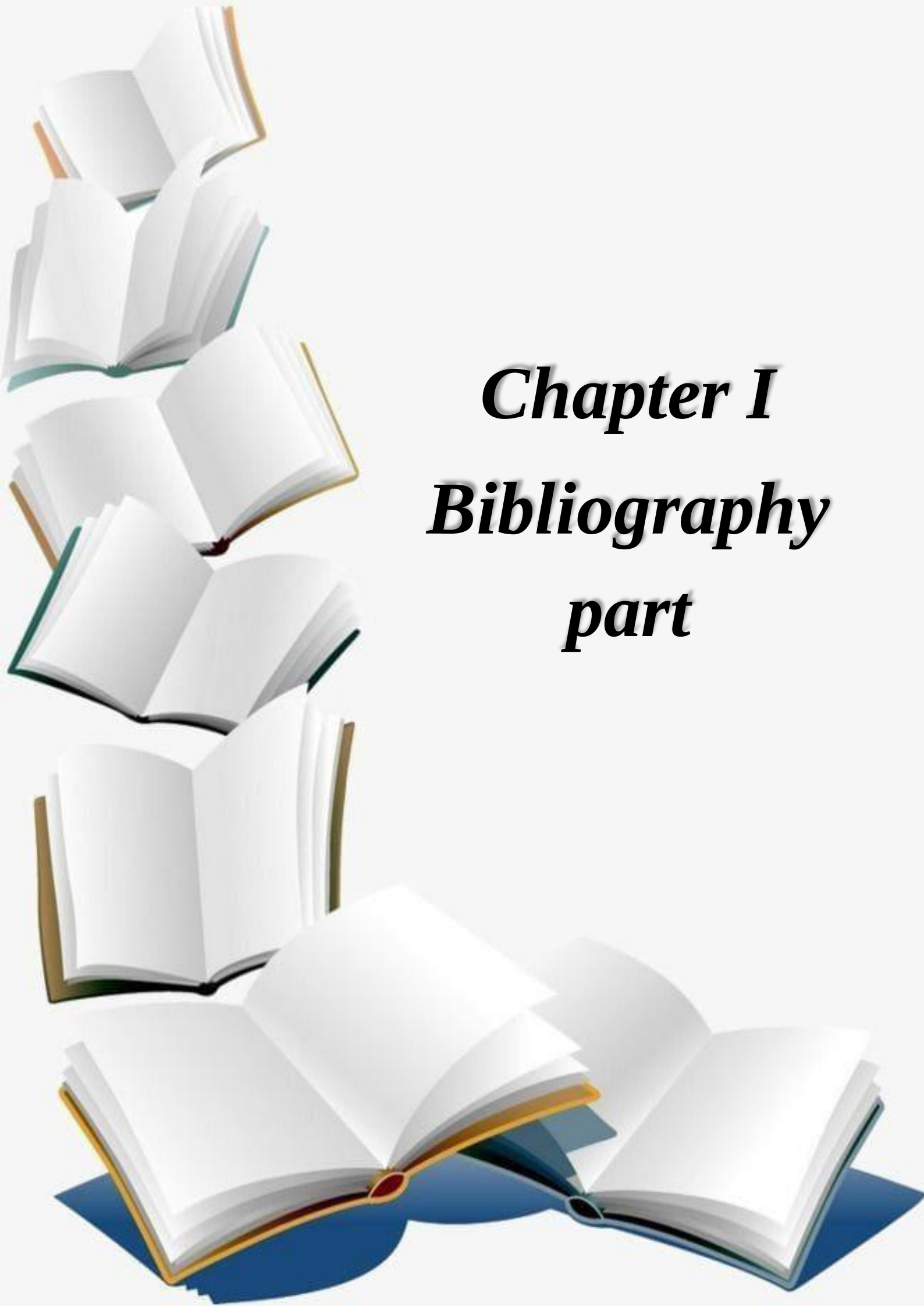

Chapter I

Bibliography part

I.1. INTRODUCTION:

Over the last years, there has been a noticeable increase in fascination with natural plants that have long been used as cures for a variety of illnesses **[1]**.

These days, there is a resurgence of interest in using plant-based remedies. With the help of new experiments and analytical methods in scientific research, the medical field is progressively realizing the effectiveness of traditional prescriptions involving medicinal plants. Ethnobotany, a scientific field that translates common knowledge into scientific understanding, plays a key role in studying traditional herbal medicine **[2]**.

I.2. DEFINITION OF MEDICINAL PLANTS:

The medicinal plant refers to a plant with therapeutic qualities that can be used whole or in plant parts. Some plants may also have used as food, condiment or cosmetic. Medicinal plants used in traditional medicine mainly contain active ingredients for the whole plant, a part of the plant or a mixture of several parts **[3]**. They are used throughout the world to support, promote, maintain and restore human health. Traditional medicines are part of the wider field of traditional medicine, which includes procedures and practitioners, as well as products **[4]**.

The World Health Organization estimates that 80% of people living in developing countries depend on traditional medicinal practices for their primary health care **[5]**.

I.3. THE BENEFITS OF MEDICINAL PLANTS:

Despite modern medicine's strides, herbal medicine offers numerous benefits, echoing historical reliance on plants for treatment. Today, a resurgence in plant-based treatments emerges as antibiotic efficacy wanes against evolving bacteria and viruses. Herbal medicine sees a revival, notably in chronic disease management, as concerns over medication side effects grow. Approximately 10 to 20% of hospital admissions stem from chemical medication side effects **[6]**.

Interest in medicinal plants has been commonplace since time immemorial. Numerous studies have been carried out in this field by renowned researchers in traditional medicine, some of which are particularly noteworthy **[6]**.

Chapter I *Bibliography part*

Youdim and Deans examined the effects of aging on antioxidant enzyme activity and fatty acid composition in rat brains, as well as the effects of dietary supplementation with thyme oil or thymol. Untreated rats showed a significant decline in antioxidant enzyme activity with age, while those fed thyme oil or thymol maintained higher levels of antioxidant activity. In addition, rats fed these supplements had higher levels of beneficial fatty acids in their brains, suggesting the beneficial potential of thyme oil as a dietary antioxidant **[7]**.

Cosentino *et al* analyzed the essential oils of different wild thyme species and their antimicrobial activity. Oil components were identified by GC/MS, revealing variable concentrations of hydrocarbon monoterpenes and phenolic monoterpenes. Tests showed antimicrobial activity similar to that of other thyme oils, attributed to their high phenol content. Carvacrol and thymol were the most effective against bacteria, suggesting their potential use in prolonging food shelf life **[8]**.

Lavender's impact on stress levels was investigated by Ghavami *et al*, analyzing 21 studies with a total of 791 intervention group participants and 804 controls. The findings demonstrated a notable decrease in stress scores among those who used lavender compared to controls. Subgroup analyses underscored the efficacy of L. angustifolia, student groups, and the perceived stress scale. Overall, the study supports lavender's significant role in stress reduction, advocating for its inclusion in stress management interventions, especially for students **[9]**. Anther research paper proved that the lavender is a rich source of phenolic acids and flavonoids, thus resulting in antioxidant and anti-inflammatory properties **[10]**.

The medicinal properties of Aloe Vera were explored by Danish *et al*, this plant is rich in beneficial compounds. Alcohol extracts from leaves and roots are tested against various bacterial and fungal strains, their results showed promising antibacterial and antifungal activity **[11]**.

I.4. PRINCIPAL INGREDIENTS OF MEDICINAL PLANTS:

Medicinal plants contain hundreds if not thousands of active chemicals, which are often difficult to identify, even though their medicinal effects are well known. Some of these active ingredients are listed in the bullet points below **[12]**:

- **Phenols:** diverse compounds in plants, from salicylic acid to phenolic glucoside acids, exhibit anti-inflammatory and antiseptic properties, serving as plant defenses. Phenolic acids like rosmarinic acid offer antioxidant benefits and may combat viruses. Certain plants yield salicylic derivatives and methyl salicylate through distillation, with therapeutic potential.
- **Flavonoids:** pigments found in most plants, have a wide range of beneficial effects. They act as antioxidants, improving blood circulation, and possess anti-inflammatory, antiviral and hepatoprotective properties. Flavonoids such as hesperidin and rutin strengthen capillary walls, while isoflavones, found in plants such as red clover, offer estrogenic effects useful in the treatment of menopausal disorders.
- **Tannins:** present in all plants at different levels, give a bitter taste to bark or leaves, making them unfit for consumption by certain animals. These compounds contract tissues by binding proteins, and are used in tanning hides. They stop bleeding, fight infection and promote healing of damaged tissue, as in the case of eczema or burns. Plants rich in tannins, such as oak and acacia, are used to strengthen supple tissues and drain excessive secretions, as in cases of diarrhea.
- **Essential oils:** obtained by distilling plants, are oxygenated aromatic compounds, sometimes of terpenoid origin. They are widely used in perfumery and possess a variety of properties, such as the antiseptic effect of tea tree.

I.5. THE STUDIED PLANTS:

The use of medicinal plants remains largely unexploited in Algeria in the cosmetics, pharmaceutical and food sectors **[14]**. As part of our efforts to enhance the value of Algerian flora, the three plants listed below pique our curiosity.

I.5.1. Thymelaea hirsuta

The Thymelaeaceae family, part of the Magnoliopsida includes some 1,200 species in 67 genera. Etymologically, the generic name "Thymelaea" comes from a species whose leaves are reminiscent of thyme and whose fruit is reminiscent of a small "olive", derived from the Greek "elaia".

The Thymelaeaceae are represented by around ten species belonging to the genera Thymelaea and Daphne. They are found in tropical and temperate zones around the world, mainly in Africa, but are absent from colder climates.

In Mediterranean regions, the Thymelaea genus comprises around 31 species of evergreen shrubs and herbs of these, Thymelaea hirsuta is considered the most representative species on the Mediterranean coast **[14, 15]**.

Thymelaea hirsuta Endel was described as a perennial shrub that can reach heights of 2 to 3 meters **[16]**. Its leaves are very small, densely imbricated, leathery, elongated ovals with sharp points. Like the stems, they are smooth on the underside and pubescent to woolly on top. The flowers, 2 to 5 in number, are found at the tip of the twigs and are characterized by a yellowish calyx that falls rapidly. Flowers can be either unisexual or polygamous, and are borne on different plants.

Thymelaea hirsuta Endel is a hermaphrodite plant. Its fruits are smooth berries that are eaten by animals, ensuring their dispersal (zoochore dispersal). The plant flowers from October to April are pollinated by insects **[17]**. Two types of flowers can be distinguished, male and female greenish male flowers have a cylindrical calyx, while female flowers are yellowish with a swollen calyx.

(a) **(b)** **(c)**

Figure I.1: a: Thymelaea hirsuta (Original Photo), b: female flowers, c: male flowers

The use of thymelaea for hair care is not new and has been passed down through generations in some cultures. Many people, including our grandmothers, have used thymelaea-infused preparations to wash and treat their hair, noticing its beneficial effects as thickening moisturizing and hair growth.

The dried flowering tops of the plant are traditionally ground and then boiled in water to obtain a viscous solution which is spread daily over the skin for treatment of cutaneous infections and noninfectious skin disorders such as eczema. It is also reported as having leishmanicidal and vermifuge properties

Badawy *et al* give The systematic classification of Thymelaea hirsute reported in below table and the geographical distribution of T. hirsuta grows in the Mediterranean coastal plains. Thymelaea.hirsuta is situated from Spain to Greece and Turkey, southern side from Morocco to Egypt and it is also founded in Lebanon and Palestine **[19]**.

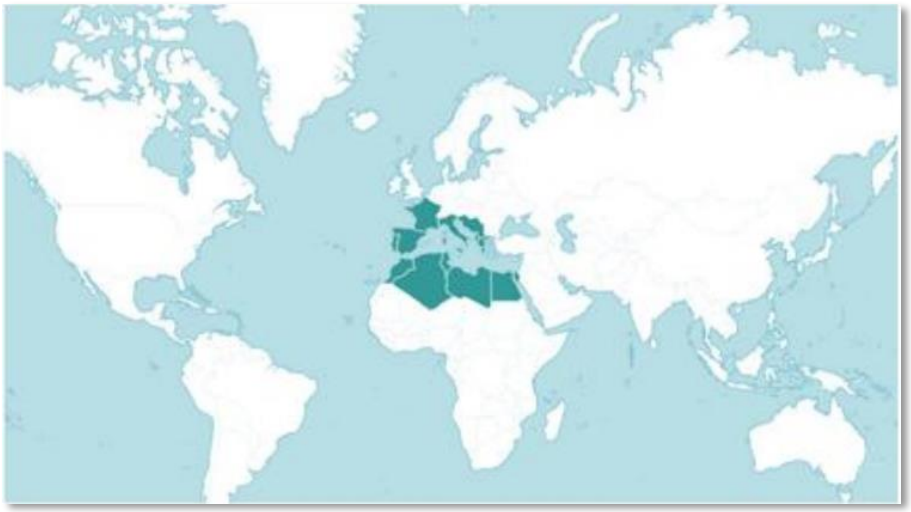

Figure I.2: Geographical distribution of Thymelaea.

Table I.1: the classification of Thymelaea hirsuta.

Division	Tracheophyta
Class	Magnoliopsida
Superorder	Rosanae
Order	Malvales
Family	Thymelaeaceae
Genus	thymelaea
Species	hirsute
Arabic name	Methnane

The main components of plants of the Thymelaeaceae family are daphnane diterpenes and coumarins, as well as lignans and phenolics. Daphnane diterpenes are the major biologically active constituents of Thymelaeaceae **[5].**

Preliminary phytochemical screening of Thymelaea hirsuta leaves, flowers and stems revealed the presence of tannins, alkaloids, steroids, saponins, coumarins, reducing compounds and anthraquinones **[18]**. so that the contents of phenolics and flavonoids in Thymelaea were

determined in high levels by Djermane on several extracts, including an aqueous extract and a methanol extract **[20]**.

Many studies have proven several biological properties characterized by the methane plant, according to the study conducted by Dehimi **[21]**. It has been shown to possess anti-cancer, anti-inflammatory, and anti-diabetic properties, it has been also used in folk medicine for its antimelanogenesis, antioxidant, hypoglycemic and antiarthritic **[22]** and antitumor characteristics **[5]**.

According to a study by Toumi *et al.*, thymelaea has demonstrated promising benefits in preventing cell growth and invasion as well as triggering cell cycle arrest in colorectal cancer cells **[23]**. The hepatoprotective qualities were also identified **[24]**.

Figure I.3: Biological proprieties of Methane.

1.5.2. Chamomile

Chamomile is a plant that belongs to the Asteraceae family and can be either annual or perennial, according to Dai *et al*. It is indigenous to Europe and Asia's temperate zones. It is grown all over the world for its excellent nutritional, therapeutic, and cosmetic value. It was in use in ancient Greece, Rome, and Egypt for thousands of years. The extensive usage of this herb in Uyghur medicine was first documented in China **[25]**.

Chamomile has a sweet, herbaceous and slightly fruity aroma, daisy-like flowers with a yellow center 1-1.5 cm long and 12-20 white petals, as described by Bayati *et al* **[26].** According to Probadh *et al*, it is a plant that grows upright to a height of 50 to 90 cm and is distributed throughout the world. Its blossoms are highly therapeutic **[27]**.

Figure I.4: Matrecaria Chamomile (Personal picture).

Traditionally, chamomile has been used for centuries as an anti-inflammatory, antioxidant, mild astringent and healing medicine. It is also used to treat wounds, ulcers, eczema, gout, skin irritations, bruises, burns, canker sores, neuralgia, sciatica, rheumatic pain, hemorrhoids, mastitis and other ailments. Externally, chamomile has been used to treat diaper rash, cracked nipples, chicken pox, ear and eye infections, disorders of the eyes including blocked tear ducts,

conjunctivitis, nasal inflammation and poison ivy. Chamomile is widely used to treat inflammations of the skin and mucous membranes, and for various bacterial infections of the skin, oral cavity, gums, and respiratory tract **[28]**. Different parts and behaviors of chamomile were described in the following figure **[29]**.

