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THÈME

Contribution to the *In Vitro* Study of the Antioxidant and Antimicrobial Activities of *Urtica dioica* L. Extracts from the Mostaganem Region for Cosmeceutical Applications.

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(من لم يشكر الناس لم يشكر الله)

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Dedication

*I dedicate this work to my persevering **self**, who overcame all challenges with patience and wisdom.*

*To my beloved **parents**, whose constant support, love, and encouragement have been the foundation of my success.*

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*And to everyone who helped me and everyone who greeted me with a **smile** and restored my **hope** this is all for you.* ☺

BOUABBACI Ậyatte

List of Abbrevisations

ABTS : 2,2'-Azino-Bis(3-ethylbenzothiazoline-6-sulfonic acid)

BPH : Benign Prostatic Hyperplasia

CFU : Colony Forming Unit

Cm : Centimetre

di : Differential index

DMAP : Distribution Mapping and Analysis Program

DMSO : Dimethyl Sulfoxide

DNA : Deoxyribonucleic Acid

DPPH : 2,2-diphenyl-1-picrylhydrazyl

Dr : Doctor

ds : Distribution coefficient

E. coli **:***Escherichia Coli*

EC_{50} : effective concentration

EM : Extract by Maceration

ES : Extract by Soxhlet

ETC : Electron Transport Chain

$FeCl_3$: Ferric Chloride

G- : Gram negative

g : Gram

G+ : Gram positive

H : Hour

H_2O_2 : Hydrogen peroxide

HCl : Hydrochloric acid

HPLC : High Performance Liquid Chromatography

IC_{50} : Half Maximal Inhibitory concentration

L : Liter

LD_{50} : Median Lethal Dose

mg : Milligram

Min : Minute

mL: Milliliter

mm : Millimeter

No : Number

NaCl : Sodium Chloride

NADPH : Nicotinamide Adenine Dinucleotide Phosphate

NaOH : Sodium Hydroxide

nm : Nanometre

O_2^- : Superoxide anion

OD : Optical Density

OH• : Hydroxyl radicals

P450 : Cytochrome P450 (Enzyme with absorption peak at 450 nm)

pH : Potential of Hydrogen

Rf : Retention Factor

ROS : Reactive Oxygen Species

TLC: Thin-Layer Chromatography

V : Volume

% : Percent

& : and

°C : Degrees Celsius

µL : Microliter

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Abstract

This study aims to enhance the value of Urtica dioica by evaluating its biological properties, particularly its antioxidant and antibacterial activities, as well as its potential use in the preparation of natural cosmetic products.

The study includes a theoretical part, presenting the main botanical, chemical, and pharmacological characteristics of this plant. The practical part involved the extraction of active compounds using both maceration and Soxhlet methods, followed by physical, chemical, and biological analyses.

This experimental work focused on assessing the biological properties of Urtica dioica extracts obtained through two methanolic extraction methods: Soxhlet extraction (ES) and maceration (EM). The study covered the identification of bioactive compounds, the evaluation of antioxidant and antibacterial activities, and the formulation of natural cosmetics based on the plant extracts.

The results showed that the Soxhlet method yielded a slightly higher extract percentage than maceration (51% vs. 50%), reflecting the influence of the extraction method on the amount of compounds obtained.

Phytochemical screening confirmed the presence of secondary metabolites such as phenols, flavonoids, alkaloids, tannins, and coumarins. Thin-layer chromatography (TLC) further confirmed these findings, revealing variations in compound composition depending on the extraction method.

Regarding antioxidant activity, the DPPH assay showed that the EM extract exhibited stronger activity compared to the ES extract. The IC_{50} value was 27.35 mg/ml for EM versus 52.8 mg/ml for ES, indicating a higher free radical scavenging capacity in the macerated extract.

Concerning antibacterial activity, the results showed that the extracts were effective against some bacterial strains, particularly *Bacillus cereus*, which was the most susceptible, while other strains, such as *Escherichia coli*, showed significant resistance. These results were explained by the characteristics of the bacterial cell wall and the varying ability of plant compounds to penetrate it.

Finally, this work culminated in the development of two natural cosmetic products: a soap and a skin cream formulated with Urtica dioica extract. Preliminary tests showed acceptable properties in terms of stability, pH, foaming, and texture, enhancing their potential for use in biocosmetics.

Keywords: *Urtica dioica.L.* Antioxidant activity; Antibacterial activity; Methanolic extract; Natural cosmetics.

Résumé

Cette étude vise à valoriser l'ortie en évaluant ses propriétés biologiques, notamment ses activités antioxydante et antibactérienne, ainsi que son potentiel d'utilisation dans la préparation de produits cosmétiques naturels.

Le travail comprend une partie théorique présentant les principales caractéristiques botaniques, chimiques et pharmacologiques de cette plante. La partie pratique a consisté en l'extraction des composés actifs par les méthodes de macération et de Soxhlet, suivie d'analyses physiques, chimiques et biologiques.

Cette étude expérimentale s'est concentrée sur l'évaluation des propriétés biologiques des extraits méthanoliques d'ortie obtenus par deux méthodes d'extraction : Soxhlet (ES) et macération (EM). L'étude a porté sur l'identification des composés bioactifs, l'évaluation des activités antioxydante et antibactérienne, ainsi que la formulation des produits cosmétiques naturels à base d'extraits de cette plante.

Les résultats ont montré que la méthode Soxhlet a permis d'obtenir un rendement légèrement supérieur à celui de la macération (51 % contre 50 %), reflétant l'influence de la technique d'extraction sur la quantité de composés extraits.

Le criblage phytochimique a confirmé la présence de métabolites secondaires tels que les phénols, flavonoïdes, alcaloïdes, tanins et coumarines. La chromatographie sur couche mince (CCM) a renforcé ces résultats en révélant des variations dans la composition selon la méthode d'extraction utilisée.

Concernant l'activité antioxydante, le test au DPPH a montré que l'extrait obtenu par macération (EM) présentait une activité plus élevée que celui obtenu par Soxhlet (ES). La valeur de l'IC_{50} était de 27,35 mg/ml pour EM contre 52,8 mg/ml pour ES, indiquant une plus grande capacité de piégeage des radicaux libres dans l'extrait macéré.

En ce qui concerne l'activité antibactérienne, les résultats ont montré que les extraits étaient efficaces contre certaines souches bactériennes, notamment *Bacillus cereus*, la plus sensible, tandis que d'autres, comme *Escherichia coli*, ont présenté une résistance notable. Ces résultats s'expliquent par les caractéristiques de la paroi cellulaire bactérienne et la capacité variable des composés végétaux à la traverser.

Enfin, ce travail a abouti à la formulation de deux produits cosmétiques naturels : un savon et une crème à base d'extrait d'ortie. Les tests préliminaires ont révélé des propriétés acceptables en termes de stabilité, de pH, de pouvoir moussant et de texture, renforçant leur potentiel d'utilisation en biocosmétique.

Mots Clés : *Urtica dioïca* L ; Activité antioxydante ; Activité antibactérienne ; extrait méthanolique ; cosmétiques naturels.

الملخص

تهدف هذه الدراسة إلى تثمين نبات القراص من خلال تقييم خصائصه البيولوجية، لا سيما نشاطه المضاد للأكسدة والمضاد للبكتيريا، بالإضافة إلى إمكانية استخدامه في تحضير مستحضرات تجميل طبيعية.

تتضمن الدراسة جزءًا نظريًا يعرض الخصائص النباتية والكيميائية والدوائية الرئيسية لهذا النبات، وجزءًا تطبيقيًا تم فيه استخراج المركبات النشطة باستخدام طريقتي النقع (المحضر البارد) والسوكسليه (الاستخلاص الحراري)، متبوعًا بتحاليل فيزيائية وكيميائية وبيولوجية.

ركز هذا العمل التجريبي على تقييم الخصائص البيولوجية لمستخلصات القراص التي تم الحصول عليها باستخدام طريقتين للاستخلاص بالميثانول: استخلاص سوكسليه (ES) والنقع (EM). شملت الدراسة تحديد المركبات النشطة بيولوجيًا، وتقييم النشاط المضاد للأكسدة والمضاد للبكتيريا، وصياغة مستحضرات تجميل طبيعية تعتمد على مستخلصات هذا النبات.

أظهرت النتائج أن طريقة السوكسليه أعطت مردودًا أعلى قليلًا من طريقة النقع (51٪ مقابل 50٪)، مما يعكس تأثير طريقة الاستخلاص على كمية المركبات المستخلصة.

أكد الفحص الكيميائي النباتي وجود العديد من المستقلبات الثانوية مثل الفينولات، الفلافونويدات، القلويدات، التانينات والكومارينات. كما أكدت تقنية التحليل بالكروماتوغرافيا على الطبقة الرقيقة (TLC) هذه النتائج من خلال الكشف عن اختلافات طفيفة في تركيبة المركبات حسب طريقة الاستخلاص المستخدمة.

فيما يخص النشاط المضاد للأكسدة، أظهر اختبار DPPH أن مستخلص النقع (EM) أظهر فعالية أعلى مقارنةً بمستخلص السوكسليه (ES)، حيث بلغت قيمة IC_{50} لمستخلص النقع 27.35 ملغ/مل مقابل 52.8 ملغ/مل لمستخلص السوكسليه، مما يدل على قدرة أعلى على تحييد الجذور الحرة في المستخلص الناتج عن النقع.

أما بالنسبة للنشاط المضاد للبكتيريا، فقد أظهرت النتائج أن المستخلصات فعالة ضد بعض السلالات البكتيرية، وخصوصًا *Bacillus cereus* التي كانت الأكثر حساسية، في حين أظهرت سلالات أخرى مثل *Escherichia coli* مقاومة كبيرة. وقد فُسرت هذه النتائج بخصائص الجدار الخلوي للبكتيريا واختلاف قدرة المركبات النباتية على اختراقه.

وأخيرًا، تُوِّج هذا العمل بتطوير منتجين تجميليين طبيعيين: صابون وكريم جلدي يحتويان على مستخلص القراص. أظهرت الاختبارات الأولية خصائص مقبولة من حيث الثبات، ودرجة الحموضة والرغوة والملمس، مما يعزز إمكانيتهما للاستخدام في مجال التجميل الحيوي.

الكلمات المفتاحية : *Urtica dioïca* L. ؛ نشاط مضاد للأكسدة ؛ نشاط مضاد للبكتيريا؛ مستخلص ميثانولي؛ مستحضرات تجميلية طبيعية.

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Introduction

Plants, particularly green plants, are well known for their biological activity due to the presence of essential bioactive compounds. In recent years, interest in these natural compounds has grown significantly, especially for their use in the pharmaceutical and cosmetic industries **(Ekor, 2014).**

Among these compounds, antioxidants are of particular importance. They play a key role in neutralizing oxidative stress caused by free radicals, which are responsible for various skin disorders and serious chronic diseases, including cardiovascular conditions and cancer **(Pham-Huy *et al.,* 2008)**. Therefore, medicinal plants are considered valuable allies in the fight against oxidative stress and its associated health risks.

At the same time, the rapid emergence of microbial resistance to conventional antibiotics has become a critical global health concern. This issue has triggered a growing interest in the search for alternative antimicrobial agents derived from natural sources, particularly those with antibacterial and antifungal properties **(Ventola, 2015)**. In this context, medicinal plants offer promising solutions owing to their complex phytochemical compositions that reduce the likelihood of resistance development.

One such plant is *Urtica dioica*, a herbaceous species from the *Urticaceae* family, commonly known as stinging Urtica dioica. It is widely recognized for its broad medicinal potential and rich phytochemical composition, including polyphenols, flavonoids, and essential minerals. Traditionally used in herbal medicine, *Urtica dioica* is known for its antioxidant, anti-inflammatory, and antimicrobial properties. Recent scientific studies have further emphasized its potential in dermatological and cosmetic applications, particularly due to its bioactive components that support skin protection and inhibit microbial proliferation.

Recent scientific studies have further highlighted the cosmeceutical potential of *Urtica dioica*, particularly in dermatological applications. Its bioactive compounds not only help protect skin cells from oxidative damage but also inhibit the proliferation of pathogenic microorganisms, making it a compelling candidate for incorporation into natural skincare and personal care formulations **(Gülçin *et al.,* 2004; Kosalec *et al.,* 2009)**.

While various studies have confirmed the biological activities of Urtica dioica extracts, additional in vitro investigations are needed to better elucidate their mechanisms of action and assess their effectiveness in targeted applications.

The present study aims to evaluate the antioxidant and antimicrobial activities of *Urtica dioica* extracts collected from the Mostaganem region in Algeria. The study involves the preparation of plant extracts using two different extraction techniques, followed by phytochemical screening, antioxidant assays (such as DPPH), and antimicrobial testing against selected bacterial strains. The ultimate objective is to explore the potential of this plant as a natural ingredient for the development of safe and effective cosmeceutical products.

This research is structured into two main parts. The first part presents a literature review outlining the general characteristics and biological activities of *Urtica dioica*. The second part details the experimental methodology and presents the results obtained, with an analysis of their relevance in the context of future cosmeceutical use.

LITERATURE REVIEW

1. Introduction

Stinging Urtica dioica (*Urtica dioica*) is a perennial herb widely known in traditional medicine for its therapeutic benefits. Historically, it has been used by peoples of various cultures to treat ailments such as arthritis, skin diseases, and urinary tract disorders **(Chrubasik *et al.,* 2007)**. In recent years, this plant has gained increasing scientific interest due to its richness in biologically active compounds with diverse pharmacological effects.

Through botanical studies, it has been found that the Urtica dioica plant consists of stinging hairs distributed throughout its leaves, stems, and roots. It also has an additional component that distinguishes it from many other medicinal plants: small green flowers.

All parts of this plant, including the roots, stems, and especially the leaves, contain essential plant compounds that enhance its antioxidant and antibacterial properties, which were evaluated in this study through laboratory analysis.

Key bioactive constituents of stinging Urtica dioica include flavonoids, vitamins (notably vitamins A and C), minerals (such as iron, calcium, and magnesium), as well as organic acids and phenolic compounds **(Gülçin *et al.,* 2004)**. These phytochemicals are believed to underlie the plant's observed biological activities, including its antioxidant, antimicrobial, anti-inflammatory, and even anticancer properties **(Upton, 2013)**.

2. Botanical Aspects

2.1. History and Traditional Uses

In ancient times, some civilizations such as those of Egypt, Greece, and Rome used stinging Urtica dioica for therapeutic purposes, including the treatment of arthritis, relief of joint pain, and stimulation of blood circulation, particularly in women.

In medieval Europe, Urtica dioica was commonly employed to treat both minor and more complex skin conditions, such as psoriasis, and was also used as a cleansing agent for the body.

Today, Urtica dioica continues to be a widely used remedy in alternative medicine, often consumed as a tonic or anti-rheumatic drink **(Upton, 2013) (Fig. 01)**.

Figure 01: *Urtica dioica* plant *(stinging Urtica dioica).*

2.2. Botanical Description

- **Leaves:** Opposite, dark green, heart-shaped leaves with serrated edges, covered with stinging hairs that inject histamine and other irritant substances **(Fig.02)**.

Figure 02 : The leaves of *Urtica dioica*.

❖ **Stem :** Stem: Erect, quadrangular stems, also covered with stinging hairs **(Fig.03)**.

Figure 03: The stem of *Urtica dioica*.

❖ **Flowers:** Small, green, unisexual flowers forming in axillary clusters **(Fig.04).**

Figure 04 : The flowers of *Urtica dioica*.

❖ **Roots :** This herbaceous plant has creeping roots with fibrous roots **(Fig.05)**.

Figure 05 : The roots of *Urtica dioica*.

❖ **Stinging hairs:** Needle-like structures containing acetylcholine, histamine, serotonin, and formic acid, responsible for the characteristic sting upon contact **(Thiem and Goslinska, 2004) (Fig.06)**.

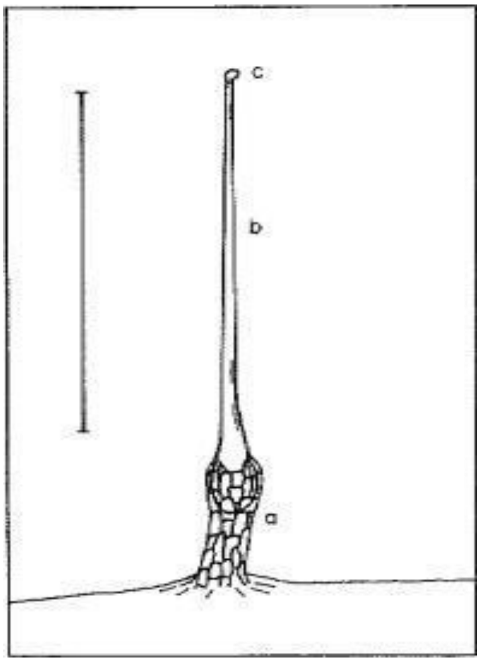

Figure 06: Stinging hairs of *Urtica dioïca* **:**

(a): multicellular pedestal; **(b):** stinging cell; **(c):** swollen, oblique tip of stinging cell.

