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To the One who grants beginnings their light and endings their peace, To Allah, the Most High, the Most Praiseworthy, I owe every ounce of strength, every spark of inspiration, and every step taken on this journey. Without His mercy and guidance, this achievement would have never seen the light of day.

To those who were my first home, my earliest teachers, and my constant guardians,

To my father, ***Mohammed****, whose presence is a quiet force,*

Thank you for being the voice of reason when I faltered, the hand that lifted me when I stumbled, and the shield that protected me through life's storms. Your sacrifices are too many to count, and your love too vast to measure.

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To my sisters and brothers in life—those not born of my mother but gifted to me by time To those who, in the harshest of years, were the clouds that brought rain: ***Houda, Sawsen, Manal, Samra, Mouna, Ilham, Amir, Katib, Ammar:***

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To the one who shared this journey with me, ***Adem****: Thank you for your patience, your kindness, and your understanding. Without your support, some stages would have been much harder to endure.*

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Finally, I dedicate this work to everyone who has been a support to my heart, warmth to my soul, and light to my path

Wafaa

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A	Absorbance
ABTS	2,2'-Azino-bis(3-ethylbenzothiazoline-6-sulfonic acid)
Al^{3+}	Aluminum(III) ion
$AlCl_3$	Potassium Acetate
$Al(NO_3)_3$	Alumium Trichloride
AQCA	Aminoquinoline carboxylic acid
BHA	Butylated hydroxyanisole
BHT	Butylated hydroxytoluene
BSA	Bovine serum albumin
°C	Degrees Celsius
CAGR	Compound annul growth rate
CCl_3COOH	Trichloroacetic acid
CH_3COOK	Potassium Acetate
DPPH	2,2-diphenyl-1-picrylhydrazyl
Eo	Essential oil
FCR	Folin –ciocalteu Reagent
$FeCl_3$	Ferric Chloride
GAE	Gallic acid equivalents
GHZ	Gigahertz
$H_3 PMo_{12} O_{40}$	Phosphotungstic acid
H_3Po_4	Phosphoric acid
$H_3 PM_{12} O_{40}$	Phosphomolybdic acid
IC_{50}	Inhibitory concentration
K3FeCN	Potassium hexacyanoferrate
LPO	Lipid peroxidation
MAE	Microwave-Assisted Extraction
MDA	Malondialdehyde
MIC	Minimum Inhibitory Concentration
MO8O23	Molybdenum oxide cluster
Nh	Imine
Oh	Hydroxyl
PBS	Phosphate-Buffered Saline
Ph	Potential of hydrogen
QE	Quercetin Equivalents
ROS	Reactive Oxygen Species
SH	Sulfhydryl
TBA	Thiobarbiyuric acid
TBARS	Thiobarbituric acid reactive substances
TCA	Trichloroacetic acid
UV	Ultraviolet
W8O23	Tungsten oxide cluster

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INTRODUCTION

INTRODUCTION

Medicinal plants constitute one of the earliest therapeutic agents utilized for millennia in traditional medical practices across diverse cultures worldwide. The understanding of their health-promoting properties has been preserved and transmitted through generations within human societies via empirical knowledge **(Marrelli, 2021).**

Traditional medicine, as defined by the World Health Organization, is the total of the knowledge, skills, and practices based on the theories, beliefs, and experiences indigenous to different cultures, whether explicable or not, used in the maintenance of health as well as in the prevention, diagnosis, improvement, or treatment of physical and mental illness. Huge volumes of literature and records of the theoretical concepts and practical skills support some traditional medicine systems; others are passed down from generation to generation-through verbal teaching **(Che *et al.*, 2023)**.

Evidence suggests that the use of medicinal plants may date back to the Paleolithic era. Today, 80% of the global population relies on traditional herbal medicine as a primary healthcare resource. Despite their widespread use, most medicinal plant species remain untested in clinical settings, and only a small proportion have been scientifically studied for their pharmacological effects. **(Davis and Choisy, 2024)**

This thesis focuses on the valorization of endemic medicinal plants by exploring their pharmacological properties and potential applications in modern medicine. Through ethnobotanical surveys, phytochemical analyses, and biotechnological approaches, this study aims to bridge the gap between traditional knowledge and scientific validation.

This work aims to study the bioactive properties of the *n*-butanol phase of four medicinal plants from Algeria: *Ephedra alata, Linaria tingitana* Boiss. & Reut, *Asteriscus graveolens*, and *Limoniastrum guyonianum*.

This study will be divided into three main parts:

The first part is devoted to a literature review. In its first chapter, we will present the definition of medicinal plants and provide a general overview. The second chapter introduces the main classes of bioactive compounds, such as polyphenols, flavonoids, terpenes, and essential oils, along with their respective conventional and innovative extraction methods. The

third chapter summarizes these compounds' biological activities, particularly their antioxidant and anti-inflammatory effects. Finally, we will present the studied plant families, detailing their characteristics and chemical compositions.

The second part concerns the practical aspect of the study, which involves an *in vitro* experimental investigation. This includes preparing plant extracts, analyzing their phytochemicals (specifically polyphenols, flavonoids, flavonols, chlorophyll, and carotenoids), and evaluating their antioxidant (via DPPH, reducing power, and O-phenanthroline methods), anti-inflammatory, anti-lipid peroxidation, anti-enzymatic and antimicrobial activities.

The third and final part is dedicated to presenting, analyzing, and discussing the results obtained from the experimental work.

Finally, the study will conclude with a general conclusion, perspectives for future research, and a list of bibliographic references.

LITERATURE REVIEW

I LITERATURE REVIEW

I.1 Definition of medicinal plants

A medicinal plant is any plant that, in one or more of its organs, contains biologically active substances capable of being used for therapeutic purposes or serving as precursors for synthesizing valuable drugs. These plants are recognized for their potential to treat or alleviate various health conditions through their bioactive compounds, which can be extracted, isolated, or processed into medicines. This definition allows us to differentiate between two categories of medicinal plants: those whose therapeutic properties and active constituents have been well-documented and scientifically validated through rigorous research and those plants that are considered medicinal in traditional practices but have not yet undergone comprehensive scientific investigation to confirm their effectiveness and safety. The distinction is crucial for advancing the understanding of these plants and ensuring that only, those with proven benefits are incorporated into modern medicine **(Sofowora *et al.,* 2013).**

I.1.1 Overview of medicinal plants

Medicinal plants have been essential in human health and healing practices throughout history. Approximately 10% of all vascular plants are used for medicinal purposes, with estimates suggesting 350.000 and 500.000 species with such properties **(Salmerón-Manzano *et al*., 2020)**. Here's an overview highlighting their significance, uses, and the evolution of research around them:

I.1.1.1 Historical context

It is well-established that all civilizations have developed medicinal practices based on the plants native to their environments. Some scholars argue that this body of knowledge forms the foundation of both medicine and pharmacy. Even today, numerous plant species are cultivated globally to extract valuable substances for use in medicine and pharmacy. The therapeutic properties of plants led to the development of medicinal drugs derived from specific plants with recognized health benefits. Until the 18th century, the therapeutic effects of many plants and their application methods were known, but the active compounds responsible for these effects remained unidentified. For instance, the *Canon of Medicine* by the Persian physician and

scholar Avicenna (Ibn Sina) remained a key reference in medical practice until the 18th century **(Salmerón-Manzano *et al.*, 2020).**

I.1.1.2 Current research trends

There has been a significant increase in research publications related to medicinal plants. From 1960 to 2019, over 110,000 studies have been published, with notable peaks in research output observed around 2011, followed by a sustained publication rate exceeding 5,000 studies per year **(Salmerón-Manzano *et al.*, 2020).**

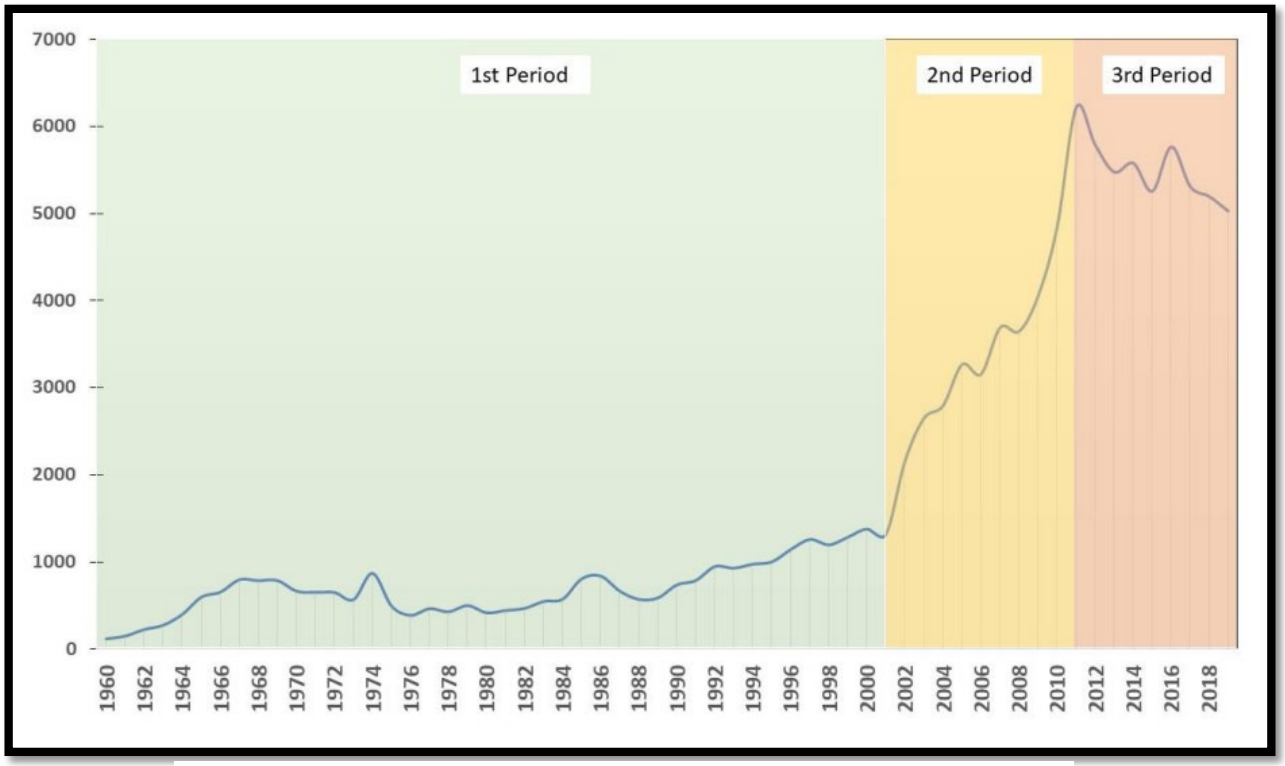

Figure 1*:* Worldwide temporal evolution of medical plants publications **(Salmerón-Manzano *et al.*, 2020).**

I.1.1.3 Futur directions

The future of medicinal plant research centers on discovering new bioactive compounds for drug development, ensuring their efficacy through rigorous scientific validation. A key aspect of this progress is the collaboration between traditional medicine practitioners and modern scientists, combining ancestral knowledge with advanced research methods to create safer and more effective herbal treatments. With pressing global health challenges like antibiotic resistance and chronic diseases, medicinal plants are being explored as potential solutions. This research involves collaboration across different fields, integrating insights from botany,

chemistry, and pharmacology for a more comprehensive understanding. Moreover, sustainability is also a priority, with growing efforts to protect plant biodiversity and promote ethical sourcing. Public health initiatives are also expected to educate communities about medicinal plants' safe use and integration into primary healthcare, particularly in developing regions **(Salmerón-Manzano *et al.*, 2020).**

I.2 Bioactive compounds of medicinal plants

I.2.1 Polyphenols

Polyphenols are natural compounds that are produced only by plants. They have a structure related to phenolic substances and are known for their bioactive properties. These properties allow polyphenols to help manage oxidative and inflammatory stress, influence the digestion of macronutrients, and even act like prebiotics to support the gut microbiota.

Polyphenols are found in nearly all plants and play several important roles. They help attract pollinators, contribute to the plant's structural functions, protect plants from ultraviolet radiation, and defend against microbial invasions and herbivores **(Bertelli *et al.*, 2021).**

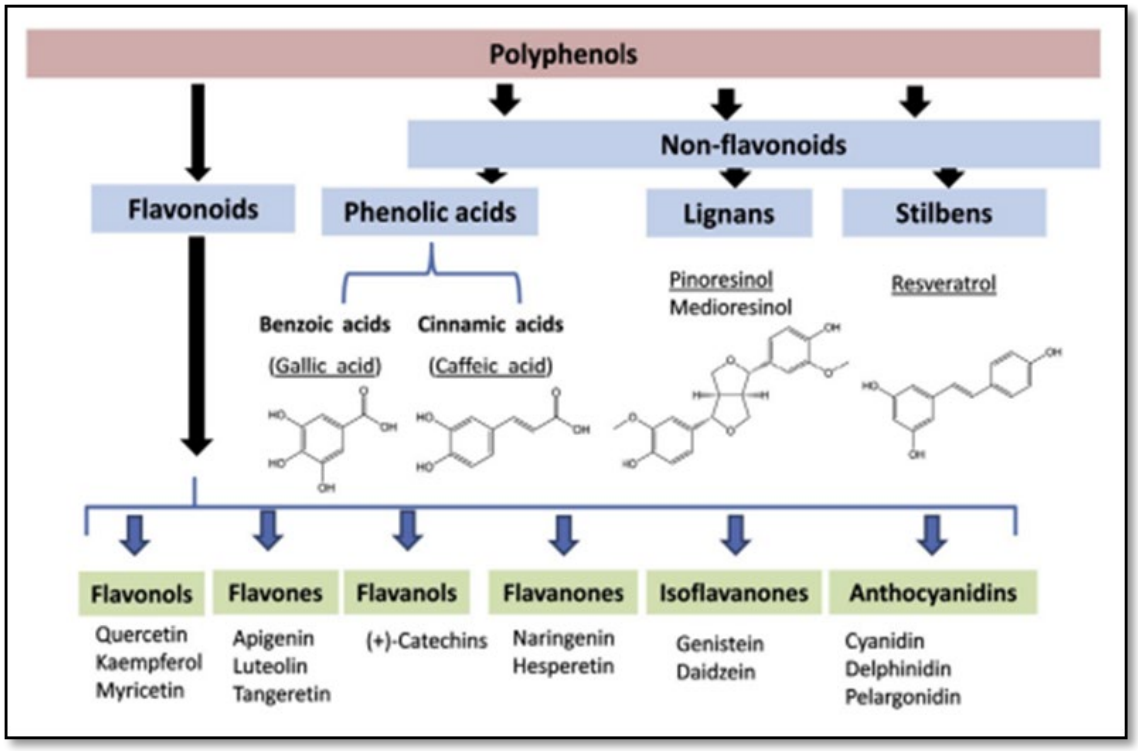

Figure 2: General classification of polyphenols **(Vaher *et al.*, 2005).**

I.2.1.1 Bioactivities of Polyphenols

Natural polyphenols offer many health benefits, helping to prevent and manage age-related diseases, cancer, heart conditions, and more **(Vaher *et al.*, 2005)**. They play a significant role in cardiovascular disease (CVD) prevention and management through various mechanisms, with a diet rich in polyphenols being associated with improved heart health and a reduced risk of cardiovascular disease **(Du *et al.*, 2015).** Their cardioprotective effects are primarily attributed to their antioxidant properties, as these compounds help lower blood pressure, enhance endothelial function, and inhibit platelet aggregation **(Erlund *et al.*, 2008; *Grassi et al.*, 2009).**

I.2.2 Flavonoids

Flavonoids are essential compounds in plants, playing key roles in their development and defense mechanisms. They are responsible for many of the colors in plants, acting as pigments such as anthocyanins (which produce red, orange, blue, and purple hues), chalcones and aurones (yellow pigments), and flavonols and flavones (white and pale-yellow pigments).

Besides adding color, flavonoids also serve as protective agents. As phytoalexins and antioxidants, they help plants defend against damage caused by various stresses, such as UV radiation, cold temperatures, pathogen infections, and insect feeding, by scavenging harmful reactive oxygen species (ROS) **(Liu *et al.*, 2021).**

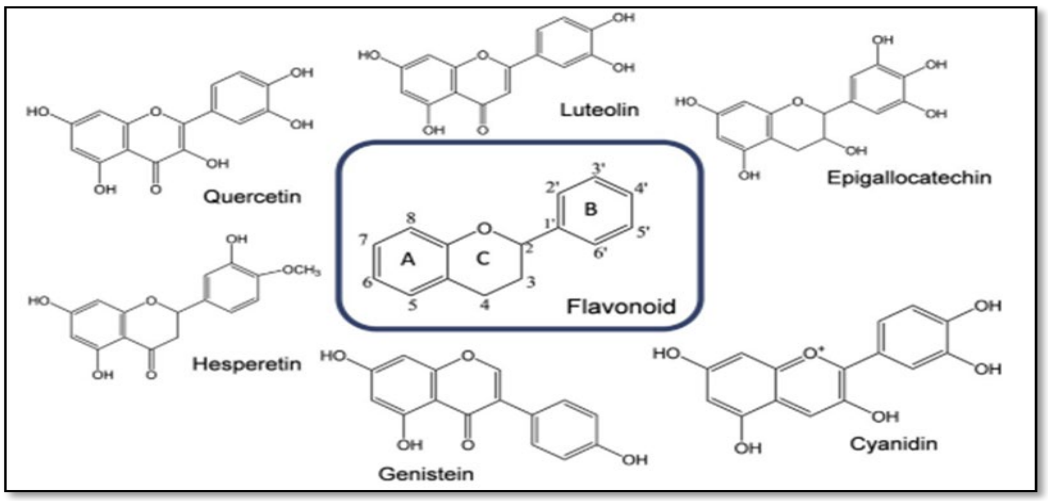

Figure 3: The flavonoids class **(Vaher *et al.*, 2005).**

I.2.2.1 Bioactivities of Flavonoids

Flavonoids have long been recognized for their health benefits, including antihepatotoxic (liver-protecting), anti-inflammatory, antiatherogenic (preventing artery hardening), antiallergic, antiosteoporotic (preventing bone loss), and anticancer properties. Many of these effects are linked to their interaction with various enzymes and their antioxidant activity. This antioxidant effect can come from their ability to neutralize free radicals, bind to metal ions, and work with other antioxidants to enhance their protective power **(Silva *et al*., 2002).**

In most flavonoids, the presence of the C2=C3 double bond facilitates the monoelectronic oxidation of the hydroxyl group at the C3 position, leading to the formation of a hydroxyl radical. The resulting unpaired electrons can delocalize across the C2 position and the B-ring, enhancing stability. Numerous studies have examined the role of the C2=C3 double bond and the 3-OH group, with most recognizing the double bond's significant contribution to the antioxidant activity of flavonoids **(Chen *et al.,* 2017)**. Also, a negative correlation between coronary disease and the consumption of flavanols, flavonols, and flavones has been observed. Additionally, the intake of flavanones and anthocyanins has been associated with a reduced mortality rate from cardiovascular disease **(Abbas *et al.,* 2016).**

I.2.3 Alkaloides

Alkaloids are a group of naturally occurring compounds found in plants, and they often contain nitrogen atoms that give them their distinctive properties. These compounds can also include other neutral or mildly acidic substances. Alkaloids are known for their wide range of effects on the human body and are used in various medicines due to their powerful biological activity **(Manske and Holmes, 2014).**

They are small and comprise about 20% of the secondary metabolites found in plants. These substances play important roles in the plant’s defense mechanisms.