Figure I.5: Different plant parts and habit of M. Chamomile **a:** Flowering plant**, b:** Flower head**, c:** Ray floret**, d:** Disk floret**, e:** Capitulum I**, f:** Teeth/petals of disc floret, **g:** Anthers, **h:** Stigma**, i:** Seed**, j:** Fresh flowers.

The chamomile genus consists of 5 species that are primarily found across Europe, Northem Africa, Macaronesia, western, south-western and central Asia, and western North America. These species are known to thrive in various environments such as disturbed land, grassland, regions adjacent to roads and railways, as well as in waste areas and unoccupied spaces **[29]**.

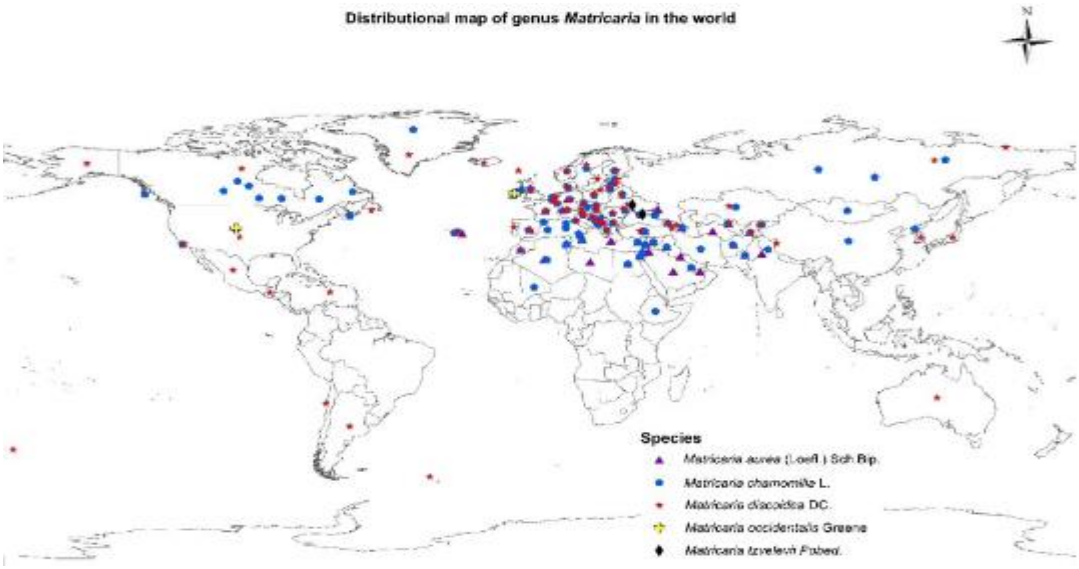

Figure I.6: Accepted species of Genus Matricaria and their distribution.

Table I.2: The scientific classification of Chamomile

Kingdom	**Plantae**
Subkingdom	Tracheobionta
Super division	Spermatophyta
Division	Magnoliophyta
Class	Magnoliopsida
Order	Asterales
Family	Asteraceae
Genus	Matricaria
Species	Chamomilla
Synonyms	Matricaria recutita L ; Chamomilla vulgaris Gray

The chamomile harbors a diverse range of therapeutically valuable compounds, among these, sesquiterpenes, flavonoids, coumarins, and polyacetylenes stand out as crucial constituents. Noteworthy coumarins in M. chamomilla include herniarin, umbelliferone, and other minor variations.

The glucoside precursor of herniarin, (Z)- and (E)-2-β-d-glucopyranosyloxy-4-methoxycinnamic acid has been identified as a native compound in chamomile. Additionally, eleven bioactive phenolic compounds, including herniarin, umbelliferone, chlorogenic acid, caffeic acid, apigenin, apigenin-7-O-glucoside, luteolin, luteolin-7-O-glucoside, quercetin, rutin, and naringenin, are present in chamomile extract **[30]**.

The obtained results proved by Stanojevic *et al* the presence of 52 components, wherein the highest content of β-farnesene (29.8 %), α-farnesene (9.3 %), α-bisabolol and its oxide (15.7 %), chamazulene (6.4 %), germacrene D (6.2 %) and spiroether (5.6 %) is determined **[31]**.

The phytochemistry composition of essential oils and extracts of M. chamomilla has been widely analyzed, showing that the plant contains over 120 constituents. Essential oils are generally composed of terpenoids, such as α-bisabolol and its oxides A and B, bisabolone oxide A, chamazulene, and β-farnesene, among other compounds. On the other hand, M. chamomilla extracts were dominated by phenolic compounds, including phenolic acids, flavonoids, and coumarins **[32]**.

The anti-inflammatory and antioxidant activities of chamomile within the analgesic action are well known, so that this medicinal plant is usually used as liquid extracts, tablets, or capsules to decrease sleep and digestive tract disorders and to diminish anxiety and neuralgic pains (headache, sciatica, rheumatic pain). Topical application is useful in treatment of epidermis and mucous membrane inflammation (burns, sunburn, insect bites, psoriasis, eczema, and conjunctivitis). In addition to the aforementioned properties, recent studies have also shown a possible role of chamomile in the prevention of tumors, cardiovascular diseases, and diabetes **[33]**.

Chamomile tea finds widespread use across Europe, South America, and Mexico, particularly among parents who rely on it to alleviate colic, digestive issues, fever, insomnia, and the discomfort associated with teething in children.

Additionally, women have historically turned to chamomile to address symptoms of menstrual cramps. Its benefits extend beyond ingestion. Chamomile is also valued for its calming properties, often recommended for muscle cramps, headaches, and soothing indigestion and colic-related discomfort.

Inhaling steam infused with chamomile is a common remedy for respiratory tract irritations. Moreover, chamomile-based creams and ointments are prized for their ability to soothe and heal various skin ailments, including burns, wounds, diaper rash, and sore nipples. Even in homeopathic form, such as tablets, chamomile is administered to babies to ease teething discomfort and fussiness **[34]**.

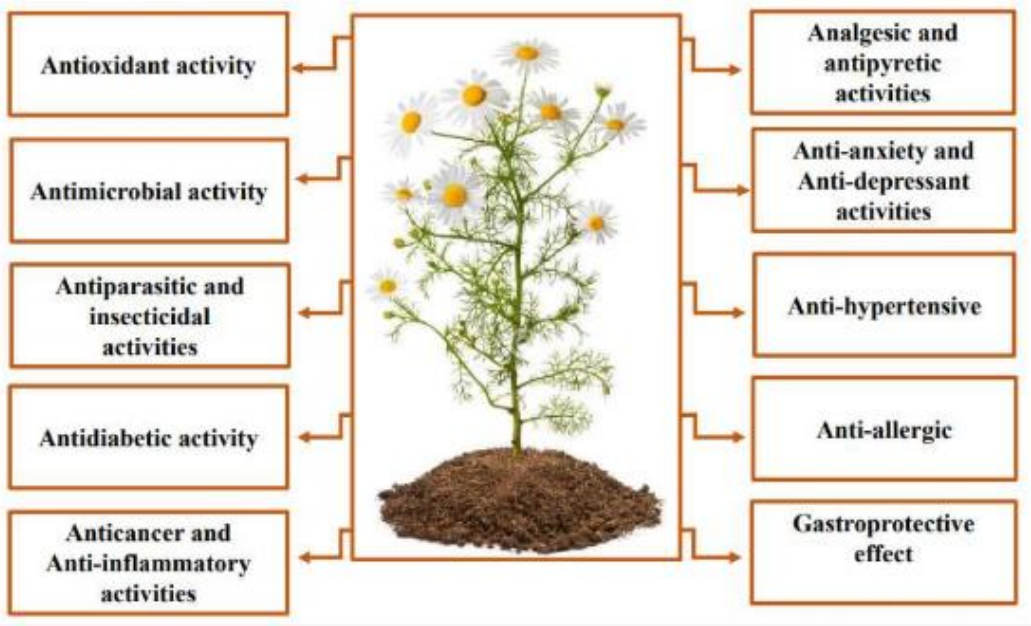

Figure I.7: Biologicals proprieties of Chamomile.

1.5.3. Curcuma longa

Turmeric was first utilized approximately 4,000 years ago in India's Vedic culture, when it was both a culinary spice and a holy herb. It most likely made its way to East Africa, West Africa, China, and Jamaica by the eighteenth century. When Marco Polo first wrote about this spice in

1280, he was amazed at how closely the vegetable resembled saffron in its properties. Turmeric has a long history of medicinal use in South Asia, according to Ayurvedic and Unani traditions, as well as Sanskrit medical treatises **[35]**.

A popular spice that has captured the attention of both the medical and culinary communities. Turmeric, a perennial herbaceous plant with rhizomes (Curcuma longa) belonging to the ginger family, has been recognized for its healing properties for centuries. While the medicinal benefits of turmeric and its active component, curcumin, have been understood for ages, scientists have only recently begun exploring its precise mechanisms of action and identifying its bioactive elements **[36]**.

Turmeric, known as haldi is a vibrant yellow-orange spice derived from the rhizome of the Curcuma longa plant. This plant typically grows up to 3 feet tall and features lance-shaped leaves. It produces spikes of yellow flowers that emerge from a fleshy rhizome or an underground stem. The medicinal powder of turmeric comes from the orange pulp found within the rhizome **[37]**. In the next, the content of turmeric is described **[39]**:

- **The Rhizome:** Curcuma longa has numerous underground stems, including a main one measuring around 5 cm x 2.5 cm. At maturity, it produces many aromatic, cylindrical, or ellipsoidal lateral rhizomes, with a gray-yellow exterior and a dark orange interior.
- **The Leaf:** The leaves are typically wide (7 to 25 cm) and long (up to 50 cm), uniformly green in color. They are arranged in two rows, alternating with each other. Their spikes can reach up to 20 cm, and the flowers are sterile

Figure I.8: The different part of Curcumin.

In traditional medicine, turmeric has been used in therapeutic preparations over the centuries in different parts of the world. In Ayurvedic practices, turmeric is thought to have many medicinal properties including strengthening the overall energy of the body, relieving gas, dispelling worms, improving digestion, regulating menstruation, dissolving gallstones, and relieving arthritis **[39]**.

The composition of turmeric encompasses a diverse array of components, with over 100 identified thus far. The primary constituents include a volatile oil rich in turmerone, alongside curcuminoids, such as curcumin, demethoxycurcumin, 5'-methoxycurcumin, and dihydrocurcumin, which exhibit natural antioxidant properties. Standard turmeric comprises moisture (>9%), curcumin (5–6.6%), minimal extraneous matter (<0.5% by weight), low levels of mold (<3%), and volatile oils (<3.5%), containing various compounds like d-α-phellandrene, d-sabinene, cinol, borneol, zingiberene, and sesquiterpenes. These sesquiterpenes, including germacrone, termerone, α-, and β-termerones, among others, often exhibit species-specific characteristics **[40]**.

Figure I.9: Some therapeutic application of Turmeric.

The aromatic essence of turmeric primarily derives from turmerone, arturmerone, and zingiberene. Additionally, the rhizomes contain polysaccharides-ukonans, stigmasterole, β-sitosterole, cholesterol, and 2-hydroxymethyl anthraquinone. In terms of nutrition, 100 grams of turmeric provides 390 kcal, 10 grams of total fat, 3 grams of saturated fat, essential minerals like calcium, phosphorus, sodium, potassium, iron, and vitamins such as thiamine, riboflavin, niacin, and ascorbic acid. Furthermore, turmeric is recognized as a notable source of the ω-3 fatty acid and α-linolenic acid **[40]**.

The main component found in turmeric is called curcumin, which is known for its diverse range of healing properties **[41]**. Curcumin presents different therapeutic action as well as hepatoprotective **[42]**, antimicrobial and cardio protective **[43]**, anticancer **[44]**, hypoglycemic **[45]** and antiarthritic properties **[46]**.

Jovanovic *et al* have determined that turmeric is a donor of proton of the central methyl group rather than phenolic groups in aqueous, acidic and acetonitrile solutions, which explains the potent antioxidant activity of curcumin, as demonstrated by its attribution to the neutralization of

free radical **[47]**. However, Barclay *et al* proposed that curcumin's antioxidant properties stem from phenolic groups that have ability to give a proton **[48]**.

According to Priyadarsin *et al*, the presence of methoxy groups enhances this property aven further and the phenolic groups play a crucial role in the curcumin's antioxidant activity **[49]**.

Another research by Mou-Tuan Huang *et al* has demonstrated that curcumin inhibits tumor growth, protease C activity, cyclooxygenase epidermal in vitro activity and epidermal inflammation **[50]**. Additionally, curcumin exhibits tremendous promise in the treatment of Alzheimer's disease **[51]**.

Since the Covid-19 pandemic struck Wuhan, numerous research projects have been launched around the world and the fight against the virus has been the focus of extensive studies. One of them, is being conducted by Khaerunnisa team **[52]**, which evaluated the bioactive components found in medicinal plants as curcumin, that may be Covid-19 inhibitors using a molecular modeling technique. They found that, when combined with other natural compounds, curcumin appears to have the most potential for acting as Covid-19 inhibitors.

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Chapter II

Experimental part

II.1. INTRODUCTION:

Plants have played an important role in medicine since ancient times, offering a vast of therapeutic compounds that have been utilised for healing purposes. These compounds include alkaloids, flavonoids, terpenes and phenolics, among others. Many of these compounds have been isolated and studied for their pharmacological effects. In our work, our plan is to attempt extractions from some plants or work with natural extracts.

II.2. MATERIALS AND METHODS:

II.2.1. Chemical products:

Table II.1: chemical products.

Almond oil	salicilic acid
coco betaine	sodium bicarbonate
copper sulfate	starch
Fenugreek oil	tri-ethanolamine
kathon CG	vasline
lavender oil	vitamin E
magnesium sterate	zinc oxide
propylene glycol	zinc sulfate

II.2.2. Vegetal materials:

Our study focused on three plants:

- The first known as “wild thyme”. The upper part of this plant was harvested in the flowering period from the Rahouia region, specifically Ain Abd Errahim, 40km from state of Tiaret, on December.

- The second is Chamomile, with its delicate flowers and gentle fragrance, it is commonly used in teas and herbal remedies for its calming effect, anti-inflammatory and antioxidant benefits. It's purchased from the market.
- The third is derived from the Curcuma longa plant, it's celebrated for its potential health benefits as anti-inflammatory and antioxidant properties. It's also purchased from the market.

A **B** **C**

Figure II.1: Presentation of the studied plants A: Thymelaea, B: Chamomile, C: Curcuma longa.

II.2.3. Methodology of work

These studies were carried out at the chemistry laboratory at the faculty of Materials Sciences of Ibn Khaldoun University of Tiaret

- ***Washing process***: the washing process of the plant involved repeated washing at least three times to ensure that all impurities are removed from the soil and sediment followed by final wash with distilled water.

Figure II.2: The washing of the thymelaea.

- ***Drying process:*** the plant was dried in an air dryer at a temperature of 40-45°C for 48 hours.

Figure II.3: Vacuum drying chamber.

- ***Grinding process:*** The plants mentioned above were milled in the same manner using the grinder depicted below.

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Figure II.4: The used grinder.

A B C

Figure II.5: The obtained powders A: Thymelaea, B: Chamomile, C: Curcuma longa.

- ***Hydrodistillation experience***

The obtained powders of Thymelaea and Chamomile was subjected to hydrodistillation for three hours. the process was repeated four times and a homogenized filtrate. However, it was impossible to extract the oil.

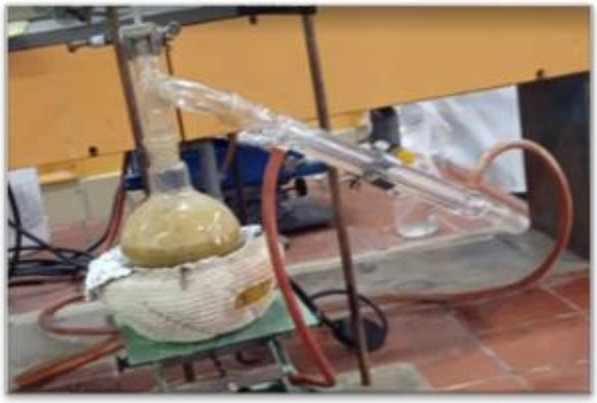


Figure II.6: The hydrodistillation experience.