Scale line=1mm **(Pollard and Briggs, 1984).**

2.3. Taxonomy and Classification

Urtica dioica, commonly known as stinging Urtica dioica, belongs to the *Urticaceae* family, which comprises about 54 genera and over 2,000 species, predominantly found in temperate and tropical regions. This species is the most widespread and well-known among the *Urtica genus*, due to its medicinal and nutritional significance **(Tadhani *et al.,* 2007)**.

The species *epithet dioica* refers to the *dioecious* nature of the plant, meaning that male and female flowers are borne on separate individuals. This distinctive feature aids in the identification and ecological understanding of the species **(Randall, 2012)**.

Members of the *Urticaceae* family are characterized by the presence of stinging hairs (trichomes) containing histamine and other irritants, which serve as a defense mechanism against herbivores. The full taxonomic classification of *Urtica dioica* is as follows:

Table 01: The classification of *Urtica dioica* **(CISEH, 2018).**

Kingdom	*Plantae*
Phylum	*Magnoliophyta*
Class	*Magnoliopsida*
Subclass	*Hamamelidae*
Order	*Urticales*
Family	*Urticaceae*
Genus	*Urtica*
Subject	*Urtica dioica L*

2.4. Geographical Distribution and Ecology

Stinging Urtica dioica is native to the continents of Europe, Asia, North America, and even North Africa, where it grows in moist, nitrogen-rich soils, typically near rivers, forest edges, and roadsides. Its preferred growing climate is temperate, with adequate sunlight **(Chrubasik *et al.*, 2007)**.

The following map, drawn by Stephanie Ames using Dr. A. Morton's **DMAP** software and based on data collected by members of the Botanical Society of the British Isles, shows the distribution of *stinging Urtica dioica* in the British Isles. Each dot represents at least one record within a 10- square-kilometer area of the national grid. A cross (●) indicates the original locations of records from 1970, and a cross (○) indicates the original locations before 1970 **(Taylor, 2009) (Fig. 07)**.

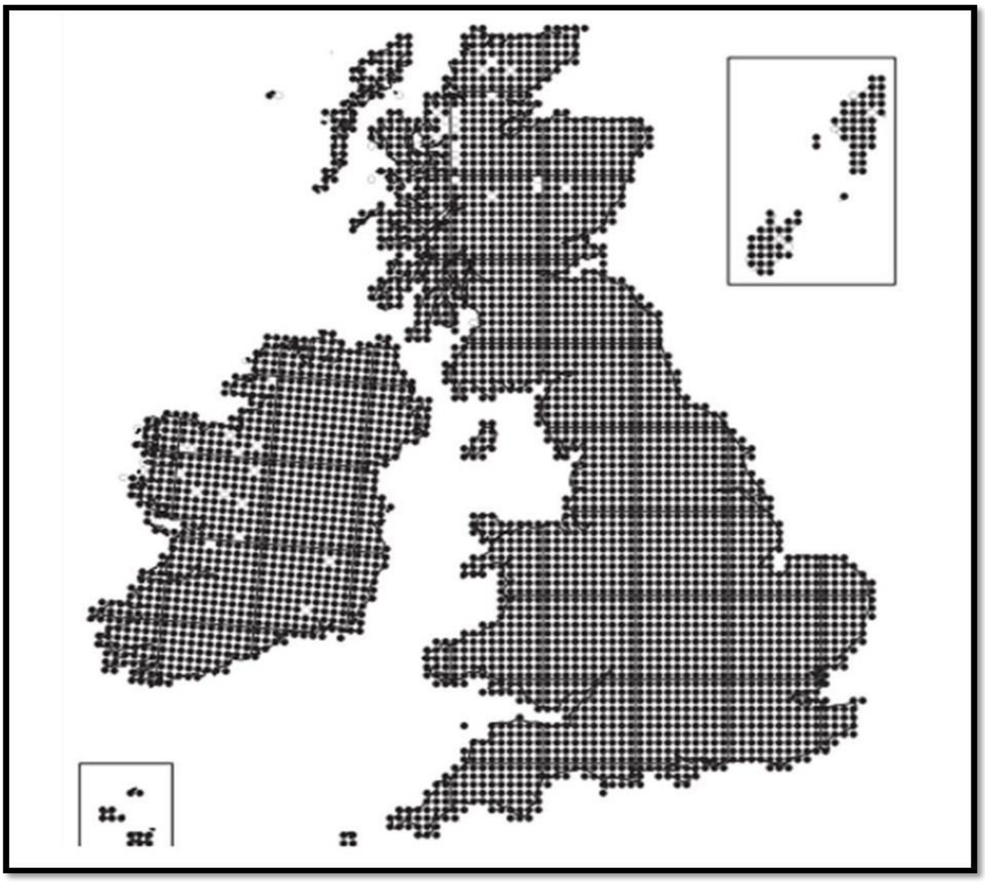

Figure 07 : Map shows the distribution of stinging Urtica dioica in the British Isles **(Taylor, 2009).**

3. Chemical Composition of *Urtica dioica*

3.1. Primary Metabolites

These include essential nutrients that contribute to the plant's nutritive value:

- **Carbohydrates:** glucose, fructose;
- **Proteins:** with all essential amino acids;
- **Lipids:** including polyunsaturated fatty acids;

A study by **Alimoddin *et al.* (2024)** investigated the fatty acid profile of *Urtica dioica*, confirming the role of these compounds in the development of therapeutic applications. The research revealed high levels of linoleic acid and alpha-linolenic acid in both young and mature leaves of *Urtica dioica* **(Fig. 08)**.

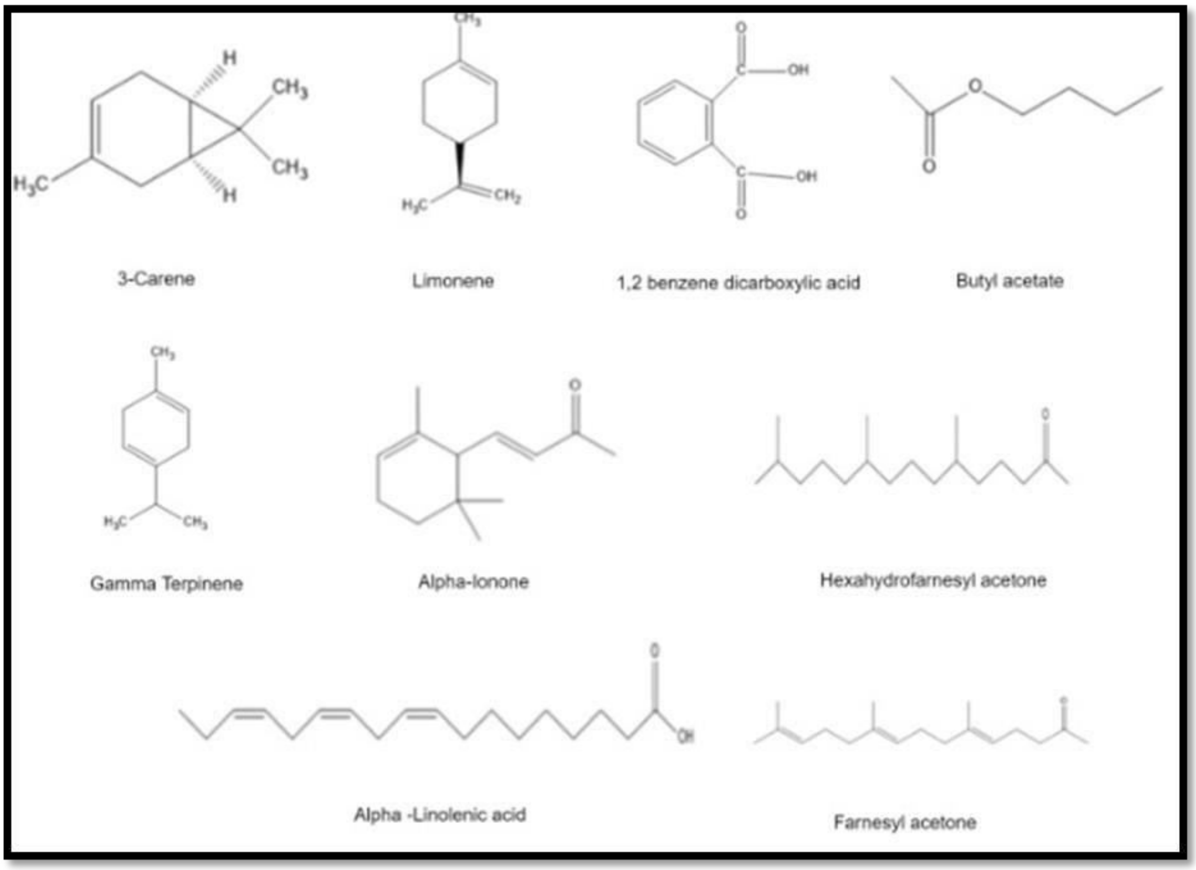

Figure 08 : The fatty acids of *Urtica Dioica* **Alimoddin *et al.* (2024).**

- **Vitamins:** A, C, K, and several B-complex vitamins;
- **Minerals:** Iron, calcium, magnesium, and potassium.

3.2. Secondary Metabolites

These are the main biologically active compounds responsible for the therapeutic effects of stinging Urtica dioica. Among these compounds are :

- ❖ Flavonoids: Quercetin, Kaempferol ;
- ❖ Phenolic Acids: Caffeic Acid, Chlorogenic Acid;
- ❖ Lignins and Coumarins ;
- ❖ Tannins and Saponins ;
- ❖ Sterols: Including Beta-Sitosterol.

These compounds have antioxidant, antimicrobial, and even anti-inflammatory properties **(Gülçin *et al.,* 2004)**.

4. Biological and Pharmacological Properties

4.1. Antioxidant Activity

Urtica dioica's ability to combat oxidation is attributed to its high content of active compounds, including polyphenols, flavonoids, and vitamins, particularly vitamins C and E. These compounds help protect cellular components from oxidative damage, prevent lipid peroxidation, and neutralize reactive oxygen species (ROS).

Several studies, including **Gülçin *et al.* (2004),** using DPPH and ABTS assays, confirmed that Urtica dioica leaf extract exhibits antioxidant activity by scavenging free radicals responsible for oxidative stress in body cells.

Regular consumption or topical application of Urtica dioica-based products may help reduce oxidative stress, which contributes to various chronic conditions, including premature aging.

4.2. Antibacterial Activity

Urtica dioica extract exhibits antibacterial activity against both Gram-positive and Gram-negative bacteria. However, its efficacy tends to be greater against Gram-negative bacteria, likely due to the structural differences in bacterial cell walls, particularly the presence of an outer membrane in Gram- negative strains.

This antimicrobial activity is mainly attributed to the presence of phenolic compounds, flavonoids, and tannins, which can disrupt bacterial membranes and inhibit key enzymatic functions.

Laboratory studies have demonstrated that ethanolic extracts of Urtica dioica are capable of inhibiting the growth of *Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas aeruginosa*, among others (**Thiem and Goslinska, 2004)**. These findings support its potential use as a natural alternative for combating microbial infections, particularly those caused by antibiotic-resistant bacteria.

4.3. Anti-inflammatory Effects

The anti-inflammatory activity of stinging Urtica dioica extract, particularly against arthritis and allergic inflammation, is well documented by comprehensive studies. By examining a number of scientific articles on this topic, we concluded that Urtica dioica is believed to contain compounds such as scopoletin and beta-sitosterol, which are responsible for its anti-inflammatory activity, such as prostaglandins and cytokines.

Clinical studies have also shown improvement in osteoarthritis patients who took Urtica dioica extracts as an adjunctive therapy **(Chrubasik *et al.*, 2007)**.

4.4. Other Pharmacological Effects

Leaf and root extracts of *Urtica dioica* exhibit a wide range of pharmacological properties, including hypoglycemic, anti-inflammatory, antiproliferative, antioxidant, antibacterial, hypolipidemic, analgesic, antirheumatic, anticancer, antiviral, anticolitic, and anti-Alzheimer activities. **Figure 09** illustrates the diverse health benefits associated with Urtica dioica **(Bhusal et al., 2022)**.

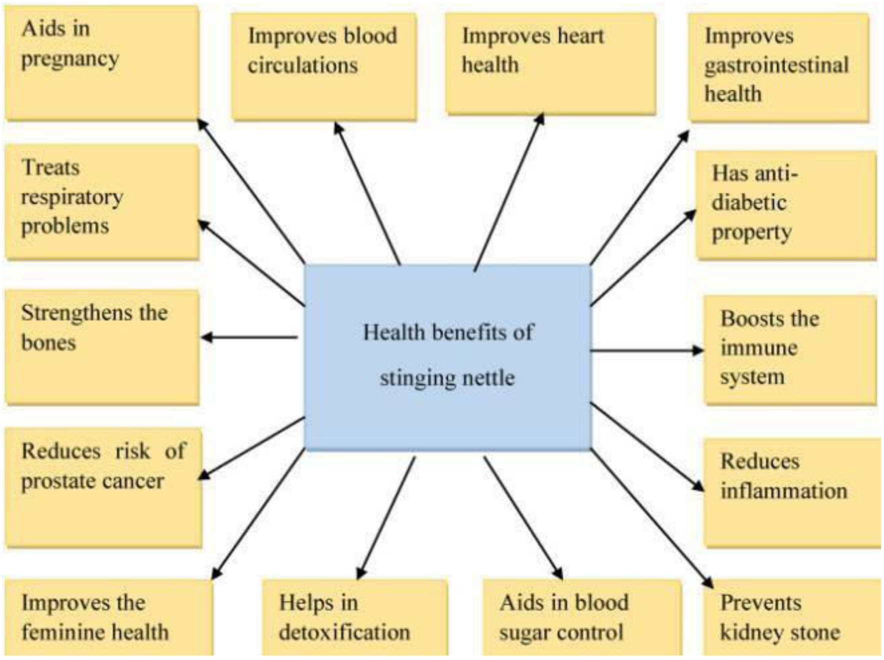

Figure 09 : The pharmacological effets of *Urtica dioica* **(Bhusal *et al.*, 2022)**.

Several pharmacological effects position *Urtica dioica* as a promising candidate in modern herbal medicine, justifying further investigation in clinical settings. These effects include:

- ❖ **Antidiabetic:** Studies have shown that Urtica dioica extract can lower blood glucose levels and enhance insulin sensitivity, supporting its potential use in diabetes management;
- ❖ **Anti-allergic:** Urtica dioica extract may reduce histamine activity and modulate immune responses. It has been reported to alleviate conditions such as eczema and severe hay fever;

- **Analgesic:** Due to its antioxidant and anti-inflammatory properties, Urtica dioica can help relieve various types of body pain;
- **Diuretic:** Urtica dioica promotes diuresis, thereby aiding in detoxification and supporting kidney function.

5. Applications of *Urtica dioica*

Urtica dioica (*Urtica dioica*) is a medicinal plant rich in bioactive compounds such as flavonoids, polyphenols, and organic acids, which are well known for their antimicrobial properties. Traditionally used in folk medicine to treat various conditions, including arthritis and skin diseases, Urtica dioica has recently gained increasing scientific interest due to its therapeutic potential.

Because of its role in purifying the skin, combating free radicals, and strengthening hair follicles, Urtica dioica extracts are now used in the preparation of natural cosmetics, known as cosmeceuticals, as we will do after completing our analysis and testing of the extract.

This diversity of uses makes Urtica dioica a promising candidate for the development of health and beauty products that combine efficacy and safety **(Tabl. 02)**.

Table 02 : The potential uses of Urtica dioicas **(Vogl & Hartl, 2003)**.

Field of application	Use	Part of the plant	Literature cited
Medicine	Hemostatic, diuretic, anti-arthritic, anti-cheumatc,anti-itch, anti-inflammatory	Dried leaves (tea) and juice made from fresh plants.	**(Lutomsky and Speicher, 1983; Monographie, 1987; Dreyer *et al.*, 1996)**
Food	Spinach and soups.	Young plants and leaves	**(Dreyer,1995)**
Cosmetics	Soaps, Shampoos, hair lotion Chlorophyll production.	-	**(Bredemann, 1959)**
Industrial use Forage Crop	Used frech, dried, milled and silaged during periods of forage shortage beforeduring and after the First and Second World Wars, for freeding poultry, cattle and horses.	*Leaves* and Whole plants.	**(Bredemann, 1959)**
Horticulture		Seeds, leaves, Urtica dioica shives and whole plant.	**(Bredemann, 1959; Dreyer, 1995)**

5.1. Medicinal Uses

For centuries, Urtica dioica has been used to treat various ailments, including arthritis, anemia, allergies, and urinary tract problems such as inflammation. The roots or leaves were boiled and drunk as tea. Its anti-inflammatory and diuretic properties are particularly valued for treating joint pain and water retention **(Upton, 2013)**.