Around 12,000 different alkaloids have been isolated from various plant species. These compounds come from many different plant families and are valued for their diverse effects and potential medicinal uses **(Kaur and Arora, 2015).**

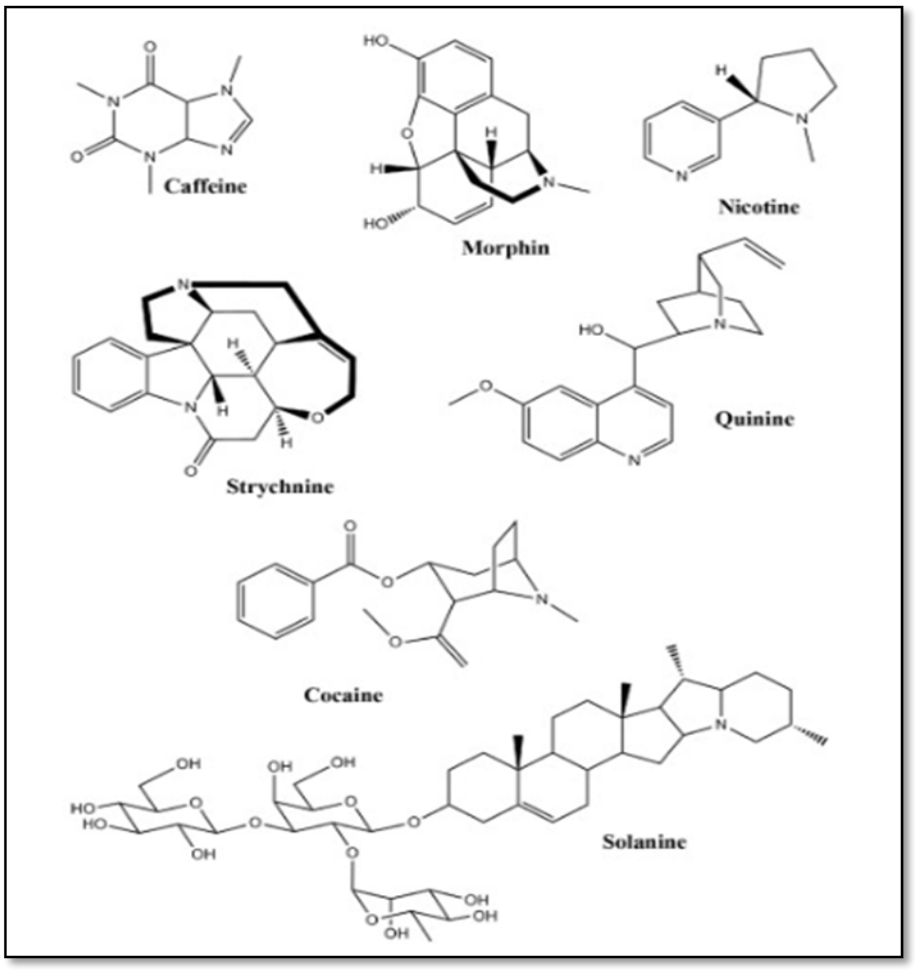

Figure 4: Example of alkaloids **(Richard *et al*., 2013).**

I.2.4 Glycosides

Glycosides are a large and diverse group of secondary metabolites throughout the plant kingdom. Due to their proven biological activities and long history of traditional use, they play a key role in pharmacognosy. However, much remains to learn about their exact roles and properties. All glycosides share a common structure of two parts: an aglycone (or genin) unit, which is mainly hydrophobic (lipophilic), and a glycone unit, which is hydrophilic and made up of one or more sugar molecules **(Bartnik and Facey, 2024).**

Based on the aglycone portion, glycosides such as triterpene glycosides, flavonoid glycosides, and saponins are further classified. Glycosides have demonstrated significant therapeutic potential, including antifungal, anticancer, and antiplatelet activities **(Khan *et al.*, 2020).**

Table 1: Classifications of glycosides according to number of saccharides, type of glycosidic linkage between the carbohydrate and the aglycone, and chemical group of the aglycone **(Soto-Blanco, 2022).**

Parameter for classification	Groups
Number of saccharides	Monodesmoside or monoside (one) Bidesmoside or bioside (two) Tridesmoside or trioside (three)
Glycosidic linkage	C-glycosides (by carbon) N-glycosides (by nitrogen of NH) O-glycosides (by oxygen) S-glycosides (by sulfur)
Aglycone	Anthraquinone Coumarin Cyanogens (cyanohydrin) Flavonoids Glucosinolates (or thioglycosides) Phenols Saponins Steroidal Terpenoids

I.2.5 Terpenes

Terpenes are a large group of natural compounds with significant structural variation, including linear hydrocarbons or carbocyclic skeletons. They are derived from isoprene units (C5H8) and can undergo modifications such as oxygenation, hydrogenation, or dehydrogenation to form terpenoids. Terpenes are abundant in higher plants, including citrus, conifers, and eucalyptus, and are found in various plant parts like leaves, flowers, stems, and roots. They are known for their antifungal, antimicrobial, antiviral, and antiparasitic properties and also play roles in deterring herbivores and acting as insecticides **(Ninkuu *et al.*, 2021).**

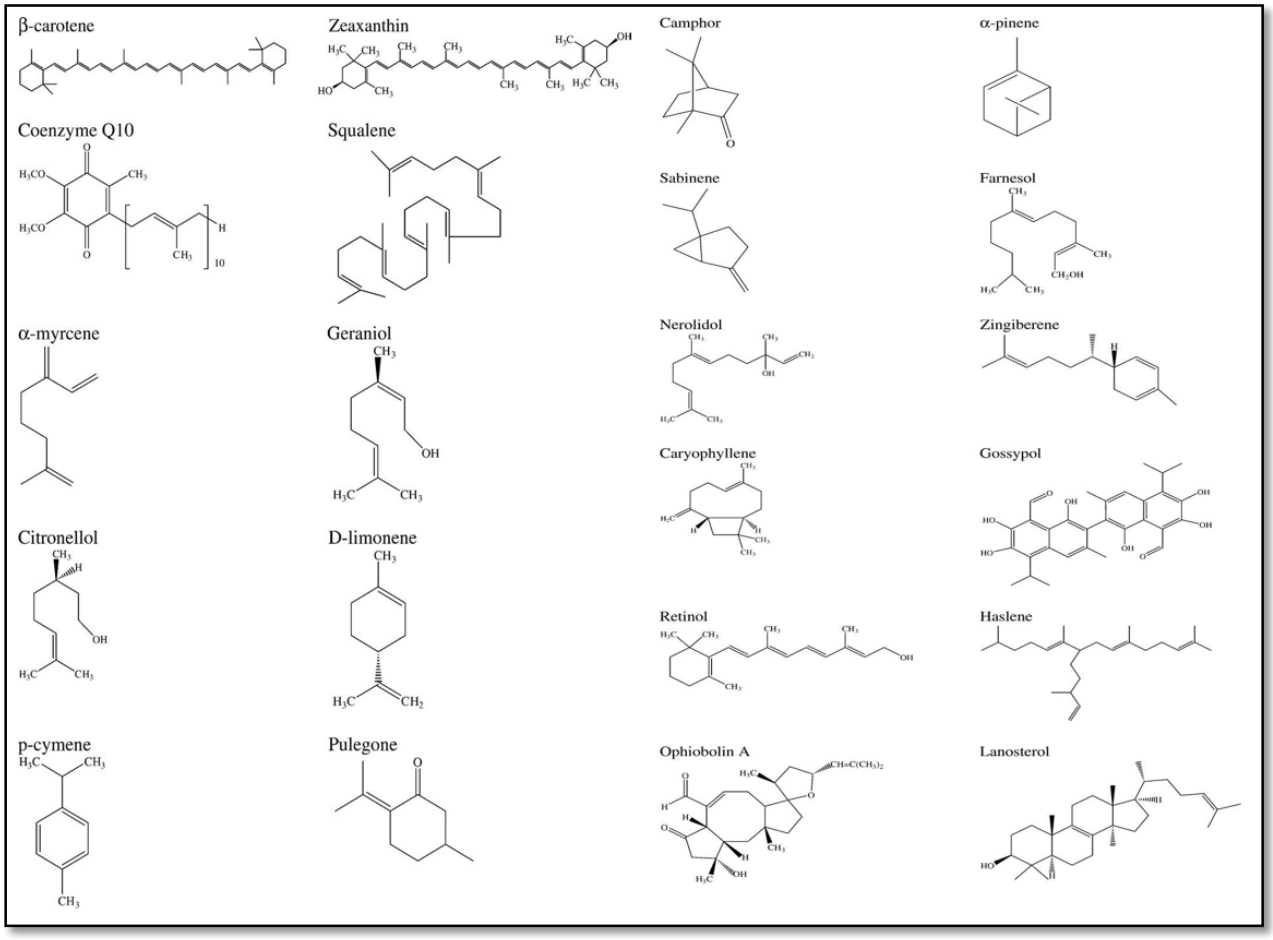

Figure 5: List of chemical structures of various terpenoids possessing pharmaceutical properties **(Reddy & Couvreur, 2009).**

I.2.6 Methods used for bioactive compound extraction

All plant components, such as leaves, roots, barks, tubers, woods, gums or oleoresin, exudates, fruits, figs, flowers, rhizomes, berries, twigs, and the whole plant, produces active chemicals in smaller quantities and at variable concentrations. Thus, selection of the right extraction process is crucial to maximize the extract from tissues. The extraction efficiency is governed by several critical elements, including extraction technique, plant component matrix properties, extraction solvent, temperature, pressure, and time **(Jha & Sit, 2022)**.

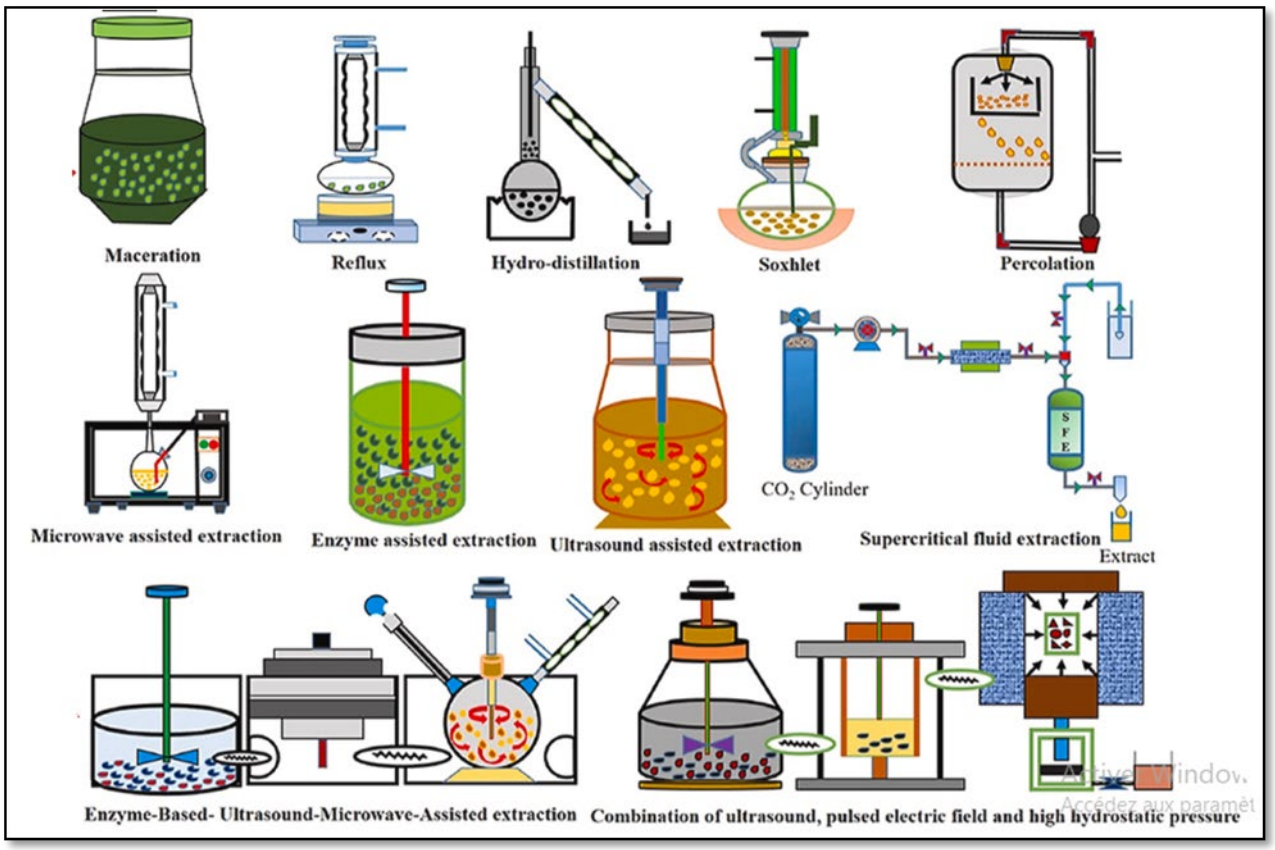

Figure 6: Extraction types **(Jha & Sit, 2022).**

I.2.6.1 Classical and Conventional Extraction Methods

The most commonly employed extraction techniques have predominantly relied on liquid-solid extraction. These methods are characterized by their simplicity and depend on solvents or heat with varying polarities **(Ghenabzia *et al*., 2023).**

I.2.6.1.1 Maceration

Maceration involves soaking plant materials, either powdered or coarse, in a solvent for two to three days at room temperature, with frequent stirring. To prevent the evaporation of the solvent under normal atmospheric pressure, the extractor is sealed [20]. Moreover, if contained in a bottle, it should be shaken occasionally to ensure thorough extraction. Upon completion, the liquid extract (micelle) is separated from the residual solid matter through filtration or decantation. The micelle is then subjected to evaporation in an oven or over a water bath to remove the solvent. This method is particularly well-suited for extracting bioactive compounds from thermolabile plant materials **(Abubakar and Haque, 2020).**

I.2.6.1.2 Digestion

Digestion is an extraction technique that involves moderate heating throughout the process. The extraction solvent is first placed in a clean container, followed by the powdered plant material. The mixture is then heated, typically around 50°C, using a water bath or an oven.

Applying heat reduces the solvent's viscosity, thereby enhancing the dissolution and extraction of secondary metabolites. This method is particularly suitable for plant materials with readily soluble components **(Abubakar and Haque, 2020).**

I.2.6.1.3 Percolation

Percolation is an extraction technique that employs a specialized apparatus known as a percolator, which is a narrow, cone-shaped glass vessel with openings at both ends. In this process, dried, ground, and finely powdered plant material is first moistened with the extraction solvent in a clean container. Additional solvent is then introduced, and the mixture is allowed to stand for approximately 4 hours.

Following this initial soaking period, the mixture is transferred to the percolator, with the lower end sealed and left undisturbed for 24 hours. After this period, the extraction solvent is gradually added from the top until the plant material is fully saturated. The lower end of the percolator is then opened, allowing the extract to drip slowly. Throughout the process, the solvent is continuously added, and extraction occurs through gravitational force, which facilitates the downward movement of the solvent through the plant material.

Solvent addition is discontinued when the total volume reaches approximately 75% of the intended quantity. The resulting extract is separated through filtration, followed by decantation. The residual plant material (marc) is then pressed to recover any remaining extract, and the final volume of solvent is adjusted to achieve the desired concentration **(Abubakar and Haque, 2020).**

I.2.6.1.4 Decoction

A decoction is a water-based extraction method to isolate bioactive compounds from medicinal plants. This process involves boiling plant materials in a specific volume of water for a defined duration (ranging from 15 minutes to 2 hours), followed by cooling and filtration. It

is particularly effective for extracting water-soluble, heat-stable phytoconstituents, especially from tougher plant structures.

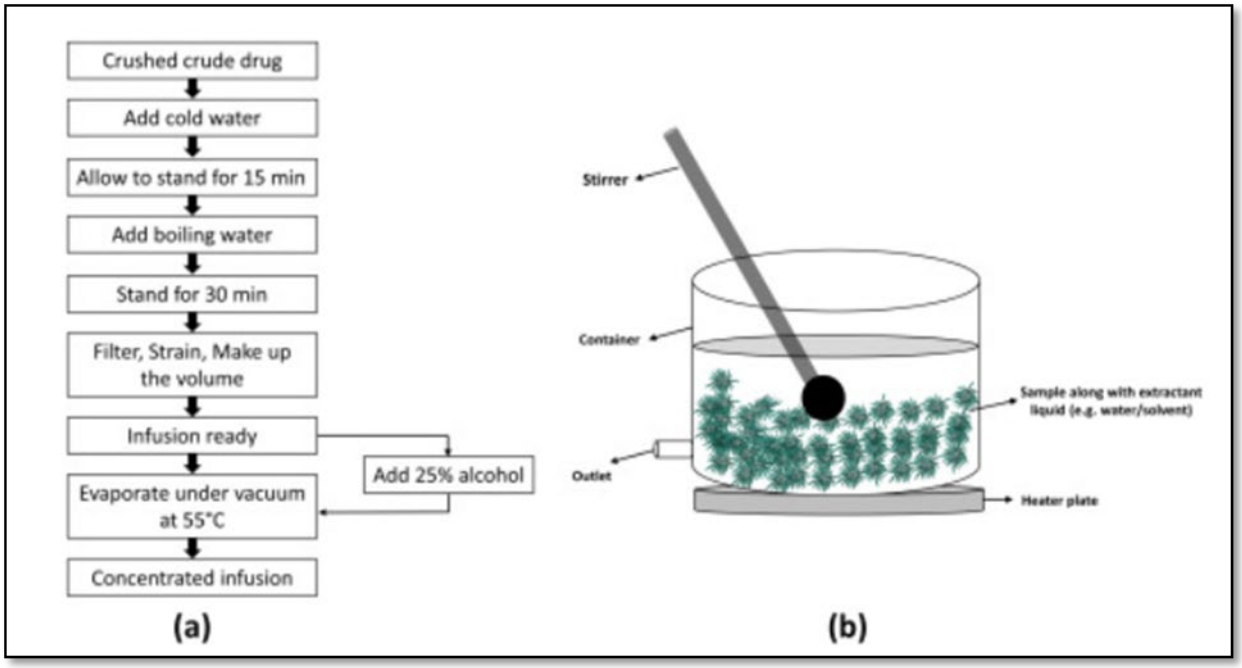

Figure 7: (A) and (B) Flow chart and a diagram for preparation of infusions using decoction **(Miralrio & Vázquez, 2020)**.

Delicate plant parts, such as leaves, roots, flowers, and tender stems, are typically boiled for 15 minutes, whereas harder plant components, including branches and tree bark, may require up to an hour of boiling. Decoction presents certain limitations. It lacks selectivity, particularly for water-insoluble compounds, results in lower yields, and requires substantial water consumption. Consequently, it raises concerns regarding safety and environmental sustainability **(Abubakar and Haque, 2020).**

I.2.6.1.5 Soxhlet extraction

Soxhlet extraction utilizes a Soxhlet apparatus to extract bioactive phytoconstituents from plant materials. This technique repeatedly exposes the plant material to a heated solvent, enhancing extraction efficiency. The plant sample is placed in a thimble holder, undergoing continuous solvent circulation from a heated distillation flask. The solvent vaporizes upon heating, travels through the distillation arm, and condenses onto the plant material in the thimble, initiating extraction.

This process operates continuously, with the solvent recirculating through the sample. When the condensed solvent reaches near full capacity in the thimble, a siphon redirects it back into the distillation flask. The extracted bioactive phytoconstituents are then separated using rotary evaporation **(Hlatshwayo *et al*., 2025).**

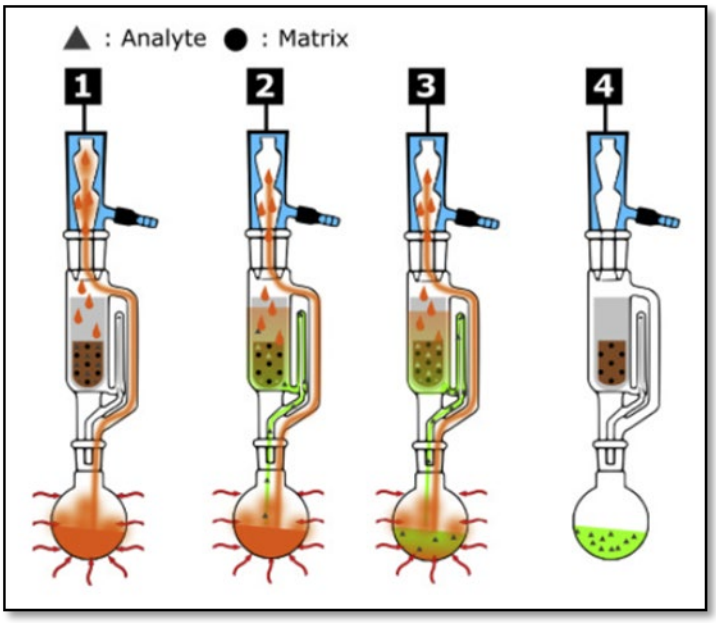

Figure 8: Schematic illustration of the workflow of SOX. 1) Solid matrix is placed in SOX thimble. Solvent is heated under reflux. 2) Condensation and extraction with "fresh" solvent. Solutes are transferred from the extraction chamber into the reservoir. 3) Cont Continuous repetition of the extraction. 4) Exhaustive extraction is complete **(Weggler *et al*., 2020).**

Soxhlet extraction offers several advantages, including extracting large quantities of active compounds using a relatively small amount of solvent. It is particularly suitable for stable plant materials under heat and does not require additional filtration, making the process more efficient. Additionally, high temperatures can be applied to enhance extraction. However, this method has limitations, such as the inability to provide continuous agitation, which may restrict its effectiveness for some materials. Moreover, it is unsuitable for thermolabile plant materials, as prolonged heating can lead to the degradation of heat-sensitive compounds **(Abubakar and Haque, 2020).**

I.2.6.2 Innovative (Non-Conventional) Extraction Techniques

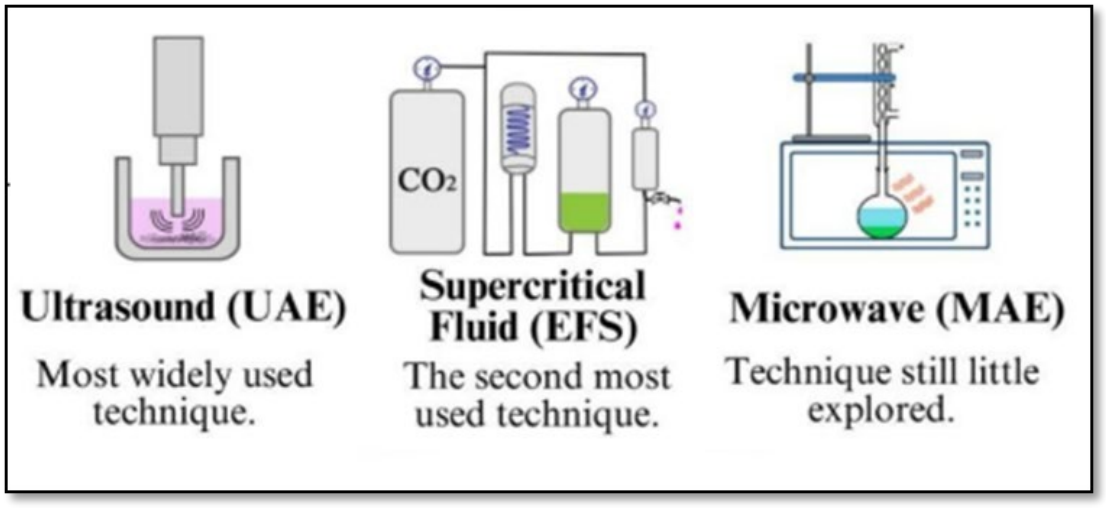

Figure 9: Innovative (Non-Conventional) Extraction Techniques **(Vilela Junior *et al.*, 2024).**

In recent years, extraction technology has continuously advanced, leading to modern and sophisticated approaches, often called advanced extraction methods **(Ghenabzia *et al.*, 2023).**

I.2.6.2.1 Microwave-Assisted Extraction

Microwaves are non-ionizing radiations consisting of electric and magnetic fields. These fields oscillate perpendicularly to one another within a frequency range of 300 MHz to 300 GHz, positioned between X-rays and infrared rays in the electromagnetic spectrum. Microwave-assisted extraction (MAE) is a process that utilizes solvents such as water or alcohol to extract bioactive phytoconstituents from medicinal plants. During MAE, microwaves transfer energy to the solvent and the plant material, facilitating extraction through structural changes in plant cell walls caused by electromagnetic waves **(Hlatshwayo *et al.*, 2025).**

Microwave radiation interacts with the dipoles of polar and polarizable materials (e.g., solvents and samples), causing heating near the surface of the materials, with heat being transferred through conduction. The dipole rotation of molecules induced by microwave electromagnetic waves disrupts hydrogen bonding, enhances the migration of dissolved ions, and facilitates solvent penetration into the matrix. In non-polar solvents, poor heating occurs as energy is transferred solely by dielectric absorption. Various factors, including the power and frequency of microwaves, the duration of microwave radiation, the moisture content and particle size of plant samples, the type and concentration of the solvent, the solid-to-liquid ratio,

extraction temperature, extraction pressure, and the number of extraction cycles can influence the MAE of plant secondary metabolites.