- ***Maceration with ethanol***

This process facilitates the extraction of active compounds but with our plants the oils weren't obtained. In this experience, the maceration for 48 hours includes immersing Thymelaea and Chamomile in ethanol maintained at 4°C was studied.

Figure II.7: The maceration with ethanol experience.

- ***Maceration with distilled water***

In this part, the maceration at room temperature for 24 hours involves immersing Thymelaea and Chamomile in distilled water with vigorous agitation was released three times as described in previous paper **[1]** but the oil wasn't also obtained.

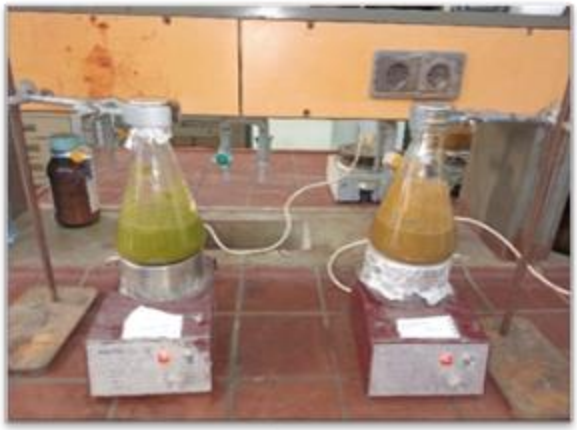


Figure II.8: The maceration with distilled water experience.

- ***Extraction of curcumin***

We placed the obtained curcuma powder with acetone in the reflux set-up for 24 hours at a temperature of 35 °C.

Figure II.9: Curcumin Extraction.

Several methods were used to extract essential oils from the studied plants, but no results were obtained. Therefore, we decided to use extract of plant in our work.

II.2.4. Preparation of the extract:

After maceration with distillated water, the mixture was filtered then the obtained solution was dried in vacuum drying chamber for 24h at 40°C.

A B C

Figure II.10: The extracted solutions A: Thymelaea, B: Chamomile, C: Curcuma longa.

A B C

Figure II.11: The extracts of plant A: Chamomile, B: Thymelaea, C: Curcuma longa.

II.3. QUALITATIVE ANALYSIS

IR analysis of extracts

Infrared (IR) spectroscopy serves as a pivotal analytical method primarily utilized for identifying functional groups in organic molecules and determining the structures of certain simple molecules. This technique relies on the absorption of infrared radiation by the sample under examination, allowing for the detection of characteristic vibrations of chemical bonds. These

vibrations aid in the analysis of the chemical functionalities present within the sample. Leaf extracts from three species were subjected to IR analysis within the range of 400 to 4000 cm^{-1}, utilizing an ALPHA-P BRUKER SPECTROMETER. The analyses took place at the materials chemistry laboratory of the University of Oran1.

II.4. PHYTOCHEMICAL SCREENING

This study highlights the presence of some groups of diverse metabolites in plants and to detect these latter we perform a series of chemical analyses of the obtained extract and among these compounds we mention: alkaloids, phenolic compounds and tannins (reaction with ferric chloride), flavonoids, saponins and coumarins **[2-5]**.

- Alkaloid test: 2mL of the extracted solution was mixed with 2 mL of concentrated hydrochloric acid (HCl) followed by the addition of a few drops of Mayer's reagent, the formation of a green or white precipitate indicates the presence of alkaloids.
- Coumarins test: 1 mL of the extracted solution was mixed with 1 mL of sodium hydroxide 10%. The formation of a yellow color indicates the presence of coumarins.
- Flavonoids test: 1 mL of sodium hydroxide solution 10% was added to 1 mL of the aqueous extract. The presence of flavonoids is indicated by the formation of a yellow to orange color.
- Phenolic test: 1 mL of extracted solution was diluted with 2 mL of distilled water, followed by the addition of a few drops of a ferric chloride solution 10%. The formation of a blue or green color indicates the presence of phenols.
- Quinone test: 1 mL of the extracted solution was merged with 1 mL of concentrated sulfuric acid. The presence of quinones is indicated by the formation of a red color.
- Saponins test: 1 mL of aqueous extract was diluted with 5mL of water, the tube was shaken for 2 minutes. The formation of stable foam after 15 min confirms the presence of saponins.
- Steroid test: 0,5 mL of the extracted solution was treated with drops of chloroform, acetic anhydride and concentrated sulfuric acid. The formation of a dark pink color indicates the presence of steroids.
- Tannin test: 2 mL of the extracted solution was diluted with distilled water and 2-3 drops of ferric chloride 5% ($FeCl_3$) were added. A green-black or blue coloration would indicate the presence of tannin.

II.5. Quantitative analysis of extracts

Colorimetric methods based on UV-visible spectrophotometry were used to assess the quantity of phenolic compounds: total polyphenols, total flavonoids and tannins present in the leaves of the studied species **[6-8]**.

II.5.1. Dosage of total polyphenols

The principle of the method: the dosage of polyphenols is released by spectral photometry using the Folin-Ciocalteu method. This method is based on the oxidreduction reactions with Folin-Ciocalteic acid which has a yellow color, it is composed of a mixture of phosphotungsthenic and Phosphomolybdic acids. The phenol reduces this reagent to a mixture of blue oxides of tungsten and molybdenum that absorb at 765 nm. The intensity of color is proportional to the content of oxidized phenolic compounds.

- *Operating mode:* Polyphenols were determined using a modified Folin-Ciocalteu photometric assay photometric assay **[9]**. The assay protocol for total polyphenols is summarized in figure II.12 below. All manipulations are carried out in triplicate. the blank is also prepared under the same conditions, replacing the quantity of extract with distilled water.
- *Expression of results:* Total polyphenol concentrations are calculated using the equation developed by Nirmala *et al.* **[9]**.

$$\text{TPC (mg/g)} = \frac{C*V}{m}$$

TPC: total polyphenols content.

C: extract concentration established from calibration curve.

V: extract volume

m: mass of dry extract

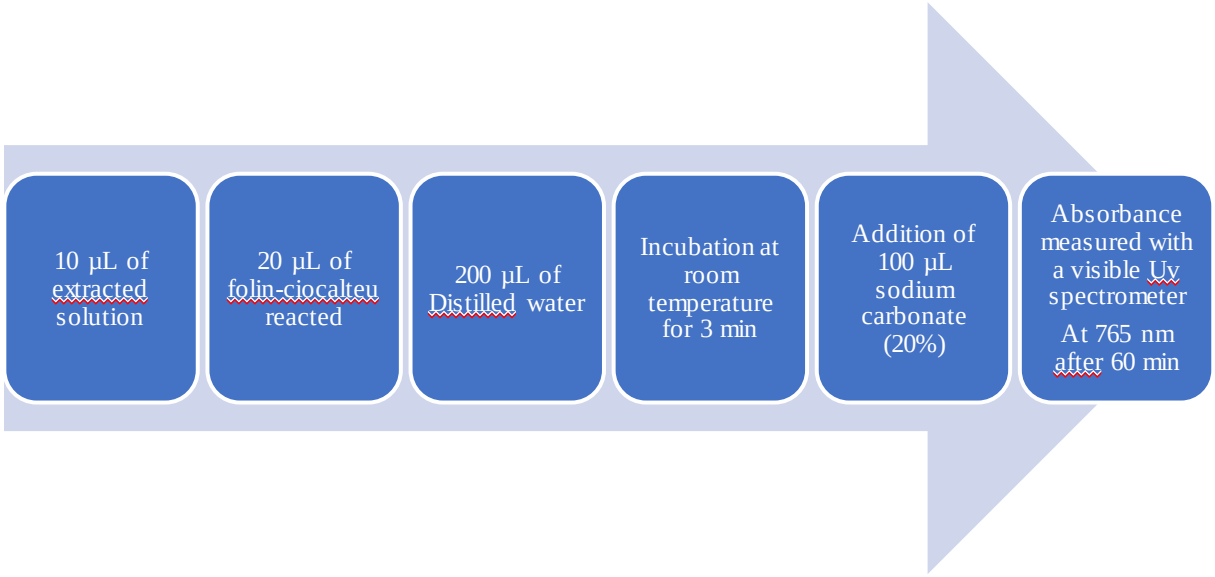


Figure II 12: Total polyphenol assay steps.

II.5.2. Determination of total flavonoids

- *Principle of the method:* The determination of total flavonoids is based on a colorimetric test using aluminum trichloride $AlCl_3$ as the reagent. Flavonoids have a free hydroxyl (OH) group in position 5, which is able to form a colored complex with aluminum chloride, together with the CO group. Flavonoids form yellow complexes by chelating metals (aluminum). The Al metal loses 2 electrons to join two oxygen atoms of the phenolic molecule acting as an electron donor.
- *Operating mode:* The total flavonoid content of the extracts was determined using the method described by Marsoul and Kim with a few modifications **[10,11].** All manipulations are carried out in triplicate. The blank is also prepared under the same conditions, replacing the quantity of extracted solution with distilled water. The assay protocol for total flavonoid is summarized in the figure below.
- *Expression of results:* Flavonoid quantification was based on the equation established by Singh *et al.* **[12]**

$$TFC\ (mg/g) = \frac{C*V}{m}$$

TFC: total flavonoid content.

C: extract concentration established from calibration curve.

V: extract volume.

m: mass of dry extract.

Figure II.13: Total Flavonoids assay steps.

II.5.3. Dosage of condensed tannins

- *Principle of Method:* Condensed tannins are determined by the vanillin method in acid medium. The principle of this assay is based on the reaction of vanillin with the terminal flavonoid group of catechins, resulting in the formation of a red complex. This is explained by the property of tannins to transform into red anthocyanidols, which absorb light at 500 nm.
- *Operating mode:* Total condensed tannin content was determined by the Sun at al procedure **[13]**. All manipulations are carried out in triplicate. the blank is also prepared under the same conditions, replacing the quantity of extract with distilled water.
  The assay protocol for total flavonoid is summarized in the figure below.
- *Expression of results:* The concentration of condensed tannins is calculated using the equation established by Madhav *et al.* **[14].**

$$TC\ (mg/g) = \frac{C*V}{m}$$

TC: tannin content (mg ECT/g of dried extract).

C: extract concentration established from calibration curve.

V: extract volume.

m: mass of dry extract.

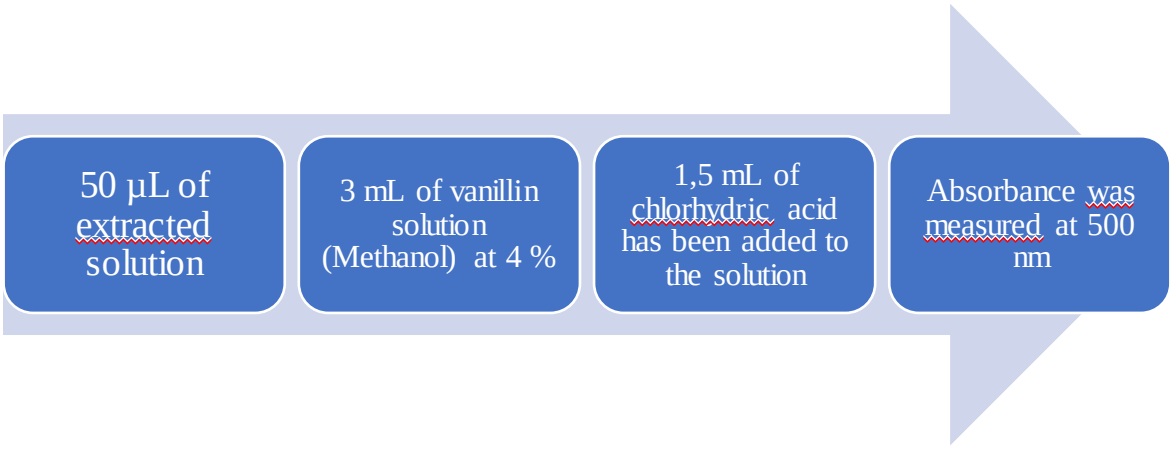


Figure II.14: The steps for the determination of condensed tannins.

II.6. GALENIC FORMS FORMULATION:

Our work's goal is to create herbal remedies based on plant extracts and assess their therapeutic potential. This section displays the composition of our prepared formulations in each table.

Table II.2. The composition of L1.

Ingredients	Curcuma	Paraffin oil	Vaseline	Magnesium stearate	Starch	CG Kathon
Quantity	0.8 g	20 mL	20 g	0.2g	4g	1 mL

Table II.3. The composition of L2.

Ingredients	Curcuma	Paraffin oil	Vaseline	Magnesium stearate	Starch	CG Kathon	Zinc oxide
Quantity	0.8 g	20 mL	20 g	0.2g	4g	1 mL	0.8 g

Table II.4. The composition of L3.

Ingredients	chamomile	Paraffin oil	Vaseline	Magnesium stearate	Starch	CG Kathon	Flower water
Quantity	0.8 g	15mL	22 g	0.2g	4g	1 mL	20mL

Table II.5. The composition of L4.

Ingredients	chamomile	Paraffin oil	Vaseline	Magnesium stearate	Starch	CG Kathon	Flower water
Quantity	1 g	20mL	15 g	0.2g	5g	1 .5mL	22mL

Table II.6. The composition of L5.

Ingredients	Curcuma	chamomile	Vaseline	Magnesium stearate	Starch	CG Kathon	Flower water
Quantity	0.8 g	0.7 g	23 g	0.2g	5.5 g	1 mL	13 mL

Table II.7. The composition of L6.

Ingredients	Curcuma	chamomile	paraffin oil	Vaseline	Magnesium stearate	Starch	CG Kathon	Flower water
Quantity	0.9 g	0.8 g	8 mL	20 g	0.2g	4 g	1 mL	13 mL

In order to prepare all galenic formulations, we mixed the ingredients in a mechanical blender as follows:

FigureII.15: Mechanical blender.

FigureII.16: Adopted cream.

II.7. QUALITY CONTROL FOR GALENIC FORMS:

Quality control for galenic forms is necessary to ensure the efficacy, consistency of the prepared formulations. the prepared formulations exhibit the following traits:

- **Consistency and Homogeneity,** Testing the consistency involved placing each product detail on a glass plate.
- **Color and odor** are tested by visual inception
- **Stability** is tested by centrifugation of galenic forms at different times (5min and 30min)
- **pH Level:** all pH galenic forms are determined by pH-meter.

II.8. DRUG RELEASE OF GALENIC FORMS:

II.8.1. Operating procedure:

In order to release the active ingredients from the prepared galenic forms, a system that resembles the Franz cell was attempted. This cell contains a donor compartment, separated from a recipient compartment by the membrane of the skin to be studied. In the donor compartment, the

formulation containing the active ingredient is placed, and successive samples taken from the recipient compartment enable the kinetic profile of release to be monitored over time.

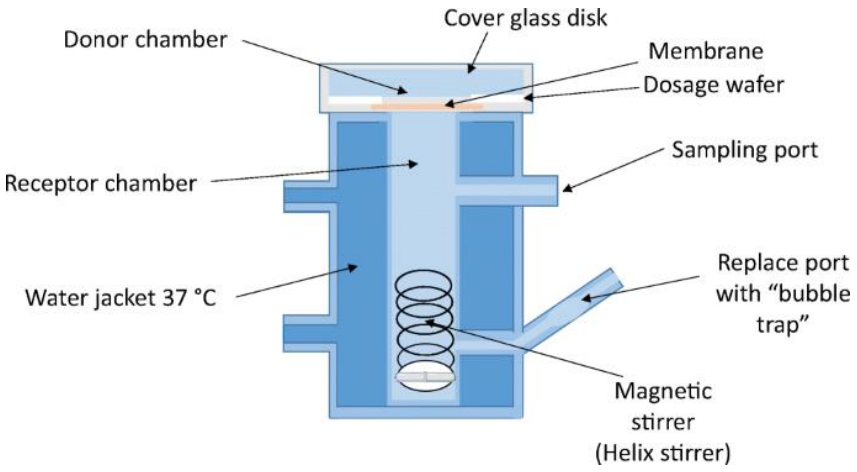


Figure II.17: Franz cell.