5.2. Pharmaceutical Applications

Among the pharmaceutical applications of Urtica dioica is the pharmaceutical industry, where it is used in the development of drugs and natural supplements. Urtica dioica roots are often used to treat benign prostatic hyperplasia (BPH), while its leaf extract is used in formulations that enhance immunity and detoxify. Due to its antioxidant content, it is also considered beneficial for liver protection and general vitality **(Riehemann *et al.,* 1999)**.

5.3. Uses in Cosmetics and Dermatology

Urtica dioica is also widely used in the cosmetic industry due to its antioxidant, anti-inflammatory, and antibacterial properties. It is incorporated into the formulation of facial cleansers, acne creams, shampoos for oily hair, anti-dandruff treatments, and moisturizing products.

Moreover, Urtica dioica contributes to skin purification by helping to reduce pimples and wrinkles, tighten pores, and stimulate blood circulation—particularly beneficial for women. These properties make it an excellent natural ingredient for facial and body skincare **(Ratz-Łyko and Arct, 2014)**.

5.4. Applications in Food and Agriculture

Urtica dioica is an edible plant rich in vitamins A and C, proteins, and iron. Urtica dioica powder can be used as a natural ingredient in soups or herbal teas. It also contains minerals and biologically active compounds, which makes it useful in agriculture as a natural insecticide or compost activator to enrich the soil **(Chevallier, 2001)**.

5.5. Industrial and Textile Uses

Historically, Urtica dioica fibers were used in the manufacture of fabrics, especially during wartime cotton shortages. Today, Urtica dioica fibers are gaining renewed interest as an environmentally friendly and sustainable textile alternative. They are durable, breathable, and biodegradable, making them ideal for producing environmentally friendly fabrics **(Buchbauer, 2010)**.

6. Toxicological Aspects

Despite its safety and diverse uses, Urtica dioica is not entirely free of toxic effects. Its stinging hairs contain histamine, acetylcholine, and formic acid, which can cause skin irritation, redness, and itching upon contact.

These effects are usually mild and short-lived but may be more irritating for sensitive individuals **(Lahiri *et al.*, 2016)**.

Urtica dioica is generally considered safe for internal use, provided it is consumed in moderate amounts. Excessive or prolonged consumption without medical supervision may lead to allergic reactions. Pregnant women are also advised to avoid large quantities, as Urtica dioica can stimulate uterine contractions **(Mills and Bone, 2000)**.

Toxicological studies have shown that both aqueous and ethanolic extracts of Urtica dioica possess a relatively high safety margin. The lethal dose (LDy_0) in animal studies is significantly high, indicating low acute toxicity. However, long-term toxicity evaluations are necessary, particularly for standardized extracts intended for pharmaceutical or cosmetic applications **(Ozen *et al.*, 2012)**.

In conclusion, stinging Urtica dioica is a valuable medicinal plant containing essential compounds with antibacterial, antioxidant, and anti-inflammatory properties. Nevertheless, despite its many benefits, it may have adverse effects on the human body. Therefore, determining the appropriate formulation, dosage, and usage guidelines is essential to ensure its safety and efficacy.

1.1. Definition of Oxidative Stress

Oxidative stress is a condition resulting from an imbalance between reactive oxygen species (ROS), oxidizing molecules such as free radicals, and antioxidants, the body's defense systems. Normally, the body has effective defense mechanisms that help inhibit the activity of these harmful molecules. However, cellular damage occurs due to the accumulation of free radicals beyond the ability of these mechanisms to control them. This damage may be the primary cause of some diseases such as cancer, heart disease, diabetes, and some diseases related to aging **(Valko *et al.,* 2007; Halliwell & Gutteridge, 2015) (Fig.10)**.

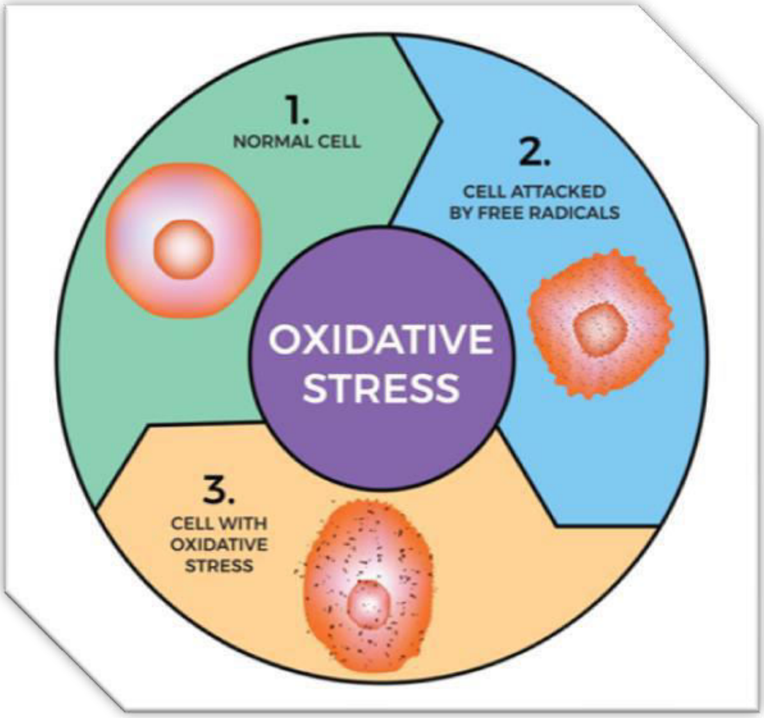

Figure 10 : The oxidative stress cycle **(Adapted from Haleva, 2024)**.

Oxidative stress is a physiological state characterized by an imbalance between the production of reactive oxygen species (ROS) and the body's ability to detoxify them using antioxidants. Thus, when ROS exceed the body's antioxidant defenses, they can cause cellular and molecular damage, particularly to lipids, proteins, and DNA **(Pham-Huy *et al.,* 2008)**.

Oxidative stress plays a crucial role in the development of a serious group of chronic diseases associated with aging. Free radicals attack essential components of cells, including DNA, which carries genetic information, lipids, and proteins, thereby disrupting vital cellular functions.

This disruption can persist over time and may limit the body's ability to repair and regenerate tissues, contributing to the accelerated onset of chronic diseases **(Sies, 2017)**.

1.2. Origin and Production of Free Radicals

Free radicals are highly chemically active molecules, possessing a single unpaired electron in their outer shell. This enables them to react easily with cellular components such as proteins, lipids, and DNA, causing damage that can lead to chronic and serious diseases such as cancer, heart disease, and neurodegenerative disorders. These molecules are produced by both internal and external factors.

Free radicals are atoms or molecules with unpaired electrons, making them highly reactive. They are naturally produced in the body during metabolic processes, particularly in the mitochondria during aerobic respiration. External sources, such as ultraviolet radiation, pollution, smoking, and certain medications, also contribute to the formation of free radicals **(Valko *et al.,* 2007) (Fig.11)**.

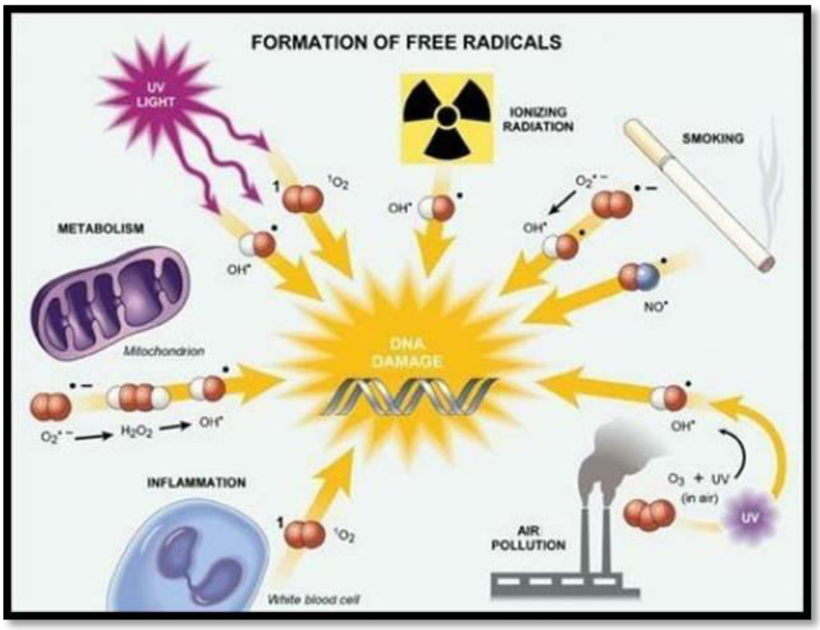

Figure 11: The external sources **(Adapted from Dickson, 2008)**.

- Among the sources of free radicals in the body:

➤ Cellular Metabolism

Cellular respiration in mitochondria is the primary process responsible for the production of free radicals within the body, where electrons are transferred along a chain of proteins. In some cases, this transfer is incomplete, resulting in the formation of reactive oxygen species such as superoxide (O_2^-), hydrogen peroxide (H_2O_2), and hydroxyl radicals (OH•) **(Finkel & Holbrook, 2000) (Fig.12)**.

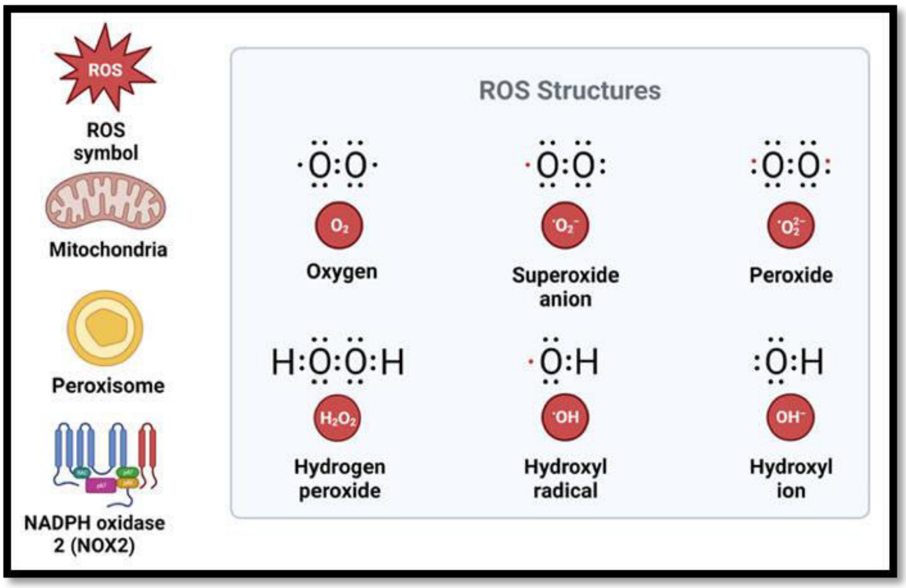

Figure 12 : Structure of reactive oxygen species and their sources. **ROS:** Reactive Oxygen Species; **NADPH:** Nicotinamide Adenine Dinucleotide Phosphate; **NOX2**: NADPH Oxidase **(Masenga *et al.*, 2023)**.

- **Immune Response**

During the immune system's interaction with pathogens, some immune cells, such as neutrophils and macrophages, release reactive oxygen and nitrogen species to eliminate microbes. However, if this activity exceeds the normal limit, it can lead to tissue damage and increased inflammation **(Kohen & Nyska, 2002)**.

- **Enzymatic Activity**

Some enzymes, such as xanthine oxidase and cytochrome P450, contribute to the production of free radicals as part of their normal functions **(Reeves, 2012)**.

- Also among the influencing environmental factors (external sources):

- **Pollutants**

Environmental factors such as cigarette smoke, air pollutants, and some types of radiation significantly contribute to stimulating the production of free radicals within the body. Compounds such as polycyclic hydrocarbons stimulate the formation of reactive oxygen species **(Marinelli *et al.*, 2015)**.

- **Ultraviolet radiation**

Direct exposure to sunlight is one of the main causes of free radical formation within skin cells, which leads to damage and also increases the risk of skin cancer and premature aging **(Lester, 2008)**.

- **Dietary habits**

Dietary habits include diets rich in fats, especially saturated fats, which can lead to the formation of free radicals during digestion, raising levels of oxidative stress in the body and increasing the risk of heart disease **(Giugliano *et al.,* 2003)**.

- The body produces free radicals through several reactions, including:

- **Oxidation-reduction reactions**

These are reactions that are part of normal metabolic processes, but if their balance is disturbed, they can lead to the formation of free radicals.

- **Fenton and Haber-Weiss reactions**

These reactions involve the conversion of hydrogen peroxide into hydroxyl radicals, which are among the most reactive and can cause severe tissue damage **(Halliwell, 1992)**.

- **Oxidative enzyme activity**

Enzymes such as xanthine oxidase and NADPH oxidase contribute to the production of free radicals as part of their normal functions **(Liu *et al.,* 2010)**.

1.3. Reactive Oxygen Species (ROS) and Their Effects

Reactive oxygen species (ROS) include species such as superoxide anion (O_2^-), hydrogen peroxide (H_2O_2), and hydroxyl radicals (OH•). At low concentrations, ROS play physiological roles in cell signaling and immune defense. However, at high concentrations, they can damage cellular components, leading to inflammation, aging, cancer, neurodegenerative diseases, and cardiovascular diseases **(Lobo *et al.,* 2010)**.

Reactive oxygen species (ROS) are chemical molecules containing oxygen and characterized by an unpaired electron in their outer shell, making them highly reactive and energetic. ROS are formed naturally within the body, particularly during metabolic processes such as cellular respiration. Although they play a vital physiological role, their overproduction or any disruption in their regulation can lead to cellular damage that contributes to the development of a range of chronic diseases.

They are produced within mitochondria during electron transport chain (ETC) activity, where electrons can be lost and react with oxygen to produce superoxide anions. They are also produced in other cellular locations, such as the cytoplasm, endoplasmic reticulum, and other organelles, through enzymatic processes involving enzymes such as NADPH oxidase, xanthine oxidase, and cytochrome P450 **(Finkel & Holbrook, 2000)**. This is in addition to some external factors such as exposure to ultraviolet radiation, smoking, pollution, and certain medications, which increase ROS production **(Marinelli *et al.,* 2015)**.

- Despite the reactive nature of ROS, at low levels they play an important role in the body, including:

➤ **Cell Signaling**

Reactive oxygen species (ROS) function in many signaling pathways as secondary messengers within the cell, regulating gene expression, the immune response, and apoptosis **(Caro *et al.*, 2014)**.

➤ **Immune Response**

In the immune response, immune cells such as neutrophils and macrophages produce ROS during "oxidative stress" to combat and eliminate pathogens **(Kohen & Nyska, 2002)**.

➤ **Regulation of Vascular Function**

ROS contribute to the regulation of vascular function by controlling vascular contraction through smooth muscle and endothelial cells **(Giugliano *et al.*, 2003)**.

Oxidative stress occurs when ROS production exceeds the body's ability to combat it with antioxidants. This leads to damage to lipids, proteins, and DNA, which is associated with the emergence and rapid progression of many diseases, such as:

➤ **Cancer**

ROS can cause DNA mutations and destabilize DNA, contributing to the emergence and development of tumors **(Jiang *et al.*, 2013)**.

➤ **Cardiovascular Diseases**

ROS lead to endothelial dysfunction, inflammation, and atherosclerosis and contribute to the development of hypertension and heart failure **(Liu *et al.*, 2010)**.

➤ **Neurodegenerative Diseases**

ROS are involved in the development of diseases such as Alzheimer's and Parkinson's by causing mitochondrial damage and protein accumulation within neurons **(Halliwell & Gutteridge, 2015)**.

➤ **Aging**

The oxidative stress theory of aging is the most common explanation, whereby ROS cause cumulative damage to cells and tissues over time, leading to a gradual decline in vital functions **(Finkel & Holbrook, 2000)**.

The body has a complex antioxidant defense system to counteract the damaging effects of ROS. These defenses include enzymes such as superoxide dismutase (SOD), catalase, and glutathione peroxidase, in addition to non-enzymatic antioxidants such as vitamin C, vitamin E, and glutathione **(Halliwell, 1992)**.

1.4. Antioxidant Potential of *Urtica dioica*

The antioxidant activity of various *Urtica dioica* fractions has been studied in numerous studies, including *In Vitro*, *In Vivo*, and human studies. Experiments have also been conducted to enhance antioxidant activity by increasing the content of active phytochemicals in *Urtica dioica* extracts through improved extraction parameters and techniques **(Fig.13).**

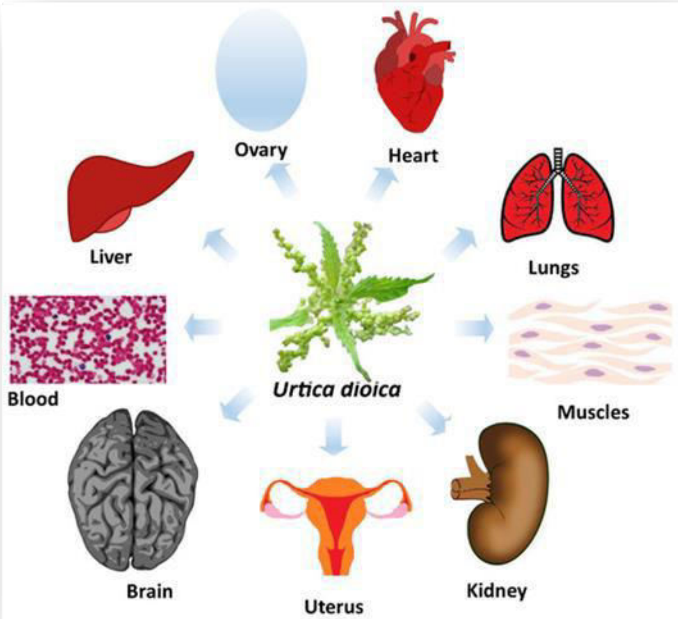

Figure 13 : Antioxidant activity of *Urtica dioica* observed in different organs **(Jaiswal and Lee, 2022)**.