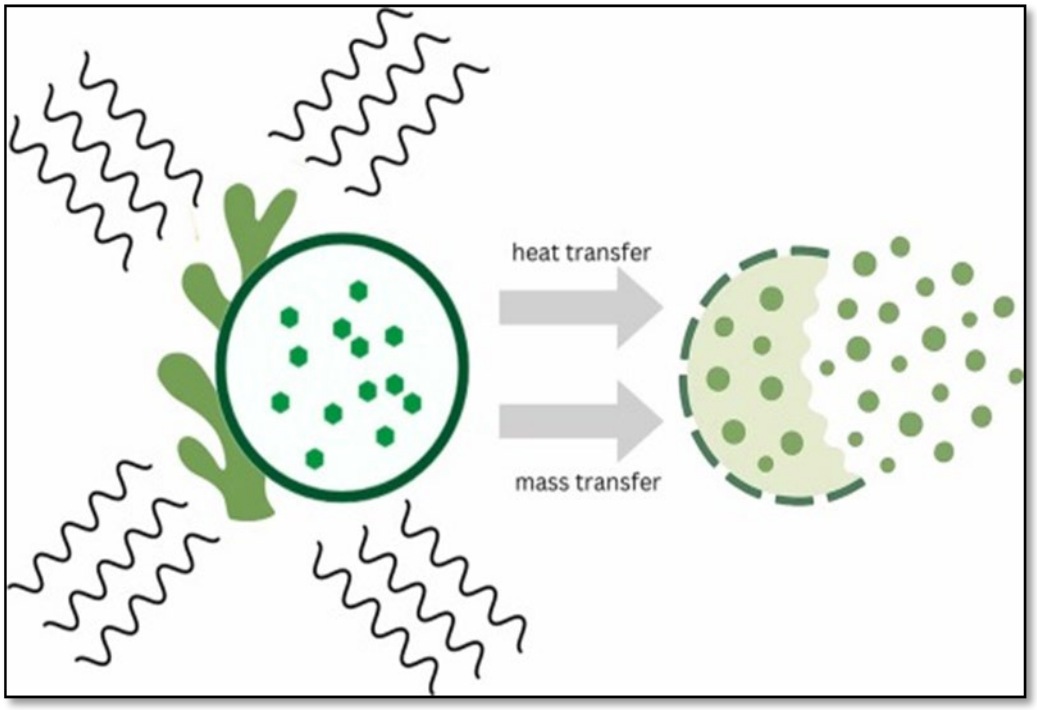

Figure 10: Mechanism of microwave-assisted extraction aiding cell rupture of seaweed cell wall **(Gonzaga *et al.*, 2025).**

Numerous studies suggest that MAE offers several significant advantages, such as shorter extraction times, higher extraction yields, and reduced solvent consumption compared to traditional extraction methods **(Abubakar and Haque, 2020).**

I.2.6.2.2 Ultrasound-Assisted Extraction

This process involves applying sound energy at a very high frequency, greater than 20 kHz, to disrupt plant cell walls and increase the compound's surface area for solvent penetration. As a result, secondary metabolites are released. The plant material should be dried first, ground into a fine powder, and properly sieved in this method. The prepared sample is mixed with an appropriate extraction solvent and placed in the ultrasonic extractor. The high sound energy applied accelerates the extraction process by reducing heat requirements **(Mapholi *et al.*, 2025)**.

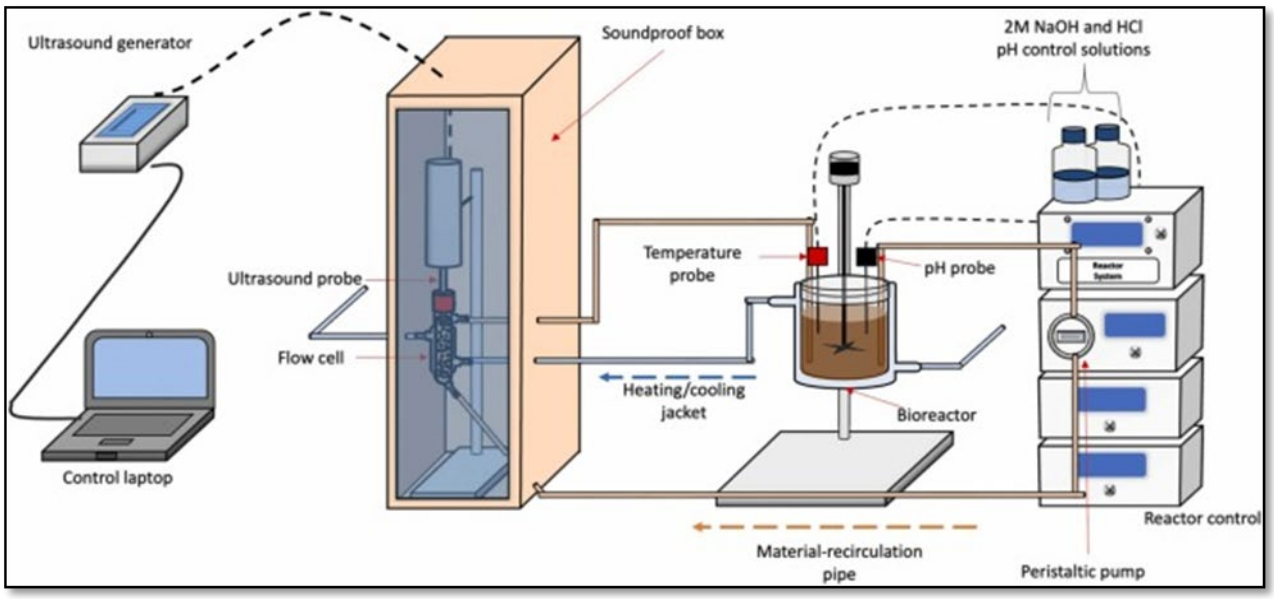

Figure 11: Experimental set-up for ultrasound-assisted extraction **(Mapholi *et al.*, 2025).**

Ultrasound-assisted extraction (UAE) offers several advantages, including suitability for small samples, reduced extraction time and solvent usage, and maximized yield **(Abubakar and Haque, 2020)**. Compared to other methods, such as microwave-assisted extraction, the ultrasound device is more cost-effective and easier to handle **(Tăbărașu *et al.*, 2022)**. However, this method has limitations, as it is difficult to reproduce, and the high energy applied may degrade phytochemicals by generating free radicals, leading to unfavorable alterations in drug molecules **(Abubakar and Haque, 2020)**. The effectiveness of UAE is influenced by various factors, including extraction time, solvent type, power, plant material characteristics, liquid-to-solid (L/S) ratio, frequency, intensity, and amplitude **(Ghenabzia *et al.*, 2023).**

I.2.7 Essential Oils

Essential oils are secondary metabolites derived from plants, distinguished by their strong aroma. These natural, multicomponent systems primarily consist of volatile terpenes and hydrocarbons **(Baptista-Silva *et al.*, 2020).**

I.2.7.1 Extraction Methods of EOs

Essential oil extraction (EO extraction) must ensure high yields and quality for optimal utilization. The efficiency of extraction methods is often evaluated based on yield, duration, and product stability.

I.2.7.1.1 Steam distillation

Steam distillation is the most commonly employed method for essential oil extraction, accounting for approximately 93% of global essential oil production **(Machado *et al.*, 2022).**

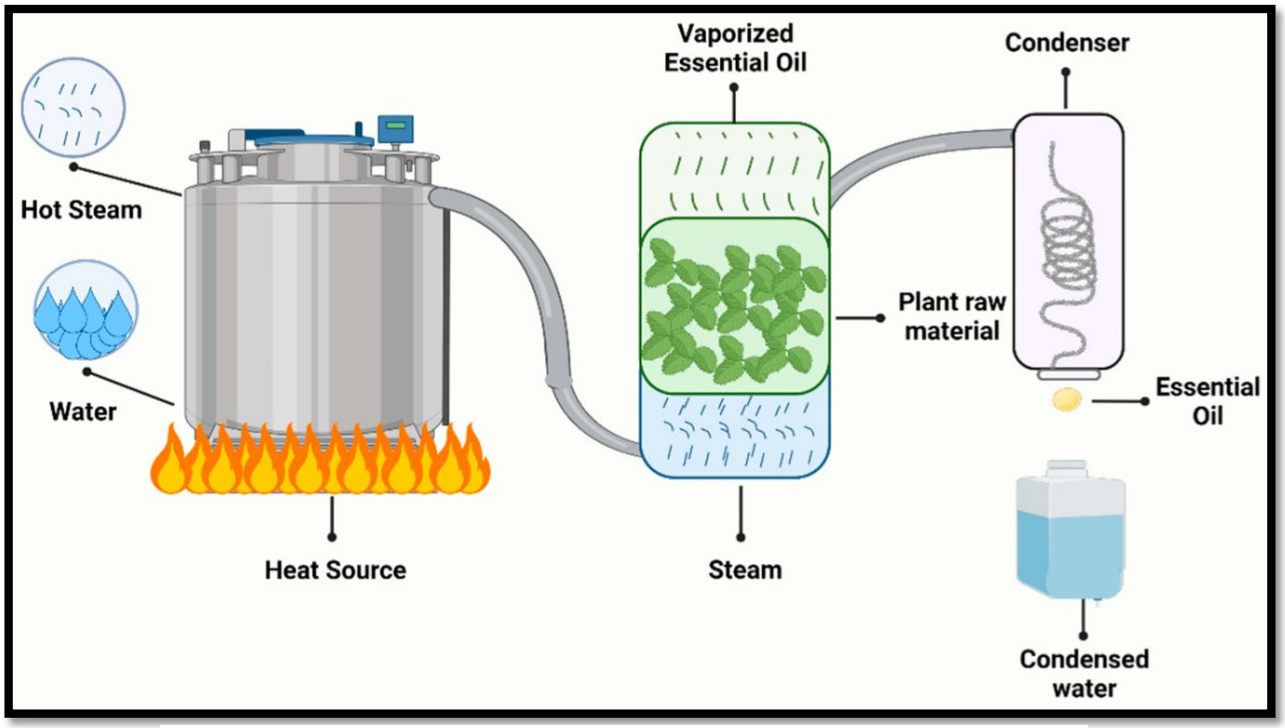

Figure 12: Overview of the steps involved in the steam distillation process **(Machado *et al.*, 2022).**

I.2.7.1.2 Cold Pressing

Cold-pressed oil is extracted without solvents, relying solely on pressing and filtration. According to the Codex Alimentarius Standard for Named Vegetable Oils, these oils are obtained through mechanical processes such as expelling or pressing, without chemical alteration or heat application, ensuring the preservation of bioactive compounds such as phytosterols and phenolics.

The extracted oil undergoes purification through physical techniques, including filtration, sedimentation, or centrifugation **(Chin and Chew, 2020).**

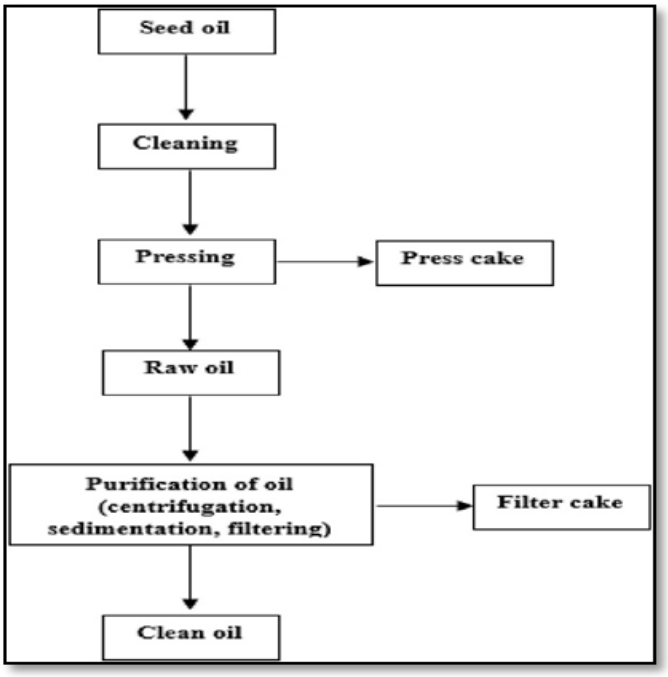

Figure 13: Cold pressed seed oil process **(Oroian, 2024).**

I.2.7.1.3 Hydrodistillation

Hydrodistillation is a traditional essential oil extraction method in which plant material is submerged in water and heated, allowing volatile compounds to vaporize. The resulting vapors are condensed and separated to obtain the essential oil **(Katekar *et al*., 2023).**

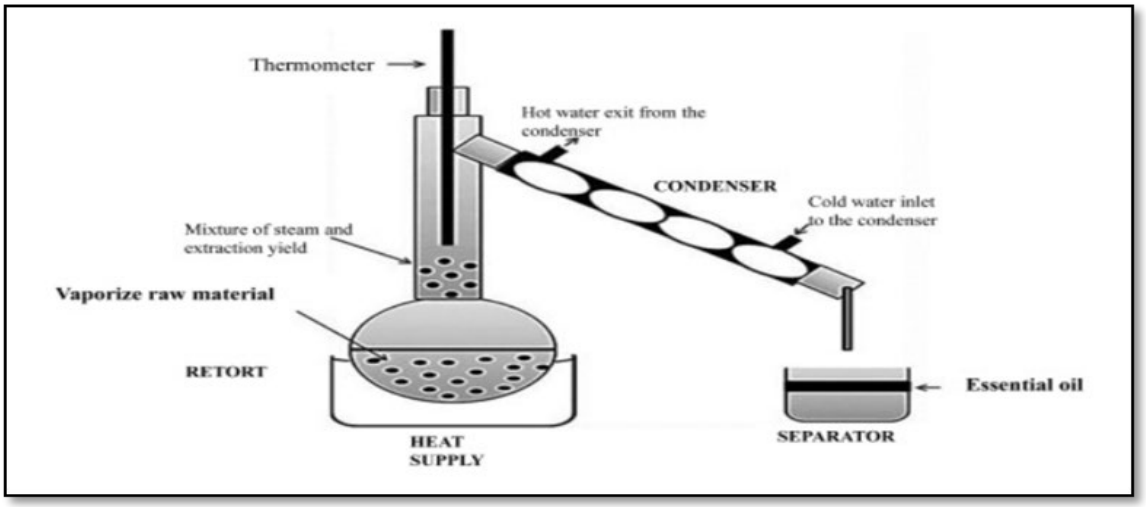

Figure 14 : Schematic representation of hydrodistillation **(Rafiq *et al*., 2024).**

I.3 Biological activities

Medicinal plants are a valuable source of a wide variety of chemical molecules having different structures and functionalities that exhibit important biological activities and are linked to a multitude of beneficial properties, such as antimicrobial, anticancer, antiviral, antioxidant and enzyme inhibitory, anti-aging, anti-inflammatory, antihypertensive, neuroprotective and anticoagulant effects **(Ali *et al*., 2019; Lesellier *et al*., 2021)**. Different parts of medicinal plants (leaves, stems, roots, seeds, flowers or fruits) are rich sources of bioactive compounds **(Knez Hrnčič *et al*., 2020)**.

The phytochemicals isolated from medicinal plants and dietary sources have shown great potential on different *in vitro* cell lines and in vivo experimental animal models **(Iqbal *et al*., 2019)**.

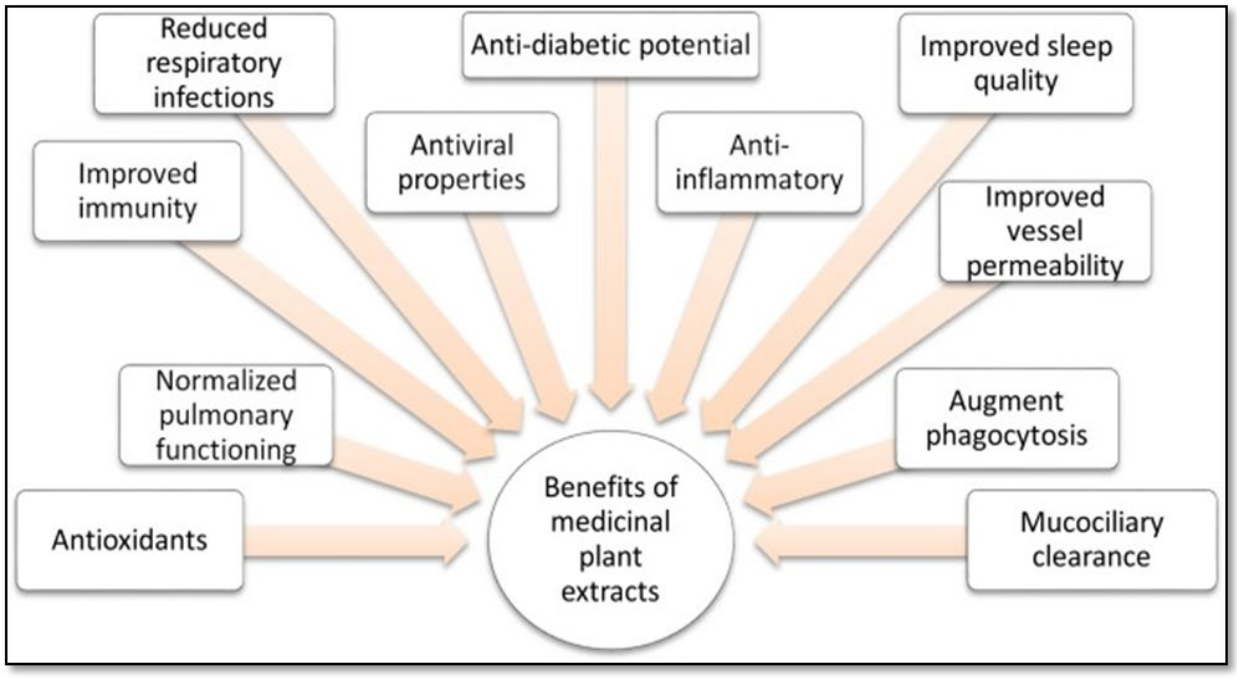

Figure 15: Schematic representation of the potential beneficial effects of medicinal plant extracts in the prevention and treatment of many diseases **(Kalyniukova *et al*., 2021).**

I.3.1 Antioxidant activity

Aromatherapy is a natural form of treatment that employs natural plant spices or aromatic essential oils as a medium to administer bioactive substances through respiration, the skin or blood circulation using massage, inhalation, fumigation, and compression, to help harmonize the body's homeostasis and to achieve the effect of preventive health care and treatment of

diseases **(Perry and Perry, 2006)**. Aromatic plants are considered the perfect source of natural antioxidants, such as polyphenols commonly found in plants; moreover, herbs are antioxidants with redox properties **(Proestos and Varzakas, 2017)**. Besides their anti-inflammatory, antiviral and antibacterial pharmacological effects, aromatic plant extracts can generate specific physiological responses for the prevention and therapy of a diverse range of diseases, including cardiovascular, neurological and cancerous disorders **(Edris, 2007, Ma *et al*., 2023)**, particularly effective in the therapy of conditions involving emotional and cognitive information processing and autonomic nervous system activity **(Haze *et al*., 2002, Villemure and Bushnell, 2009)**. Therefore, this review focuses on the role of oxidative stress in the pathogenesis of Alzheimer's disease, Parkinson's disease, Huntington's disease, and Amyotrophic lateral sclerosis, and the antioxidant effects of aromatic plant extracts used in the treatment of these diseases.

I.3.2 Anti-inflammatory activity

Inflammation is an underlying complex mechanism in many chronic diseases and disorders, including diabetes, Alzheimer's, Parkinson's, cardiac, respiratory, renal, hepatic, and cancer. Oxidative stress and excessive production of reactive oxygen species (ROS) are associated with inflammation, leading to the synthesis and release of proinflammatory cytokines **(Gupta *et al*., 2021)**. Polyphenols are secondary metabolites produced by many plant species. More than 5000 polyphenols have been identified in plants. Many of them are associated with a wide spectrum of health effects **(Cheynier *et al*., 2015)**, including antiinflammatory, antimicrobial, anticarcinogenic, anti-HIV, cardioprotective, and neuroprotective activity.

Among the most active polyphenols are flavonoids **(Ullah and Khan, 2008)**. Flavonoids are known to have strong antiinflammatory activity. A number of *in vitro* studies have shown that quercetin is capable of inhibiting lipopolysaccharides (LPSs)-induced TNF-α production in macrophages and LPS-induced IL-8 production in lung cells (A549), as well as reduce mRNA levels of TNF-α and IL-1 **(Nathiya *et al*., 2014)**.

The ability of polyphenols in general and flavonoids in particular to have antiinflammatory effect probably relies on their ability to act as antioxidants. Reactive oxygen species (ROS) are a major contributor to many diseases, including those associated with inflammation **(Blaser *et al*., 2016)**. ROS were shown to damage many biomolecules, including

lipids, proteins, DNA, and small molecules. Natural antioxidant molecules found in food can either counter ROS directly or enhance the body antioxidant capacity **(Shahidi and Ambigaipalan, 2015)**. In the case of polyphenols, their antioxidant activity is mainly due to their redox properties and their ability to block the production of ROS. This ability is based probably on their free radical scavenging activity, transition metal-chelating activity, and/or singlet oxygen quenching capacity **(Bernstein *et al*., 2018)**.