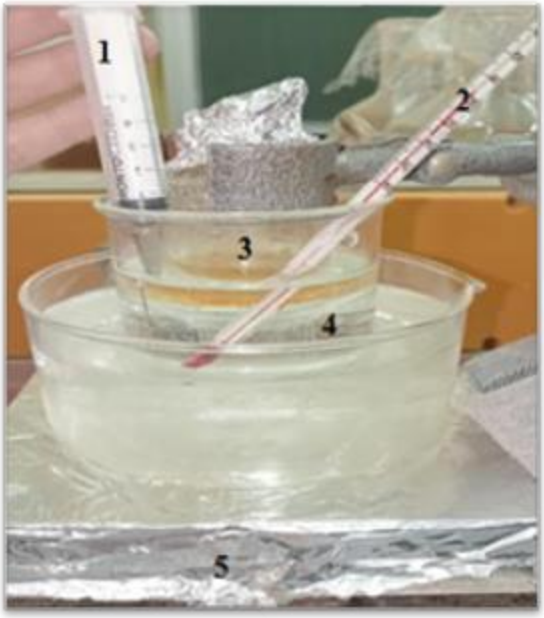


Figure II.18: system for drug release (**1.** Syringe for sampling, **2.** Thermometer**, 3**. Membrane**, 4.** pH= 5.5**, 5.** Stirrer).

The formulations are placed in the donor compartment, while the recipient compartment contains a medium thermostated at 37°C with a pH=5.5 simulated to the skin medium, and magnetic stirring. The system is carefully sealed with paraffin paper and aluminum foil to prevent evaporation of the medium. The active ingredient is thus released by contact of the formulations with the reconstituted medium through the membrane. Over time, 1 mL samples are taken from the recipient compartment using a syringe, then they are diluted in the same medium to a total volume of 10 mL, maintained at a pH of 5.5, and analyzed by UV spectrophotometry. For all experiments, samples are taken over seven hours.

The controlled conditions in all experiments are:

- ***Stirring the medium:*** To ensure uniform concentration of the solution at all points, a magnetic stirrer is used in the flask. The speed of rotation is set at 500 rpm for all experiments. This is crucial, as the absence of stirring would lead to the formation of a concentration gradient in the solution, which could induce errors in UV spectrophotometric analyses.
- ***Temperature:*** Temperature plays an essential role in diffusion phenomena, influencing the solubility of the active ingredient and facilitating diffusion. All experiments are carried out at a constant temperature of 37°C, which corresponds to human body temperature. A heated stirrer is used to keep this temperature
- ***The nature of the medium, its pH and volume:*** Like temperature, the nature of the medium plays a crucial role in the solubility of the active agent, which in turn affects the diffusion process. In addition, the pH of the medium has a significant impact on the hydrolysis rate and solubility of the active ingredient.

II.8.2. Principe active analysis

λ max research : λ max is determined by performing a U.V. spectrum at a concentration of 10^{-3} g/L of each extract in the study medium at pH= 5.5.

The λ max values thus found (λ1 max = 273 nm / λ2 max =272nm) are correspond respectively to cam and cur. The instrument used is the U.V JENWAY spectrophotometer (Synthesis and Catalysis Laboratory, Ibn Kaldoun University, Tiaret).

- **Calibration line and Calculation of ε max:** Solutions at known concentrations were analyzed at λ max. The spectral absorption curve of the extract in a medium at pH=5.5 is shown below. Optical density (O.D.) or absorbance (A) is related to concentration (g/L) by the Beer-Lambert law:

$$(D.O)_{max} = \log (I_{max}/I_0) = \varepsilon_{max}. l. C$$

$$(D.O)_{max} = \varepsilon_{max}. l.C$$

- Imax/I0: is the transmittance.
- ε max: the specific absorption coefficient ($L.cm^{-1}. mole^{-1}$).
- C: concentration of the solution in mole/l.
- l: length of quartz cell (1cm).

The value of ε max corresponds to the value of the tangent of the line (D.O) max=f(C). the calibration lines are summarized in annex.

- **Analysis of transferred quantities**

Release kinetics were studied using a UV-VIS spectrometer (UV 1650PC SCHIMADZU). This device is first calibrated to the wavelength λmax of the active ingredient in the specific medium at pH 5.5 and 37°C. The quantity of active ingredient released is then determined as a function of time in the dissolution medium. At each time t, a sample of volume Vp is taken from the solution under study, then diluted with a medium of the same pH in a 10 cm^3 volumetric flask. The optical density (O.D.) of the diluted solution is then measured on the instrument calibrated at the corresponding wavelength (the dilution volume is chosen to obtain an O.D. value within the linear range of the calibration curve).

The following equation is used to calculate the mass of the liberated active agent (mt) at each instant t:

$$mt = DO.Vp.Vf / \varepsilon V_F l$$

Vp = taken volume (1 mL).

Vf = volume of solution after dilution (10 mL).

VF = volume of residual solution in the flacon following each precipitation.

ε: the specific absorption coefficient.

l: length of quartz cell (1cm).

II.9. BIOLOGICAL STUDY:

Medicinal plants exhibit significant biological activities such as antibacterial, antifungal, and cytotoxic properties **[15,16]**. These plants contain bioactive compounds that contribute to their medicinal properties and therapeutic applications **[17]**. Studies have shown that extracts from plants like Acorus calamus, Artemisia annua, and Euphorbia helioscopia demonstrate promising antibacterial and antifungal activities against various pathogens **[18]**. Additionally, research highlights the effectiveness of medicinal plant extracts from Catharanthus roseus, Bixa orellana, Azadirachta indica, and Dracaena sanderiana in combating bacterial and fungal infections **[19]**.

The utilization of medicinal plants for their biological activities is rooted in their traditional use as natural remedies, offering a safer and cost-effective alternative to synthetic drugs. These findings underscore the importance of exploring the bioactive potential of medicinal plants for developing novel therapeutic interventions.

Antibacterial activity refers to the ability of a substance to either kill bacteria (bactericidal) or inhibit their growth (bacteriostatic) **[20]**. Various antimicrobial agents, including antibiotics, and plant extracts, exhibit antibacterial activity **[21, 22]**. For instance, plant extracts like Imperata cylindrica have demonstrated potent antibacterial activity against pathogens like *Staphylococcus aureus* and *E. coli.*

In our study, we examined the antibacterial activity of creams prepared and our aqua extracts in vitro against various bacterial strains, including *Staphylococcus aureus*, *Escherichia coli, Candida albicans* and *Pseudomonas aeruginosa*. To ensure their purity, these strains were seeded

on selective media. The strains were then grown in Mueller Hinton (MH) agar medium and incubated at 37°C for 24 hours to obtain a young culture (24 hours old). Strain identification was carried out by the laboratories of the Faculty Material Sciences, Ibn Khaldoun University.

Table II.08: The tested bacterial stains.

Reference strains	***Escherichia coli***	***Pseudomonas aeruginosa***	***Staphylococcus aureus***	***Candida albicans***
Code	ATCC 25922	ATCC 27853	ATCC 25923	ATCC 10231
Gram	Negative (-)	Negative (-)	Positive (+)	Positive (+)

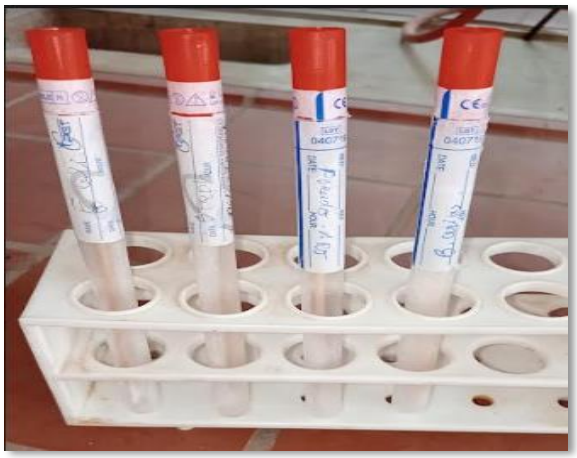


Figure II.19: Bacterial strains.

Figure II.20: Mueller Hinton Medium.

20 milliliters per Petri dish were aseptically prepared for the super cooled Mueller Hinton (MH) agar culture media. Let the medium cool and become solid on the surface of the work. After soaking a sterile swab in the bacterial solution, twist and press the swab firmly against the tube's inner wall to squeeze out any extra liquid. Using the swab, create tight ridges from top to bottom over the whole agar surface. Do this twice more, rotating the Petri dish through 60 degrees each time to cross the ridges and remembering to move the swab around on itself. Complete the seeding process by swabbing the agar's perimeter. As many Petri plates were inoculated, refill the swab was always necessary.

The analysis was released for the extracted plants and the prepared whites and their fresh creams.

For the extracted plants, discs are prepared by compression and were dropped on the Muller-Hinton surface while for the creams and their whites, they are placed in the created holes in Petrie dishes. Each test was release in triplicate.

Figure II.21: Preparation of the petri dishes.

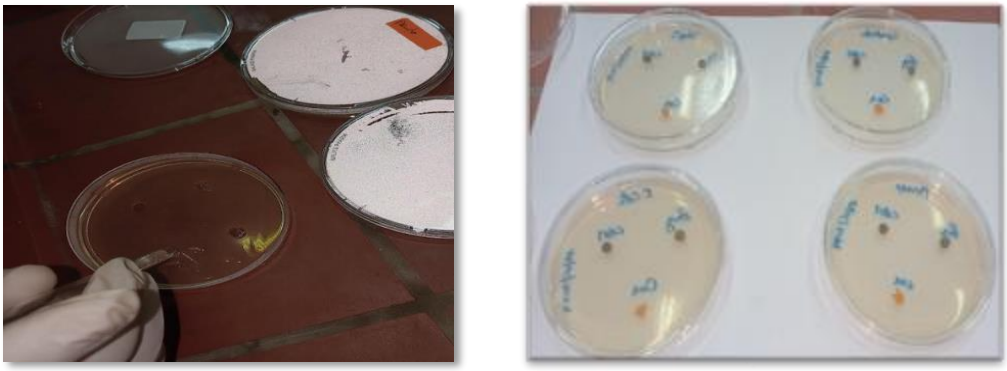


Figure II.22: Methods of product's statement.

Petri dishes were incubated at 37°C for 18 to 24 hours. Then the results were reading by measuring the diameter of the transparent zone of inhibition against the test microorganisms using a caliper.

Figure II.23: Bacterial dishes after incubation.

II.10. Minimal Concentration of Inhibitory

The minimum inhibitory concentration (MIC) is defined as the lowest concentration of an antimicrobial agent capable of effectively inhibiting bacterial growth **[23]**. Research has shown that various natural extracts exhibit antibacterial properties at specific concentrations, making them potential alternatives for combating bacterial diseases in aquaculture **[24, 25]**. Determining how well these extracts prevent bacterial growth requires knowing their minimum inhibitory concentration.

II.10.1. Principe of the method:

The minimum inhibitory concentrations (MICs) of plant extracts were initially determined using Mueller–Hinton broth microdilution . MIC determination was performed by a serial dilution technique using 96-well microtiter plates. Plant extract (100 µL) was placed into the well/plate. Then, 100 µL bacterial cell suspensions were placed in each well/plate. Microplates were incubated for 24 h at 37 °C. The lowest concentrations without visible growth completely inhibited the bacteria (MICs) **[26]**.

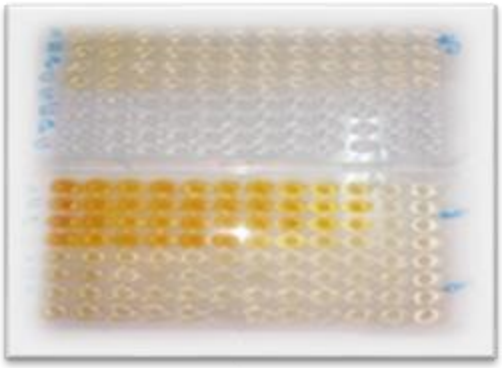
Figure II.24: Microplates for the MCI method.

II.10.2. Protocol of the MCI Determination:

The extracted solutions were prepared to be tested with a mass of 0.01g of extract solubilized in 1mL of DMSO. The spaces of the microplates were filled with 100µL of Mueller-Hinton microdilution broth (96 spaces). The prepared solution was deposited in the first space and homogenized with micropipettes, then a second dilution was released from the first solution. The process was repeated as well as to fill the 10 spaces. 20µLs were deposited in all spaces and the last space was considered as reference.

(1) : 100µL of Mueller-Hinton microdilution broth

(2) : Extract plants (100 µL) were placed into the microplate.



(3): Addition of 20 µL of bacterium medium

(4) : Microplates were incubated for 24 h at 37 °C hours

Figure II.25: Protocol of the MCI determination.

II.11. Antioxidant activity

II.11.1. Principle of the method:

Numerous methods are currently used to assess this activity; the antioxidant activity of a compound corresponds to its ability to resist oxidation **[27]**. The 2,2-diphenyl-1-picrylhydrazyl radical has been widely used to study the free radical scavenging activity of various extracted plants. The chemical compound 2,2-diphenyl-1-picrylhydrazyl (DPPH) was one of the first free radicals used to study the structure-antioxidant activity of phenolic compounds **[28]**. It possesses an unpaired electron on one atom of the bridge, the reduction of this radical is accompanied by its from the characteristic violet color of the DPPH solution to the yellow color measurable spectrophotometrically at 514-518 nm **[29]**.

DPPH is a stable free radical with a dark purple dark violet color that turns pale yellow when reduced in the presence of an antioxidant **[30]**.

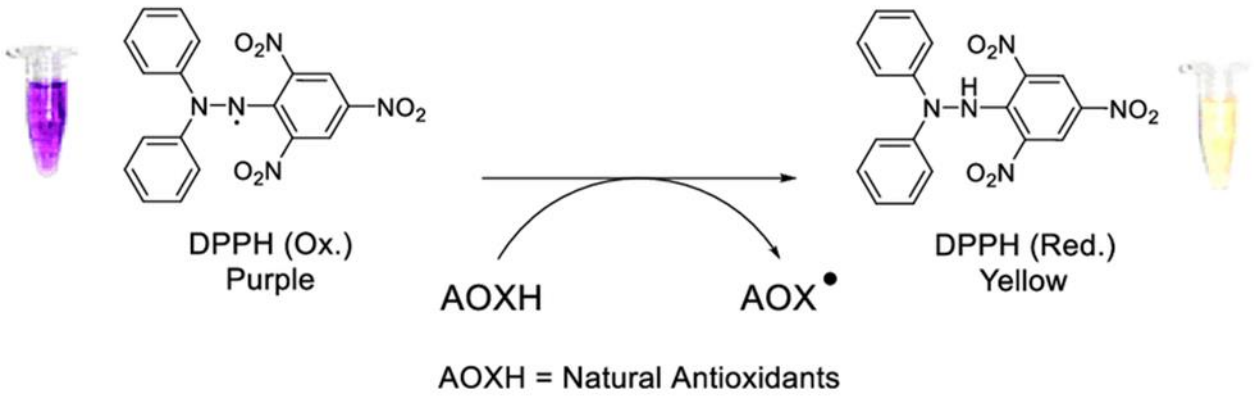


Figure II.26: Structure of the DPPH radical and its reduced form.

II.11.2. Antioxidant experiment:

The antioxidant activity of the extracted plants and the standard was assessed on the basis of the radical scavenging effect of the stable 1, 1-diphenyl-2-picrylhydrazyl (DPPH)-free radical activity by modified method. The diluted solutions of the extracted plants were prepared in methanol 0.002% of DPPH which was prepared in methanol. 1.5 mL of this solution was mixed with 1 mL of prepared and standard solutions separately. These mixtures were kept in dark for 30 min and optical density was measured at 517 nm. Methanol (1 mL) with DPPH solution (0.002%, 1.5 ml) was used as blank, and the same method of ascorbic acid at different concentration was used as reference **[31]**.

The difference in the absorbance between the test solution and the control (DPPH in methanol) was calculated and expressed as scavenging percent or inhibition percent of DPPH radical. The inhibition percent of DPPH radical was calculated by using the equation; Percent inhibition of DPPH.

$$I = \left(\frac{Ac - As}{Ac}\right) * 100$$

Whereas: I %: Trapped DPPH rate or inhibition rate.

As: Is the absorbance of the test solute

Ac: Is the absorbance of the control.