Antioxidants are molecules capable of inhibiting oxidation and neutralizing free radicals. They are classified into enzymatic antioxidants (e.g., superoxide dismutase, catalase, glutathione peroxidase) and non-enzymatic antioxidants (e.g., vitamin C, vitamin E, flavonoids). Their mechanisms include scavenging free radicals, chelating metal ions, and regenerating other antioxidants **(Prior *et al.,* 2005)**.

1.5. The role of *Urtica dioica* in oxidative stress regulation

Numerous studies have demonstrated the antioxidant potential of Urtica dioica due to its rich content of polyphenols, flavonoids, carotenoids, and vitamins. Plant extracts, particularly methanolic and ethanolic, have demonstrated remarkable activity in scavenging DPPH radicals and lipid peroxidation **(Gülçin *et al.,* 2004)**. These effects make Urtica dioica a promising natural antioxidant for use in health and cosmetic applications **(Fig.14).**

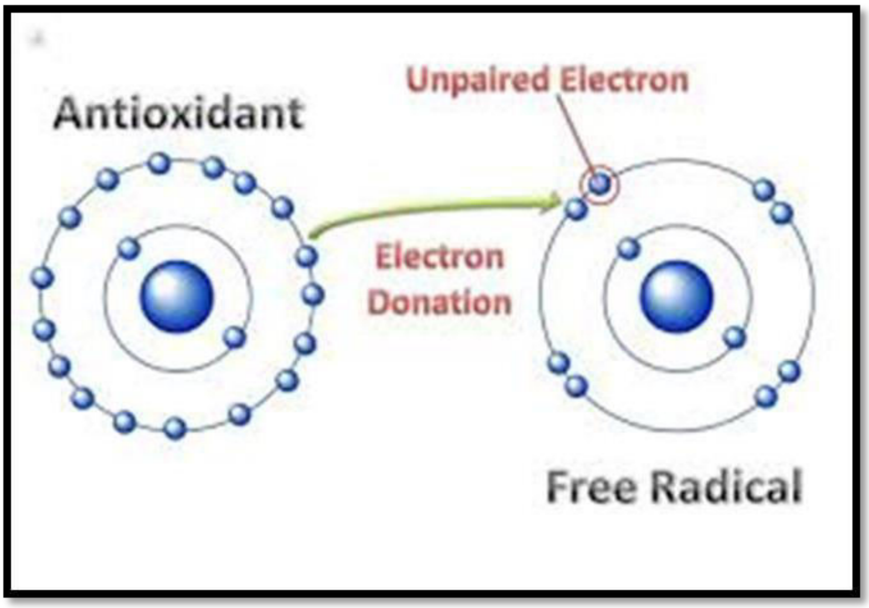

Figure 14 : Antioxidants fight free radicals **(Adapted from Alexandrou, 2013)**.

Oxidative stress is one of the factors contributing to the development of many chronic diseases such as heart disease, cancer, neurological disorders, and premature aging. This stress causes damage to cells and tissues, whether as a result of the accumulation of free radicals or reactive oxygen species (ROS). In this context, stinging Urtica dioica is among the medicinal plants known for effectively combating this phenomenon, due to its richness in natural antioxidant compounds.

Oxidative stress occurs when the production of free radicals exceeds the body's ability to eliminate them through its antioxidant systems. Radicals such as superoxide (O_2•) and hydroxyl radicals (OH•) are among the most destructive, attacking proteins, lipids, and even DNA, causing cellular damage that contributes to the development of serious diseases **(Halliwell, 2007)**.

Stinging Urtica dioica contains a range of essential plant compounds with biological activity, including flavonoids such as quercetin, kaempferol, and rutin, as well as polyphenols and tannins. These compounds work to combat free radicals and reduce their negative effects in the body **(Biesalski *et al.*, 2007; Demircan *et al.*, 2009)**.

- In order to reduce oxidative stress using Urtica dioica, there are biological mechanisms, including:

➤ **Neutralizing free radicals**

Urtica dioica has the ability to inactivate free radicals and prevent their interaction with cellular components, helping to protect cell membranes and DNA from damage.

➤ **Activating antioxidant enzymes**

Urtica dioica stimulates enzymes responsible for cellular defense, such as SOD and catalase, helping to convert ROS into less harmful compounds.

- **Preventing lipid peroxidation**

Thanks to its ability to prevent lipid oxidation reactions, Urtica dioica helps reduce the risk of atherosclerosis and heart disease **(Youdim *et al.,* 2003)**.

- **Protecting the nervous system**

Studies have shown that Urtica dioica reduces the harmful effects of free radicals in the brain, enhancing its role in preventing diseases such as Alzheimer's and Parkinson's.

- One of the health benefits associated with reducing oxidative stress using Urtica dioica is:

- **Cancer Prevention**

Reducing DNA damage caused by oxidation reduces the likelihood of the development of genetic mutations that cause cancer **(Demircan *et al.,* 2009)**.

- **Improving Cardiovascular Health**

Urtica dioica protects blood vessel walls from oxidative damage, thus helping to prevent atherosclerosis and high blood pressure.

- **Anti-inflammatory Properties**

Although inflammation is associated with oxidative stress, Urtica dioica helps reduce chronic inflammation such as acute arthritis **(Barthel *et al.,* 2012)**.

2. Antibacterial Activity

2.1. Overview of Bacteria

Bacteria and fungi are among the most common pathogenic microorganisms that infect humans, animals, and plants. While some are beneficial, others can cause infection and damage. The overuse of synthetic antibiotics has led to increased microbial resistance, making the search for alternative natural agents even more important **(Davies and Davies, 2010)**.

Bacteria are among the most widespread and diverse microorganisms on Earth, playing a pivotal role in various ecosystems. They are prokaryotic organisms, lacking a true nucleus; however, their simple structure belies the complexity of their vital functions. Bacteria vary in shape, structure, and feeding mechanisms, enabling them to adapt to a wide variety of environmental conditions, from the depths of the oceans to harsh environments such as acidic or salty water.

Although often associated with disease, many bacterial strains are essential for life, contributing to nitrogen fixation, the decomposition of organic matter, and being used in vital industries such as the production of antibiotics and enzymes. This balance between beneficial and harmful roles reflects the great biological importance of bacteria in nature, medicine, and industry **(Fig. 15)**.

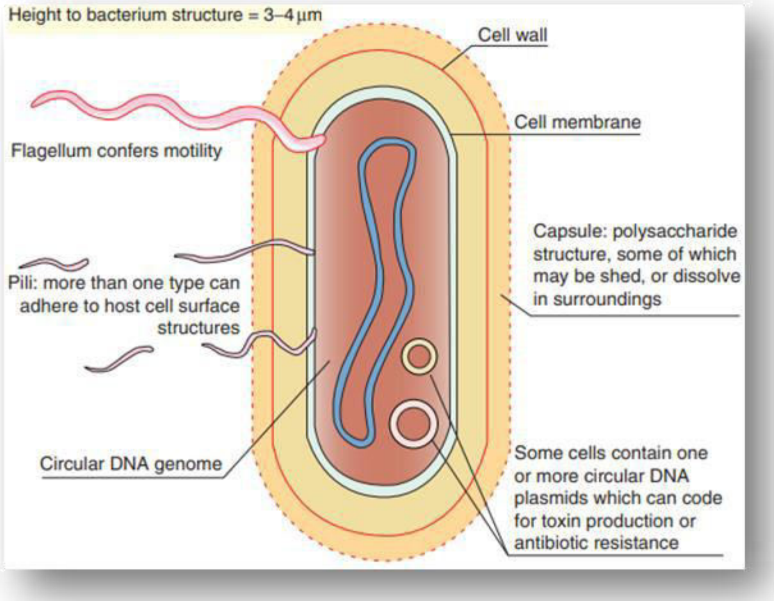

Figure 15 : Structure of a bacterium **(Doron and Gorbach, 2008)**.

2.2. Mechanisms of antimicrobial action

Natural plant extracts, such as Urtica dioica, can combat microbes through specific mechanisms, including disrupting the cell wall or outer membrane of microbes, such as those of Gram-negative bacteria; inhibiting nucleic acid synthesis, preventing microbes from reproducing; interfering with energy metabolism; and chelating essential nutrients for microbes **(Cowan, 1999)**. These mechanisms reduce the development of resistance compared to conventional antibiotics.

2.3. Antibiotic Resistance and Natural Alternatives

Antibiotic resistance is now a global health concern. Plants such as Urtica dioica are being explored as natural alternatives due to their bioactive compounds that exhibit antimicrobial properties without significantly contributing to resistance. The complexity of plant extracts, which contain multiple active molecules, makes it difficult for microbes to develop resistance **(WHO, 2014)**.

2.4. Potential of *Urtica dioica* extract as antimicrobial agents

Several studies have demonstrated the effectiveness of Urtica dioica extracts, particularly ethanolic and methanolic, against Gram-positive (e.g., *Staphylococcus aureus*) and Gram-negative (e.g., *Escherichia coli*) bacteria, as well as various fungal strains such as *Candida albicans*. These effects are mainly attributed to compounds such as flavonoids, phenolic acids, and tannins **(Akbay *et al.,* 2003; Ghaima *et al.,* 2014)**. These findings support their use in traditional medicine and their potential in the development of natural antimicrobial products for cosmetic and pharmaceutical uses.

EXPERIMENTAL STUDY

1. Objectives of the Experimental Study

The objective of this study was to investigate the biological activities of methanolic extracts (EM and ES extracts) derived from Urtica dioica (*Urtica dioica* L.).

During our study, we identified and characterized the main bioactive compounds present in the plant extracts, and evaluated their antioxidant and antibacterial properties.

The phytochemical and antioxidant activity studies were conducted in Biochemistry Laboratory N°02, while the antibacterial activity tests were conducted in Microbiology Laboratory No°03, Department of Biology, Faculty of Natural and Life Sciences, Abdelhamid Ibn Badis University, Mostaganem, Algeria.

2. Plant Material and Extraction of Bioactive Compounds

2.1. Collection and Preparation of plant Material

2.1.1. Plant Material

Our aim was to contribute to the valorization of *Urtica dioica* L., a medicinal plant that grows naturally in the Mediterranean basin, particularly in the region of Mesra, located in Mostaganem, Algeria. This plant was selected based on previous studies highlighting its pharmacological potential, its frequent use in traditional medicine, and its recognition as a medicinal herb by physicians, researchers, and herbalists alike **(Fig.16)**.

Figure 16: Leaves of *Urtica dioica* L.

2.1.2. Collection and Preparation of Plant Material

- **Plant Collection:** Urtica dioica (*Urtica dioica*) leaves were collected from the local environment in the ear- ly morning;
- **Cleaning:** The leaves were gently wiped with a paper towel to remove dust and impurities, rather than washed, and were then left to air dry for 30 minutes.
- **Drying:**
  - ✓ **Shade Drying:** The leaves were placed in a well-ventilated room, away from direct sunlight, and left to dry for 7 to 10 days. They were turned occasionally to ensure uniform drying ;
  - ✓ **Oven Drying:** The leaves were then transferred to an oven and dried at 37–40 °C for 24 to 48 hours until fully dehydrated **(Fig. 17)**.
- **Grinding and Storage:**
- The dried leaves were ground into a fine powder using a mortar and pestle. The resulting powder was weighed and stored in a sterile reagent beaker, placed in a dry and dark location until further use **(Fig. 18)**.

Figure 17: Drying *Urtica dioica* leaves.

Figure 18: *Urtica dioica* leaf powder.

2.2. Laboratory Materials and Equipment

- **Glassware:** Beakers, test tubes, Pasteur pipettes, flasks, conical flasks, funnels, watch glasses, and glass containers;
- **Other Laboratory Items:** Sterile filter paper, test tubes, platinum loops, Bunsen burners, and silica gel plates;
- **Culture Media:** Nutrient Agar and Nutrient Broth;
- **Instruments and Equipment:** Analytical balance, Soxhlet extractor, rotary evaporator, autoclave, drying oven, hot plate, water bath, and UV-visible spectrophotometer.

2.3. Methanol Extraction Procedures

2.3.1. Methanol Extraction Using the Soxhlet Method

- Forty grams of dried plant powder were placed in a thick filter paper cartridge and inserted into a Soxhlet extractor containing 450 mL of methanol as the extraction solvent **(Fig. 19)**;
- The extraction process was monitored continuously and allowed to proceed for six successive cycles. During each cycle, the solvent mixed with plant material returned to the flask and was siphoned off through the side arm of the apparatus;
- The resulting extract was concentrated by solvent evaporation using a rotary evaporator at 40–45 °C;
- The remaining residue was dissolved in a 1:10 DMSO (dimethyl sulfoxide) solution;
- The extraction process was repeated several times to obtain a sufficient quantity of extract for the planned laboratory analyses;
- The final methanolic extract (ES) was stored at 4 °C, protected from light.

Figure 19: Soxhlet Methanol Extraction.

2.3.2. Methanolic Maceration Extraction

- A 38-gram sample of *Urtica dioica* powder was soaked in 400 mL of methanol for 24 hours at room temperature **(Fig. 20)**;
- The mixture was then filtered to remove any particulate matter that could interfere with the evaporation process;
- The filtrate was concentrated using a rotary evaporator under reduced pressure at 45 °C until complete solvent evaporation was achieved **(Fig. 21)**;
- The final extract (EM) was collected and stored for further analysis.

Figure 20: Methanolic Extraction of Urtica dioica by Maceration Technique.

Figure 21: Methanolic extract by maceration in the Rotavapor apparatus.

3. Phytochemical Analysis of *Urtica dioica* Extracts

3.1. Qualitative Detection of Bioactive Metabolites

- **Phenolic Compounds:** Based on the method described by **Rosine and Momo (2009),** 0.1% of the extract was dissolved in 3 mL of ethanol. Five drops of ferric chloride ($FeCl_3$) were added, and the solution was shaken thoroughly. The appearance of a green coloration indicated the presence of phenolic compounds.
- **Flavonoids:** Following the method of **Ciulel (1982),** 5 mL of hydrochloric acid (HCl) were added to 3 mL of the extract, followed by a small amount of magnesium sulfate. After shaking, the development of an orange color confirmed the presence of flavonoids.
- **Tannins:** According to **Hadduchi *et al.* (2016)**, 8 drops of a diluted ferric chloride solution were added to 1 mL of the extract. After shaking and allowing the mixture to stand at room temperature, a color change was observed. A green coloration indicated the presence of catechin tannins, while a bluish coloration indicated the presence of gallic tannins.
- **Coumarins:** In this test, 3 mL of 10% sodium hydroxide (NaOH) were added to 2 mL of the plant extract. The solution was shaken well. The appearance of a yellow coloration indicated the presence of coumarins.
- **Alkaloids:** Five milliliters of the extract were evaporated to dryness. The residue was dissolved in 2 mL of 2% hydrochloric acid, heated in a water bath, and then filtered. The filtrate was divided into two test tubes:
  - In the first tube, a few drops of **Mayer's reagent** were added. A yellowish-white precipitate indicated the presence of alkaloids.
  - In the second tube, **Wagner's reagent** was added. The appearance of a reddish-orange coloration also indicated the presence of alkaloids.

3.2. Thin-Layer Chromatography (TLC) Analysis

- **Characterization of Alkaloids and Flavonoids on TLC Plates**

The objective of this analysis was to identify the main phytochemical groups present in the *Urtica dioica* extract, particularly alkaloids and flavonoids. The method involves depositing a small amount of the extract onto a TLC plate and developing it in a suitable solvent system. As the solvent ascends the plate via capillary action, it carries the compounds at different rates depending on their solubility and affinity for the stationary phase. The resulting migration patterns provide preliminary insights into the chemical composition of the extract, which can be correlated with its biological activities.