I.3.3 Antimicrobial activity

Traditionally, medicinal plants have been used across the globe for various therapeutic purposes, including the treatment of microbial diseases **(Safarzadeh *et al*., 2022, Alfuraydi *et al*., 2024)**. In 2022, the global herbal medicine market was valued at $170 billion, with projections suggesting it will reach $600 billion by 2033, reflecting a compound annual growth rate (CAGR) of 15 % from 2023 to 2033 **(Sarkar *et al*., 2024)**. Indeed, the ability of plant extracts to inhibit the growth of pathogenic bacteria has become a focus of recent research, particularly for their potential to modulate bacterial drug resistance. These studies could serve as a valuable reference for guiding future research aimed at reversing microbial resistance **(Yap *et al*., 2014, Chikowe *et al*., 2020).**

According to the literature, thousands of plant species have been tested *in vitro* against many bacterial strains, and a good number of medicinal plant extracts and pure compounds have now been proven to be active against Gram-positive and Gram-negative bacteria. Nevertheless, only a few antibacterial plant extracts exhibited significant antibacterial activity against the tested bacterial isolates as judged by their higher minimum inhibitory concentration (MIC) values **(Sharma *et al*., 2017)**. MIC value is defined as the lowest concentration of the assayed antimicrobial agent that inhibits the visible growth of the microorganism tested, and it is usually expressed in µg/mL or mg/L **(Balouiri *et al*., 2016).**

The potential antibacterial activity is observed in some flavonoid compounds including apigenin, galangin, and flavonol glycosides, isoflavones, and chalcones . The prowess of bioactive flavonoids obtained from edible and medicinal plants provides potent antibacterial property **(Kandar, 2022)**.

I.4 Description of the plants studied

I.4.1 Ephedra alata

The Ephedra genus includes more than 65 species and is widely distributed across various regions, including Europe, Asia, the Americas, and Africa. These plants have been utilized in traditional medical practices for centuries, particularly within the Chinese, Arab, African, and Japanese systems of medicine. *Ephedra alata*, referred to in Arabic as "*Alanda*," is native to several countries in the Middle East and North Africa, including Saudi Arabia, Tunisia, Algeria, and Egypt. It has traditionally been used to manage conditions like bronchial asthma, digestive ailments, cancer, and infections of fungal or bacterial origin. Numerous studies have confirmed that *E. alata* possesses various biological properties, including anti-inflammatory, antiproliferative, antioxidant, antimicrobial, and antidiabetic activities **(Mohammed *et al.*, 2024).**

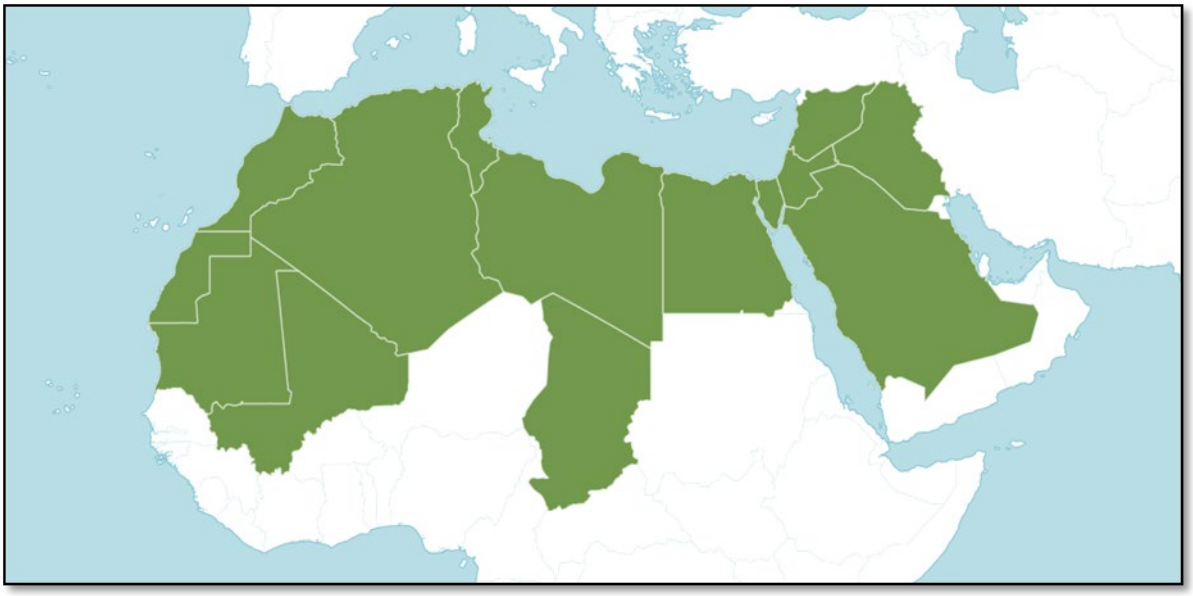

Figure 16: Geographical distribution of Ephedra alata in North Africa and the Middle East **(Mohammed *et al*., 2024).**

I.4.1.1 Botanical description

Ephedra species are perennial herbaceous plants that may grow taller than one meter. They are characterized by a pronounced pine-like aroma and a distinctly astringent flavor. In traditional medicinal practices, dried green stems are commonly utilized. They are typically prepared as a decoction and consumed as a hot infusion. The standard daily dosage of the decocted material ranges from 1.5 to 9 grams **(Abourashed *et al.*, 2003).**

Ephedra species are primarily wind-pollinated, though some species also attract insects, a dual strategy known as homophily. While wind is the main pollination method, traits like sticky pollen and sugary pollination drops suggest that insects may also play a role. Insects and lizards visit some species, but these are considered secondary to wind regarding reproductive success **(González-Juárez *et al.*, 2020).**

Figure 17: *Ephedra alata*

I.4.1.2 Scientific Classification

Kingdom: Plantae

Phylum: Spermatophyta

Subphylum: Gymnosperms

Class: Gnetopsida

Order: Ephedrales

Family: Ephedraceae

Genus: Ephedra

Species: *Ephedra alata*

Subspecies: *Ephedra alata* subsp.

Local name: *Alanda*

I.4.2 Linaria tingitana Boiss. & Reut

The genus Linaria, which has more recently been classified within the family Plantaginaceae rather than Scrophulariaceae, encompasses approximately 200 species primarily distributed across the Northern Hemisphere, particularly in the Mediterranean region and Eastern Asia. Traditionally, various Linaria species have been employed in folk medicine for their tonic, laxative, anti-scorbutic, anti-diabetic, and diuretic effects and in treating wounds, hemorrhoids, and vascular conditions **(Cheriet *et al.,* 2015).** Moreover, it is recognized for its diverse phytochemical profile, including iridoids, terpenoids, flavonoids, and alkaloids. *L.tingitana* is a species typically found in coastal sandy environments characterized by its succulent, linear leaves and generally leafless flowering stems. Phytochemical investigations of its aerial parts have revealed the presence of triterpenoids and flavones, while other studies have reported its *in vitro* antioxidant and antimicrobial properties **(Hanfer *et al.,* 2017).**

I.4.2.1 Scientific Classification

Kingdom: Plantae

Phylum: Streptophyta

Class: Equisetopsida

Subclass: Magnoliidae

Order: Lamiales

Family: Plantaginaceae

Genus: *Linaria*

Species: *Linaria tingitana*

Local name: /

Figure 18: *Linaria tingitana* Boiss & Reut

I.4.3 Asteriscus graveolens

The name Aster, derived from the Greek word meaning "star," reflects the flower's characteristic star-shaped morphology. Martynov formally designated the botanical family name Asteraceae in 1820, whereas Giseke proposed the earlier synonym Compositae in 1792. Asteraceae constitutes the largest family within the dicotyledonous angiosperms, encompassing a vast diversity of species **(Belouahad and Bensammar, 2022).**

Commonly referred to as the sunflower family, Asteraceae ranks among the largest families of angiosperms, encompassing more than 1.600 genera and around 25.000 species globally. This family includes a variety of economically and medicinally significant plants, such as chicory, sunflower, lettuce, coreopsis, dahlias, and daisies, as well as herbal species like wormwood, chamomile, and dandelion. Members of the Asteraceae family are widely distributed across all continents except Antarctica, occupying a broad range of ecological zones, including forested regions and high-altitude grasslands.

Asteriscus graveolens (Forsk) Moench also known by several synonyms, including *Bubonium graveolens*, *Buphthalmum graveolens* (Forsk), *Nauplius graveolens*, and *Bubonium odorum* (Schoub) is an aromatic herbaceous species belonging to the Asteraceae family. It is indigenous to arid Saharan regions, with a natural distribution primarily in the southwestern territories of Algeria **(Belouahad and Bensammar, 2022).**

The genus Asteriscus, native to the Mediterranean region and classified within the Asteraceae family, is morphologically defined by its inflorescence composed of numerous florets aggregated into a capitulum, which is encased by an involucre of bracts. The florets are uniformly yellow, with ligulate forms situated peripherally and tubular disc florets occupying the central position. Leaves are typically simple, alternate, and lanceolate in shape. The bracts, often resembling the upper leaves in appearance, commonly end in a pointed or spiny tip.

Anatomical analysis of the stems and leaves of *Asteriscus graveolens* confirms features characteristic of dicotyledonous species. In cross-section, the stem displays a periderm (suber), an underlying collenchyma layer, and cortical parenchyma that houses continuous vascular bundles (pachytes). Additionally, a limited number of secretory cells are evident, consistent with the anatomical profiles observed in other members of the Asteraceae, such as Helianthus tuberosus. The stem surface also bears non-glandular trichomes (tectorial hairs), some of which may possess secretory activity **(Belouahad and Bensammar, 2022).**

The plant's leaves are usually collected in the spring and are often used to make infusions or decoctions. The sap from fresh leaves is traditionally used as nasal drops and applied as a poultice to help relieve headaches **(Hamidi, 2013).** The local population extensively uses of the plant's aerial parts due to their aromatic and medicinal qualities.

Traditionally, the plant is used as a natural remedy for various conditions, including pain, gonorrhea, diabetes, hypertension, inflammation, fever, and stomach and intestinal issues. It is also used for its purgative effects **(Belhadi *et al.*, 2020).**

Figure 19: A bush and a flower of *Asteriscus graveolens*

I.4.3.1 Scientific Classification

Kingdom: Viridiplantae

Phylum: Tracheophyta

Subphylum: Euphyllophytina

Class: Magnoliopsida

Subclass: Asteridae

Superorder: Asteranae

Order: Asterales

Family: Asteraceae

Genus: Asteriscus

Species: *Asteriscus graveolens*

Local name: *Negued*

I.4.3.2 Geographical distribution

This perennial sub-shrub, a member of the Asteraceae family, is widely distributed across the Algerian Sahara. In southern Algeria, where the Tamahaq language is spoken, it is locally known as tamayout, and it is also commonly referred to by the vernacular names nougued and tafss **(Belhadi *et al.*, 2020)**. *The Asteraceae* family is found all over the world, thriving in almost every type of habitat except Antarctica. These plants are highly adaptable, growing in tropical and subtropical semi-arid areas, alpine, arctic tundra, and temperate climates. However, they are less common in tropical rainforest ecosystems **(Belouahad and Bensammar, 2022).**

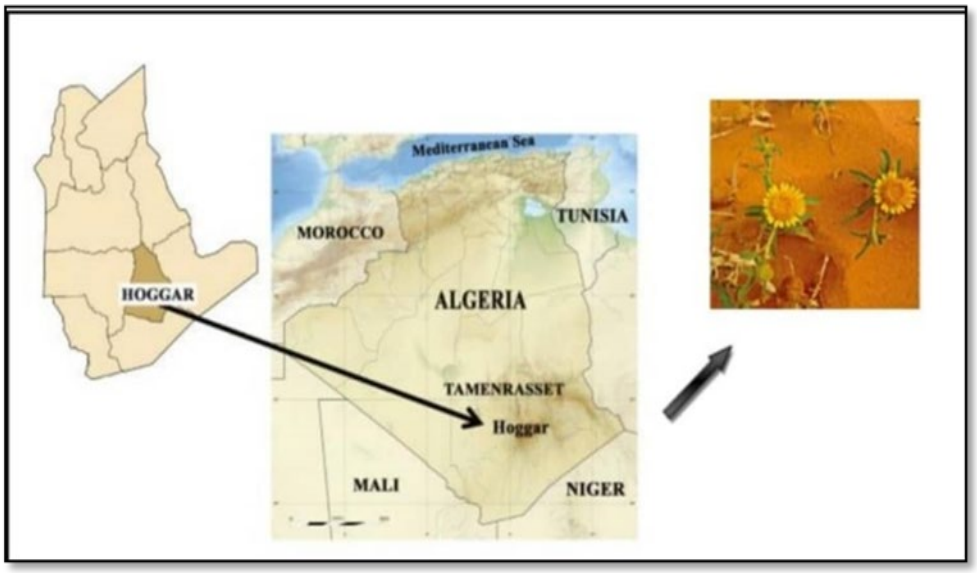

Figure 20: Geographical distribution of *Astériscus graveolens*

I.4.4 Limoniastrum guyonianum

Limoniastrum guyonianum is a shrub that grows between 0.5 and 1 meter tall, with grey-green, highly branched stems often marked by large galls. The leaves are long, linear, or almost cylindrical, tough, and have slightly pointed tips. They contain calcareous deposits and are covered in salt. The flowers are purplish-pink with five petals. This plant is classified as a halophyte. It also belongs to the Plumbaginaceae family, which includes 775 species across 14 to 24 genera. These plants are usually herbaceous but can also be shrubs or vines. The Plumbaginaceae family is found worldwide in various environments, including saline areas, and ranges from cold to tropical regions. *L. guyonianum*, in particular, is native to the Sahara **(Hamidi, 2013).**

As a halophyte, it is characterized by salt glands, essential for the plant's ability to excrete excess salt.

This species is evergreen, maintaining its foliage year-round. Its flowering period typically occurs in the spring, from April to May. The plant has simple, entire-margined leaves, which lack glands and are arranged alternately along the stem. The flowers are generally leathery, covering the entire plant, and are relatively large, with a length of up to 8 mm and a diameter of up to 10 mm. They are radial, hermaphroditic, and consist of five petals. The calyces are fused with distinct lobes, while the petals are partially fused, presenting a pinkish or purplish coloration. The stamens are positioned above the petals, and the ovary is unilocular with basal placentation. The flowers develop from the axils of small, membranous bracts, forming clusters within a branched terminal inflorescence **(Singleton and Rossi, 1965).**

The fruit contains numerous seeds covered in fine hairs known as galls. The characteristics of the stem type influence the origin of the fruit **(Singleton and Rossi, 1965).**

Figure 21: A bush and a flower of *Limoniastrum guyonianum*

I.4.4.1 Scientific Classification

Kingdom: Plantae

Sub-kingdom: Tracheobionta

Phylum: Spermatophytes

Sub-phylum: Angiosperms

Division: Magnoliophyta

Class: Magnoliopsida

Subclass: Caryophyllidae

Order: Plumbaginales

Family: Plumbaginaceae

Genus: Limoniastrum

Species: *Limoniastrum guyonianum*

Local name: *Hanet al-ibel*

I.4.4.2 Traditional Uses and Pharmacological Properties

In southern Tunisia, infusions prepared from the leaves, branches, and galls of *L.guyonianum* have traditionally been utilized in folk medicine to treat dysentery **(Hamidi, 2013).**

In addition, *L.guyonianum* has also been traditionally employed in treating of gastric infections and as an antibacterial agent for managing bronchitis. Similarly, *L.feei* has been utilized in folk medicine to treat bronchitis and gastrointestinal disorders.

MATERIALS AND METHODS

II MATERIALS AND METHODS

II.1 Preparation of extracts

The aerial parts of four plants (*L.guyonianum*: 1500 g, *E.alata*: 1500 g, *L.tingitana*: 1500 g, and *A.graveolens*: 1500 g) are cut into small pieces and macerated in a Methanol/water (80/20) mixture for 24 to 48 hours. This operation is repeated three times with solvent renewal. After concentration at a temperature not exceeding 35°C, we obtained a syrupy residue. The latter is diluted with distilled water at a rate of 600 mL per 1 kg of dry matter and then treated with lead acetate [(CH3COO) 4Pb] to remove chlorophyll by precipitation. After filtration, the aqueous phase obtained is successively exhausted by liquid-liquid extraction in a separatory funnel using water-immiscible solvents of increasing polarity (chloroform, ethyl acetate, and *n*-butanol). The organic phases thus obtained are dried with anhydrous sodium sulfate to remove all traces of water, then filtered and finally concentrated to dryness under reduced pressure and weighed.

II.2 Phytochemical study

II.2.1 Polyphenols

Total polyphenol content is determined using the Folin-Ciocalteu reagent (**Müller *et al.*, 2010)**, according to a microplate assay method described by Müller *et al.* (2010).

In the total phenolic content assay, 20 µL of plant extract is mixed with 100 µL of diluted Folin-Ciocalteu reagent (1:10) and 75 µL of 7.5% sodium carbonate solution. The mixture is then incubated in the dark for 2 hours. After incubation, the absorbance is measured at 765 nm using a spectrophotometer. A blank sample is prepared following the same procedure but replacing the plant extract with methanol.

II.2.2 Flavonoids

The determination of flavonoids in extracts is based on the formation of a complex between Al+3 and flavonoids. The method of Topçu et al. (2007) is used, with a few modifications for determination on 96-well microplates **(Topçu *et al.*, 2007).**

50 µL of plant extract (S2) is mixed with 130 µL of methanol, 10 µL of potassium acetate (CH_3COOK), and 10 µl (Al(NO3)2, 9H2O). The mixture is left to react for 40 minutes, and the

absorbance is then measured at 415 nm using a spectrophotometer. A sample blank is prepared by replacing the reagents with 150 µL of methanol instead (50µl extract + 150µl methanol).

II.2.3 Flavonols

The flavonol content of extracts was determined using the aluminum trichloride ($AlCl_3$) colorimetric method **(Kumaran and Karunakaran, 2007).**

50 µL of plant extract is mixed with 50 µL of aluminum trichloride ($AlCl_3$) and 150 µL of sodium acetate. The mixture is then incubated in the dark for two and a half hours. After incubation, the absorbance is measured at 440 nm using a spectrophotometer. A sample blank is prepared by replacing the reagents with 200 µL of methanol instead of $AlCl_3$ and sodium acetate.

II.2.4 Chlorophyll and Carotenoids

Total chlorophyll and carotenoids are calculated using the method described by Lichtenthaler and Wellburn (1983) **(Lichtenthaler and Wellburn, 1983).**

10 mg of plant is dissolved in 10 ml of distilled water, and then the absorbance is measured at 450, 645 and 663 nm.

The following equation calculates the total Chlorophyll and Carotenoid:

Chlorophyll a = 12.7 A663 - 2.69 A645

Chlorophyll b = 22.9 A645 - 4.68 A663

Total Chlorophyll = 20.2 A645 - 8.02 A663

Total Cartenoid= 4.07 A450 - [(0.0435 * Chlorophyll a) + (0.367 * Chlorophyll b)]

II.3 Antioxidant activity

II.3.1 DPPH free radical

Free radical scavenging activity is determined spectrophotometrically using the DPPH assay **(Blois, 1958).**

The DPPH scavenging assay proved to be a promising technique due to its simplicity, rapidity, and affordability, and it also provides precise information on the total antioxidant capacity of the test system. The test measures the scavenging capability of antioxidants towards

DPPH free radicals. The odd electron of the nitrogen atom in DPPH is reduced by obtaining a hydrogen atom from antioxidants and the associated hydrazine (**Sohel Rana *et al.*, 2024**).