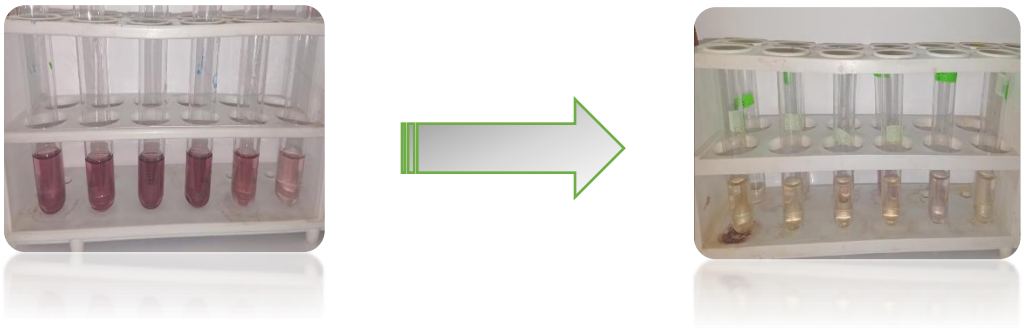


Figure II.27: Antioxidant activity protocol.

II.11.3. IC50 estimation:

IC50 is defined as the concentration of the sample required to reduce 50% of the DPPH radical, at IC50s are determined graphically by linear regression of the plotted graphs (inhibition rate as a function of different concentrations).

II.12. Anti-inflammatory activity

The anti-inflammatory activities of the crude and fractionated plant extracts were determined using a modified version of the BSA assay reported by Williams et al.11 BSA solution (0.4%, w/v) was prepared in Tris Buffered Saline, pH 6.4 at 25 o C. Stock solutions of each plant extract were prepared at a concentration of 50 µg/mL or 0.005%, w/v. Respective aliquots of 5.0 µL, 10 µL and 20 µL representing concentrations of 200ug/ml and 1.00 µg/mL of the stock solutions were added to test tubes containing 1 mL of 0.4%, w/v BSA buffer solution. Both negative (methanol) and positive (diclofenac) controls were assayed in a similar manner. The solutions were then heated in a water bath at 72 °C for 10 minutes, and cooled for 20 minutes under laboratory conditions. The turbidity of the solutions (level of protein precipitation) was measured at 660 nm in a Hatch Spectrophotometer using an air blank. The experiments were conducted in duplicate and the mean absorbance values were recorded. The percentage inhibition of precipitation (protein denaturation) was determined on a percentage basis, relative to the negative control using the following equation: **[32]**.

- Anti-Denaturation Activity% = (Absorbance of control – Absorbance of sample)/ (Absorbance of control) *100.

- Anti-Denaturation Activity%= % Inhibition of Protein Denaturation = % Anti-inflammatory Activity.

II.13. In vivo tests

In this part, two types of experience are described. The first is to test the anti-inflammatory activity whoever the second is to test the ability to progressive the hair of rats.

- ***First experience:***

The first in vivo experiment was conducted on a total of twenty-four (24) Wistar rats, sourced from the Pasteur Institute of Algiers. The rats, aged 4 months with weights ranging from 220 to 322g (mean weight=261±5g) at the beginning of the study, had undergone a 4-month adaptation period at the Hygiene and Animal Pathology Laboratory, Tiaret. Where they were housed at room temperature and provided with ad libitum access to food and water.

Rats are housed individually in plastic cages with wood shavings bedding, which is changed daily. Each cage was fitted with a tag bearing the name of the batch corresponding to the treatment. The rats were divided into 8 distinct groups each group consist 3 males.

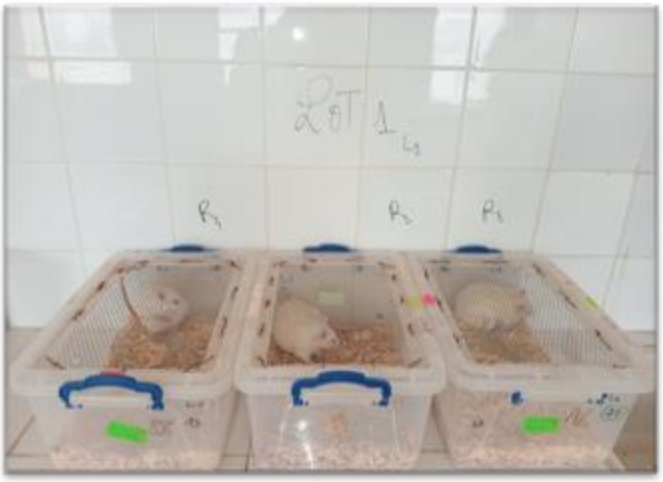


Figure II.28: Presentation of rat's group.

After that the rats were fasted for 18 hours, measurements of the right paw were taken then, an injection of 1 mL of a 1% formaldehyde solution is administered into the plantar aspect of the right paw to induce edema.

Figure II.29: Injection to induce edema.

We premeasured the paw's diameter two hours after the injection since we saw redness and swelling at the injection site.

Figure II.30: Apparition of edema and their measurement.

The edematous paw was treated by prepared galenic forms. The treatments were given to test rats for 5 days with free access to water and food each day, the description of the groups is summarized in the next table.

Table II.09: Description of rat's group of the first experience.

Number of groups	1	2	3	4	5	6	7	8
Number of Rats /group	R16, R2, R8	R1, R14, R19	R5, R7, R22	R4, R11, R21	R3, R12, R20	R8, R15, R24	R9, R10, R23	R13, R6, R17
Treatment	L1	L2	L3	L4	L5	L6	L7	Positive control

Every day prior to the tendering of prepared galenic forms, the diameter of the rats' paws is measured with a digital caliper to track the progression of the edema.

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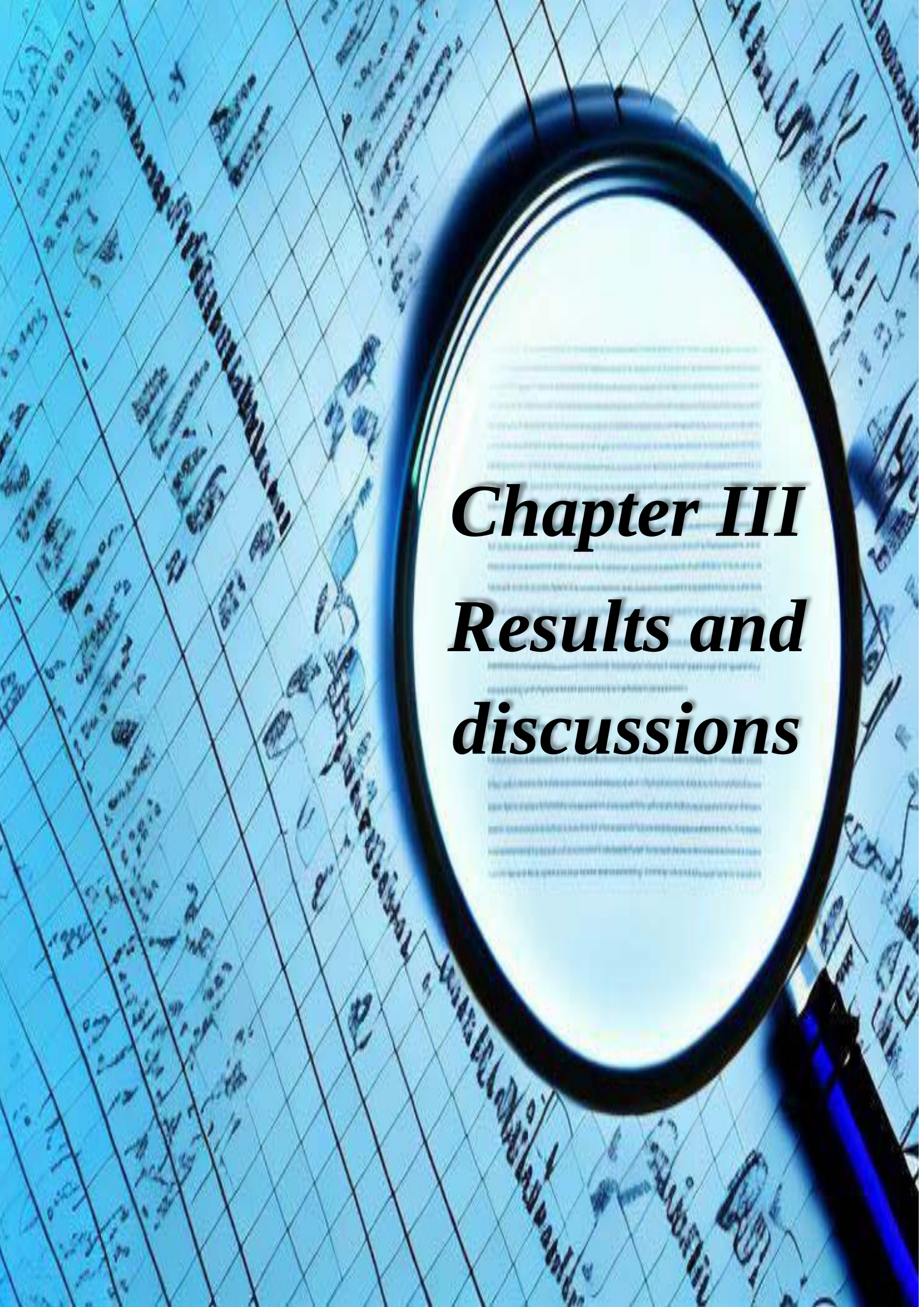
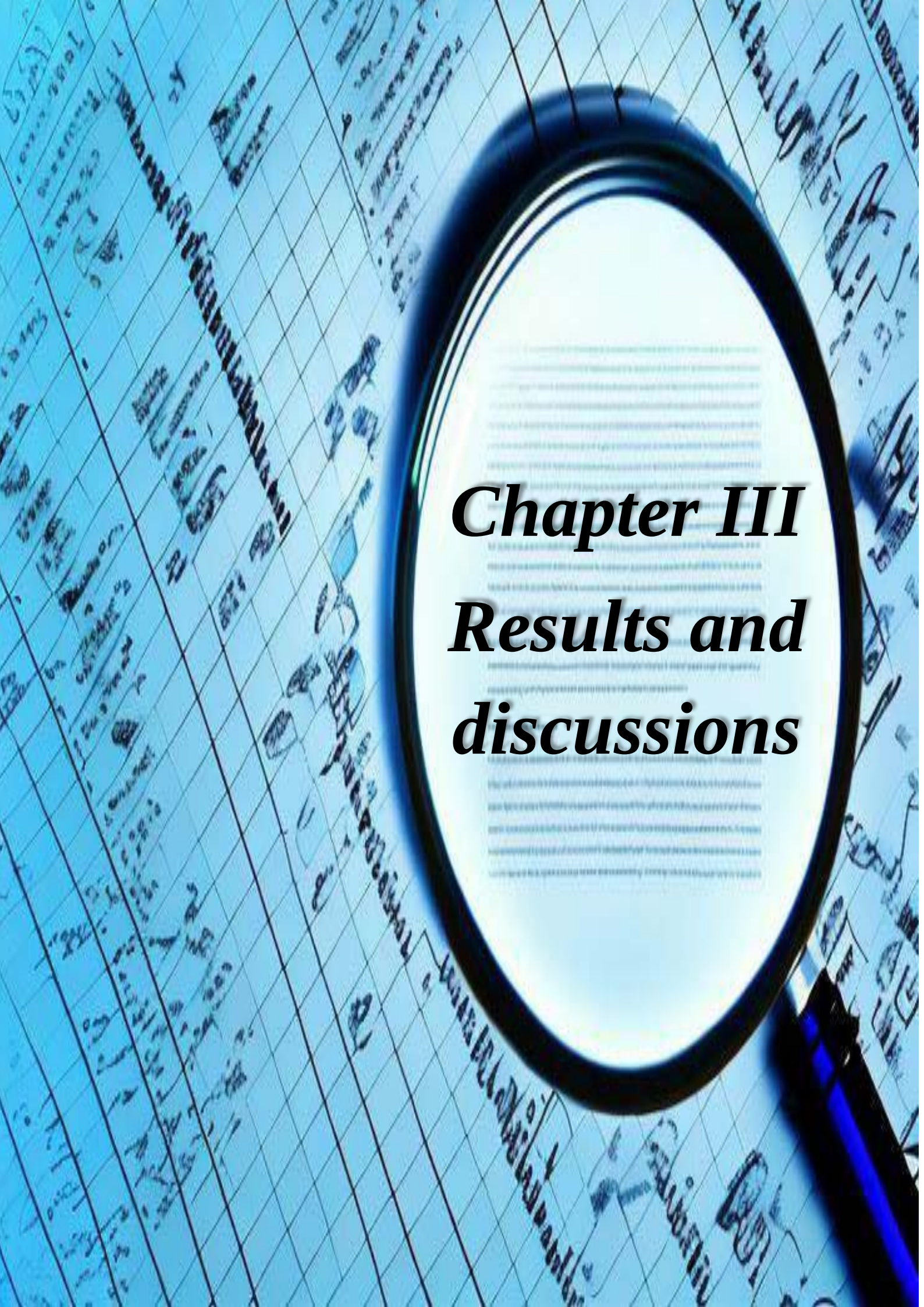

Chapter III
Results and
discussions


Chapter III
Results and
discussions

III.1. INTRODUCTION:

This section will cover the results and the characterization of prepared galenic forms and plant extracts. Bacterial tests and medication release investigations will also be covered. In vivo studies were used to confirm their activity.

III.2. CARACTERISATION OF EXTRACTS

III.2.1 PHYSICO-CHEMICAL CARACTERIZATION OF EXTRACTS PRODUCT:

- **Yield:** the yield of our experiment is calculated compared with the initial quantity of the plant as described the next equation.

$$\textbf{Yield} = (\boldsymbol{the\ mass\ of\ obtained\ extract / plant's\ initial\ mass}) * \mathbf{100}$$

The calculated values were summarized in the table III.1. The obtained values were compared with previous research and they showed interesting differences in values when organic solvents were used as extracted medium **[1]**.

Table III.1: The calculated yield.

Plants	yield %
Thy	15.73
Cam	14.254
Cur	0.03

The yield of the extracted curcumin was little and the obtained mass was analyzed in FTIR and the solubility test.

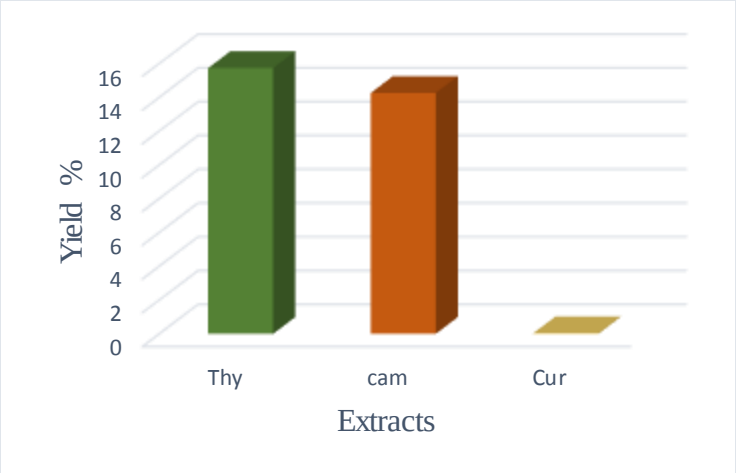


Figure III.1: Extraction yields.

- **Solubility:** it's important to test the solubility of our extracts product to choose initially the composition of our galenic forms. The obtained results are summarized in the table III.2.

Table III.2: The solubility's test.

Product	Thy	Cam	Cur
water	+++++	+++++	-------
alcohol	-----	++++	------
propylene glycol	-------	-------	-------
paraffin oil	------	------	+++++
Vaseline oil	------	------	+++++
coco betaine	++++	++++++	------
DMSO	+++	+++	+++++

- **Refractive index:** the refractive index was determined using the refractometer and the obtained values are 1.338 for the chamomile extract and 1.337 for the thymeleae hirsute extract.