- **Procedure:**
  - ✓ The chromatography chamber was prepared **(Fig. 22)**;
  - ✓ A solvent mixture was prepared using methanol, ethanol, and distilled water in a 4:4:2 (v/v/v) ratio and poured into a small beaker to serve as the mobile phase;
  - ✓ The level of the solvent was adjusted to approximately 1 cm from the bottom of the TLC plate;
  - ✓ Using a pencil, a baseline was drawn 1 cm from the bottom edge of the plate to mark the sample application point;
  - ✓ A few drops of the plant extract were carefully applied to the baseline using a capillary tube or micropipette;
  - ✓ The sample was air-dried using a desiccator to avoid diffusion;
  - ✓ The TLC plate was then placed vertically in the chamber, which was immediately sealed to allow solvent saturation;
  - ✓ Once the solvent front had risen to approximately 1 cm from the top edge of the plate, the plate was removed and the solvent front was marked **(Fig. 23)**;
  - ✓ The TLC plate was then allowed to dry completely before analysis;

Figure 22 : Preparing the chromatographic cell.

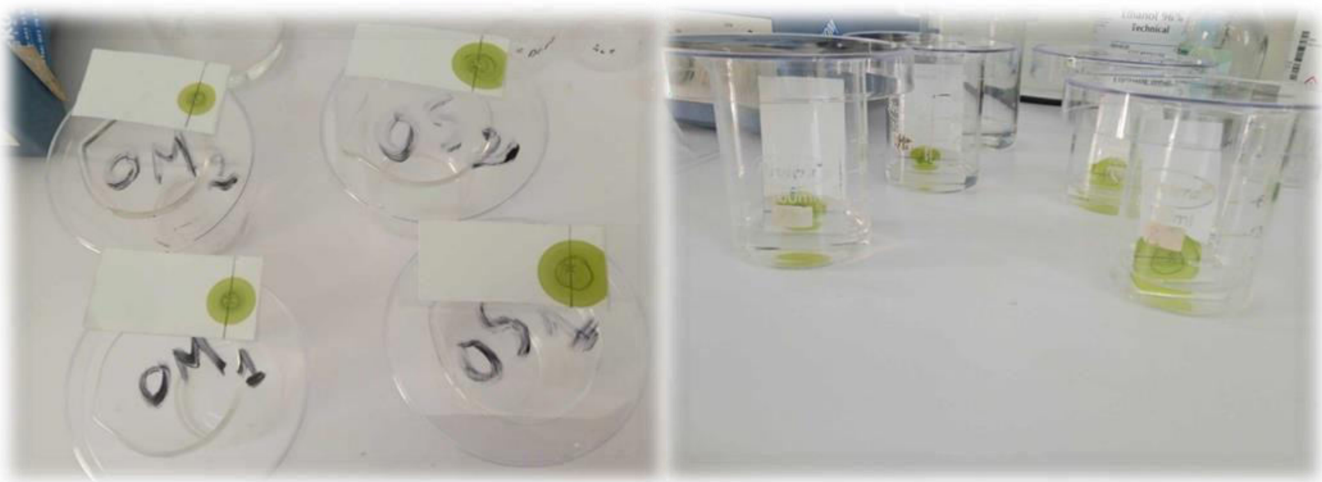

Figure 23 : Development of the Chromatogram.

✓ Based on previous experiments (developer's methodology), Dragendorff's reagent was used to detect alkaloids, while ferric chloride ($FeCl_3$) was employed to identify flavonoids, as follows:

- After pre-drying the chromatographic plates, Dragendorff's reagent was sprayed on the plates **(Fig. 24)**. The appearance of an orange color indicated the presence of alkaloids. Similarly, $FeCl_3$ was sprayed onto the other plates; the development of a blue coloration confirmed the presence of flavonoids;
- The plates were left to dry for 10 minutes, and the retention factor (Rf) was calculated for each compound.

Figure 24 : Dragendorff's Reagent.

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4. Evaluation of Biological Activities

4.1. Antioxidant Activity Test

4.1.1. DPPH Free Radical Scavenging Assay

To assess the ability of natural compounds to inhibit and neutralize free radicals responsible for oxidative stress, particularly those rich in hydroxyl and amino groups, the DPPH (2,2-diphenyl-1-picrylhydrazyl) assay was performed, as outlined by **Blois (1958)**. This test is a widely used, simple, and reliable method for evaluating the free radical scavenging capacity of plant extracts, such as *Urtica dioica*, and their antioxidant potential.

The DPPH assay has been validated by previous studies, including **Brand-Williams *et al.* (1995) and Prior *et al.* (2005),** who confirmed its applicability in identifying extracts with high polyphenol content and evaluating their antioxidant properties.

In this assay, a DPPH solution, characterized by its deep purple color, is mixed with a sample containing antioxidants. Following the reaction, the free radical DPPH is neutralized by the antioxidants present in the plant extract, resulting in a color change from purple to yellow, which is accompanied by a decrease in absorbance at 517 nm.

Procedure:

1. Prepare the DPPH solution by dissolving 2.4 mg of DPPH powder in 100 mL of methanol;
2. Take 2.4 mL of the DPPH solution as a negative control;
3. Add 1 mL of the DPPH solution to the test tube, followed by 25 µL of the plant extract at varying concentrations;
4. Incubate the mixture in the dark for 30 minutes;
5. Measure the absorbance at 517 nm using a spectrophotometer **(Fig. 25)**;
6. Calculate the percentage of free radical scavenging activity using the following formula:

$$\% \text{ Scavenging} = \frac{(A_c - A_s)}{A_c} \times 100$$

Where:

- **Ac :** Absorbance of the negative control sample;
- **As :** Absorbance in the presence of the extract.

Figure 25 : UV-Visible Spectrophotometer Used in the Analysis.

4.2. Antibacterial Activity Test

4.2.1. Bacterial Strains and Culture Conditions

- **Preparation of Culture Medium**

The culture medium used for this study was nutrient agar, prepared as follows: 10 g of peptone, 5 g of meat extract, 5 g of sodium chloride, and 15 g of agar-agar were dissolved in 1 L of distilled water. The mixture was brought to a boil with constant stirring until complete dissolution. The pH was adjusted to 7.2. The medium was then sterilized in an autoclave at 121°C for 20 minutes and subsequently poured into sterile Petri dishes under aseptic conditions.

- **Equipment Sterilization**

All materials, including distilled water, culture media, test tubes used for bacterial suspensions, and platinum loops (wrapped in aluminum foil), were sterilized in an autoclave at 121°C for 15 minutes **(Fig. 26)**.

Figure 26 : The autoclave.

- **Preparation of stinging Urtica dioica extract dilutions**

Stinging Urtica dioica leaf extracts (prepared in DMSO for EM and ES extracts) were used to pre- pare the different concentrations with successive one-half dilutions, keeping in mind that the concen- tration of the stock solution of each extract is 10 mg/ml **(Table 3)(Fig.27).**

Table 3: Different concentrations used to prepare dilutions.

Concentrations	Extracts ES/EM (mg/ml of DMSO)
1	100
2	50
3	25
4	12.5

Figure 27 : The dilutions

- **Reference Strains :**

Reference strains are collected in GN slant agar tubes and stored in a refrigerator at 4-6°C. (stock on slant agar) **(Table 4)**.

Table 4: Bacterial Strains and Their References Used in This Study.

Bacterial Strains	Forme	Gram	Familly	Code
Escherichia coli	Bacille	Negative	Enterobacteriaceae	ATCC25922
Staphylococcus aureus	Cocci	Positive	Micrococcaceae	ATCC25923
Pseudomonas aeruginosa	Bacille	Negative	Pseudomonadaceae	ATCC27853
Bacillus cereus	Bacille	Positive	Bacilaceae	ATCC11778
Bacillus subtilis	Bacille	Positive	Bacilaceae	ATCC6633

- **Strain reactivation (screening) :**

✓ **Preparation of Petri dishes:** prepare 5 petri dishes with nutrient agar;

✓ **Solidification :** Allow the medium to solidify at room temperature;

✓ **Surface plating:** By platinum loop, pick colonies (from test tubes) and inoculate;

✓ **Incubation:** Incubate for 24 hours at 37 °C **(Fig. 28)**;

✓ **Storage:** Keep refrigerated at 4 °C until use **(Fig. 29)**.

Figure 28 : Incubation in laboratory oven.

Figure 29 : Reactive Strains.

- **Inoculum preparation:**
  - ✓ Prepare 5 tubes with nutrient broth;
  - ✓ Search and pick a single colony with the platinum loop and place it in the tube;
  - ✓ Incubate at 37°C for 24 hours **(Fig 30).**

Figure 30 : The Inoculum

- **Preparation of Bacterial Suspensions**
  - ✓ Prepare 5 sterile swabs or tubes and add 5 mL of 0.9% sodium chloride (NaCl) solution (physiological saline) to each.
  - ✓ Add a few drops of bacterial inoculum to each suspension tube.
  - ✓ Adjust the turbidity of the suspension by one of the following methods:

Spectrophotometrically, by measuring optical density at 600 nm (OD_{600});

- ▪ Using the McFarland standard, where 0.5 McFarland corresponds to approximately 1.5×10^8 CFU/mL;
- ▪ If the suspension is too diluted, add more inoculum; if too concentrated, dilute with additional NaCl solution.

4.2.2. Well Diffusion Method

In this method, agar plates were first inoculated with the bacterial strains under study. Small wells were then made in the agar using a sterile cork borer or pipette tip.

Different concentrations of the plant extract were introduced into the wells, and the plates were incubated at 37 °C for 24 hours.

Following incubation, antibacterial activity was evaluated by the appearance of clear zones surrounding the wells, referred to as inhibition zones. These zones indicate areas where bacterial growth was inhibited by the extract. The larger the diameter of the inhibition zone, the greater the anti-bacterial efficacy of the extract **(Figs. 31 and 32)**.

Figure 31 : Inoculation Using the Well Diffusion Method.

Figure 32 : Petri Dishes Prior to Incubation.

- **Reading the Results**

After 24 hours of incubation, the Petri dishes were removed, and the presence of transparent, circular inhibition zones around the wells was observed. The appearance of these halos indicates the absence of bacterial growth, demonstrating the antibacterial activity of the plant extract.

To evaluate the antibacterial effect, the diameter of each inhibition zone was measured using a ruler. A larger inhibition zone diameter indicates greater bacterial sensitivity and, therefore, a stronger inhibitory effect of the extract.

According to **Celeikel and Kavas (2008)**, the bacterial sensitivity is interpreted based on the inhibition zone diameter as follows:

- **Resistant**: less than 8 mm
- **Susceptible**: 9–14 mm
- **Sensitive**: 15–19 mm
- **Very sensitive**: greater than 20 mm

5. Development of Biocosmetic Products Based on *Urtica dioica* Extracts

5.1. Formulation and Characterization of a Bio-Soap with *Urtica dioica*

- **Materials Used**:
  - ✓ Olive oil: 300 g ;
  - ✓ Coconut oil: 200 g ;
  - ✓ Castor oil (optional): 50 g ;
  - ✓ Sodium hydroxide (NaOH): 75 g ;
  - ✓ Distilled water: 190 ml ;
  - ✓ Concentrated *Urtica dioica* extract (alcoholic or aqueous): 2–3 tablespoons;
  - ✓ Dried *Urtica dioica* powder (optional): 1 teaspoon.

- **Preparation Method**:
  - ✓ **Preparation of Urtica dioica Extract**: A quantity of dried *Urtica dioica* powder was boiled in distilled water to obtain an aqueous extract **(Fig. 33)**.

Figure 33 : Urtica dioica plant powder.

✓ **Prepare the Lye (Soda) Solution:**

- In a heat-resistant container, gradually add **75 g of sodium hydroxide (NaOH)** to **190 ml of distilled water** (never the reverse), stirring continuously until fully dissolved;
- Allow the solution to cool to a temperature of **40–45 °C**.

✓ **Prepare the Oils:**

- Gently heat the oils until the solid fats are completely melted;
- Let the oil mixture cool down to **40–45 °C**.

✓ **Mixing and Molding:**

- Slowly pour the lye solution into the oils while blending with an immersion blender;
- Once the mixture reaches a trace (starts to thicken), add a small amount of the aqueous Urtica dioica ex- tract**;**
- Optionally, incorporate a small quantity of dried Urtica dioica powder for decorative or exfoliating pur- poses;
- Mix thoroughly and pour the soap mixture into molds;
- Cover with a towel and allow it to rest undisturbed for 24 to 48 hours**.**

✓ **Curing Process:**

- After solidification, remove the soaps from the molds and let them cure in a dry, well-ventilated area for 4 to 6 weeks, allowing excess water to evaporate and the soap to harden fully **(Cavitch, 1995) (Fig. 34)**.

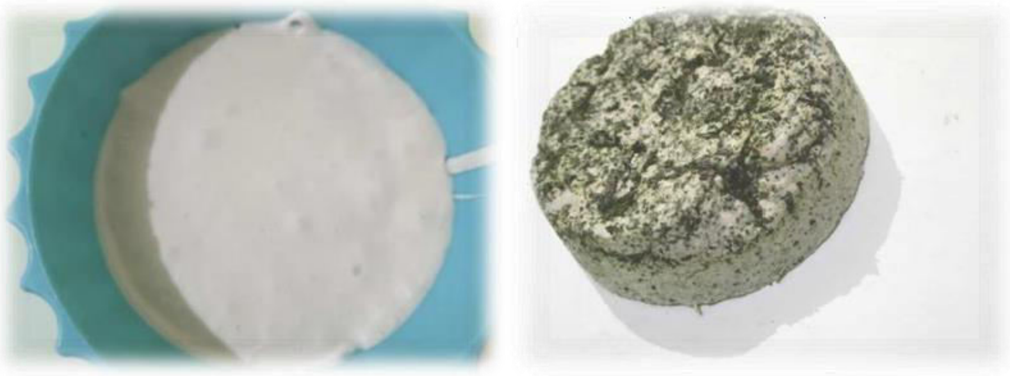


Figure 34: Visual comparison between basic soap and soap formulated with added Urtica dioica powder.

- **Properties Study of the Soap**
  - **pH Determination:** Dissolve 0.5 g of soap in 150 ml of distilled water, stir for 2 minutes, and measure the pH using a pH meter;
  - **Foaming Ability Test:** Add a small piece of the soap to 10 ml of distilled water and stir until foam is formed to evaluate the lathering properties.

5.2. Formulation and Characterization of a Dermal Cream with *Urtica dioica*

- **Ingredients:**

  a) **Aqueous Phase:**
    - ✓ *Urtica dioica* Extract: 40 ml;
    - ✓ Natural Aloe Vera Gel: 20 ml **(Fig. 35)**;
    - ✓ Glycerin: 10 ml.

Figure 35 : Aloe Vera Leaf Segment Utilized for the Recovery of Natural Gel.

  b) **Oil Phase:**
    - ✓ Olive Oil: 10 ml;
    - ✓ Coconut Oil: 10 ml;
    - ✓ (Optional: Light oil such as almond or jojoba oil, 5 ml).

  c) **Optional Ingredients:**
    - ✓ Vitamin E: 3 drops.

- **Preparation Method:**
  - ✓ Prepare the Urtica dioica infusion as usual and allow it to cool completely;
  - ✓ In a water bath, gently heat the oils until just lukewarm;
  - ✓ In a separate bowl, mix the aloe vera gel, Urtica dioica infusion, and glycerin (aqueous phase);
  - ✓ Slowly pour the aqueous phase into the oil phase while continuously mixing with a hand whisk or immersion blender to form an emulsion;
  - ✓ Finally, add vitamin E and mix thoroughly.

6. Data Processing and Statistical Analysis

The data were entered, processed, and presented in tables and graphs using "**Microsoft Excel 2013 software.**"

1. **Yield of Methanolic Extracts from *Urtica dioïca* Leaves**

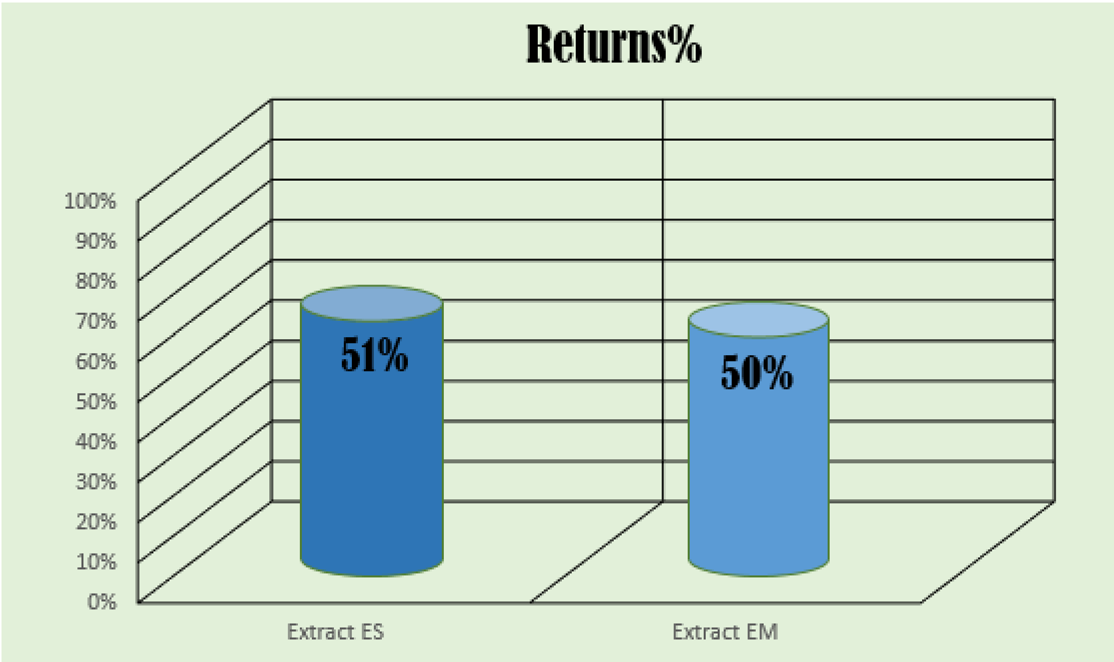


Figure 36: Yield of Methanolic Extracts from *Urtica dioica* L. Leaves.