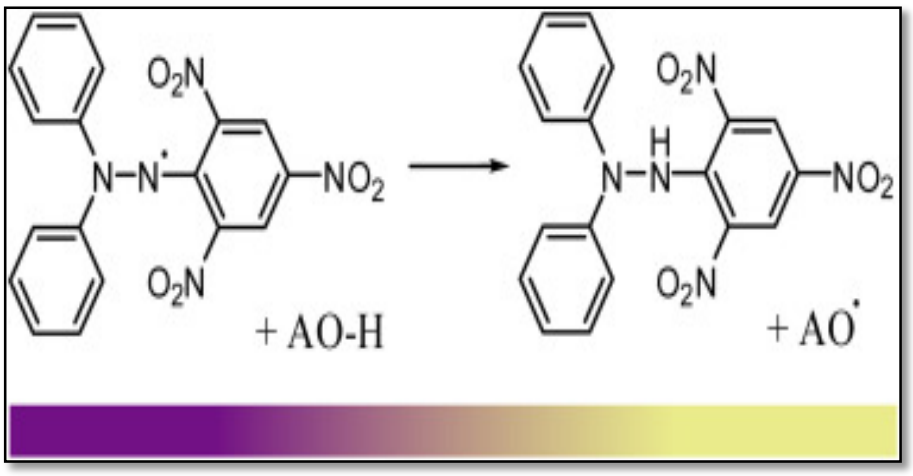

Figure 22: Mechanism of DPPH Assay **(Zhan *et al.*, 2016).**

In the DPPH assay, 160 µL of DPPH solution is mixed with 40 µL of the plant extract. The reaction mixture is then incubated 30 minutes, and the absorbance is measured at 517 nm using a spectrophotometer to evaluate the antioxidant activity. BHT, BHA, and ascorbic acid were used as antioxidant standards. The percentage of DPPH free radical scavenging activity was calculated as follows:

$$\text{Inhibition (\%)} = [(A_{\text{Contrôle}} - A_{\text{Echantillon}}) / A_{\text{Contrôle}}] \times 100$$

II.3.2 ABTS assay

The ABTS assay is considered one of the most sensitive techniques to identify antioxidant activity, because the response of antioxidants involves faster reaction kinetics. This method was initially reported by Miller and colleagues, and is based on the ability of an antioxidant to stabilize the ABTS colored cation radical, which can be previously formed by the oxidation of ABTS (2,2-azinobis-(3-ethylbenzthiazoline-6-sulfonic acid)) by methemoglobin and hydrogen peroxide . The modified technique for the generation of the ABTS cation radical involves direct production of the green-blue ABTS chromophore through the reaction between ABTS and potassium persulfate. This chromophore has three absorption maxima at wavelengths of 645, 734, and 815 nm. The addition of antioxidants to this previously obtained radical follows an electron transfer mechanism (Fig. 23), which is visualized as a discoloration corresponding to when the radical ABTS it is reduced by antioxidant. In this way, the degree of discoloration

makes it possible to evaluate the percentage of inhibition of the ABTS cation radical, which is determined as a function of the antioxidant concentration and the reaction time **(Do Prado *et al.*, 2013 ; Maqsoudlou *et al.*, 2019 : Lang *et al.*, 2024).**

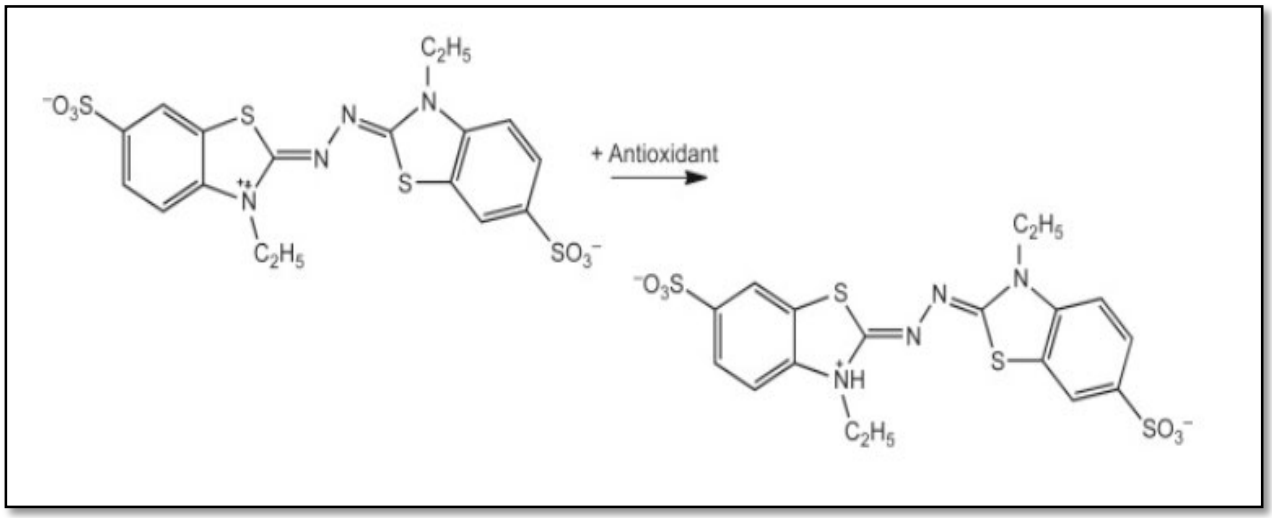

Figure 23: ABTS chemical reaction with antioxidant compound **(Hernández-Rodríguez *et al.*, 2018).**

ABTS activity is determined using the method of **Re *et al.* (1999).** From ABTS and potassium persulfate $K_2S_2O_8$: the two products in aqueous solution are mixed and protected from light for 12-16 hours; the absorbance of the resulting solution is adjusted with (ethanol or H2O) to 0.700 ± 0.020 at 734 nm before use.

(ABTS+) → 19.2 mg (7 mM) ABTS + 5 ml H2O + 3.3 mg (2.45 mM) ($K_2S_2O_8$) + 5 ml H2O+ wait 16 hours away from light.

In the ABTS assay, 160 µL of ABTS solution is mixed with 40 µL of the plant extract. The reaction mixture is then incubated 10 minutes, and the absorbance is measured at 734 nm using a spectrophotometer to evaluate the antioxidant activity. BHT, BHA, and ascorbic acid were used as antioxidant standards. The percentage of antiradical activity is calculated using the following formula:

$$\text{Inhibition (\%)} = [(A_{\text{Contrôle}} - A_{\text{Echantillon}})/A_{\text{Contrôle}}] \times 100$$

II.3.3 O-phenothroline

Phenanthroline activity is determined by the Szydlowska-Czerniaka (2008) method **(Szydłowska-Czerniak *et al.*, 2008).**

10 µL of plant extract is mixed with 50 µL of 0.2% ferric chloride ($FeCl_3$), 30 µL of 0.5% phenanthroline, and 110 µL of methanol. The mixture is then incubated in the dark at 30°C for 20 minutes. After incubation, the absorbance is measured at 510 nm using a spectrophotometer. BHT, BHA, and ascorbic acid is used as the standard for comparison. The result was given as $A_{0.5}$ value.

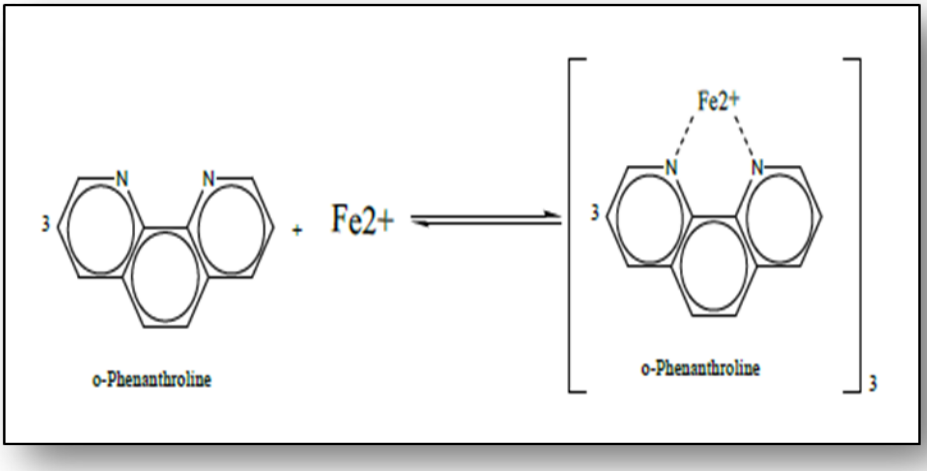

Figure 24: Formation of the Fe+2-phenanthroline complex

II.3.4 Reducing power

Reducing power activity is determined by the method of Oyaizu (1986) with a slight modification **(Oyaizu, 1986).**

In the reducing power assay, 10 µL of plant extract is mixed with 40 µL of phosphate buffer (pH 6.6) and 50 µL of 1% potassium ferricyanide \((K_3Fe(CN)_6)\). The mixture is then incubated at 50°C for 20 minutes, followed by the addition of 50 µL of 10% trichloroacetic acid (TCA) and 40 µL of distilled water. Finally, 10 µL of 0.1% ferric chloride ($FeCl_3$) is added to the reaction mixture, and the absorbance is measured at 700 nm using a spectrophotometer. BHT, BHA, and ascorbic acid is used as the standard for comparison. The result was given as $A_{0.5}$ value.

II.4 Anti-Alpha Amylase Potential

The anti-alpha amylase potential was assessed using the iodine/potassium iodide method, with few modifications (***Zengin et al., 2014***). To begin, the sample (25 µL) was mixed with an α- amylase solution (1U (50 µL)) and then incubated at 37 °C for 10 min. After that, the reaction was started by adding a starch solution (50 µL, 0.1%). Concurrently, a control was prepared without putting the enzyme solution. After another incubation of 20 min at 37 °C, 25 µL HCl (1 M) and 100 µL iodine-potassium iodide solution were added successively to stop and assess the reaction by measuring the absorbances at 630 nm, and result was given as IC_{50} value.

II.5 Inhibition of albumin denaturation

In vitro anti-inflammatory activity was determined using the method of Sunmathi et al. (2016) with slight modifications. The principle is the inhibition of BSA denaturation induced by heat (72°C) by *n*-BuOH plant extracts. The reaction mixture (2.5 mL) consisted of 0.1 mL of egg albumin (from fresh chicken eggs), 1.4 mL of phosphate-buffered saline (PBS, pH 6.4), and 1 mL of sample concentration to obtain a final concentration of 100–500 µg/mL. A similar volume of double-distilled water served as a control, then the mixtures were incubated at 37 °C in an incubator for 15 min and then heated to 70 °C for 5 min. After cooling, their absorbance was measured at 660 nm by a vehicle used as a blank, acetylsalicylic acid was used as a reference drug and treated similarly for absorbance determination. Each experiment was performed in triplicate and the results were recorded as mean (values ± SD). Diclofenac sodium was used as the reference drug and treated similarly for absorbance measurement. The percentage inhibition of protein denaturation was calculated using the following formula:

$$\text{Inhibition (\%)} = 100 \times [(A_{\text{Echantillon}} / A_{\text{Contrôle}} - 1)]$$

II.6 Lipid Peroxidation (LPO) Inhibition

The ability of *n*-butanol extracts from plants to inhibit lipid peroxidation was assessed by assaying the concentration of MDA, which results from lipid peroxidation, using the method of Banerjee et al. (2005), which is based on the egg yolk and thiobarbituric acid reaction. 50 µL of $FeSO_4$ (0.07 M) was added to 0.5 mL of 10% egg yolk homogenate. The mixture was then incubated with increasing concentrations of both extracts or vitamin C (0.1, 0.2, 0.3, 0.4, and

0.5 mg/mL) at 37°C for one hour. After incubation, 1 mL of 20% TCA (trichloroacetic acid) and 1.5 mL of 1% TBA (thiobarbituric acid) were successively added. The samples were mixed and then incubated a second time for 15 minutes at 95°C. After centrifugation of the samples at 400 g for 20 min, the resulting thiobarbituric reactive substances (TBARS) were measured in the supernatant at 532 nm. Vitamin C was used as a positive control and the percentage inhibition of lipid peroxidation (I%) was calculated using the following equation:

$$\text{Inhibition (\%)} = [(A_{\text{Contrôle}} - A_{\text{Echantillon}}) / A_{\text{Contrôle}}] \times 100$$

The median inhibitory concentrations (IC_{50}) of the *n*-BuOH extracts of plants and vitamin C were calculated from the equations of the linear trend curves obtained from the graphs of the variation of the percentage of inhibition of lipid peroxidation (I%) as a function of increasing concentrations of the *n*-BuOH extracts and vitamin C.

II.7 Anti-bacterial activity

II.7.1 Preparation of the Culture Medium

The agar medium was prepared by dissolving the powder in distilled water, according to the laboratory instructions.The medium and the paper discs were sterilized in an autoclave at 121°C. After sterilization, the medium was poured into sterile Petri dishes under aseptic conditions (e.g., inside a laminar flow hood) and left to solidify

II.7.2 Preparation of Microbial Suspensions

A bacterial suspension of *Escherichia coli* (ATCC 25922) and *Staphylococcus aureus* (ATCC 25923) were prepared by culturing each in nutrient broth overnight. Using sterile cotton swabs, each suspension was spread on the surface of the solidified agar in Petri dishes.

II.7.3 Application of Plant Extracts

n-BuOH extracts of four medicinal plants were tested. Sterile paper discs were soaked in the extracts as follows: for *E. alata* and *L. tingitana*, the extracts were diluted and tested at four concentrations, including the stock concentration of 100 mg/mL. Each Petri dish contained discs soaked in the stock on the extract and three diluted concentrations. However, the extracts *A. graveolens* and *Li.guyonianum* were used undiluted at a single concentration of 250 mg/mL.

The discs were left to dry under sterile conditions and then placed on the inoculated agar surface using sterile forceps. Two controls were used in the experiment: a positive control, a disc containing Gentamicin antibiotic. Moreover, a negative control (a disc soaked with methanol only, without any plant extract). All treatments, including the controls, were performed in triplicate to ensure the reliability of the results. The plates were incubated for 24 hours in the incubator

II.7.4 Evaluation of Antimicrobial Activity

After incubation, the diameter of the inhibition zone (in millimeters) around each disc was measured using a ruler. The presence of a clear zone around the disc indicated antimicrobial activity. The results were compared between the plant extracts, the positive control (Gentamicin), and the negative control (Methanol) to determine the relative effectiveness of each plant.

II.8 Statistical Analysis

Statistical analysis was performed using *GraphPad Prism 5* software. *In vitro* test results are expressed as mean ± SD. Correlations between test data were calculated using Pearson correlation coefficients (r^2). Significance levels were determined using the Student t-test. Differences are considered significant when $p < 0.05$:

ns; $p > 0.05$ = the difference is not significant ·

*; $0.05 > p > 0.01$ = the difference is significant ·

**; $0.05 > p > 0.001$ = the difference is highly significant ·

***; $p < 0.001$ = the difference is very highly significant.

RESULTS AND DISCUSSION

III RESULTS AND DISCUSSION

III.1 Extraction yield

The yield percentages of the plant extracts varied among the four species studied, ranging from 2.79% to 4.32% (g/g DW). *A. graveolens* exhibited the highest yield (4.32%), followed by *L. guyonianum* (4.06%), *E. alata* (3.55%), and *L. tingitana* (2.79%).

Table 2: The yield of plant extracts

Samples	Yield % (g/g DW)
L.guyonianum	4.06
E.alata	3.55
L.tingitana	2.79
A.graveolens	4.32

The differences in extraction yield between the species could be due to variations in their chemical composition, the ease with which their compounds dissolve, or the effectiveness of the extraction process. The higher yields seen in *A. graveolens* and *L. guyonianum* suggest that these plants might contain more compounds that are easily extracted under the conditions used, whether they are polar or non-polar. On the other hand, the lower yield from *L. tingitana* may indicate that it contains fewer of these extractable substances or that its cellular structure makes extraction more challenging. (**Jha & Sit, 2021**).

III.2 Phytochemical analysis

III.2.1 Total content of phenol, flavonoids and flavonols

The total phenol contents of *L.guyonianum, E.alata, L.tingitana* and*, A.graveolens* were quantified. The regression equation of calibration curve of gallic acid was y = 0.0034x + 0.1044with R^2 = 0.9972. (Fig 1, Appendix 1)

The total phenol content was highest and nearly equal *in L. guyonianum* (412.60±3.72 µg GAE/mg extract) and *E. alata* (407.65±4.10 µg µg GAE/mg extract*)*, followed by *L.tingitana* (391.12±2.27 µg GAE/mg extract*)*, with *A.graveolens* (241.31±4.30 µg GAE/mg extract*)* showing the lowest levels.

The content of flavonoids in plant extracts from *L.guyonianum, E.alata, L.tingitana* and, *A.graveolens* is determined using spectrophotometric method with $AlCl_3$ and expressed in terms of quercetin equivalent, QE (the standard curve equation: y = 0.0048x, R^2= 0.997), µg QE/mg extract. (Fig 2, Appendix 1)

The total flavonoid content was highest and nearly equal *in L. guyonianum* (162.87±3.15 µg QE/mg extract) and *L. tingitana* (163.25±5.3 µg QE/mg extract), followed by *A. graveolens* (116.26±6.10 µg QE/mg extract), with *E. alata* (92.34±5.24 µg QE/mg extract) showing the lowest levels. The similar flavonoid amounts in *L. guyonianum* and *L. tingitana* suggest common biosynthetic patterns, potentially linked to taxonomic proximity or environmental factors. The low flavonoid content observed in *E. alata* suggests a metabolic allocation toward other classes of secondary metabolites.

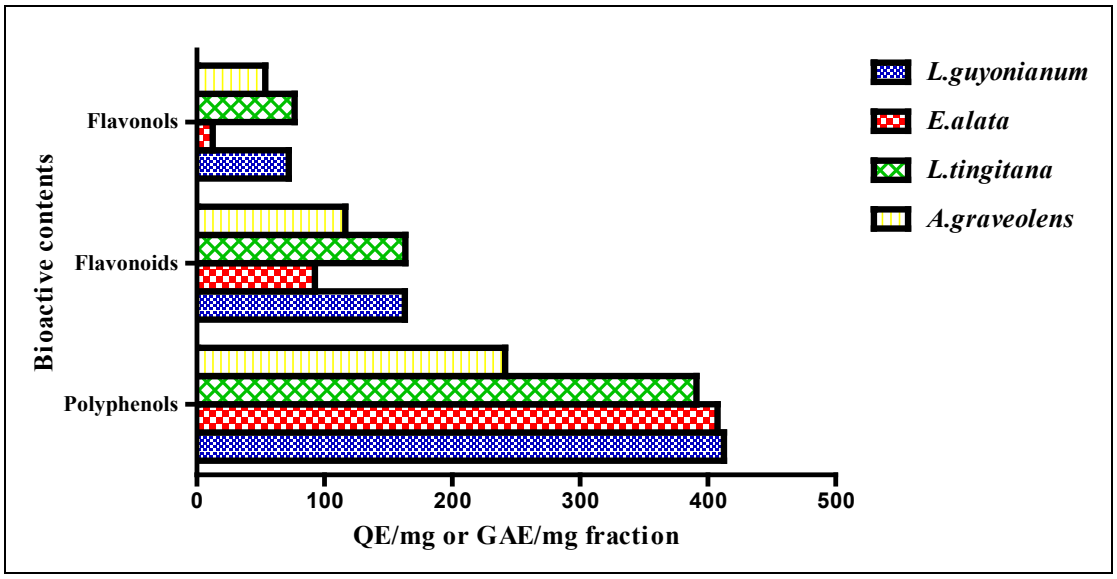

Figure 25: Total content of phenol, flavonoids and flavonols

The total flavonol content, expressed in QE (mg), followed the order: *L. tingitana* (76.50±1.99 µg QE/mg extract) was the highest, followed by *L. guyonianum* (72.04±3.02 µg QE/mg extract), then *A. graveolens* (53.51±2.28 µg QE/mg extract), and the lowest *E. alata* (12.38±0.71 µg QE/mg extract). The high flavonol levels in *L. tingitana* indicate its potential as a significant source of specific flavonol subclasses. In contrast, the low flavonol content in *E.*

alata suggests differences in its secondary metabolic pathways that may be subject to further investigation. (Fig 3, Appendix 1)

L. guyonianum consistently ranked high in both phenolics and flavonoids, indicating its potential for antioxidant applications. In contrast, *E. alata* showed low flavonoid and flavonol levels but moderate phenolic content, suggesting a divergent chemotype among the studied species.

III.2.2 Total chlorophyll and carotenoid content

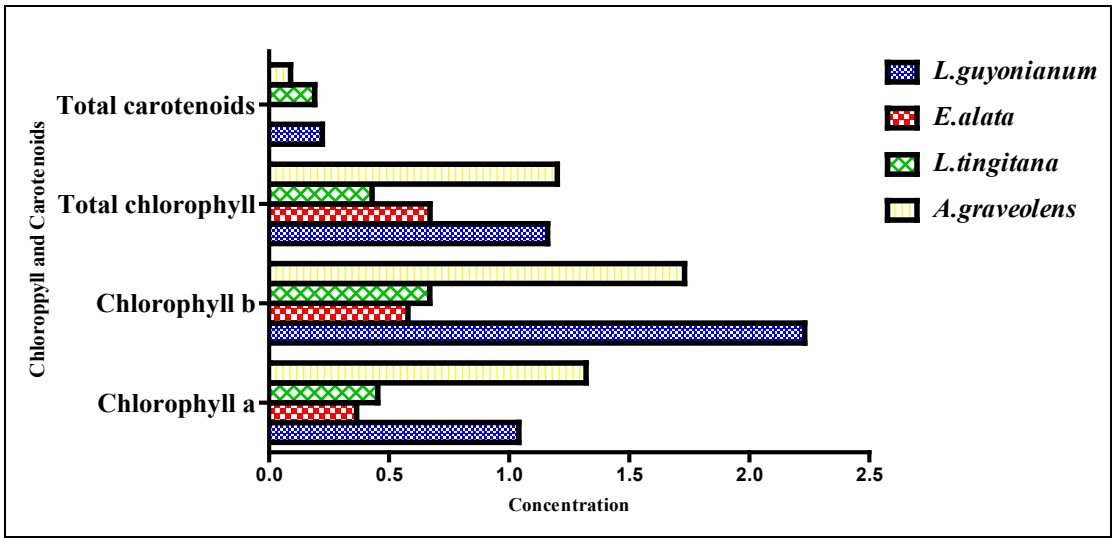

Figure 26: Total chlorophyll and carotenoid content

The figure presents a comparative analysis of total chlorophyll (the sum of chlorophyll a and b) and total carotenoid content in four plant species. The analysis of pigment composition revealed notable differences in chlorophyll and carotenoid content, which may reflect distinct physiological adaptations.

In terms of chlorophyll a content, *A. graveolens* (1.32 µg/mg extract) recorded the highest levels, followed by *L. guyonianum* (1.04 µg/mg extract), *L. tingitana* (0.45 µg/mg extract), and finally *E. alata* (0.37 µg/mg extract), which exhibited the lowest concentration. Since chlorophyll a plays a central role in the light-dependent reactions of photosynthesis, the elevated levels observed in *A. graveolens* suggest a higher photosynthetic capacity.Conversely, the reduced chlorophyll a content in *E. alata* may be associated with a lower density of chloroplasts or reduced efficiency in light absorption **(Croft *et al.*, 2017).**

For chlorophyll b, the highest concentration was found in *L. guyonianum* (2.23 µg/mg extract), followed by *A. graveolens* (1.73 µg/mg extract), *L. tingitana* (0.67 µg/mg extract), and again, *E. alata* (0.58 µg/mg extract), which had the lowest level. The abundance of chlorophyll b in *L. guyonianum* implies the presence of well-developed light-harvesting antenna systems, while the consistently low pigment levels in *E. alata* may indicate an adaptation to shaded habitats or environmental stress conditions **(Cao *et al.*, 2022).**

When considering total chlorophyll content (chlorophyll a + b), *A. graveolens* (1.20 µg/mg extract) ranked highest, confirming its overall richness in photosynthetic pigments. It was followed by *L. guyonianum* (1.16 µg/mg extract), *E. alata* (0.67 µg/mg extract), and *L. tingitana* (0.43 µg/mg extract), which showed the lowest total chlorophyll level. The relatively low total chlorophyll in *L. tingitana* may suggest a physiological shift toward the accumulation of other pigments, such as carotenoids, or an adaptation to low-light or stressful environments. **(Zhang *et al.*, 2016).**

Carotenoid levels, which are essential for photoprotection and oxidative stress mitigation, were most abundant in *L. guyonianum* (0.22 µg/mg extract), followed by *L. tingitana* (0.19 µg/mg extract) and *A. graveolens* (0.09 µg/mg extract). Carotenoids were absent or below detection limits in *E. alata*. The high carotenoid concentrations in *L. guyonianum* and *L. tingitana* suggest a greater investment in antioxidant defense mechanisms, possibly as a response to environmental stress. The absence of detectable carotenoids in *E. alata* is notable and may indicate reliance on alternative protective strategies or growth in relatively stable environments with low oxidative stress **(Uarrota *et al.*, 2018).**

In summary, *A. graveolens* appears to be the most photosynthetically active species, based on its high chlorophyll a and total chlorophyll content. *L. guyonianum* exhibits a balance between chlorophyll b and carotenoids, indicating adaptability to varying light conditions and potential stress. *L. tingitana* shows a greater investment in carotenoids relative to chlorophyll, which may be linked to stress tolerance. In contrast, *E. alata* possesses the lowest pigment content overall, suggesting unique metabolic or ecological adaptations that warrant further investigation.