- **pH:** the pH of the extracted solution of the studied plants are measured and the obtained values are 5.5 and 5 of thymeleae and chamomile respectively. The pH of extracted curcumin in acetone is 4.5.
- **FTIR analysis:** FTIR gives an idea about the composition of the analyzed product. The characterized bands were compared to another work **[2]**. The obtained IR spectra confirm the presence of characteristic bands corresponding to determined compounds in phytochemical analysis. The spectra are shown in the figures below and the bands values are reported in the table **III.3**:

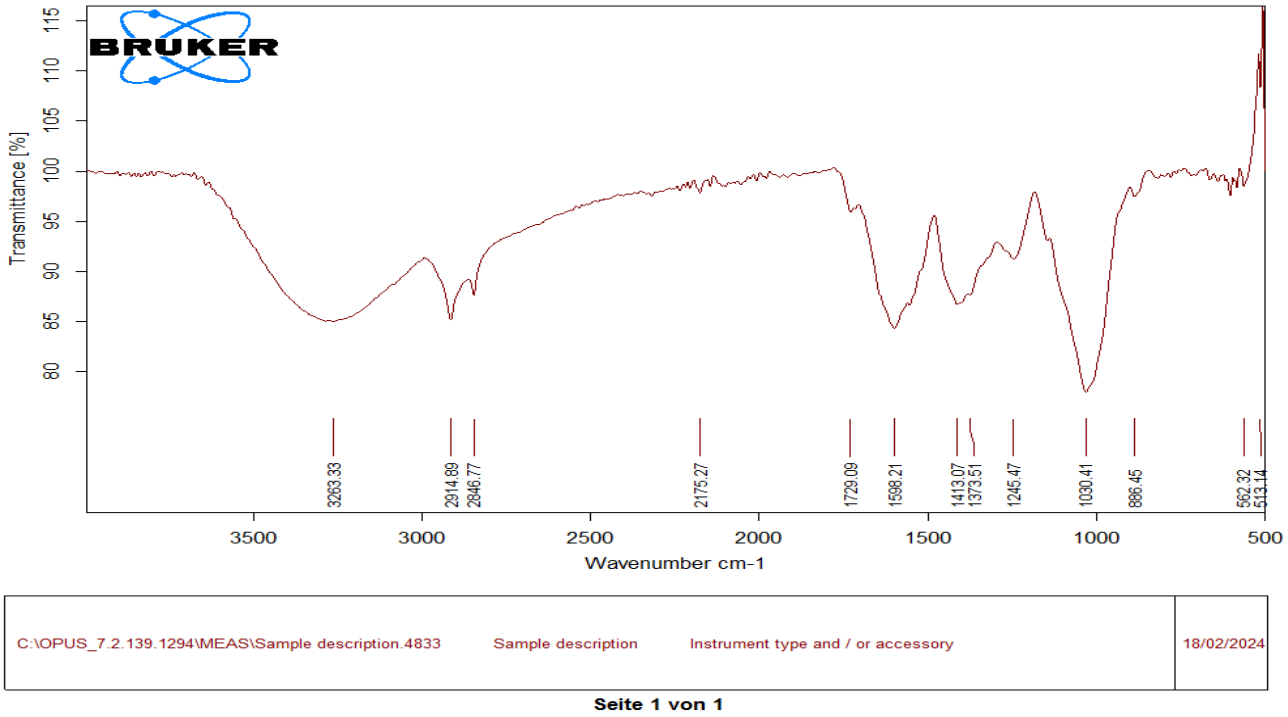


Figure III.2: Infrared spectrum of thymeleae.

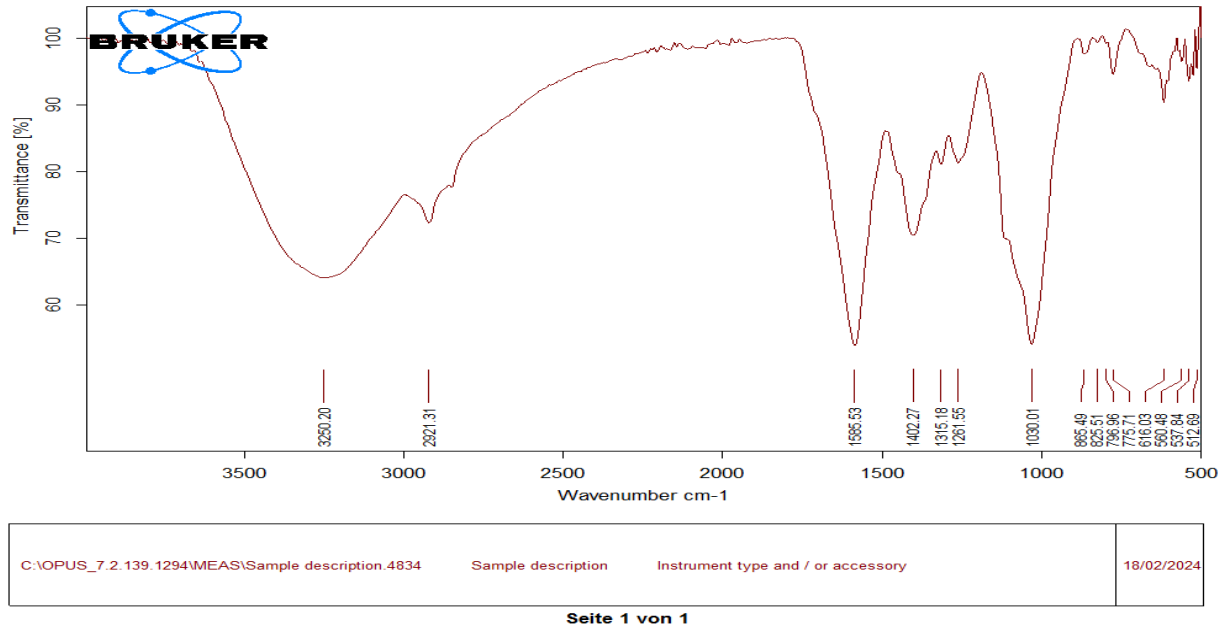


Figure III.3: Infrared spectrum of chamomile**.**

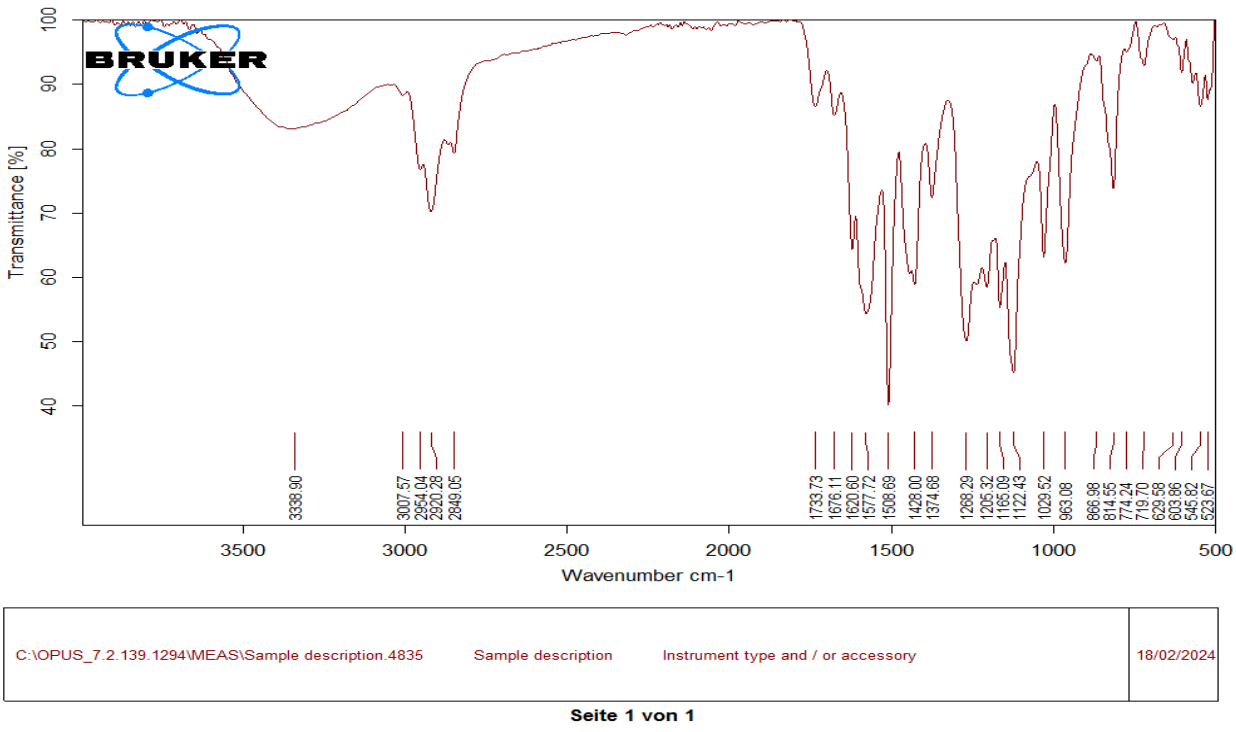


Figure III.4: Infrared spectrum of curcumin**.**

Table III.3: Characteristic bands of extract plants.

plants	Bands
Thymeleae	1030.41 cm^{-1} correspond to C-O function.
	1598.21 cm^{-1} correspond to C=C function.
	2846.77 cm^{-1} correspond to unsaturated C-H function of aldehyde.
	2914.77 cm^{-1} correspond to saturated C-H function.
	3263.33 cm^{-1} correspond to O-H function.
Chamomile	1030.01 cm^{-1} correspond to C-O function.
	1402.27 cm^{-1} correspond to (CH_2), (CH_3) function.
	1585.53 cm^{-1} correspond to C=C aromatic function.
	2921.31 cm^{-1} correspond to saturated C-H function.
	3250.20 cm^{-1} correspond to O-H function.
Curcumin	1268.29 cm^{-1} correspond to C-O function.
	1577.72 cm^{-1} correspond to C=O function.
	2920.28 cm^{-1} correspond to C-H function.
	3338.90 cm^{-1} correspond to O-H function.

The first two spectra present bands around 3263–3250 cm^{-1} in a row corresponding to the OH stretching vibrations characteristic of organic acids. Additionally, there are two medium-intensity bands at 2914 cm^{-1} and 2921 cm^{-1}, indicative of the symmetric C-H stretching vibrations of aliphatic CH_2 and CH_3 groups, respectively. A band in the range of 1598 to 1585 cm^{-1} are attributed to the C=C stretching vibrations of aromatic rings and the bands in the vicinity of 1413-1402 cm^{-1} are assigned to OH deformation vibrations. A strong band at approximately 1030 cm^{-1} is attributed to phenolic C–O stretching, however the bands in the range of 616 cm^{-1} are associated with the deformation vibrations of unsaturated CH bands. The presence of these characteristic absorption bands confirms the richness of the leaf extracts in phenolic compounds in the two studied extracts.

The FTIR spectrum of Cur shows a weak band at 3338.80 cm^{-1}, attributed to the O-H stretching. The band appearing at 1626.11 cm^{-1} corresponds to the stretching vibration of the aromatic C=C band and a moderately intense band at 1577.72 cm-1 is associated with benzene ring stretching. The sharp and intense band at 1508.69 cm^{-1} corresponds to the C=O and C=C vibrations of Cur. The band located at 1268.29 cm^{-1} is attributed to the C-O bond vibration.

III.2.2. PHYTOCHEMICAL ANALYSIS:

The phytochemical evaluation of various phytoconstituents in the studied extracts were graded as high (+++) and nil (-) based on the visual colored reaction product of the test.

Table III.4: Result of phytochemical tests.

test	aqua extract of Thy	aqua extract of Cam
tannin	+++	+++
saponins	+++	+++
quinones	+++	+++
poly phenol	+++	+++
flavonoids	+++	+++
steroids	-	-
alkaloids	+++	+++
coumarins	+++	+++

The employed phytochemical tests indicate the presence of all tested products except the steroid product. For this the obtained products were analyzed and the result will be presented.

III.2.3. QUANTITATIVE ANALYSIS

Determination of total polyphenols

The total polyphenol content of the different extracts is determined using the regression equation y=0.001x-0.031 from the calibration curve of gallic acid which is classed in annex. The results are expressed in milligrams of gallic acid equivalents per gram of dry matter (mg GAE/g) **[3]**.

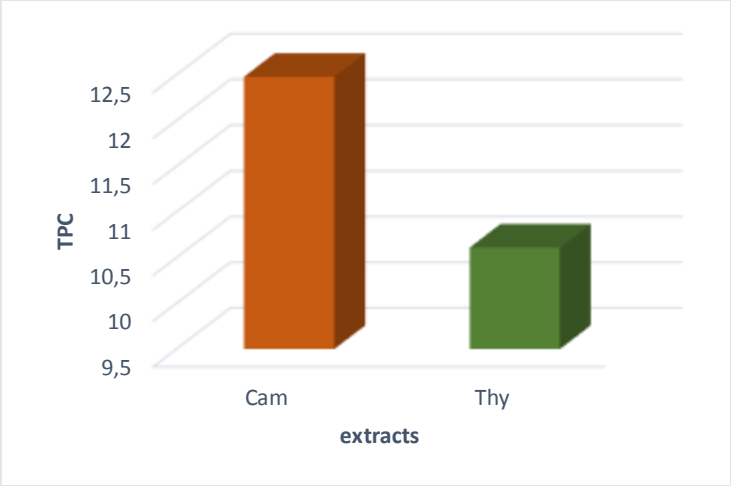


Figure III.5: Total polyphenol content of extracts.

Thy and Cam had a total phenolic content of 10.61 mg GAE/mg extract and 12.48 mg GAE/mg respectively, these values are lower than obtained values with alcoholic solvent in previous works **[4]**.

Determination of total flavonoids

The total flavonoids content of the different extracts is determined using the regression equation y=0.271x+0.0004 from the calibration curve of quercetin. The results are expressed in milligrams of quercetin equivalents per gram of dry matter (mg GAE/g) **[5]**.

,

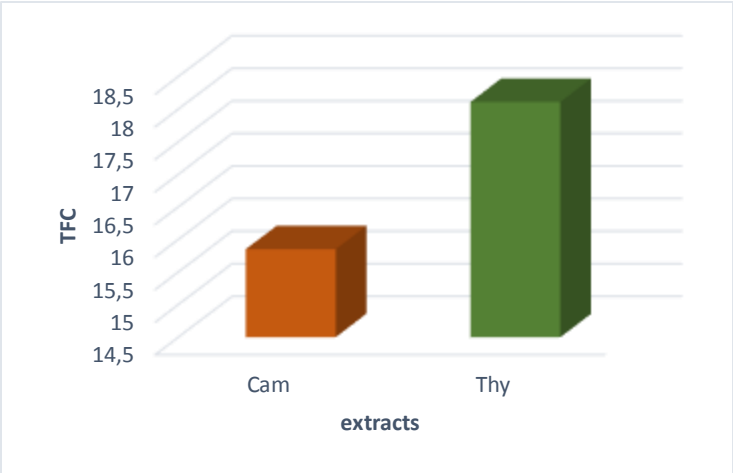


Figure III.6: Flavonoid content of various extracts.

The histogram showed the total content flavonoids in thy and cam extracts. Thy extract had a total flavonoid of 18.11 mg QE/mg and the cam extract had 15.85 mg QE/mg. The obtained values are compared to literature, and they are close **[6]**.

Determination of total tannin content

The tannin content is calculated using the calibration curve of catechin, y = 0.00076 x - 0.0076 **[7]**. It is expressed in milligrams of catechin equivalents per grams of extract (mg CE/g).

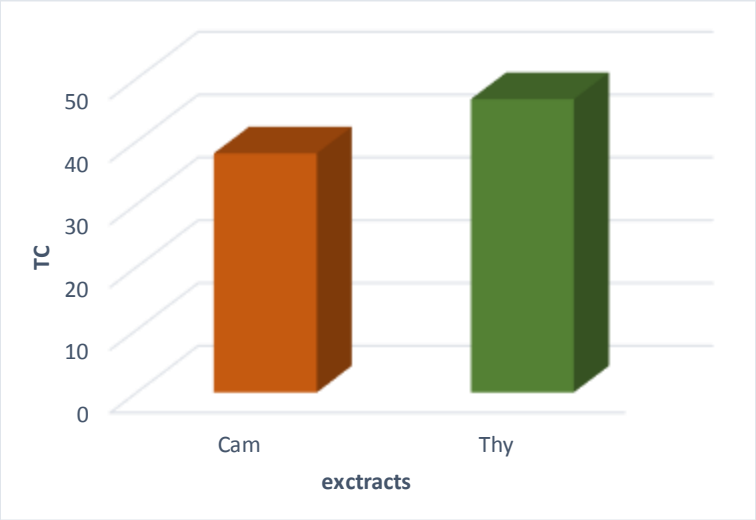


Figure III.7: Tannin content of different extracts.