Based on the initial dry mass of the Urtica dioica (*Urtica dioica*) leaves, the extraction yield was calculated as a percentage using Excel. The results indicated that the highest yield was obtained with methanolic extraction using the Soxhlet apparatus, reaching **51%**. A slightly lower yield of **50%** was observed when the same solvent was used with conventional maceration.

These findings emphasize the significant influence of the extraction technique on the quantity of bioactive compounds obtained, even when utilizing the same solvent. The superiority of the Soxhlet method can be attributed to its capacity to maintain a consistent temperature and enable continuous recycling of the solvent. This enhances the solvent's penetration into plant tissues and facilitates a more effective release of phytoconstituents **(Azwanida, 2015)**.

The minimal difference in yield between the two methods may be due to differences in solvent diffusion and interaction with intracellular compounds during maceration. As supported by **Naczk and Shahidi (2004)**, both the nature of the solvent and the applied technique critically influence extraction efficiency and the chemical profile of the resulting extract.

Table 5 : Comparative Advantages and Disadvantages of Soxhlet and Maceration Extraction Techniques.

	Avantages	Disadvantages
Soxhlet	-Efficient extraction with minimal solvent volumes due to continuous solvent recycling, which helps avoid early saturation of the solvent **(Azwanida, 2015)**. -Eliminates the need for filtration post-extraction since the extract accumulates in the flask directly. -Simple setup, cost-effective, and widely used for extracting bioactive compounds from solid materials **(Pandey and Tripathi, 2014)**. -Enhances mass transfer through repeated exposure of the sample to fresh hot solvent, which improves the yield.	-The process is time-consuming and can take several hours to complete. -Requires finely ground samples to increase surface area for better extraction. -The use of organic solvents poses toxicity risks depending on the compound and solvent used. -High temperatures involved may lead to degradation or loss of thermolabile or volatile compounds **(Wang and Weller, 2006)**.
Maceration	-Conducted at room temperature, which is ideal for preserving heat-sensitive constituents such as phenolic compounds or essential oils. -Stirring or shaking during maceration can improve extraction efficiency by promoting better interaction between solvent and plant matrix **(Azmir *et al.*, 2013)**.	-Extraction is generally slow and may take several hours or even days to achieve desired yield. -Requires fine particle size for optimal extraction surface area. -Filtration is necessary to separate the extract from the solid residue. -Large volumes of solvent are needed due to saturation effects, especially for low-solubility compounds. -As with Soxhlet, solvent toxicity is a consideration depending on the application and target compounds.

2. Phytochemical Analysis of the Extracts

2.1. Detection of Bioactive Metabolites (Phytochemical Screening)

Phytochemical screening refers to the qualitative detection of major classes of bioactive compounds found in green plants. This process involves characteristic colorimetric or precipitation reactions triggered by the addition of specific reagents.

The results of the phytochemical screening revealed the presence or absence of various groups of secondary metabolites, as illustrated in **Figures 37 and 38** and summarized in **Tables 6 and 7.**

Figure 37: Staining obtained by screening ES extract.

Table 6 presents the results of the phytochemical screening of the Methanolic extract of *Urtica dioica* leaves obtained via the Soxhlet method (ES). A pronounced color change was observed for phenols, tannins, and alkaloids following the application of Wagner's reagent, indicating their strong presence.

Additionally, a slight color change was noted for flavonoids and alkaloids upon treatment with Mayer's reagent, suggesting a weaker or moderate presence of these compounds.

Table 6 : Preliminary test results for some secondary metabolites of ES extract by Soxhlet.

Metabolites tested	Reagents	Results
Phenolic compounds	Ethanol, $FeCl_3$	+++
Flavonoids	HCl, Mg	+
Tannins	$FeCl_3$	++
Coumarins	NaOH	-
Alkaloids	HCl, dragendroff, Mayer	+
Alkaloids	HCl, dragendroff,Wagner	+++

+++ : Strongly positive; **+ :** Weakly positive; **-:** Negative.

Figure 38: Staining obtained by screening EM extract.

Table 7 presents the results of the phytochemical screening of the second methanolic extract of *Urtica dioica* leaves, obtained via the maceration method (EM). This extract demonstrated a notable richness in alkaloids, flavonoids, coumarins, and tannins, as evidenced by characteristic color changes following the application of specific reagents.

Table 7 : Preliminary test results for some secondary metabolites of EM extract (by maceration).

Metabolites tested	Reagents	Results
Phenolic compounds	Ethanol, $FeCl_3$	++
Flavonoids	HCl, Mg	++
Tannins	$FeCl_3$	+++
Coumarins	NaOH	+++
Alkaloids	HCl, dragendroff, Mayer	+
Alkaloids	HCl, dragendroff, Wagner	+++

+++ : Strongly positive; **+ :** Weakly positive; **-:** Negative.

- **Comparison Between EM and ES Extracts**

Following the phytochemical screening of both *Urtica dioica* leaf extracts, a comparative analysis was conducted. Notable differences were observed between the two extracts, primarily based on the intensity or absence of color changes during the tests. These colorimetric changes indicate the presence or absence of specific secondary metabolites.

- **Explanation of the Observed Differences**

From both a scientific and logical standpoint, and supported by findings from previous studies, it can be concluded that these differences arise from variations in extraction conditions particularly temperature. Such parameters can positively or negatively influence the total content of secondary metabolites in the extracts. Consequently, the darker the color developed upon reaction with a specific reagent, the higher the concentration of the corresponding compound in the extract.

- **Comparison with Previous Studies**

Our results were compared with those reported by **Afif-Chaouche (2015)**, who conducted a similar phytochemical analysis. Overall, there was strong agreement between our findings and theirs regarding the presence of several key metabolites. However, a notable difference was observed: saponins were detected in their samples but were absent in ours.

This discrepancy could be attributed to geographical variation in plant origin. While our *Urtica dioica* sample was collected from the Mostaganem region, **Afif-Chaouche (2015).** Sourced their material from the Tizi Ouzou region, which may influence phytochemical composition due to environmental and ecological factors.

- **Conclusion on Major Compounds**

Several studies, including that of **Toubal (2018)**, have identified the primary constituents of *Urtica dioica* leaves as flavonoids, tannins, anthocyanins, saponosides, alkaloids, coumarins, glycosides, and mucilaginous substances. Furthermore, **Chaurasia (1986)** confirmed the presence of flavonoids, sterols, and tannins in *Urtica dioica* through laboratory investigations.

2.2. Thin-Layer Chromatography (TLC) Results

Thin-layer chromatography (TLC) is a valuable and widely used technique for the phytochemical analysis of crude plant extracts, fractions, and isolated pure compounds. Due to its simplicity, low cost, and minimal laboratory requirements, TLC remains an essential tool in phytochemical investigations.

Following the development and drying of the chromatographic plate, yellow spots indicative of specific compounds were observed, as illustrated in **Figure 39**.

Figure 39: TLC Plate After Development and Drying.

As previously mentioned, after the extracts are applied to the chromatography plates, the specified reagents are sprayed onto them. Upon spraying with $FeCl_3$ reagent, blue-black spots appear, indicating the presence of flavonoids. In contrast, after spraying with Dragendorff's reagent, an orange color emerges, confirming the presence of alkaloids in the plant extracts. The results of the thin-layer chromatography are presented in **Figure 40**.

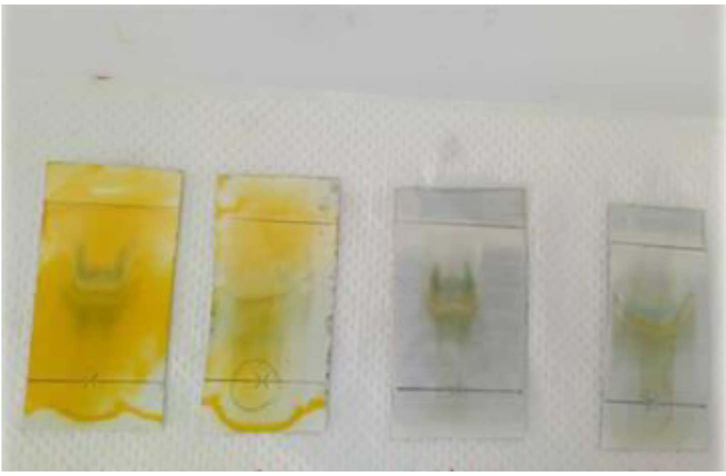


Figure 40: Separation study. EM1/ES1: alkaloids. EM2/ES2: flavonoids.

- **Calculation of Rf:** The results are usually expressed as the retention factor (Rf), which is defined as follows:

$$Rf = di / ds$$

Where:

Rf: Retention factor or interface ratio

di: Distance traveled by the compound

ds: Distance traveled by the solvent **(as shown in Tables 8 and 9)**.

Table 8: TLC results for the methanolic extract of ES.

	Stinging Urtica dioica (ES methanolic extract)			
	ds (cm)	**di (cm)**	**color**	**RF**
After $FeCl_3$ developer	6.3	d1 =1.2	Light green	0.19
		d2=1.9	Dark green	0.30
		d3=2	Orange	0.32
		d4=2.2	blue	0.35
		d5= 2.5	Light yellow	0.40
After Dragendroff developer	6.3	d1 =1.2	Brown trace	0.19
		d 2=1.5	Light green	0.24
		d3=2	Green trace	0.32
		d4=2.4	light green	0.38
		d5=2.7	Orange trace	0.43

Table 9: TLC results for the methanolic extract of EM.

	Stinging Urtica dioica (EM methanolic extract)			
	ds (cm)	**di (cm)**	**color**	**Rf**
After $FeCl_3$ developer	**6**	d1 =1.2	Green trace	0.2
		d2=1.7	Light brown	0.28
		d3=2	Orange green	0.33
		d4=2.3	Orange	0.38
		d5= 2.5	Blue	0.42
		d6=2.7	Green trace	0.45
After Dragendroff developer	**6**	d1 =1.8	Light brown	0.3
		d2=1.9	Orange	0.32
		d3=2.1	Blue green	0.35
		d4=2.3	Yellow	0.38
		d5= 2.4	Green	0.4
		d6=2.7	Blue green	0.45

Thin-layer chromatography (TLC) confirmed the presence of flavonoids in both Urtica dioica extracts through the appearance of colored fluorescent spots under ultraviolet light, typically violet, brown, or blue. These spots indicate a predominance of flavonoids in the extracts.

Our results are broadly consistent with those reported by **Harborne (1998),** who established a correlation between specific fluorescence responses and flavonoid structures.

The migration of compounds on a TLC plate is primarily influenced by their polarity. Flavanones, flavonols, and methoxylated flavonoids tend to migrate more rapidly, exhibiting higher Rf values typically ranging from 0.5 to 0.75 **(Justesen, 2000; Mabry *et al.,* 1970)**. These Rf values serve as chemical markers for the classification of flavonoid subclasses in plant extracts.

In our study, the methanolic extracts displayed variations in Rf values across different fractions, suggesting the presence of multiple flavonoid subtypes. Notably, the aqueous phase of the methanolic extract revealed a large number of distinct spots-up to five-within the Rf range of 0.5 to

0.75, indicating a high concentration of polar flavonoids. This observation is consistent with previous phytochemical studies of Urtica dioica, which identified flavonoids such as rutin, quercetin, and kaempferol derivatives as major components **(Gülçin *et al.,* 2002; Kregiel *et al.,* 2018)**.

The consistency of these findings with prior research supports the hypothesis that the *Urtica dioica* extract contains a broad array of flavonoids, which may contribute to its antioxidant and pharmacological properties.

3. Biological Activities of the Extracts

3.1. Antioxidant Activity (DPPH test)

The absorbance was measured spectrophotometrically at 517 nm, using the values obtained **(Tab.10)**.

Table 10: Absorbance measurements of the two extracts with vitamin C.

Dilution	1	2	3	4
Extract EM	0.455	0.081	0.014	0.002
Extract ES	0.530	0.156	0.071	0.069
Vitamin C	0.021	0.020	0.018	0.016

From the table below, a marked decrease in absorbance values is observed. This decline reflects a reduction in the intensity of the purple coloration, which is associated with the presence of free radicals. Therefore, the observed decrease in color intensity indicates that free radicals were scavenged, suggesting an antioxidant effect of the *Urtica dioica* extracts.

The percentages of radical inhibition were calculated using the previously mentioned formula and are presented in **Table 11.**

Table 11: Percentage of Free Radical Inhibition by Both Extracts Compared to Vitamin C.

Dilution	1	2	3	4
Extract EM	43.13	89.88	98.25	99.75
Extract ES	33.75	80.50	91	91.38
Vitamin C	72.38	88.75	94	98

Furthermore, the data in this table reveal an increase in the percentage of inhibition, which supports the observations made in the previous table. These results confirm that the *Urtica dioica* extract effectively scavenges free radicals, thereby demonstrating its antioxidant potential.

The obtained values were used to plot the curves showing the variation of the percentage of inhibition as a function of the concentration of the methanolic extract **(Fig.41.42.43)**.

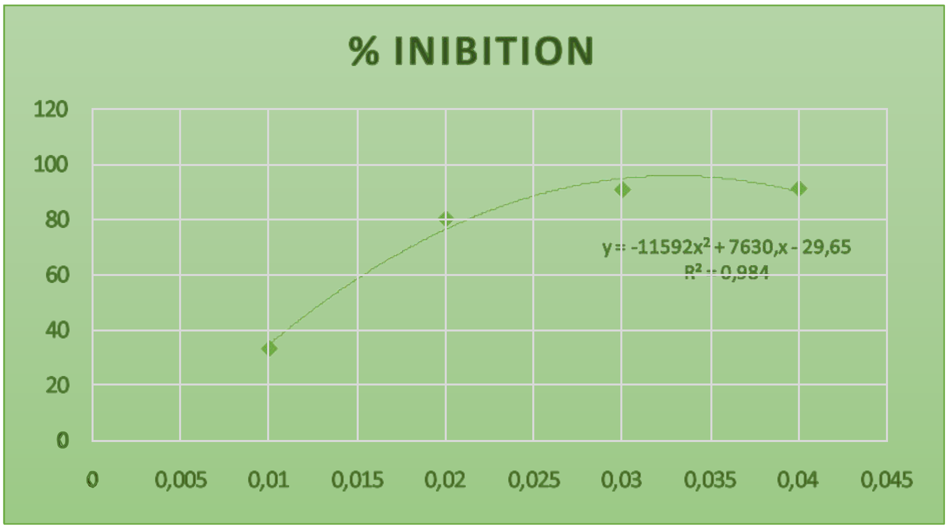


Figure 41 : % Inhibition of ES extract free radical.

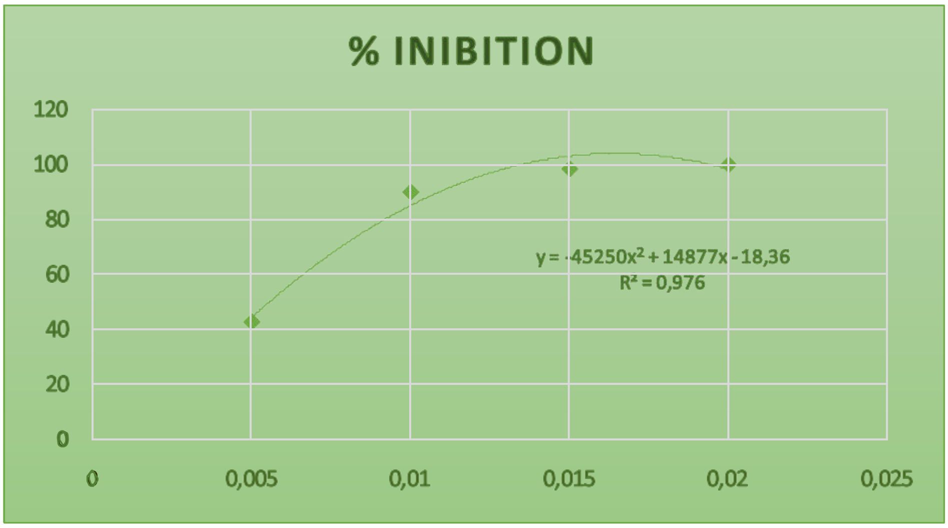


Figure 42 : % Inhibition of EM extract free radical.