III.3 Antioxidant activity

Antioxidative effects of natural products could be considered as a first insight in detecting their ethnopharmacological relevance and potential. To this end, a certain antioxidant profile could be provided by comparing different chemical assays representative of alternative mechanisms (**Zengin *et al.*, 2018**). For this reason, the antioxidant ability of *.guyonianum, E.alata, L.tingitana* and*, A.graveolens* was evaluated using four complementary *in vitro* tests: free radical scavenging (DPPH and ABTS), reducing power, phenanthroline assays.

III.3.1 DPPH antiradical activity

The DPPH free radical assay is widely used to evaluate the antioxidant capacity of compounds by measuring their ability to act as free-radical scavengers and hydrogen donors. It provides a rapid, simple, and cost-effective method for assessing antioxidant activity. The assay relies on the reduction of DPPH, a stable free radical with a deep purple color. When reduced to DPPH-H by an antioxidant, the solution's color changes to colorless or pale yellow, indicating radical scavenging activity **(Baliyan *et al.*, 2022).**

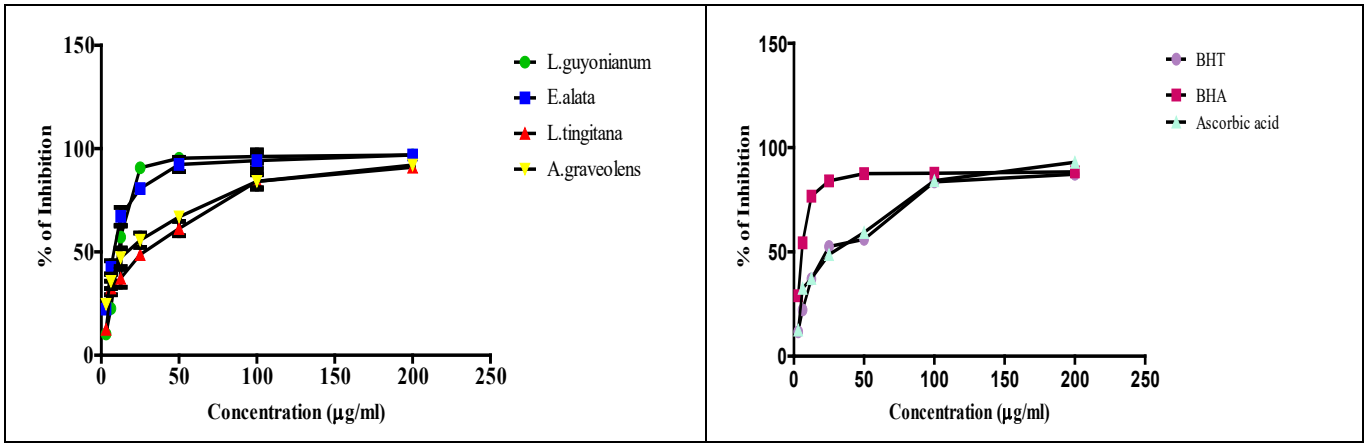

Figure 27: DPPH radical scavenging activity of plant extracts and standard antioxidants at various concentrations (µg/mL)

This figure (fig. 27) illustrates the antioxidant activity of four plant extracts and standard antioxidants, assessed through their capacity to scavenge DPPH free radicals. The percentage of inhibition represents the effectiveness of each extract and standard antioxidant in neutralizing free radicals across increasing concentrations (µg/mL).

At lower concentrations (0–50 µg/mL), the antioxidant activity of the plant extracts, as measured by DPPH radical scavenging, showed notable variation. *L. guyonianum* exhibited the highest inhibition percentages, indicating the presence of highly bioavailable and reactive antioxidant compounds, *E. alata*, despite its low pigment content, performed well at these concentrations, which may suggest the involvement of non-pigment antioxidants. In contrast, *A. graveolens* and *L. tingitana* demonstrated relatively weaker activity at lower doses, implying that their active constituents may require higher concentrations to exert measurable effects.

At higher concentrations (100–200 µg/mL), all four extracts achieved similarly high levels of DPPH inhibition, converging around 90–100%. This saturation effect suggests that, at sufficient doses, all extracts are capable of effectively neutralizing available free radicals. However, the mechanisms underlying this effect may vary. For instance, the data imply that *E. alata* may possess less potent but more abundant antioxidants, requiring higher concentrations to achieve inhibition levels comparable to the early efficiency observed in *L. guyonianum*.

A. graveolens and *L. tingitana* both exhibited a dose-dependent antioxidant response, with increased DPPH radical scavenging observed at higher extract concentrations. The relatively low activity of *A. graveolens* at lower doses, despite its high chlorophyll content, indicates that chlorophyll-related compounds may play a limited role in antioxidant activity under the specific conditions of this assay. This suggests that other non-chlorophyll constituents may be more influential in determining its radical-scavenging capacity.

The DPPH assay results for the standard antioxidants BHA, BHT, and ascorbic acid revealed differences in their activity profiles across concentrations. At lower concentrations, BHA exhibited the most rapid and potent antioxidant activity, indicating high efficiency at minimal doses. In contrast, BHT and ascorbic acid followed similar patterns, both demonstrating moderate initial activity with a gradual increase in radical scavenging capacity. As the concentration increased, all three standards approached 100% inhibition, indicating that, despite differences in early response, each standard is capable of fully neutralizing DPPH radicals at higher concentrations. This trend mirrors the behavior observed in the plant extracts under the similar assay conditions.

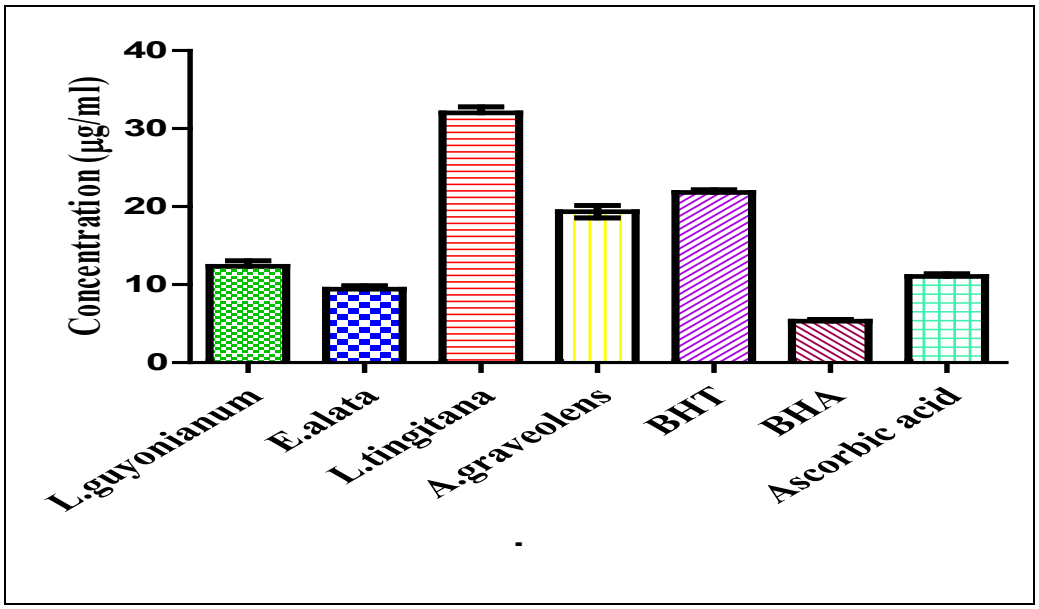

Figure 28: Graphical representation of IC_{50} values (µg/mL) of DPPH radical scavenging activity for plant extracts and standard antioxidants

Based on the IC_{50} values obtained, the antioxidants were ranked from most to least potent as follows: BHA (5.32±0.40 µg/mL) demonstrated the highest efficiency, consistent with its status as a synthetic standard. Among the natural plant extracts, *E. alata* exhibited the strongest activity (9.40±0.75 µg/mL), surpassing ascorbic acid (11.05±0.59 µg/mL), a well-established natural antioxidant. *L. guyonianum* followed closely (12.36±1.17 µg/mL), indicating good antioxidant potential though slightly less efficient than *E. alata*. *A. graveolens* (19.34±0.80 µg/mL) presented moderate activity, while *L. tingitana* showed the weakest performance (32.03±1.32 µg/mL). BHT a synthetic antioxidant recorded a relatively high IC_{50} (21.81±0.61 µg/mL), placing it below both ascorbic acid and *L. tingitana* in potency.

Certain observations need particular consideration. The notable antioxidant activity of *E. alata*, despite its low levels of pigments, indicates that non pigment secondary metabolites such as phenolic compounds may significantly contribute to its free radical scavenging ability. In contrast, The limited antioxidant response of *L. tingitana* is consistent with previous DPPH assay results and may reflect a reliance on alternative defense mechanisms that are not primarily based on radical scavenging.

III.3.2 ABTS antiradical activity

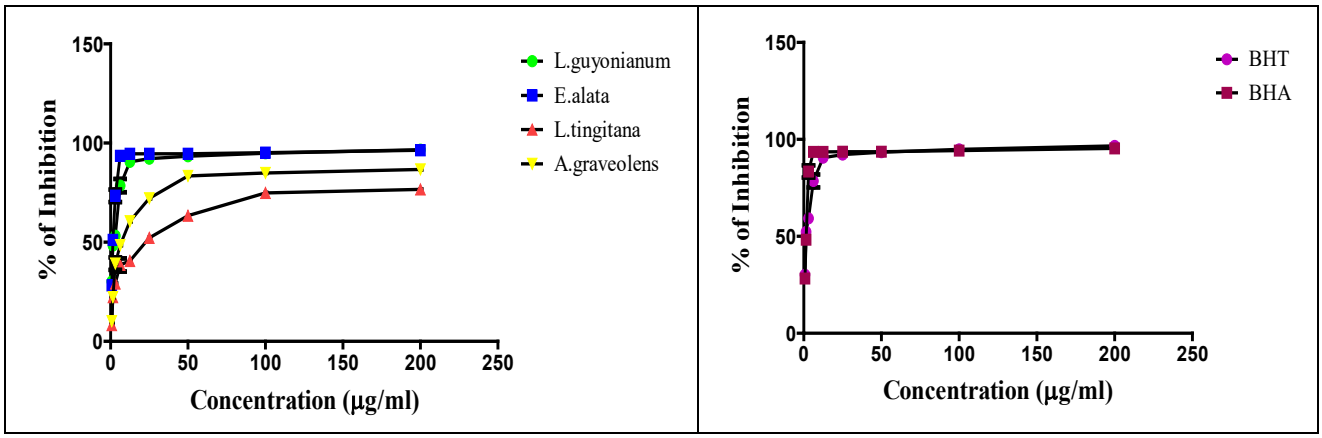

Figure 29: ABTS Radical scavenging activity of plant extracts and standards at varying concentrations

All tested plant extracts exhibited a dose-dependent scavenging effect against ABTS radicals, with percent inhibition progressively increasing as the concentration rose from 0 to 200 µg/mL. At higher concentrations, the inhibition values approached saturation levels, nearing 100% activity. This trend is consistent with the pattern observed in the DPPH assay, indicating that the antioxidant capacity of the extracts becomes fully expressed at elevated doses due to the complete neutralization of available ABTS radicals. Based on overall efficiency, the extracts ranked as follows: *L. guyonianum* and *E. alata* demonstrated the highest and nearly equivalent antioxidant activity, followed by *A. graveolens*, with *L. tingitana* showing the lowest performance in the ABTS assay.

For the standads, BHT and BHA displayed nearly identical antioxidant activity profiles across the full range of tested concentrations (0–200 µg/mL). Both synthetic antioxidants achieved comparable levels of percent inhibition at each dose, in contrast to the DPPH assay where BHA exhibited greater potency. This suggests that under ABTS assay conditions, BHT and BHA possess similar radical-scavenging efficiency. At higher concentrations, both compounds appear to approach a saturation point, with inhibition values nearing 100%, reflecting a maximum scavenging capacity similar to that observed for the plant extracts.

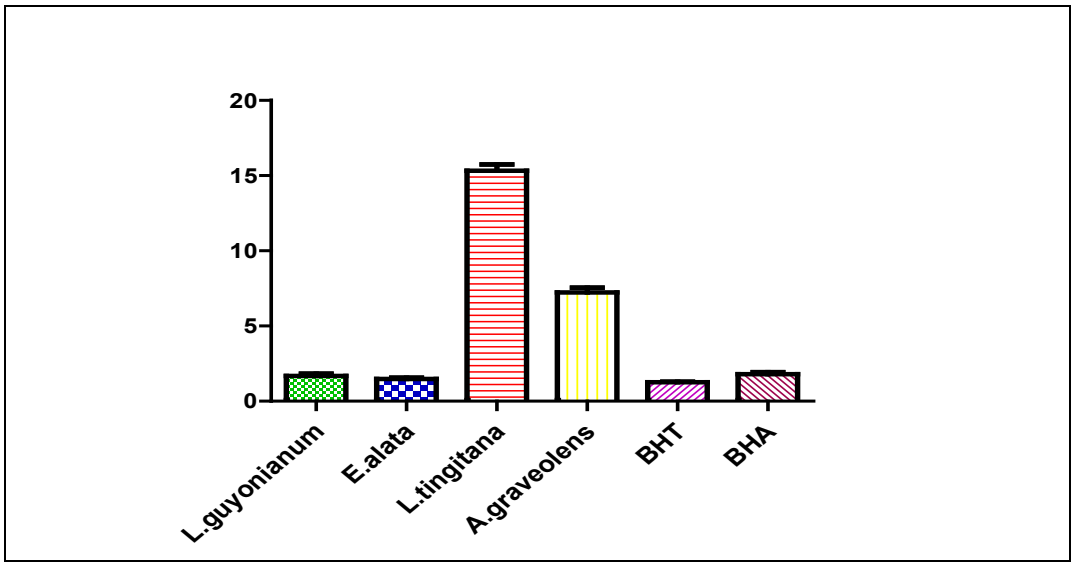

Figure 30: Graphical representation of IC_{50} values of plant extracts and standards in ABTS radical scavenging assay

The antioxidant capacities of the evaluated samples, based on their ABTS radical scavenging activity, show clear variations in effectiveness, as reflected by their IC_{50} values. Among the tested compounds, BHT demonstrated the highest activity, with the lowest IC_{50} value of (1.26±0.04 µg/mL), followed by BHA, which had a higher IC_{50} value of (1.79±0.14 µg/mL). This outcome contrasts with findings from the DPPH assay, where BHA generally exhibited greater activity than BHT, suggesting that antioxidant performance can differ depending on the assay used. **(Boulebd, 2020)**.

Among the tested plant extracts, *E.alata* exhibited the highest antioxidant activity, with an IC_{50} value of (1.49±0.07 µg/mL), which is comparable to that of BHT (1.26±0.04 µg/mL). This suggests that *E. alata* may be a relevant natural source of antioxidant compounds. *L.guyonianum* showed slightly lower activity (1.69±0.14 µg/mL). *A.graveolens* displayed moderate activity (7.22±0.33 µg/mL), whereas *L.tingitana* had the lowest activity among the samples, with a significantly higher IC_{50} value (15.32±0.40 µg/mL).

III.3.3 Reduction activity by the formation of the Fe+2-phenanthroline complex

Table 3: Reduction activity via Fe^{2+}-Phenanthroline complex formation: Comparative analysis of four flant extracts and standard antioxidants

Concentration (µg/ml)	**0.78125**	**1.56250**	**3.12500**	**6.25000**	**12.5**	**25**	**50**
L.guyonianum	*nt*	0.14±0.03	0.26±0.04	0.36±0.01	0.42±0.01	0.74±0.04	0.91±0.07
E.alata	*nt*	0.20±0.01	0.33±0.01	0.47±0.01	0.52±0.03	0.69±0.01	0.74±0.01
L.tingitana	*nt*	*nt*	*nt*	0.17±0.03	0.23±0.01	0.36±0.02	0.67±0.03
A.graveolens	*nt*	0.13	0.27	0.37±0.01	0.41±0.06	0.48±0.01	0.62±0.01
BHT	0.13±0.01	0.43±0.03	0.53±0.01	1.23±0.02	1.84±0.03	3.48±0.05	4.84±0.05
BHA	0.43±0.06	0.62±0.05	0.73±0.04	0.93±0.01	1.25±0.02	2.10±0.01	4.89±0.03
Ascorbic acid	0.25±0.03	0.42±0.04	0.51±0.01	0.72±0.03	0.91±0.01	1.23±0.01	3.23±0.02

In this assay, absorbance at 510 nm was used as an indicator of reducing power. Higher absorbance values correspond to greater antioxidant activity based on the reduction capacity of the samples.

Among the tested plant extracts, at a concentration of 50 µg/mL, *L.guyonianum* showed the highest reducing power with an absorbance of (0.91±0.07). This was followed by *E.alata* (0.74±0.01), *L.tingitana* (0.67±0.03), and *A.graveolens* (0.62±0.01), *E. alata* demonstrated strong activity at lower concentrations, but its response reached a plateau at higher concentrations.

All tested samples showed dose-dependent increases in reducing power, confirming the concentration-dependent nature of their antioxidant activity. BHA and BHT consistently exhibited the highest activity across all concentrations, with BHT showing a notable increase between 6.25 and 25 µg/mL. *L.guyonianum* displayed a gradual but consistent rise in absorbance, eventually surpassing *E. alata* at higher concentrations, indicating a slower yet cumulative effect. In contrast, *A.graveolens* maintained moderate and relatively stable activity across the tested concentrations, with no significant changes in response.

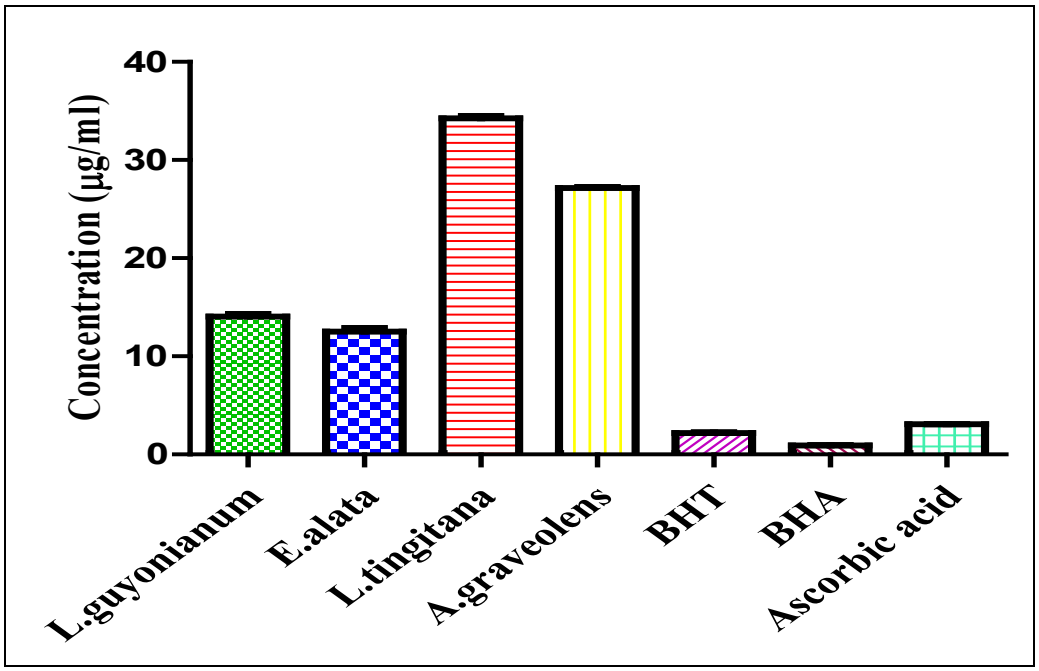

Figure 31: Graphical representation of reducing activity of plant extracts and standards measured by Fe^{2+}-phenanthroline complex formation

The $A_{0.5}$ parameter represents the concentration required to achieve 50% of the maximum reducing activity and is used as a measure of antioxidant effectiveness in reducing power assays. Similar in concept to the IC_{50} used in inhibition assays, lower $A_{0.5}$ values indicate greater potency.

Based on $A_{0.5}$ values, BHA was the most potent reducing agent among the tested substances (0.91±0.04 µg/mL). BHT followed with an $A_{0.5}$ of (2.18±0.06 µg/mL), still indicating notable reducing activity. Ascorbic acid, the strongest natural antioxidant standard, had an $A_{0.5}$ of (3.08±0.02 µg/mL), reinforcing its role as an effective reference compound for natural antioxidants.

Among the plant extracts, *E. alata* exhibited the highest reducing power, with an $A_{0.5}$ value of (12.51±0.39 µg/mL), followed by *L.guyonianum* (14.07±0.24 µg/mL). *A. graveolens* (27.14±0.09 µg/mL) and *L. tingitana* (34.25±0.23 µg/mL) showed lower potencies. Based on this parameter, *L. tingitana* was the least effective extract.

These findings support the potential of certain plant extracts, particularly *E. alata* and *L.guyonianum*, as relevant candidates for inclusion in natural antioxidant formulations.