The studied extracts showed the tannins content of 38.08mg/g for cam and 46.71 mg/g for thy. The use of water as extract solution gives a notable value of chemical compounds as previously determined.

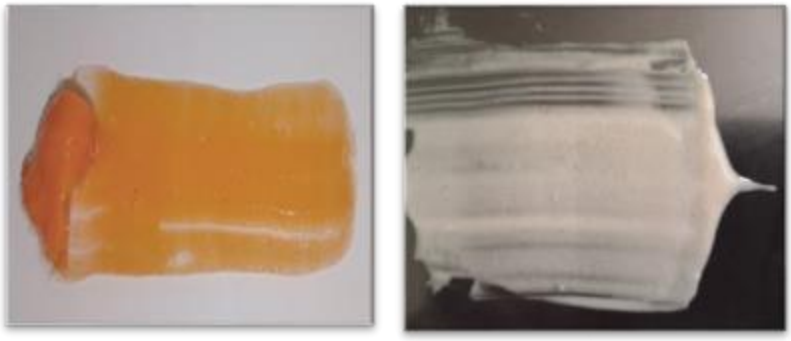
III.3. CARACTERISATION OF GALINICS FORMS:

- **Color:** our creams adapted the color of the extract.
- **Oder:** the odor of our creams is fragrant from the paraffin oil and the other corposants.
- **consistency:** All ointments display a semi-solid texture.
- **Stability:** Ointments maintain stability at 27°C and liquefy at 35°C.
- **Homogeneity:** Our ointments demonstrate excellent uniformity upon spreading several samples on a flat surface.
- **Centrifugation:** we do the test of separation with the centrifugation method. So, the centrifugation was release at 5 min and 30 min, the results are shown in the table III.5:

Table III.5: Result of centrifugation of creams**.**

Creams Time	L1	L2	L3	L4	L5	L6	L7
5 min							
30 min							

- **Spreading:** the creams were passed over a glass to identify their consistence and their morphology when using on the skin. The represented photos are summarized in figure III.8.



A **B**

Figure III.8: Spreading of some creams. (**A**: Cur cream, **B**: Cam cream).

- **pH Level:** Consistency is observed in the pH levels across all formulation so that we obtain the following results:

  The pH of prepared galenic forms was measured and the obtained values were classified in the table III.6., they present a pH close to the skin pH.

Table III.6: The galenic forms pH.

Cream	pH
L1	5.5
L2	5.5
L3	5
L4	5
L5	5.5
L6	5.5

III.4. RELEASE STUDY

The release of extracted plants from various prepared formulations has been evaluated by measuring the amount of PA that is released over time using a UV-visible spectrophotometer. The results are presented in the figures III.9-III.15.

The obtained data indicates a low release percentage, not exceeding 50% for the majority of prepared galenic forms. This is primarily justified by the lipidic composition of the prepared galenic forms, which makes the liquid's penetration (pH=5.5) into the interior of the galenic form difficult. However, the lipid-rich environment of the skin facilities their penetration.

The obtained release curves indicate that the release rate is highest at the beginning and then gradually decreases that is explained by the slow diffusion of the active ingredient. After 50mn, a continuous release pallier achieved the diffusion.

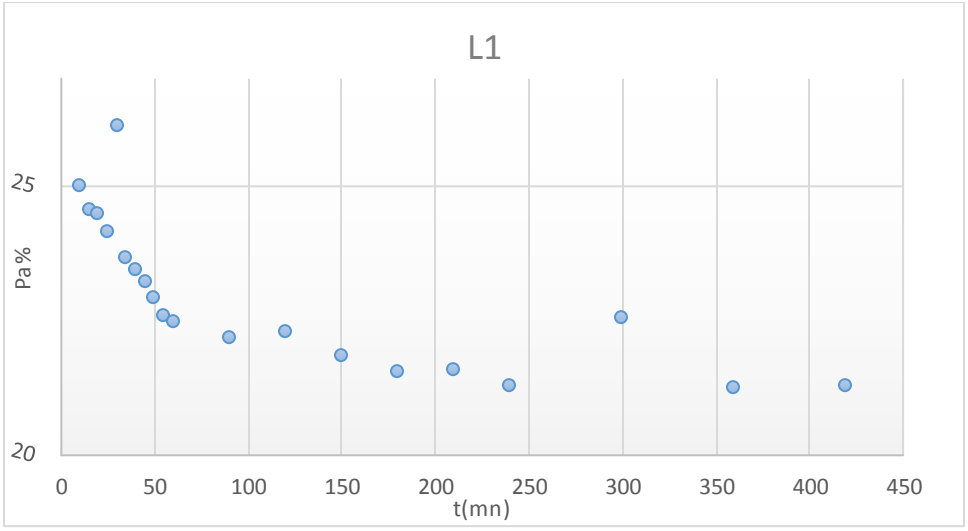


Figure III.9: Liberation curve of Cur from L1.

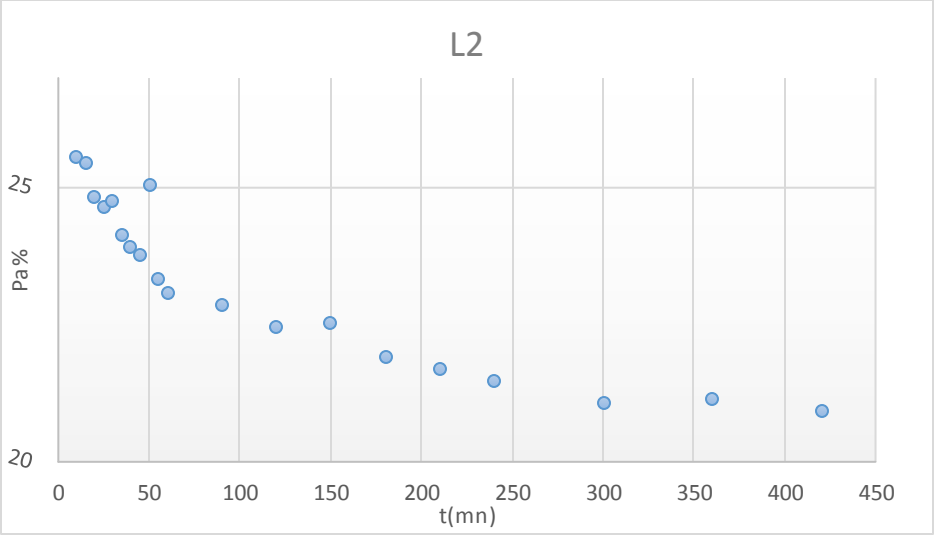


Figure III.10: Liberation curve of Cur from L2.

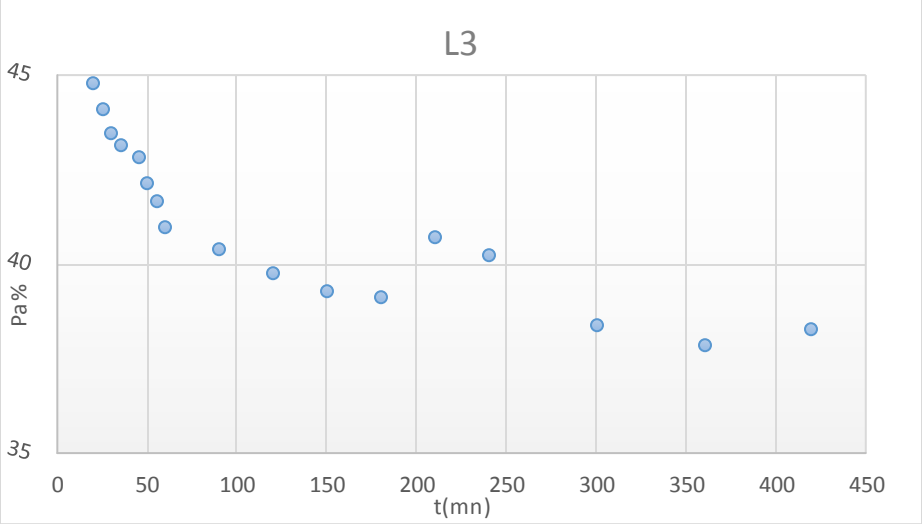


Figure III.11: Liberation curve of Cam from L3.

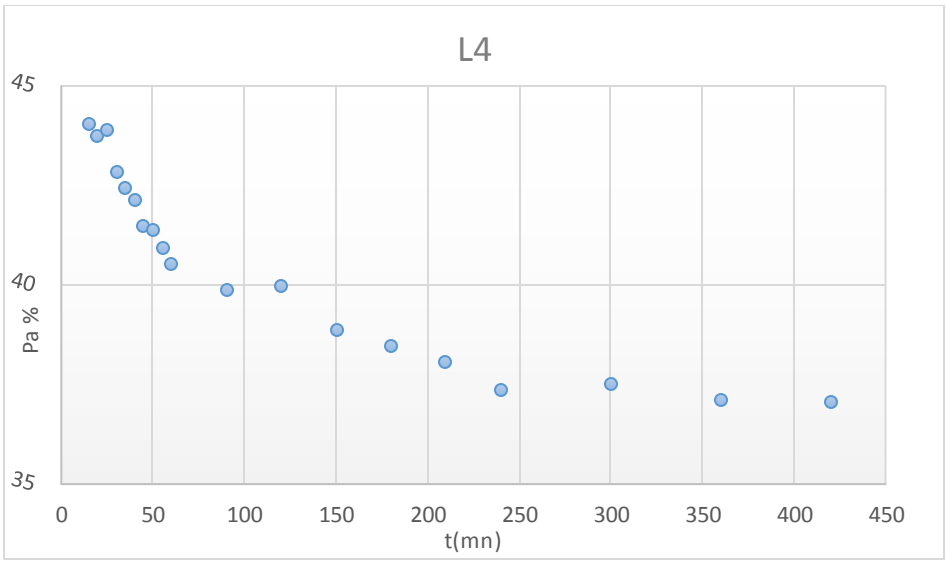


Figure III.12: Liberation curve of Cam from L4.

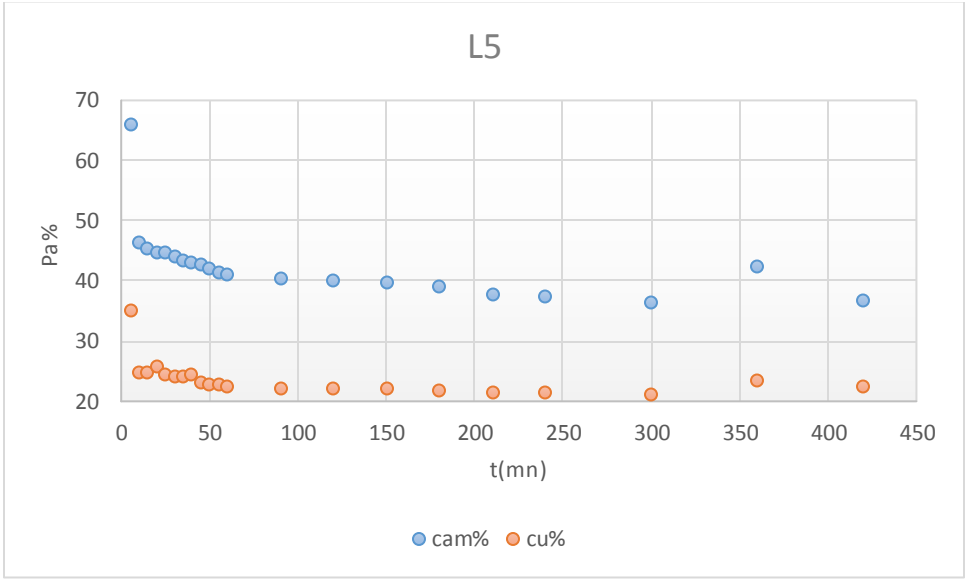


Figure III.13: Liberation curve of active agents from L5.

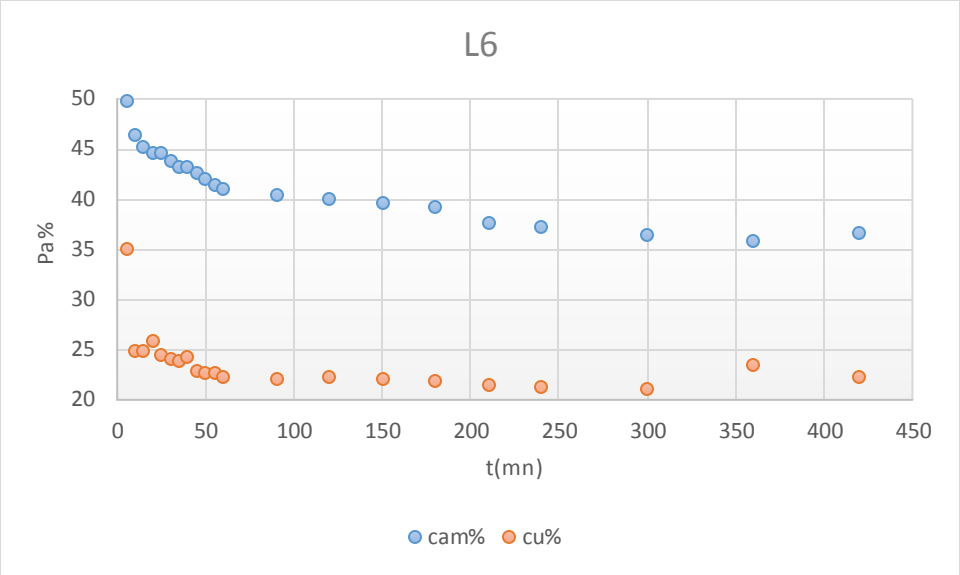
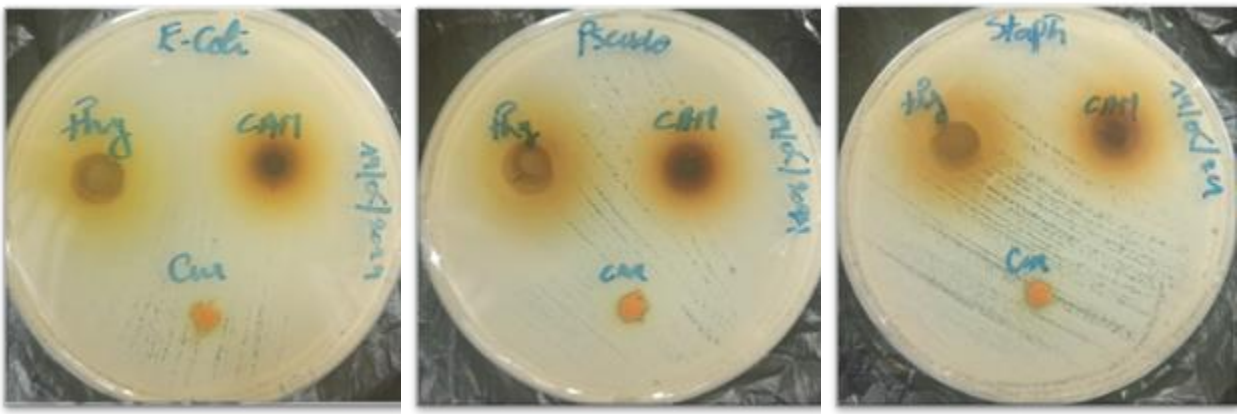


Figure III.14: Liberation curve of active agents from L6.

III.5. BACTERIAL TEST

The activity of the studied extracts and their formulations were assessed based on the presence or absence of bacterial growth inhibition. The presence of antibacterial activity was indicated by clear zones around the discs soaked with the extracts, while the absence of inhibition was reflected by the absence of such halos around the wells. This evaluation was conducted after 24 hours of incubation at 37°C. The obtained results are presented in the following figures:



a **b** **c**

d **e** **f**

Figure III. 15: Results of antibacterial activity against different strains (a, b, c, d: extracted plants, e: L5 and L6, f: L3 and L4).