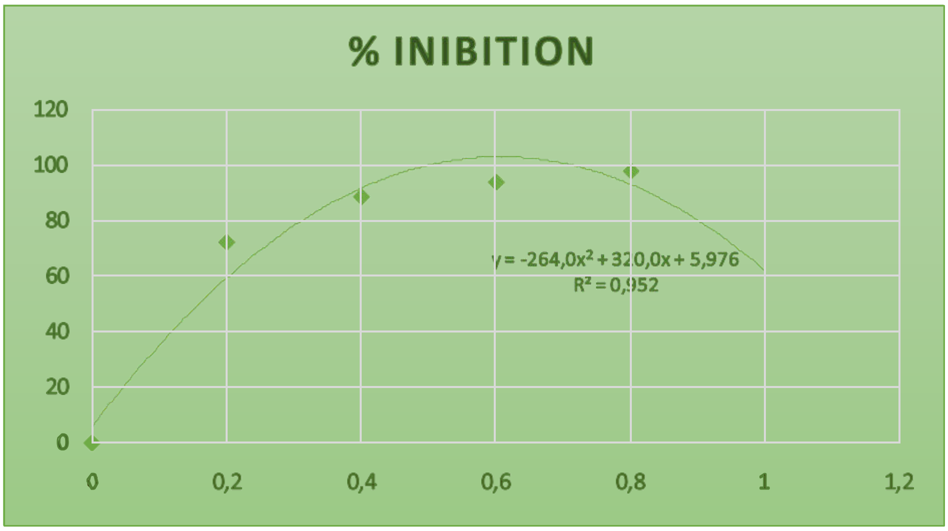


Figure 43 : % Inhibition of vitamin C free radical.

The IC50 is determined graphically from the curve representing the antioxidant activity of the methanolic extract of *Urtica dioica* L. **(Tabl.12)**

Table 12: Anti-free radical activity of leaf extracts of *Urtica dioica* L.

	IC 50(mg /ml)	% Maximum	% Minimum
Vitamin C	211.95	98	72.38
Extract ES	52.8	91.38	33.75
Extract EM	27.35	99.75	43.13

- **Calculation and Interpretation of EC_{50} and Antioxidant Activity**

The EC_{50} value was calculated by taking into account the concentration of DPPH in the reaction medium, using the following formula: $EC_{50} = IC_{50}$ / [DPPH concentration in mg/ml]

The IC_{50} value is inversely proportional to the antioxidant capacity of a compound, as it represents the concentration of extract required to reduce the initial DPPH radical concentration by 50%. Thus, the lower the IC_{50} value, the stronger the antioxidant activity.

Our results showed that the methanolic extract obtained by maceration (EM) exhibited stronger antioxidant activity than that obtained by Soxhlet extraction (ES), with IC_{50} values of approximately 27.35 mg/ml for EM and 52.8 mg/ml for ES.

This indicates that the EM extract was more effective in neutralizing free radicals than the ES extract. Several studies support these findings. For example, **Ahmed *et al.* (2019)** reported that both methanolic and ethanolic extracts of Urtica dioica leaves exhibited significant DPPH scavenging activity, with inhibition rates exceeding 75%, in agreement with our observations.

Moreover, the antioxidant capacity of extracts has been shown to depend on the extraction solvent. **Alam *et al.* (2020),** in a comparative study, found that the ethyl acetate extract had a notably low ICy_0 value (~79 µg/ml), demonstrating superior antioxidant activity compared to butanol, petroleum ether, and ethanol extracts, which exhibited higher IC_{50} values, reflecting weaker free radical scavenging potential.

Similarly, **Singh and Sreenivasulu (2021)** emphasized that extraction conditions particularly solvent polarity and extraction method strongly influence phenolic and flavonoid content, and thus antioxidant activity. **Rezaei *et al.* (2018)** also demonstrated significant variations in antioxidant potential in *Citrus reticulata* (Mandarin orange) depending on geographic origin and growth stage, highlighting the importance of phytochemical variability.

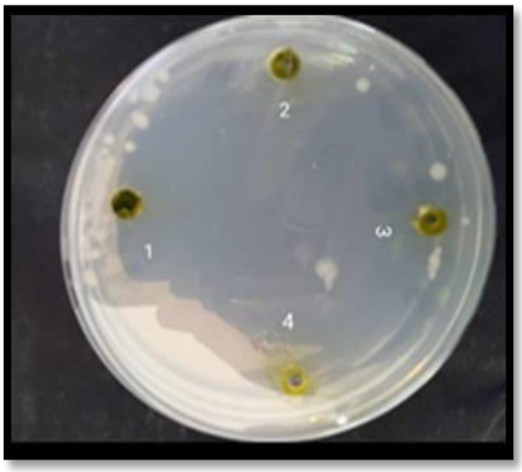
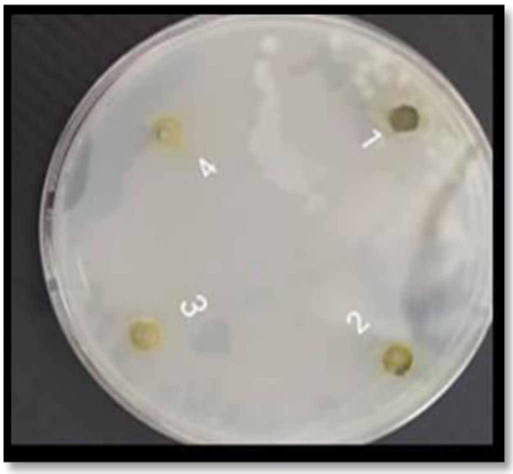
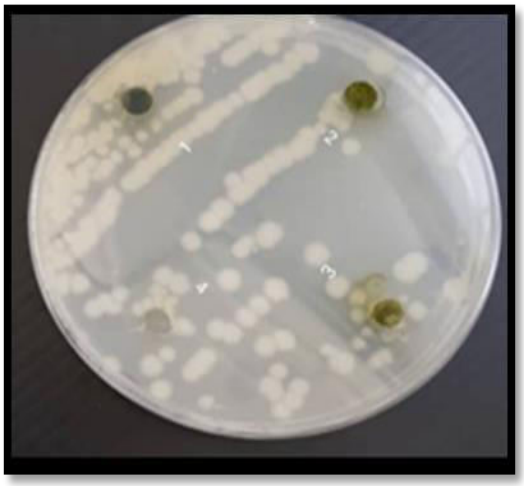
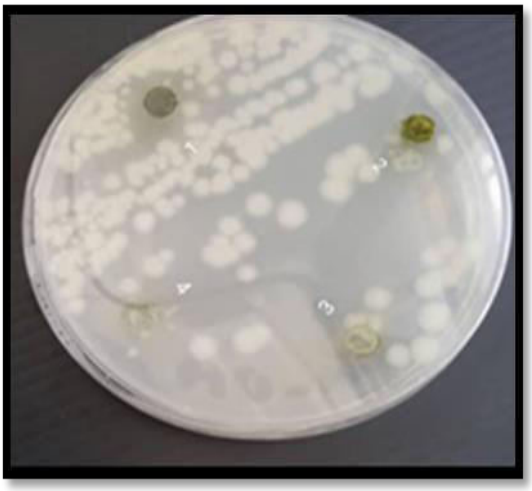
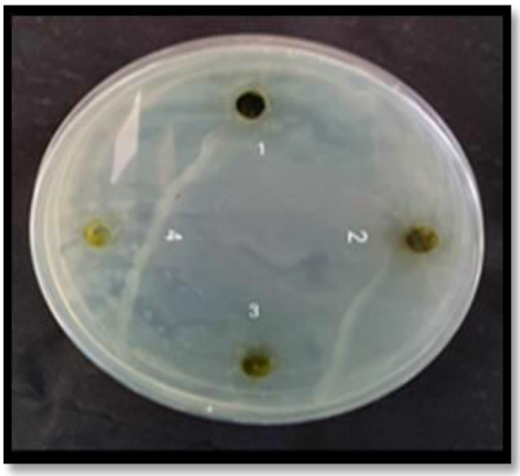
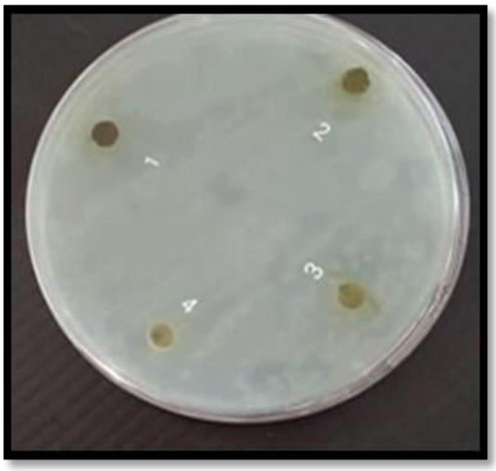
In contrast, decoction-based extracts, particularly from stinging Urtica dioica, were found to exhibit minimal activity against DPPH, with inhibition rates not exceeding 22%, as demonstrated by **Kaur and Arora (2017)**. This variation underscores the importance of extraction technique, concentration, and phytochemical composition in determining antioxidant activity.

3.2. Antibacterial Activity

With the increasing emergence of antimicrobial resistance, the exploration of alternative antibacterial agents especially those derived from natural sources like *Urtica dioïca* has become crucial. Natural compounds may offer specific modes of action and fewer side effects compared to conventional antibiotics, thereby contributing to the global effort to mitigate antibiotic resistance and improve public health outcomes.

In this study, we tested the antibacterial activity of Urtica dioica extracts using agar diffusion techniques against five bacterial strains (*Escherichia coli*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Bacillus cereus* and *Bacillus subtilis*) commonly associated with human diseases, belonging to two different classes (Gram-positive and Gram-negative). The results are interpreted based on the presence or absence of inhibition zones.

Testing the sensitivity of our extracts allowed us to determine the diameters of inhibition around wells saturated with different concentrations of each extract (EM and ES) **(Fig.44**, **45** and **46)**.

Bacterial Strains	**Extract ES**	**Extract EM**
Eschrichia coli		
Bacillus cereus		
Pseudomonas aeruginosa		

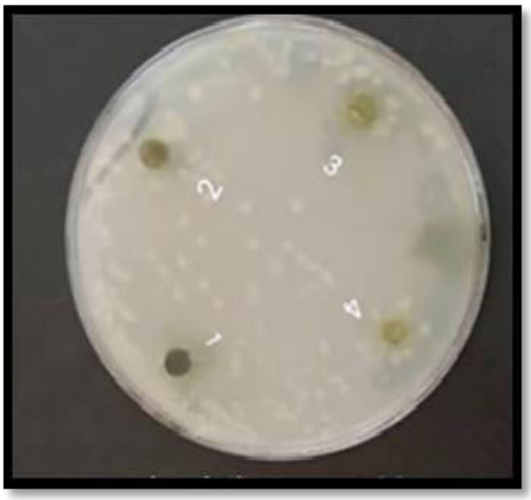
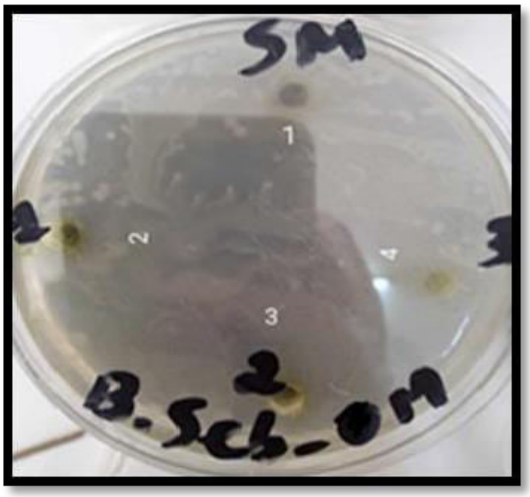
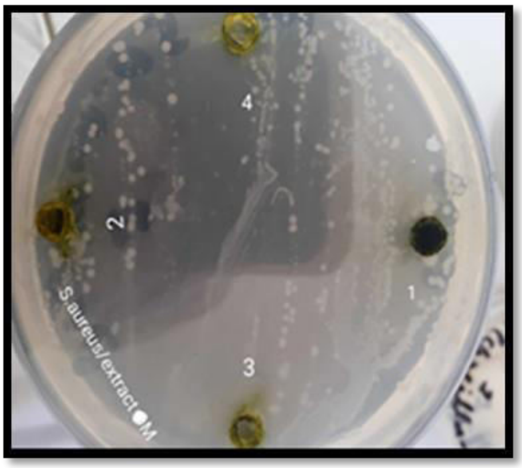
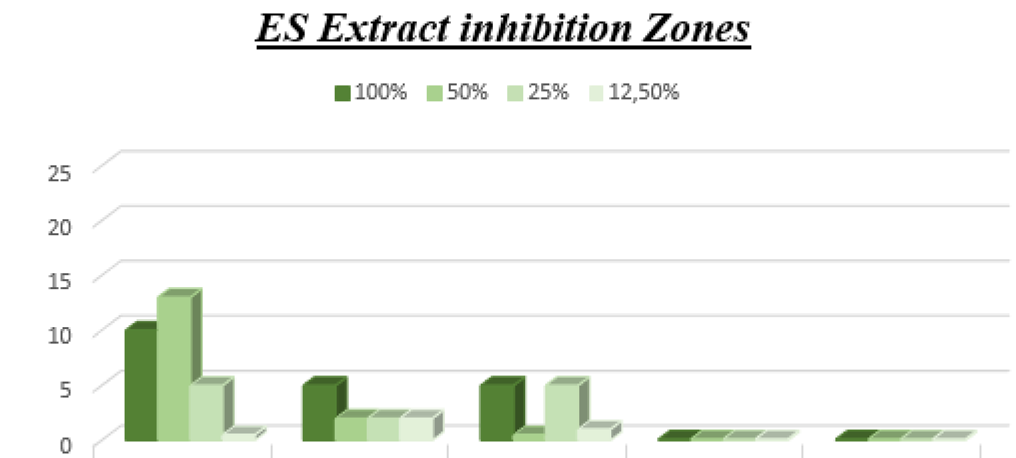
Bacillus subtilis		
Staphylococcus aureus		

Figure 44: Observation of the inhibition zones of ES and EM extracts of *Urtica dioica* L.

Both extracts demonstrated antibacterial activity against at least two of the tested microbial strains, confirming that *Urtica dioica* possesses notable antibacterial properties. However, strains such as *Escherichia coli*, *Pseudomonas aeruginosa*, *Bacillus subtilis*, and *Staphylococcus aureus* exhibited high levels of resistance to the extracts. In contrast, *Bacillus cereus* was found to be highly sensitive to both EM and ES methanolic extracts of *Urtica dioica* leaves.

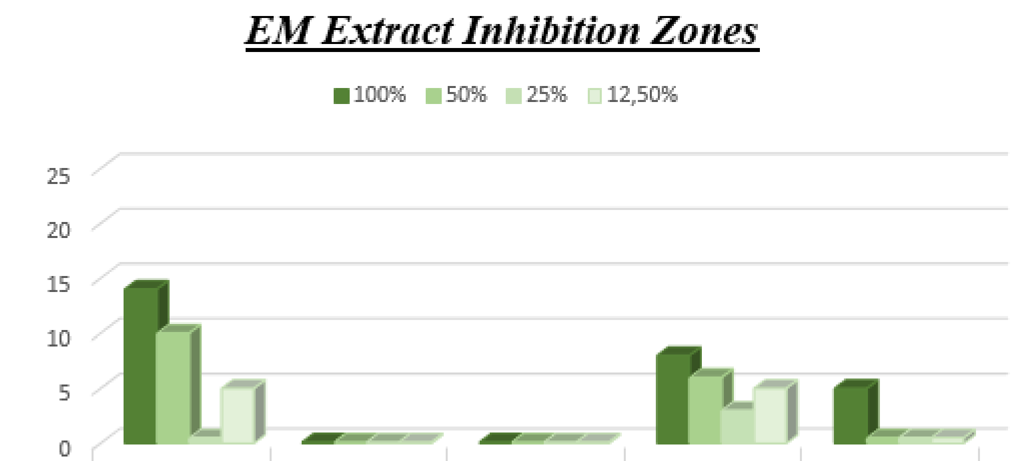
The interpretation of these results is based on the antibacterial activity scale proposed by **Celeikel and Kavas (2008),** which categorizes inhibition zone diameters to assess the degree of antimicrobial effectiveness:

- **Resistant:** Diameter less than 8 mm.
- **Sensitive:** Diameter between 9 and 14 mm.
- **Highly sensitive:** Diameter between 15 and 19 mm.
- **Extremely sensitive:** Diameter greater than 20 mm.



	B.cereus	B.subtilis	S.aureus	P.aerugenosa	E.coli
100%	10	5	5	0,1	0,1
50%	13	2	0,5	0,1	0,1
25%	5	2	5	0,1	0,1
12,50%	0,5	2	1	0,1	0,1

Figure 45: Inhibition Zone Diameters of Five Bacterial Strains Treated with the ES Extract of *Urtica dioica* L.