III.3.4 Activity of reducing power

The reducing power assay is a widely recognized method for evaluating antioxidant potential, as it reflects the ability of compounds to act as electron donors, this mechanism highlights their role as both primary and secondary antioxidants **(Jayanthi & Lalitha, 2011).**

Table 4: Reducing power activity of plant extracts and standard antioxidants at various concentrations

Concentration (µg/ml)	**3.12500**	**6.25000**	**12.5**	**25**	**50**	**100**
L.guyonianum	0.27±0.02	0.35±0.02	0.52±0.03	0.74±0.04	0.91±0.06	1.55±0.07
E.alata	*nt*	0.19±0.01	0.31±0.03	0.39±0.02	0.48±0.02	0.69±0.03
L.tingitana	0.10±0.01	0.27±0.04	0.33±0.01	0.56±0.02	0.77±0.03	0.99±0.01
A.graveolens	0.19±0.01	0.29±0.02	0.32±0.04	0.58±0.03	0.82±0.01	1.23±0.06
BHT	0.10±0.01	0.13±0.02	0.22±0.04	0.28±0.05	0.43±0.02	0.51±0.02
BHA	0.18±0.02	0.36±0.04	0.78±0.07	1.74±0.07	3.53±0.19	*nt*
Ascorbic acid	0.16±0.01	0.33±0.04	0.76±0.16	2.02±0.23	3.87±0.27	*nt*

At the highest measured concentrations, a comparative assessment of reducing power indicated considerable differences among the standards and plant extracts. Ascorbic acid displayed the greatest reducing activity, with an absorbance value of (3.87 ± 0.27) at 50 µg/mL, making it the most potent natural antioxidant in this assay. BHA followed closely with (3.53 ± 0.19) at the same concentration, making it the most powerful synthetic standard under these conditions.

Among the plant extracts examined at 100 µg/mL, *L.guyonianum* demonstrated the highest reducing activity (1.55 ± 0.07), identifying it as the most effective natural extract in this context. *A.graveolens* (1.23 ± 0.06) and *L.tingitana* (0.99 ± 0.01) also indicated moderate activity, whereas *E.alata* recorded a significantly lower value of (0.69 ± 0.03). BHT despite its recognized antioxidant properties showed the lowest performance among the synthesized substances at this dosage (0.51 ± 0.02), suggesting a decreased efficacy in this particular experimental setup.

A broad dose-dependent trend was seen across all samples, with absorbance values increasing with greater doses, consistent with expected behavior for reducing power assays. However, certain variances were identified in the patterns of increase. *E. alata* demonstrated minimal action at lower dosages and maintained relatively modest performance even at higher

levels. In contrast, *L. tingitana* and *A. graveolens* demonstrated considerable increases in reduction power at elevated concentrations, indicating stronger reactions at the upper end of the dose range

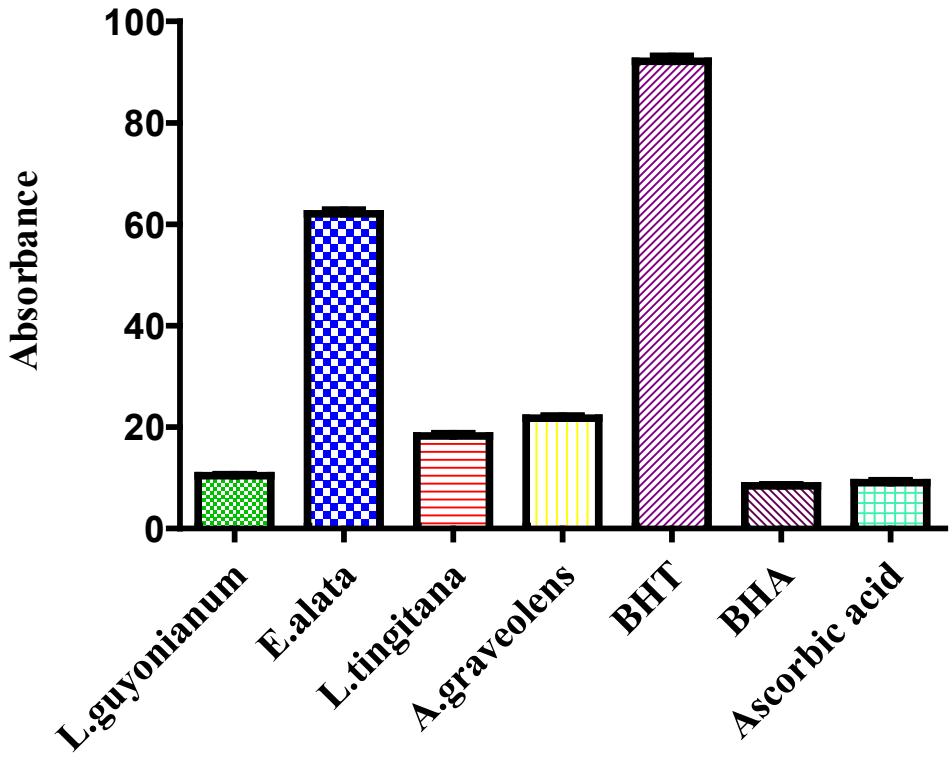

Figure 32: Graphical representation of reducing activity of plant extracts and standards measured by FRAP assay

Among the standard antioxidants studied, ascorbic acid and BHA displayed the strongest reducing power, with $A_{0.5}$ values of (9.02±0.40 µg/mL) and (8.40±0.20 µg/mL), respectively. These low $A_{0.5}$ values imply that only a tiny concentration of these chemicals is needed to provide considerable antioxidant activity, showing their great efficacy. In contrast, BHT demonstrated the poorest reducing power, with an $A_{0.5}$ value of (92.16±0.88 µg/mL). This shows that BHT requires a concentration approximately ten times greater than that of ascorbic acid to exhibit a comparable effect. The low performance of BHT accords with its recognized

role as a lipid-soluble, chain-breaking antioxidant rather than a powerful reducing agent **(Yehye *et al.*, 2015).**

Among the plant extracts studied, *L. guyonianum* demonstrated the most strong reducing activity (10.40±0.24 µg/mL), placing it on level with ascorbic acid and much more effective than BHT. This result refers to a high content of electron-donating substances in the extract, such as polyphenols, which contribute to its antioxidant activity. *A. graveolens* (21.79±0.38 µg/mL) and *L. tingitana* (18.27±0.40 µg/mL) demonstrated moderate reducing capacities, which may suggest an intermediate presence of reductive components. *E. alata* displayed the lowest reducing power among the plant extracts, with an $A_{0.5}$ value of (62.08±0.67 µg/mL). This shows that its antioxidant action might operate primarily through other processes, such as radical scavenging, rather than direct electron donation.

When analyzing all samples, the pattern in antioxidant reduction capacity from greatest to weakest is as follows: BHA, Ascorbic acid, *L. guyonianum*, *L. tingitana*, *A. graveolens*, *E. alata* and lastly BHT. This rating illustrates the noteworthy potency of both certain plant extracts and conventional substances.

Table 5: Pearson correlation coefficients (r^2) between antioxidant (IC_{50} and $A_{0.5}$) and phytochemical content of *n*-BuOH extract of *L. guyonianum*

	TPC	TFC	FC	DPPH	ABTS	PHEN	FRAP
TPC	1						
TFC	0.9914895	1					
FC	0.9999865**	0.9921516	1				
DPPH	0.9857814	0.9555164	0.9848964	1			
ABTS	0.9863085	0.9564455	0.9854397	0.999995**	1		
PHEN	0.9578773	0.9123387	0.9563745	0.9925129	0.9921212	1	
FRAP	0.9048005	0.8416622	0.9025791	0.9634898	0.9626372	0.9889784	1

Statistics: Values are significantly different (* p <0.05, ** p <0.01 and *** p <0.001) by Pearson correlation coefficient. TPC, Total phenol content; TFC, Total flavonoid content; FC, Flavonol content, PHEN, Reducing activity by formation of Fe+2-phenanthroline complex; FRAP, Reducing power activity

Among the correlations analyzed, only two were explicitly marked as statistically significant at the $p < 0.01$ level, indicating a high degree of confidence in the observed relationships:

TPC and FC (r^2 = 0.9999865, p = 0.003), this correlation is highly significant, indicating a strong and consistent association between Total Phenolic Content and Flavonol Content.

DPPH and ABTS (r^2 = 0.999995, p = 0.002), this correlation is also highly significant, reflecting a robust relationship between the two antioxidant assays.

Table 6: Pearson correlation coefficients (r^2) between antioxidant (IC_{50} and $A_{0.5}$) and phytochemical content of *n*-BuOH extract of *E. alata*

	TPC	TFC	FC	DPPH	ABTS	PHEN	FRAP
TPC	1						
TFC	0.9866498	1					
FC	0.9999918**	0.9873013	1				
DPPH	0.954465	0.8931388	0.9532489	1			
ABTS	0.9967402	0.9965724	0.9970588*	0.9272856	1		
PHEN	0.9378672	0.9818568	0.939265	0.791645	0.9628047	1	
FRAP	0.9383796	0.8695679	0.9369721	0.9987528*	0.907438	0.7601521	1

Statistics: Values are significantly different (* p <0.05, ** p <0.01 and *** p <0.001) by Pearson correlation coefficient. TPC, Total phenol content; TFC, Total flavonoid content; FC, Flavonol content, PHEN, Reducing activity by formation of Fe+2-phenanthroline complex; FRAP, Reducing power activity

Among the correlations analyzed, three pairs were explicitly marked as statistically significant, indicating varying levels of association and confidence in the observed relationships:

TPC and FC (r^2 = 0.9999918, p < 0.01), this correlation is highly significant (p = 0.003), indicating a strong and consistent association between Total Phenolic Content and Flavonol Content.

ABTS and FC (r^2 = 0.9970588, p < 0.05), this correlation is significant (p = 0.049), suggesting a strong relationship between ABTS antioxidant activity and Flavonol Content.

FRAP and DPPH (r^2 = 0.9987528, p < 0.05), this correlation is also significant (p = 0.032), reflecting a close association between reducing power (FRAP) and DPPH radical scavenging activity.

Table 7: Pearson correlation coefficients (r^2) between antioxidant (IC_{50} and $A_{0.5}$) and phytochemical content of *n*-BuOH extract of *L. tingitana*

	TPC	TFC	FC	DPPH	ABTS	PHEN	FRAP
TPC	1						
TFC	0.9805502	1					
FC	0.9845603	0.9997668*	1				
DPPH	0.9976266*	0.9917372	0.9942765	1			
ABTS	0.9196797	0.8247234	0.8367449	0.8904593	1		
PHEN	0.9274215	0.9827913	0.9785727	0.9509737	0.7060653	1	
FRAP	0.992083	0.9481389	0.9547825	0.9810812	0.9617116	0.8731083	1

Statistics: Values are significantly different (* p <0.05, ** p <0.01 and *** p <0.001) by Pearson correlation coefficient. TPC, Total phenol content; TFC, Total flavonoid content; FC, Flavonol content, PHEN, Reducing activity by formation of Fe+2-phenanthroline complex; FRAP, Reducing power activity

Among the correlations analyzed, two pairs were explicitly marked as statistically significant, each indicating a strong relationship supported by statistical evidence:

FC and TFC ($r^2 = 0.9997668$, $p < 0.05$), this correlation is significant ($p = 0.014$), showing a strong association between Flavonol Content and Total Flavonoid Content.

DPPH and TPC ($r^2 = 0.9976266$, $p < 0.05$), this correlation is also significant ($p = 0.044$), suggesting a robust relationship between Total Phenolic Content and DPPH radical scavenging activity.

Table 8: Pearson correlation coefficients (r^2) between antioxidant (IC_{50} and $A_{0.5}$) and phytochemical content of *n*-BuOH extract of *A. graveolens*

	TPC	TFC	FC	DPPH	ABTS	PHEN	FRAP
TPC	1						
TFC	0.8352224	1					
FC	0.9881431	0.9097505	1				
DPPH	0.8905901	0.9939447	0.9498596	1			
ABTS	0.9629012	0.9526327	0.9929165	0.9802818	1		
PHEN	0.9993147*	0.8550049	0.993149	0.9068144	0.97223	1	
FRAP	0.7614981	0.9924554	0.8519859	0.9729735	0.908158	0.784968	1

Statistics: Values are significantly different (* p <0.05, ** p <0.01 and *** p <0.001) by Pearson correlation coefficient. TPC, Total phenol content; TFC, Total flavonoid content; FC, Flavonol content, PHEN, Reducing activity by formation of Fe+2-phenanthroline complex; FRAP, Reducing power activity

Among the correlations analyzed, only one pair was explicitly marked as statistically significant, indicating a meaningful relationship supported by the data:

TPC and PHEN (r^2 = 0.9993147, $p < 0.05$), this correlation is significant (p = 0.024), suggesting a strong association between Total Phenolic Content and reducing activity measured by the phenanthroline assay. This may imply that phenolic compounds contribute substantially to the reducing capacity of the extract.

III.4 *In vitro* anti-inflammatory activity

In vitro anti-inflammatory activity was determined using the method of Kandikattu K, (2013) with slight modifications.

In vitro anti-inflammatory effects of *n*-BuOH extracts from four plants, *L. guyoniamum*, *E. alata*, *L. tingitana* and *A. graveolens* against the standard drug diclofenac across a concentration range (50–500 µg/mL).

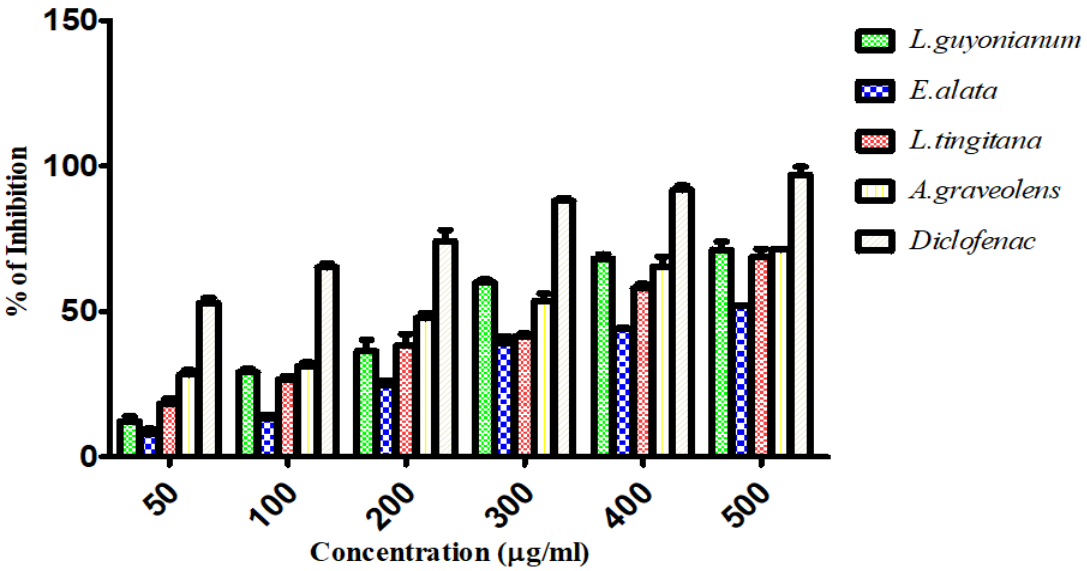

Figure 33: *In vitro* anti-inflammatory effect of *n*-BuOH extracts of the four plants; and diclofenac.

All plant extracts, as well as the reference drug diclofenac, demonstrated a concentration-dependent increase in anti-inflammatory activity within the tested range (50–500 µg/mL). Diclofenac, used as a positive control, exhibited the highest level of inhibition, thereby serving as a benchmark for evaluating the efficacy of the extracts.

Based on the percentage of inhibition across the tested concentrations (50–500 µg/mL), *A. graveolens* and *L. guyonianum* exhibited the highest anti-inflammatory activity from 50 to 400 µg/mL. However, at 500 µg/mL, the activities of *A. graveolens*, *L. tingitana*, and *L. guyonianum* became nearly equivalent. In contrast, *E. alata* and *L. tingitana* showed moderate to low activity throughout the 50–400 µg/mL range. These findings suggest that while *L. guyonianum* may initially appear among the most potent extracts, its advantage diminishes at higher concentrations, resulting in comparable efficacy with *A. graveolens* and *L. tingitana* at the highest tested dose.

Due to external or internal factors, proteins may undergo disruptive processes such as misfolding, unfolding, or aggregations. In these cases, proteins reach denatured state, i.e., protein denaturation occurs, this is the process in which proteins lose their secondary and tertiary structures due to the alteration of their hydrogen, hydrophobic, electrostatic, and disulfide bonds. Most proteins lose their biological functions after denaturation and induce the production of autoantigens causing several autoimmune dysfunctions including rheumatic and inflammatory diseases **(Khan & Khan, 2022).**

III.5 Lipid Peroxidation (LPO) Inhibition

Lipid peroxidation (LPO) refers to the autocatalytic chain reaction involving the oxidation of bis-allylic bonds within the aliphatic chains of fatty acids, leading to cell membrane damage and oxidative stress **(Aureliano *et al*., 2023)**. To evaluate the protective effect of plant *n*-BuOH extracts and vitamin C against this oxidative process, their ability to inhibit LPO was measured.

The median inhibitory concentrations (IC_{50}) of plant *n*-BuOH extracts and vitamin C were calculated from the equations of linear trend curves obtained from plots of the variation in percentage inhibition of lipid peroxidation (I%) as a function of increasing concentrations of *n*-BuOH extracts and vitamin C.

The percentage of lipid peroxidation (LPO) inhibition by the plant extracts and ascorbic acid was evaluated across doses ranging from 50 to 500 µg/mL, with higher values indicating stronger protection against oxidative lipid damage.

At the highest tested dose of 500 µg/mL, ascorbic acid had the highest inhibitory activity, achieving 98% inhibition and acting as the reference standard for natural antioxidants due to its near-complete inhibition of peroxidative processes.

Among the plant extracts, *A.graveolens* displayed the most significant efficacy, reaching (89.32%) inhibition. This result surpasses that of *L.guyonianum* (70.74%), positioning *A. graveolens* as the most effective candidate in this assay. *E.alata* achieved a moderate inhibition level of (59.33%), while *L.tingitana* exhibited the weakest activity at (30.68%), even at the highest concentration tested.

All samples demonstrated a dose-dependent increase in inhibition, consistent with typical antioxidant behavior. Ascorbic acid exhibited a rapid increase in activity, reaching a plateau at approximately 200 µg/mL with 97.5% inhibition, indicating saturation of its antioxidant capacity at relatively low concentrations. *A.graveolens* showed a marked increase in inhibitory activity from 19.32% to 89.32% as the concentration increased, which may reflect additive or synergistic effects among its phytochemical components. In contrast, *L.tingitana* maintained low inhibition values across the tested concentration range, suggesting limited antioxidant efficacy.

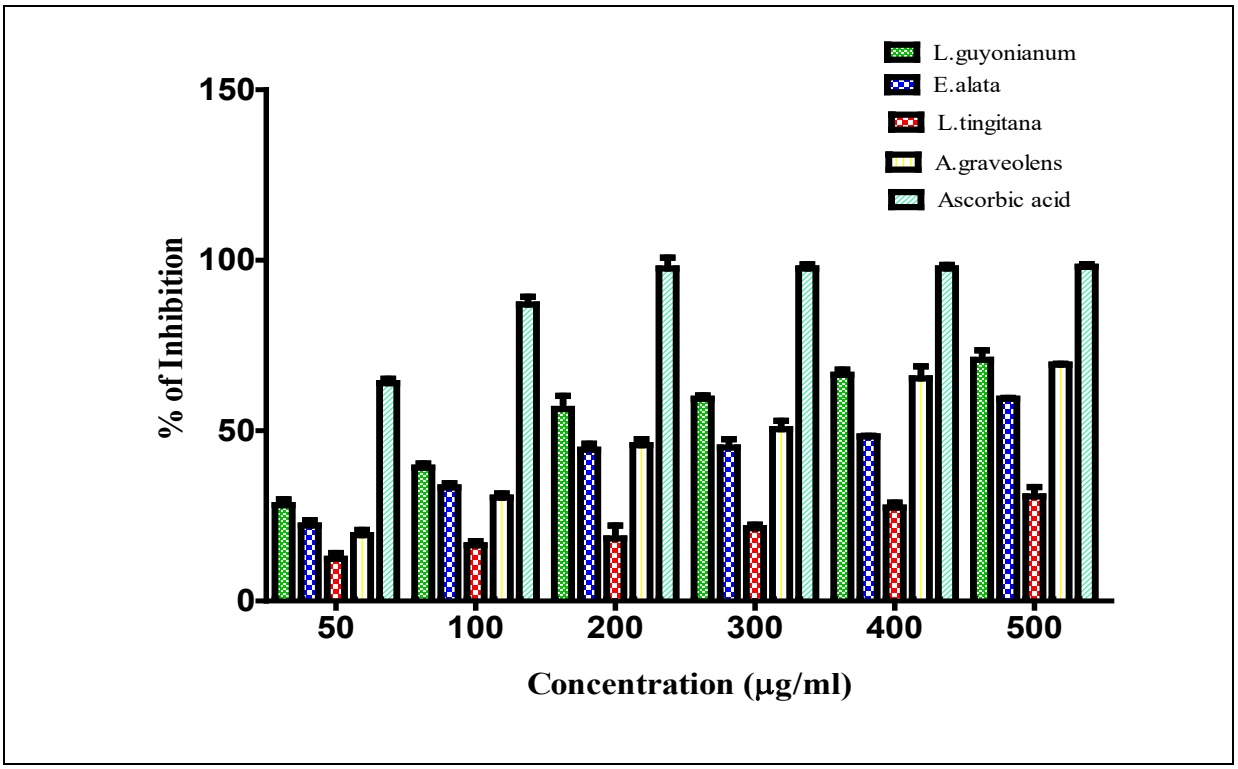

Figure 34: Concentration-dependent inhibition of lipid peroxidation by plant extracts and ascorbic acid

III.6 Anti-Alpha Amylase

The anti-alpha amylase potential was assessed using the iodine/potassium iodide method, with few modifications **(Zengin *et al.*, 2014)**.