The antibacterial activity tests revealed different activities against the various tested strains. The inhibition zones and their diameters are presented in the tables (Annex II.4) and in the figures below.

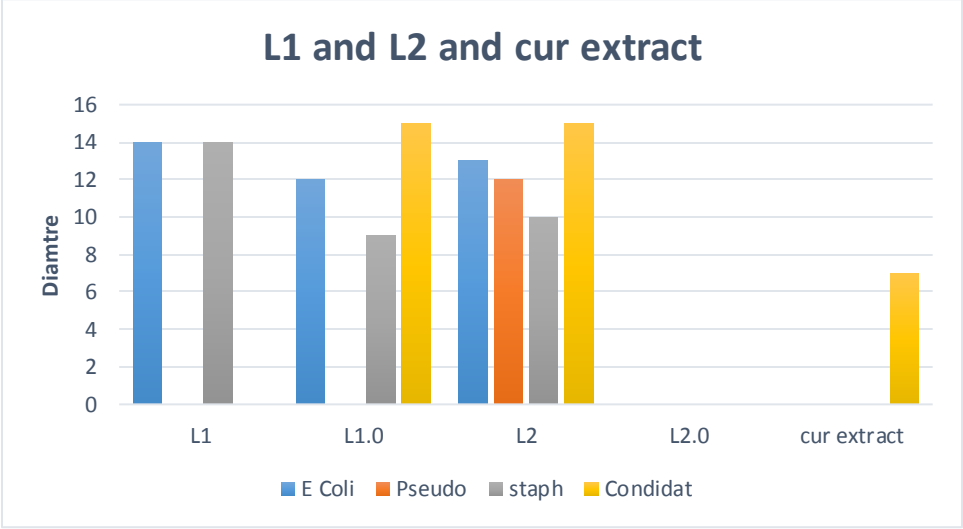


Figure III.16: Inhibition diameters of L1.0, L2.0, L1, L2, and the cur extract against the studied strains.

According the figure (**III.17),** it is evident that cur extract demonstrates antifungal activity; however, when formulated as L1, it exhibits an antibacterial activity against *E. Coli* and *Staph*,

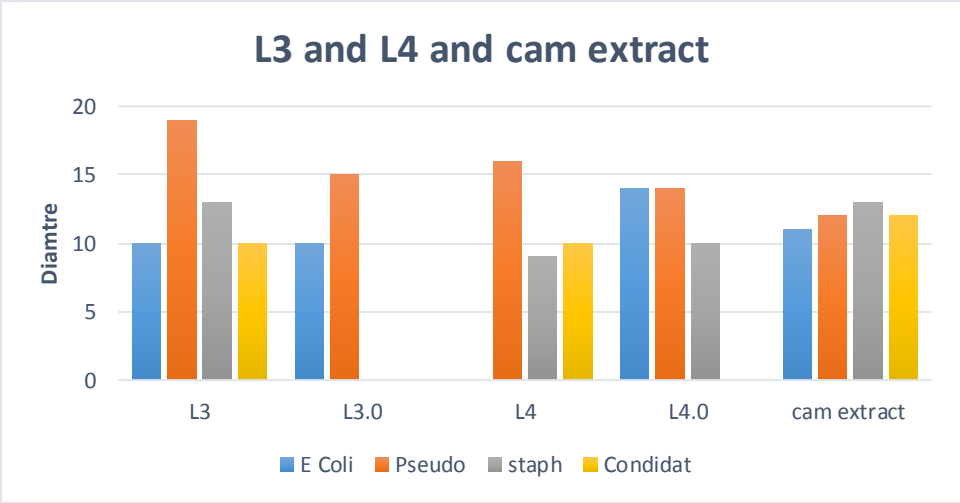
This can be explained by the associative effect between L1.0 and cur extract against these strains, but for the *Candidat Albicans*, the antagonist effect is noted.

The L2 ointment has shown a very good antifungal and bacterial activity of the core extract compared to L1, the composition of the L2 ointment has a considerable antifungal effect and an important antibacterial effect once associated with cur extract.

According to the figure **III.17**, the cam extract presents an important activity against all studied strains. For L3, the antibacterial activity was preserved after formulations, but when comparing the ointment L3 and L3.0, an associative effect is noticed for the *pseudo* and an antagonist effect for the *E. Coli* and *Candidat.*

On the other hand, For the L4 and L4.0 ointment, an associative effect is noted for the *Pseudo*, and an antagonistic effect for the *E. coli, staph* and *Candidat strains.*

The formulation of cam (L3, L4 ointments) gave an antifungal activity, which is not found in the base ointment L3.0 and L4.0.



FigureIII.17: Inhibition diameters of L3.0, L4.0, L3, L4, and the cam extract against the studied strains.

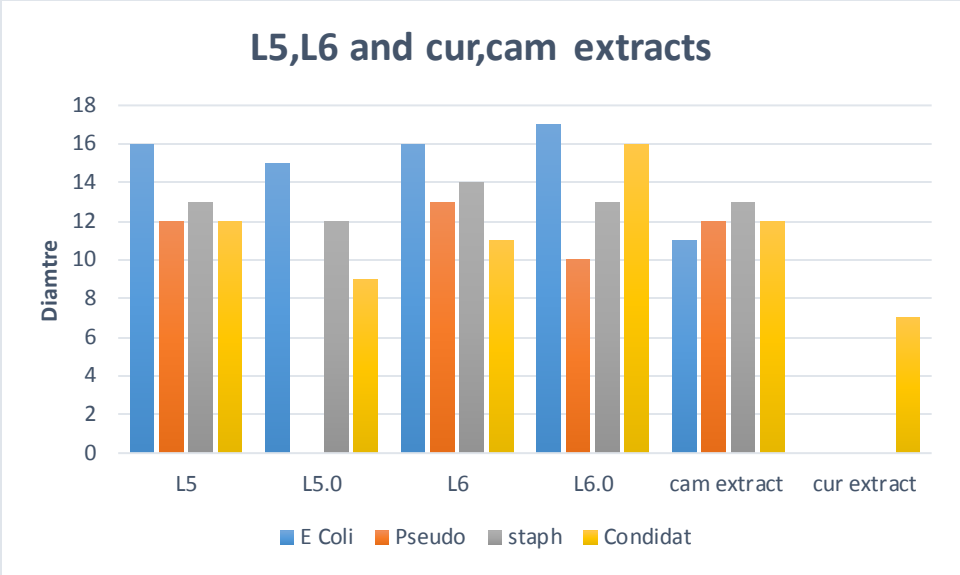


Figure III.18: Inhibition diameters of L5.0, L6.0, L5, L6, and the cam, cur extracts against the studied strains.

By comparing the biological activity of curcumin and the cam extracts with the ointment L5 and L6 loaded in different percentages, we note, that the two ointments have a good biological activity once the extracts are combined.

Comparing the composition of L5 and L6 formulation, the antifungal activity is accentuated when cur present 2% (L5) and the bacterial activity is important against *pseudo* and *staph* when cam present 2% (L6).

It is also noted that the base ointment L6.0 has a very good biological activity without being associated with any active extract.

III.6. MINIMAL CONCENTRATION OF INHIBITORY

For the evaluation of the effectiveness of the prepared formulations, it is necessary to determine the minimum inhibitory concentration. The obtained results of well plates and the tested concentration are summarized in the next tables.

Table III.7: Result of antibacterial activity of Cam extract with the method of MIC.

Concentrations (mg /ml) Of Chamomile extract												
Strains	50	25	12.5	6.25	3.12	1.56	0.78	0.39	0.19	0.09	Positive control	Negative control
Pseudo	-	-	-	-	MCI -	+	+	+	+	+	+	-
Staph	-	-	-	-	MCI -	+	+	+	+	+	+	-
E-coli	-	-	-	-	MCI -	+	+	+	+	+	+	-
Candida	-	-	-	-	MCI -	+	+	+	+	+	+	-

Table III.8: Result of antibacterial activity of Cur extract with the method of MIC.

Concentrations (mg /ml) Of Turmeric												
Strains	50	25	12.5	6.25	3.12	1.56	0.78	0.39	0.19	0.09	Positive control	Negative control
Pseudo	-	-	-	MCI -	+	+	+	+	+	+	+	-
Staph	-	-	MCI -	+	+	+	+	+	+	+	+	-
E-coli	-	-	-	MCI -	+	+	+	+	+	+	+	-
Candida	-	-	-	MCI	+	+	+	+	+	+	+	-

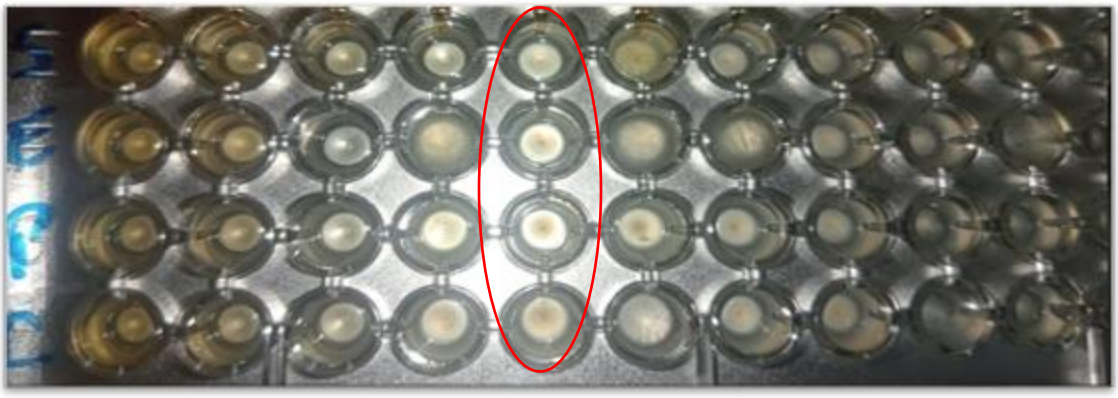


Figure III.19: MIC results for Chamomile extract.

By comparing the non-rigid parity of gram strains, the results are similar for the extract of turmeric as far as chamomile is concerned, the minimum inhibition of concentration is 3.12mg/mL for the entire strain. The least values of MIC of tested extracts correspond to the cam so, the most effective extract is chamomile, a lower MIC.

III.7. ANTIOXYDANT ACTIVITY

The obtained curve of Cur demonstrates a negative value which show that Cur hasn't an antioxidant activity.

the ascorbic acid and Cam activities represent 44.8 mg/mL and 74.24mg/mL respectively.

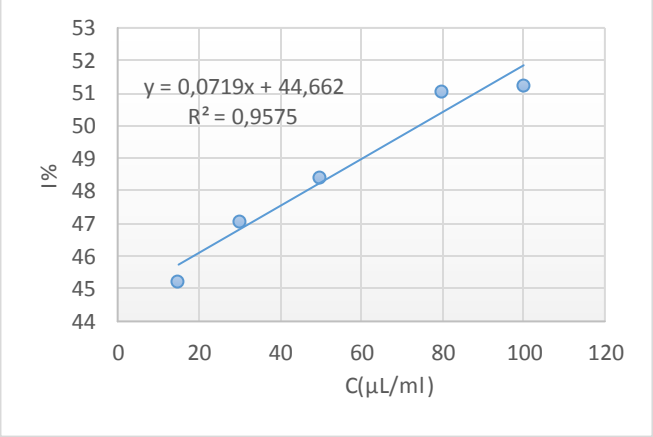


Figure III.20: Inhibition percentage of free radical DPPH in function of concentration of Cam extract.

III.8. ANTIINFLAMATORY ACTIVITY

The maximum inhibition and denaturation effect of the cam extract at the concentration of 100µg/ mL was 39.97%, whereas the diclofenac standard at 100 µg/mL showed a 98.19% inhibition of the denaturation of the SAB. The correlation between the concentration and the percentage of inhibition showed that the inhibition capacity of the denaturation of the BSA that the extract of chamomile which weaker than diclofenac.

Table III.9: % inhibition of denaturation of extracted plant.

Concentration	Extracted plants	% Inhibition of denaturation
100µg/mL	Cam	39.97%
100µg/mL	Diclofenac (reference)	98.19%

III.9. IN VIVO TEST

First experience:

In order to assess the anti-inflammatory properties of prepared galenic forms, it was tested on Wistar rats that had been experimentally injected with 1% formol at the level of their right paw.

Figure III.21: paw of rat before treatment.

Figure III.22: rat paw after treatment.

The evolution of the patten diameters is illustrated in figure **III.23.**

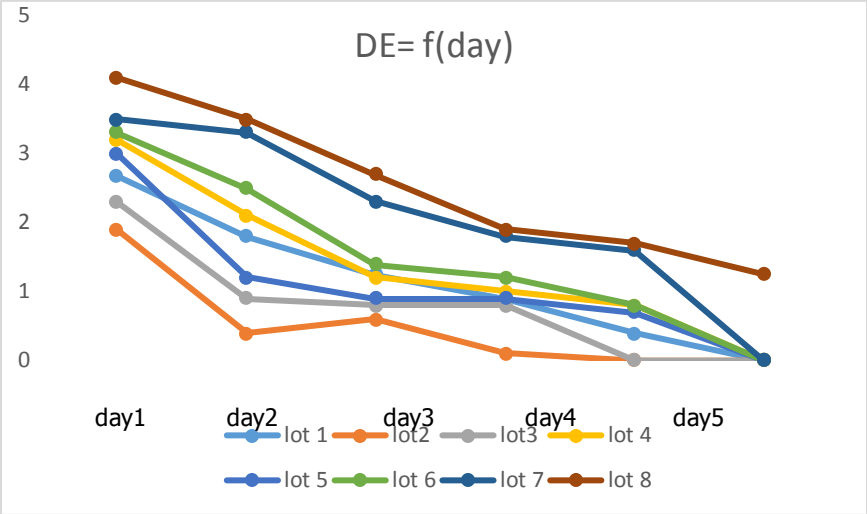


Figure III.23: Evolution of the patten diameters.

The oedema appears within the first hour of the first hour of the 1% formol injection.

The observations indicate that batch 8 (rats injected with 1% formol without treatment) presents a low decrease of the paw's diameter due to the immune system of the tested rats without returning to normal state at the end of the experiment.

The rats treated with prepared galenic forms showed a significant reduction in oedema and were back to its normal state at the sixth day. On the other hand, rats given L2 and L3, which contains Cur1%+ZnO 1% and Cam 1% respectively, their paws returned to normal state at the fifth day and the increasing is notable at the second and third days.

The galenic forms containing Cam 1% (L3 and L5) show an important reducing in oedema compared to the others with 1,5% and 2% (L4 and L6).

Result and Discussion references

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General conclusion

Medicinal plants have many established therapeutic uses because they can contain numerous active chemicals. For this purpose, this work concentrates on some extracted plants and their formulation into galenic forms (creams and shampoo).

Initially, the extracted of essential oil was proposed and since it was difficult to obtain, one moved on to plant extracts. These last were attained through hydro-maceration and they were characterized by UV, IR-spectrum, and their phytochemicals analysis were also studied and quantified. The results show that extracted plants using water gives a notable value of active chemical compounds.

Many galenic forms were prepared and they were tested to confirm their stability, the effectiveness of these creams and were analyzed by anti-bacterial, antioxidant, anti-inflammatory tests. The obtained fallouts were compared of extracted plants' results.

The extracted plants and their formulation present and important bacterial and antifungal activities with noticed difference between a formulation to other, this remark is justified by the antagonist and additional effects of some excipient or combination between extracted plants. Consuming the same used strains (*staph, pseudo, E. Coli and candida*), the minimal inhibitory concentration was determined. The least value of MIC is 3.12mg/mL correspond to the cam which present the most effective extract.

The antioxidant activity of cam extract is being noticed by the calculated IC50 of 13.68 mg/mL.

The in vitro anti-inflammatory shows that chamomile remains weaker activity than diclofenac and the in vivo test illustrates that all creams present an anti-inflammatory activity demonstrating a difference of reducing oedema.

General conclusion

As conclusion, the galenic forms (creams) give important upshots in studied biological activities as so as their extract plants.