	B.cereus	B.subtilis	S.aureus	P.aerugenosa	E.coli
100%	14	0,1	0,1	8	5
50%	10	0,1	0,1	6	0,5
25%	0,5	0,1	0,1	3	0,5
12,50%	5	0,1	0,1	5	0,5

Figure 46: Inhibition Zone Diameters of Five Bacterial Strains Treated with the EM Extract of *Urtica dioica* L.

The antibacterial activity of *Urtica dioica* extracts was evaluated using two distinct extraction methods: Soxhlet extraction (ES) and maceration (EM).

- **Antibacterial activity of Urtica dioica extract (EM) (maceration)**

The results demonstrated that the *Urtica dioica* extract exhibited notable antibacterial activity against *Bacillus cereus*, with an inhibition zone diameter of 13 mm at 100% concentration. Moderate activity was observed against *Pseudomonas aeruginosa* (8 mm), while *Escherichia coli* showed weak susceptibility (5 mm). These findings suggest that the extract contains bioactive compounds, particularly effective against Gram-positive bacteria.

Maceration-based extracts are known to primarily yield polar compounds such as phenols and flavonoids, which are well-documented for their antimicrobial properties **(Gülçin *et al.,* 2010)**. However, this method may be less effective than Soxhlet extraction for isolating a broader range of bioactive constituents, especially non-polar compounds.

- **Antibacterial activity of the ES extract (using the Soxhlet method)**

In contrast, the Soxhlet-derived extract (ES) exhibited moderate antibacterial activity against *Bacillus cereus*, with an inhibition zone reaching up to 10 mm at 100% concentration, and weaker activity against *Staphylococcus aureus* and *Bacillus subtilis*. Interestingly, an inhibition diameter of 13 mm was recorded at the 50% concentration, which could be attributed either to enhanced diffusion of the active compounds at this dilution or to partial degradation of thermolabile constituents at higher concentrations. While the Soxhlet method is generally more efficient in extracting a broader range and higher quantity of phytochemicals, the elevated temperature involved may compromise the stability of certain heat-sensitive bioactive compounds. This could explain the relatively lower antibacterial activity observed compared to the maceration-derived extract **(Azwanida, 2015)**.

- **Effect of Extraction Method**

When comparing the two extraction methods, maceration appeared to better preserve the antibacterial activity of the *Urtica dioica* extracts in this study. This is likely due to the stability of the bioactive compounds at room temperature, as opposed to the thermal conditions involved in Soxhlet extraction. Previous studies have indicated that Soxhlet extraction can alter some phenolics and antioxidants **(Do *et al.,* 2014)**.

- **Variation in Bacterial Response**

The differences observed in the antibacterial activity of the extracts across the tested bacterial strains can be attributed to the structural characteristics of their cell walls. Gram-negative bacteria, such as *Escherichia coli*, generally exhibit higher resistance to plant-derived antimicrobial agents due to the presence of an outer membrane, which acts as a barrier against the penetration of bioactive compounds **(Nazzaro *et al.,* 2013)**. In contrast, Gram-positive bacteria lack this outer membrane, rendering them more susceptible to such compounds.

4. Evaluation of Biocosmetic Formulations

4.1. Properties and Stability of the Bio-Soap Formulated with *Urtica dioica*

Based on the well-documented chemical and biological properties of *Urtica dioica*, we developed a natural antibacterial soap intended for topical use. The soap was formulated following a standardized experimental protocol and was subsequently evaluated for key characteristics such as texture, homogeneity, color, and foaming capacity. These features were visually assessed, as illustrated in **Figure 47**.

Figure 47 : The Urtica dioica soap.

- **Determination of pH**

The pH of cosmetics is a crucial parameter, as it directly affects skin tolerance and overall product stability.

To assess the quality of soap and its compatibility with skin physiology, we measured the pH. **Figure 48** shows the pH value of soap. **Table 13** also shows the pH values obtained for soap made with Urtica dioica extract.

Figure 48: The pH measurement of Urtica dioica soap.

Table 13: pH Measurement of the *Urtica dioica* Soap.

Duration	After 2 hours	After 48 hours	After one week
Value	6.5	6.5	7

The pH of the formulated soap ranged from 6.5 to 7.0, indicating a slightly acidic to neutral composition. This pH range aligns closely with the natural pH of human skin, which generally falls between 4.7 and 5.75, thus supporting its potential dermatological compatibility and minimizing the risk of skin barrier disruption **(Lambers *et al.,* 2006)**. A soap formulation within this range is often preferred for sensitive skin to avoid irritation and maintain the skin's natural protective layer.

- **Foam Test**

Additionally, a Foam test was conducted to verify the success of the saponification process a critical step in transforming oils into effective cleansing agents. The Results demonstrated the production of a dense and stable Foam, which is a key indicator of proper formulation and functional surfactant content **(Ananthapadmanabhan *et al.,* 2004)**, as illustrated in **Figure 49**.

Figure 49: Urtica dioica soap foam.

- **Organoleptic Characteristics**

The organoleptic characteristics of the soap formulated with *Urtica dioica* extract were evaluated through practical handwashing tests, which allowed us to observe a range of physical and Sensory attributes indicative of the product's quality and its potential application in natural cosmetics.

- **Appearance :** The soap exhibited a cohesive and homogeneous form, with no signs of layer separation or crystallization, indicating good physical stability of the formulation.
- **Color :** The soap has a faint natural green color, which reflects the presence of active plant compounds such as chlorophyll and flavonoids, known for their antioxydant properties **(Dweck, 2002).**
- **Scent :** The soap emits a light and pleasant herbal scent, resembling that of fresh plants, providing a refreshing sensation during and after use **(Souto *et al.,* 2022)**.
- **Texture :** Upon touch, the soap presents a firm and cohesive texture with slight roughness, which can be attributed to the presence of unrefined plant components in the traditional formulation.
- **Foaming Ability :** During use, we observed the production of a moderate and relatively stable amount of foam, sufficient to ensure effective cleaning while maintaining skin balance and avoiding dryness **(Barel *et al.,* 2014)**.

These characteristics confirm the Sensory quality of the soap and support its integration into the field of natural cosmetics. Sensory evaluations remain a crucial step in product development, as they directly influence consumer satisfaction and reception of the product.

4.2. Properties and Stability of the Dermal Cream Formulated with *Urtica dioica*

In parallel, a natural skin cream was developed with the aim of producing a moisturizing, anti-inflammatory, and antibacterial product for topical application. The cream was prepared using a unified protocol, and its physical properties and performance were evaluated, as depicted in **Figure 50**.

Figure 50 : Urtica dioica Moisturizing Cream.

- **Determination of pH**

To assess the quality and skin compatibility of the Urtica dioica cream, its pH was measured using pH test strips.

After measuring the pH of the Urtica dioica cream, we observed a value of 5, which remained stable over the course of one week. This pH is in close alignment with the natural pH of human skin, typically ranging between 5 and 6. When compared to the pH values of shea butter cream as reported by **Toé (2004),** our results show a similar pH. Thus, the slightly acidic pH of 5 may contribute to the antibacterial properties of the Urtica dioica cream, potentially offering protection against certain skin conditions, particularly infectious diseases **(Amouroux, 2017).**

- **Allergy Test**

To further assess product safety, a sensitivity test was performed by applying a small amount of the *Urtica dioica*-based cream to a localized area of skin. The test site was monitored for visible signs of adverse reactions, such as erythema, irritation, or itching. The absence of such symptoms confirmed the topical safety and skin tolerance of the cream, as depicted in **Figure 51**. These results suggest that the formulation may be appropriate for regular cosmetic or therapeutic use.

Figure 51: Application of *Urtica dioica* cream to the skin of the hand.

- **Sensory Properties (Alergy properties)**

The following table summarizes the sensory analysis results of the Urtica dioica cream, including its Consistency, Appearance, Color and Odor.

Table 14: Sensory Properties of the Final Urtica dioica Cream.

	Consistency	**Appearance**	**Color**	**Odor**
The sample	Milky	Transparent	Beige green	Odorless

GENERAL CONCLUSION AND PERSPECTIVES

This study falls within the framework of the valorization of local medicinal plants, with a particular focus on *Urtica dioica* L. (stinging Urtica dioica), a species known for its distinctive biological properties and broad therapeutic and cosmeceutical potential. The research responds to the increasing scientific interest in this plant, which has long been used in traditional medicine and is now supported by modern studies highlighting its rich composition of bioactive compounds.

The theoretical component of this work involved an extensive review of the botanical, phytochemical, and pharmacological characteristics of *Urtica dioica*. Literature findings confirmed the plant's richness in secondary metabolites such as flavonoids, phenolic compounds, tannins, saponins, organic acids, and minerals. These compounds are known to confer a range of biological properties including antioxidant, antibacterial, anti-inflammatory, and anticancer activities.

In the experimental phase, methanolic extracts of *Urtica dioica* leaves were obtained using two extraction methods: maceration and Soxhlet. The Soxhlet technique yielded a higher extraction output, while the maceration method preserved greater antioxidant activity, highlighting the impact of heat on thermolabile compounds. Phytochemical screening confirmed the presence of major bioactive constituents, and TLC analysis visually supported these findings by indicating the diversity of phytochemical profiles in both extract types.

Antioxidant activity assessed by the DPPH radical scavenging assay demonstrated that the maceration extract (EM) had a stronger capacity to neutralize free radicals than the Soxhlet extract (ES), as indicated by a lower ICy_0 value. These results suggest that *Urtica dioica* is a promising candidate for applications aimed at reducing oxidative stress and potentially preventing age-related skin damage and diseases linked to oxidative mechanisms.

Regarding antibacterial activity, both extracts were tested against five bacterial strains, both Gram-positive and Gram-negative. The results showed that both extracts possessed interesting activity, particularly against the *Bacillus cereus* strain, which was the most susceptible, highlighting the potential of Urtica dioica as a natural antibiotic. The efficacy also varied depending on the concentration and extraction method. The maceration method appeared to preserve heat-sensitive components better than the Soxhlet method, which, while effective in extraction, can lead to the degradation of some active compounds due to heat.

This study clearly demonstrates that Urtica dioica *(Urtica dioica)* possesses promising biological and therapeutic potential, making it one of the most important medicinal plants deserving of further research and development, whether in the pharmaceutical, cosmetic, or even industrial fields. The results of the analyses and tests conducted in this memorandum, both at the chemical and biological levels, clearly demonstrate that the methanolic extracts of *Urtica dioica leaves* possess remarkable antioxidant and antibacterial activity, opening up broad prospects for their use as a natural ingredient in cosmetic formulations and health supplements.

The production of two natural cosmetic products (soap and skin cream) from Urtica dioica extracts demonstrated their practical application value. The suitable properties of the prepared products (pH, foaming, and stability) were confirmed, along with the absence of side effects upon external application, making them safe and effective for daily use, especially for sensitive and irritated skin.

To further develop the therapeutic and cosmetic potential of *Urtica dioica*, the following perspectives are proposed:

- Expand the range of solvents used in extraction (e.g., ethanol, acetone) to optimize the recovery of different classes of bioactive compounds;
- Conduct in vitro and in vivo studies to assess the biological activity and safety of the extracts at the cellular and clinical levels;
- Formulate a wider variety of cosmeceutical products (such as gels, serums, sprays) using different concentrations of extracts tailored to various skin types and needs;
- Characterize the chemical composition of extracts in detail using advanced analytical techniques such as HPLC or GC-MS;
- Encourage interdisciplinary collaboration between departments of biology, pharmacy, and chemical engineering to develop standardized, stable, and market-ready products derived from *Urtica dioica*.

In conclusion, it can be said that investing in medicinal plants such as Urtica dioica is an investment in both health and the environment, as it offers natural and effective solutions to health and beauty problems at a time when demand for safe and natural alternatives is growing.

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APPENDICES

Appendix 1: Composition of physiological saline.

- 17 g sodium chloride
- 1000 ml distilled water

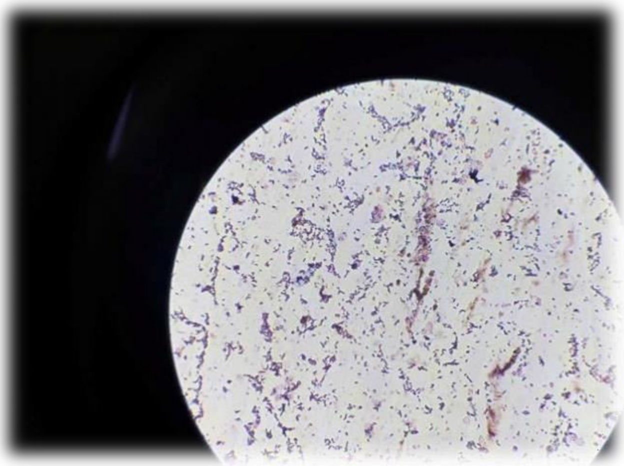
Appendix 2: Composition of nutrient broth.

- 5 g peptone
- 3 g beef extract
- 5 g sodium chloride (NaCl)
- 1 l distilled water
- pH = 7.0–7.4

Appendix 3 :

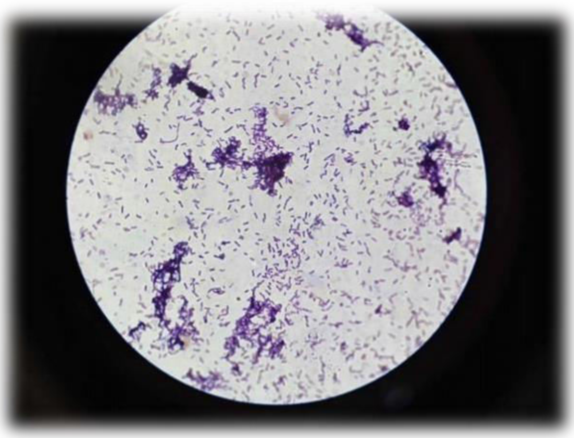
E.coli observed under 10X magnification.

Appendix 4 :



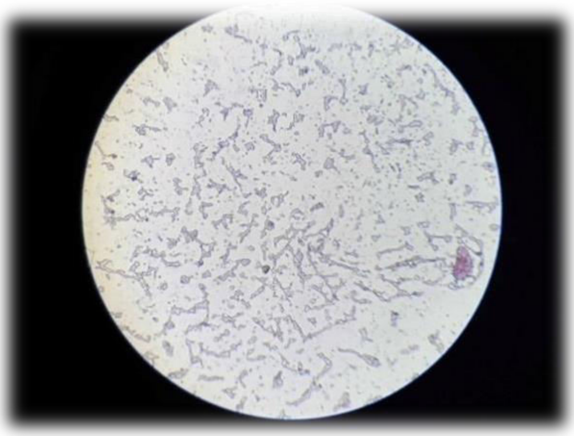
E.coli observed under 40X magnification.

Appendix 5 : Microscopic observation of *E.coli* before and after treatment with *Urtica dioica* extract (EM diluted).



E.coli observed under 100X magnification before treatment.

The bacterial cells appear dense, numerous, and well-structured, indicating normal growth conditions.



E.coli observed under 100X magnification after applying a few drops of *Urtica dioica* EM diluted extract.

A notable reduction in bacterial density is observed, along with disruption of cell stucture in some areas .This suggests an initial antibacterial effect of the extract, even with a short contact time of 2 minutes.

الجمهورية الجزائرية الديموقراطية الشعبية
وزارة التعليم العالي والبحث العلمي
جامعة عبد الحميد بن باديس-مستغانم-
كلية علوم الطبيعة والحياة

تصريح شرفي خاص بالالتزام بقواعد النزاهة العلمية لإنجاز البحث

أنا الممضي أدناه،

الطالب(ة): بـوعباسي آيات **رقم التسجيل الجامعي:** 202038023141

الحامل لبطاقة التعريف الوطنية رقم: 110020735033520002 **والصادرة بتاريخ:** 2024.09.17

عن: بلدية سعيدة -سعيدة

المسجل بـكلية علوم الطبيعة والحياة / قسم العلوم البيولوجية

شعبة: علوم بيولوجية / **التخصص:** علم الأحياء الدقيقة الأساسية

والمكلف بإنجاز مذكرة ماستر بعنوان:

Contribution to the In Vitro Study of the Antioxidant and Antimicrobial Activities of *Urtica dioica* L. Extracts from the Mostaganem Region for Cosmeceutical Applications.

أصرح بشرفي أني ألتزم بمراعاة المعايير العلمية والمنهجية ومعايير الأخلاقيات العلمية والنزاهة الأكاديمية المطلوبة في إنجاز البحث ، وأتحمل المسؤولية الشخصية عن كل المحتوى المتضمن في البحث المذكور أعلاه .

التاريخ: 2025/06/17

إمضاء المعني

* ملحق القرار الوزاري رقم 933 المؤرخ في 28 جويلية 2016 الذي يحدد القواعد المتعلقة بالوقاية من السرقة العلمية ومكافحتها.