The inhibitory effects of the four plant extracts (*L. guyonianum, E. alata, L. tingitana, and A. graveolens*) on α-amylase activity were compared to those of acarbose.

The results reveal that *E. alata* exhibited the strongest α-amylase inhibitory activity with the lowest IC_{50} value of (64.62±1.34 µg/mL), followed by *L. guyonianum* (119.58±14.38 µg/mL) and *A. graveolens* (230.82±10.20 µg/mL). *L. tingitana* showed moderate inhibition (355.31±29.58 µg/mL), while acarbose, the reference drug, presented the weakest activity with a notably higher IC_{50} value of (3650.61±10.34 µg/mL).

Our findings are consistent with Jaradat *et al*. (2021), who reported that the methanolic extract of *Ephedra alata* fruits showed α-amylase inhibitory activity (9.43±0.6 µg/mL), surpassing the potency of acarbose (28.84±1.22 µg/mL) **(Jaradat *et al.*, 2021).**

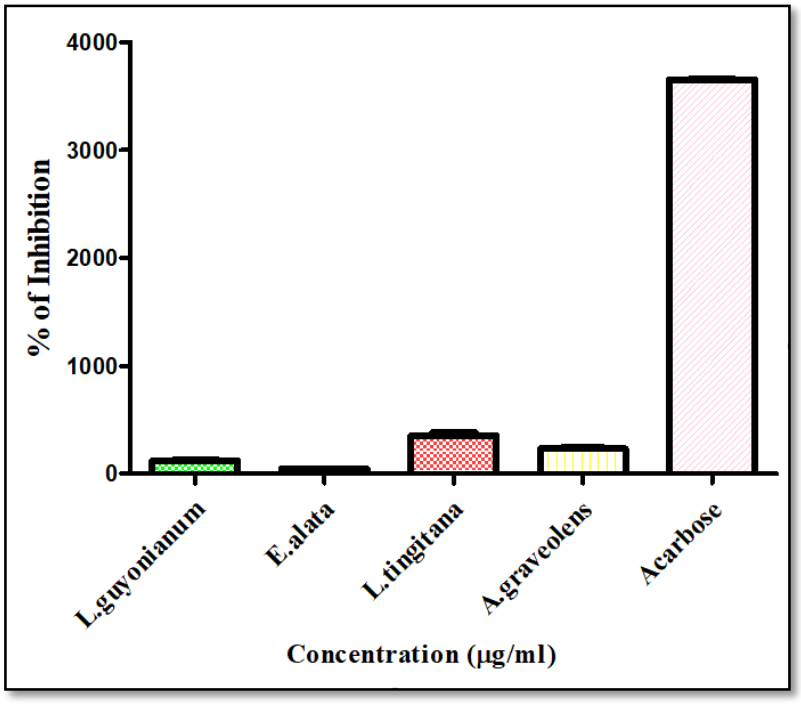

Figure 35: Inhibitory effects of four plant extracts and acarbose (standard) on α-amylase activity, expressed as percentage inhibition.

The inhibition of α-amylase prevents starch from breaking down and results in lower blood glucose levels. **(Khattabi *et al.*, 2022).** The inhibitory activity of a polyphenol against α-amylase is highly related with the phenolic molecular structure, and the structure-inhibition relationships have been partially studied. Specifically, for flavonoids, the existence of hydroxyls (-OH) at 5-, 6-, 7-positions of ring A and at 4′-position of ring B are able to enhance the inhibitory activity because –OH plays an important role in formation of hydrogen bondings with the active site of the enzyme **(Sun *et al.*, 2020**).

III.7 Antibacterial Activity

Medicinal plants contain antimicrobial compounds that act through mechanisms different from standard drugs, making them promising for treating drug-resistant microbes. Some compounds have both antibacterial effects and can modulate antibiotic resistance, while others enhance the effectiveness of conventional antibiotics when combined with them **(Vaou *et al.*, 2021).**

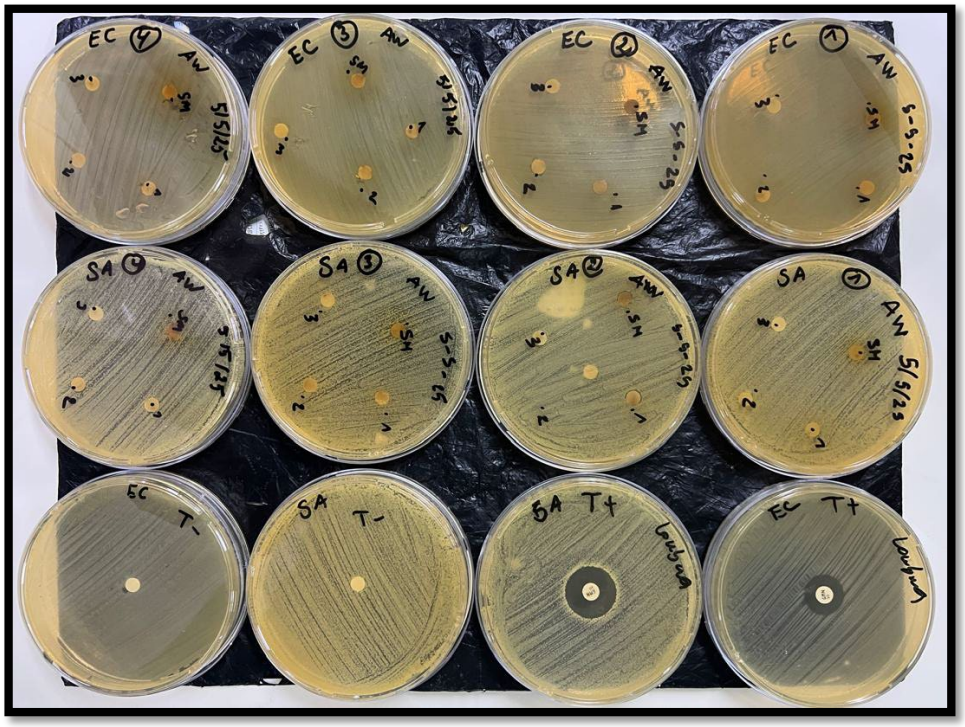

Figure 36: Antibacterial activity of *n*-butanol extracts of the four plants against Escherichia coli and Staphylococcus aureus, including positive (Gentamicin) and negative (Methanol) controls

The results revealed that none of the tested plant extracts exhibited antibacterial activity under the conditions used. Specifically, no inhibition zones were observed around the discs impregnated with any of the plant extracts, regardless of concentration or bacterial strain. In contrast, the positive control (Gentamicin) produced clear and measurable inhibition zones, confirming that the bacterial strains were sensitive and the experimental procedure was valid. The negative control (methanol) produced no inhibition zones, indicating that the solvent itself had no antibacterial effect and did not interfere with the assay.

The absence of antibacterial activity suggests that the *n*-BuOH fractions of the tested extracts were not active against either of the two bacterial strains used. This may be due to the absence or low concentration of antimicrobial compounds in the *n*-BuOH phase, or to the poor diffusibility of active constituents in the agar medium. Additionally, it is possible that the bioactive molecules with antibacterial potential were not efficiently extracted with *n*-butanol, a solvent of intermediate polarity. Compounds with stronger antibacterial effects might be more abundant in fractions obtained using solvents of different polarities, or may require higher concentrations to exert detectable activity **(Zouine *et al.*, 2024).**

CONCLUSION

Conclusion

This research aimed to investigate the phytochemical composition and biological activities of four endemic Algerian medicinal plants: *Ephedra alata*, *Linaria tingitana*, *Asteriscus graveolens*, and *Limoniastrum guyonianum*. Through a combination of quantitative phytochemical assays and in vitro biological evaluations, the study aimed to explore the pharmacological potential of these plants as sources of bioactive compounds. The results revealed substantial variations in both phytochemical content and biological performance among the studied species, confirming the value of scientific validation in the use of traditional medicinal plants.

Among the four species, *Limoniastrum guyonianum* and *Asteriscus graveolens* stood out for their rich phytochemical profiles and pronounced antioxidant and anti-inflammatory activities. These findings position them as strong candidates for the development of natural therapeutic agents. *L. guyonianum* demonstrated consistently strong activity across multiple assays, while *A. graveolens* showed potent effects, particularly at higher concentrations. Conversely, *Ephedra alata*, despite its low flavonoid and pigment content, displayed unexpectedly high antioxidant activity, suggesting the presence of alternative antioxidant mechanisms. *L. tingitana* presented modest bioactivity overall, except for its significant α-amylase inhibition.

These results not only provide scientific support for the pharmacological use of certain Algerian medicinal plants but also contribute to the broader effort of conserving and valorizing the country's rich endemic flora. The demonstrated bioactivities open the door to potential applications in natural health products, dietary supplements, and even the pharmaceutical and cosmetic industries. Such valorization promotes the sustainable use of local plant resources and supports the integration of traditional knowledge into modern therapeutic approaches.

Looking ahead, further studies should aim to isolate and characterize the active compounds responsible for the observed effects, assess their safety through in vivo testing, and explore their mechanisms of action. Developing extracts or formulations from the most promising species could enhance their commercial and therapeutic value. This research lays a solid foundation for future collaborations and for integrating Algerian phytodiversity into national and international strategies for natural product development.

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APPENDIX

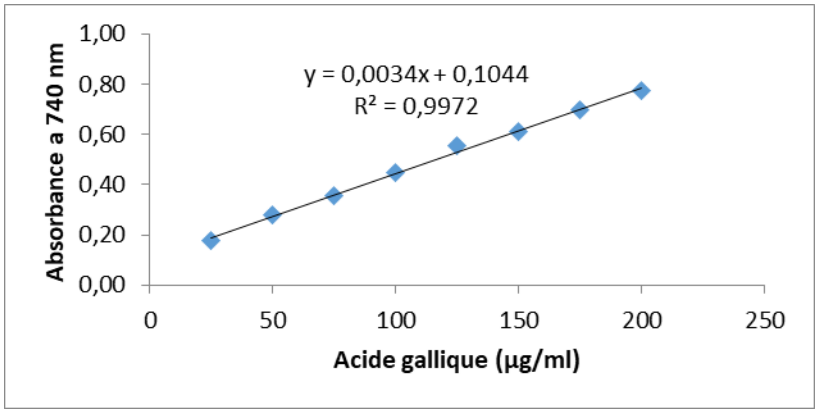

Figure 1: Calibration curve for the determination of total polyphenols (gallic acid as a standard) (Average of 3 tests)

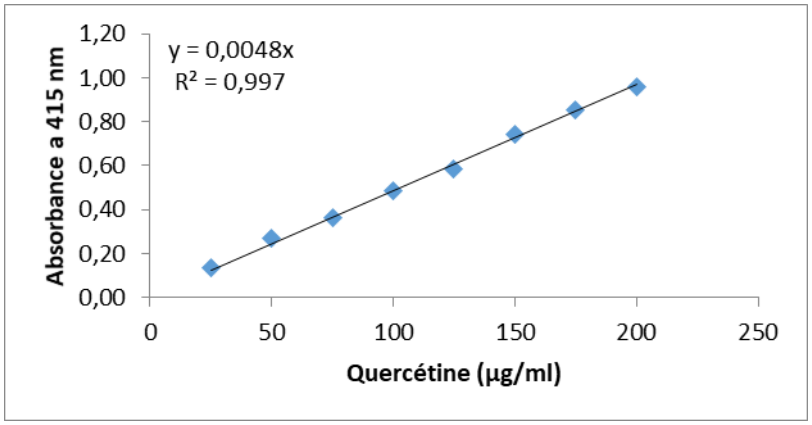

Figure 2: Calibration curve of total flavonoids assay (quercetin as a standard) (Average of 3 trials)

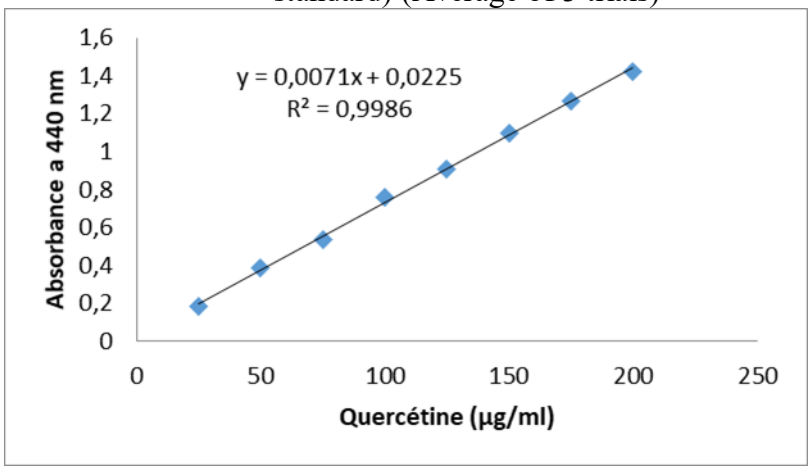

Figure 3: Calibration curve for flavonol content assay (quercetin as a standard) (Average of 3 tests)

Abstract

This study examines the phytochemical composition and biological properties of four *n*-butanol fractions from endemic Algerian medicinal plants: *Ephedra alata* and *Linaria tingitana* Boiss. & Reut, *Asteriscus graveolens*, and *Limoniastrum guyonianum*. Phytochemical analyses included total phenolic, flavonoid, flavonol, chlorophyll, and carotenoid contents. Biological assays assessed antioxidant activity using different methods: 2, 2-diphenyl-1-picrylhydrazyl (DPPH), 2,2′-casino-bis (3-ethylbenzothiazoline)-6-sulfonate (ABTS), O-phenanthroline (PHEN), and FRAP assay. The anti-inflammatory potential is estimated by measuring the denaturation of protein. Moreover, the anti-lipid peroxidation and anti-α-amylase activities were evaluated. Finally, antibacterial ability was assessed on Escherichia coli and Staphylococcus aureus.

The four plant extracts revealed high amounts of flavonoids, flavonols, and total phenolics. Furthermore, the results indicate that they were highly effective in scavenging DPPH and ABTS, with IC_{50} values ranging from 9.40±0.75 to 32.03±1.32 µg/mL for DPPH and from 1.69±0.14 to 15.32±0.40 µg/mL for ABTS. In the FRAP and PHEN assays, the antioxidant activity was important compared with the standard solution. In addition, the plants exhibit moderate anti-lipid peroxidation and anti-denaturation of protein potentials. However, using the alpha amylase test, the reaction of the four plants gave the best response, with a much lower IC_{50} value than that of acarbose. On the other hand, the results show an absence of antibacterial activity against *Escherichia coli* and *Staphylococcus aureus*.

Finally, the results obtained from this study demonstrate that the four extracts possess several biological activities, which justify their traditional use and suggest potential therapeutic applications.

Keywords: *Ephedra alata*, *Linaria tingitana* Boiss. & Reut, *Asteriscus graveolens*, *Limoniastrum guyonianum*, Phytochemistry, Biological activity.

Résumé

Cette étude examine la composition phytochimique et les propriétés biologiques de quatre fractions de *n*-butanol issues de plantes médicinales algériennes endémiques : *Ephedra alata* et *Linaria tingitana* Boiss. & Reut, *Asteriscus graveolens* et *Limoniastrum guyonianum*. Les analyses phytochimiques ont porté sur les teneurs totales en composés phénoliques, flavonoïdes, flavonols, chlorophylles et caroténoïdes. L'activité antioxydante a été évaluée par différentes méthodes : dosage du 2,2-diphényl-1-picrylhydrazyl (DPPH), du 2,2′-casino-bis(3-éthylbenzothiazoline)-6-sulfonate (ABTS), de l'O-phénanthroline (PHEN) et du FRAP. Le potentiel anti-inflammatoire est estimé par la mesure de la dénaturation des protéines. De plus, les activités anti-peroxydation lipidique et anti-α-amylase ont été évaluées. Enfin, le pouvoir antibactérien a été évalué sur *Escherichia coli* et *Staphylococcus aureus*. Les quatre extraits de plantes ont révélé des concentrations élevées de flavonoïdes, de flavonols et de composés phénoliques totaux. De plus, les résultats indiquent une grande efficacité pour piéger le DPPH et l'ABTS, avec des valeurs de IC_{50} comprises entre 9.40 ± 0.75 et 32.03 ± 1.32 µg/mL pour le DPPH et entre 1.69 ± 0.14 et 15.32 ± 0.40 µg/mL pour l'ABTS. Dans les dosages FRAP et PHEN, l'activité antioxydante était importante par rapport à la solution standard. De plus, les plantes présentent des potentiels modérés d'antiperoxydation lipidique et d'antidénaturation des protéines. Cependant, lors du test à l'alpha-amylase, la réaction des quatre plantes a donné la meilleure réponse, avec une IC_{50} bien inférieure à celle de l'acarbose. En revanche, les résultats montrent une absence d'activité antibactérienne contre *Escherichia coli* et *Staphylococcus aureus*. Enfin, les résultats obtenus dans le cadre de cette étude démontrent que les quatre extraits possèdent plusieurs activités biologiques, justifiant leur utilisation traditionnelle et suggérant des applications thérapeutiques potentielles.

Mots-clés : *Ephedra alata*, *Linaria tingitana* Boiss. & Reut, *Asteriscus graveolens*, *Limoniastrum guyonianum*, Phytochimie, Activité biologique.

الملخص

تبحث هذه الدراسة في التركيب الكيميائي النباتي والخصائص البيولوجية لأربعة أجزاء من الن-بيوتانول من نباتات طبية جزائرية متوطنة: *Ephedra alata* et *Linaria tingitana* Boiss. & Reut, *Asteriscus graveolens* و*Limoniastrum guyonianum*شملت التحاليل الكيميائية النباتية محتوى الفينول الكلي، والفلافونويد، والفلافونول، والكلوروفيل، والكاروتينويد. قيّمت الاختبارات البيولوجية النشاط المضاد للأكسدة باستخدام طرق مختلفة: 2، 2-ثنائي فينيل-1-بيكريل هيدرازيل(DPPH) ، و2،2′-كازينو-بيس (3-إيثيل بنزوثيازولين)-6-سلفونات(ABTS) ، و-Oفينانثرولين (PHEN)، واختبار FRAP قُدّرت القدرة المضادة للالتهابات عن طريق قياس تحلل البروتين. كما قُيّمت الأنشطة المضادة لأكسدة الدهون والمضادة لإنزيم ألفا-أميليز. أخيرًا، تم تقييم القدرة المضادة للبكتيريا على بكتيريا *الإشريكية القولونية والمكورات العنقودية الذهبية*. أظهرت المستخلصات النباتية الأربعة كميات عالية من الفلافونويدات والفلافونولات والفينولات الكلية. علاوة على ذلك، تشير النتائج إلى أنها كانت فعالة للغاية في إزالة DPPH وABTS، حيث تراوحت قيم IC_{50} من 0.75±9.40 إلى 1.32±32.03 ميكرومتر/مل لـDPPH ، ومن 0.14±1.69 إلى 0.40±15.32 ميكرومتر/مل لـ ABTS.في اختبارات FRAP وPHEN، كان النشاط المضاد للأكسدة مهمًا مقارنةً بالمحلول القياسي. بالإضافة إلى ذلك، أظهرت النباتات إمكانات معتدلة مضادة لأكسدة الدهون ومضادة لتغير طبيعة البروتين. ومع ذلك، باستخدام اختبار ألفا أميليز، أعطى تفاعل النباتات الأربعة أفضل استجابة، مع قيمة IC_{50} أقل بكثير من قيمة الأكاربوز. من ناحية أخرى، أظهرت النتائج غياب النشاط المضاد للبكتيريا ضد *الإشريكية القولونية والمكورات العنقودية الذهبية*.

وأخيرًا، أظهرت نتائج هذه الدراسة أن المستخلصات الأربعة تمتلك العديد من الأنشطة البيولوجية، مما يبرر استخدامها التقليدي ويشير إلى تطبيقات علاجية محتملة.

الكلمات المفتاحية *Ephedra alata* :، *Linaria tingitana* Boiss. & Reut، *Asteriscus graveolens*، *Limoniastrum guyonianum*، الكيمياء الحيوية، النشاط البيولوجي.

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Phytochemical content, biological properties of endemic Algerian plants

Thesis submitted for the Master's Degree in biochemistry

Abstract:

This study examines the phytochemical composition and biological properties of four *n*-butanol fractions from endemic Algerian medicinal plants: *Ephedra alata* and *Linaria tingitana* Boiss. & Reut, *Asteriscus graveolens*, and *Limoniastrum guyonianum*. Phytochemical analyses included total phenolic, flavonoid, flavonol, chlorophyll, and carotenoid contents. Biological assays assessed antioxidant activity using different methods: 2, 2-diphenyl-1-picrylhydrazyl (DPPH), 2,2′-casino-bis (3-ethylbenzothiazoline)-6-sulfonate (ABTS), O-phenanthroline (PHEN), and FRAP assay. The anti-inflammatory potential is estimated by measuring the denaturation of protein. Moreover, the anti-lipid peroxidation and anti-α-amylase activities were evaluated. Finally, antibacterial ability was assessed on Escherichia coli and Staphylococcus aureus.

The four plant extracts revealed high amounts of flavonoids, flavonols, and total phenolics. Furthermore, the results indicate that they were highly effective in scavenging DPPH and ABTS, with IC_{50} values ranging from 9.40±0.75 to 32.03±1.32 µg/mL for DPPH and from 1.69±0.14 to 15.32±0.40 µg/mL for ABTS. In the FRAP and PHEN assays, the antioxidant activity was important compared with the standard solution. In addition, the plants exhibit moderate anti-lipid peroxidation and anti-denaturation of protein potentials. However, using the alpha amylase test, the reaction of the four plants gave the best response, with a much lower IC_{50} value than that of acarbose. On the other hand, the results show an absence of antibacterial activity against *Escherichia coli* and *Staphylococcus aureus*.

Finally, the results obtained from this study demonstrate that the four extracts possess several biological activities, which justify their traditional use and suggest potential therapeutic applications.

Keywords: *Ephedra alata*, *Linaria tingitana* Boiss. & Reut, *Asteriscus graveolens*, *Limoniastrum guyonianum*, Phytochemistry, Biological activity.

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