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Master's thesis

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Specialty: Applied Biochemistry

THEME

Phytochemical screening and pharmaceutical effect of *Teucrium polium* L.

Presented by:

HANI Sabrine
OUAGHED Amna
GHERBI Brahim

Examining committee:

Presidente	**NADJI Nassima**	**M.A.A**	**El-Oued University**
Examinatrice	**OTMANI Hadjer**	**M.C.A**	**El-Oued University**
Supervisor	**MAHBOUB Nasma**	**M.C.A**	**El-Oued University**
Co-supervisor.	**CHERFI Inasse**		**El-Oued University**

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DEDICATION

In the name of Allah the Most Compassionate the Most Merciful.

I dedicate this memoir.

To my dear mother: Mrs. Bouchoul Saida To the source of tenderness. O candle that lit my path, O star that guided my steps, O you who gave me your life, O you who stayed up nights praying for me in secret and in public, O you who instilled in me the most beautiful values and meanings, my beloved mother... this work is the fruit of your good planting.

To my dear father: **Mr. Hani Larouci**. O you who have carried so much for me and bestowed upon me the most precious things you possess, this work is the least I can offer as an expression of my love and eternal gratitude to you.

To my very dear brother, the jewel of the family Abd elhamid, And

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DEDICATION

In the name of Allah the Most Compassionate the Most Merciful.

I dedicate this memoir

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To my dear mother: Hayet Said For his kindness, patience, constant presence and listening. And his unparalleled support during the most difficult times of my life. Today I have reached your home, my dear parents. I must do this, God bless you.

To my brothers: Anas and Adam I wish you a future filled with joy and happiness.

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I dedicate this humble work to all of you... as a token of love and gratitude.

All praise is due to Allah

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Abstract :

Phytochemical screening and pharmaceutical effect of *Teucrium polium L.*

Medicinal plants are among the most important natural resources that humans have used since ancient times to treat diseases and promote health, playing a pivotal role in traditional medicine across various cultures. Among these plants, our study focused on *Teucrium polium L.*, which is notable for its morphological diversity and multiple medicinal properties, including antibacterial, anti-inflammatory, antioxidant, wound healing, anti-diabetic, and anticancer activities. Chemical and chromatographic analyses have shown a high presence of therapeutically important compounds. Antioxidant assays such as DPPH and FRAP, along with anti-inflammatory tests, demonstrated significant efficacy of the plant extracts. The DPPH assay revealed an activity of 41.26 mg/mL for the aqueous extract of *Teucrium polium L.*, the FRAP assay showed 71.07 mg/mL, and the anti-inflammatory test recorded 28.10 µg/mL, indicating strong bioactivity. In this context, a novel natural cream was developed based on *Teucrium polium L.*, which exhibited high effectiveness in accelerating wound healing, reducing inflammation, combating infections, stimulating tissue regeneration, and enhancing collagen production in clinical and experimental trials—without any reported side effects—making it a safe and effective natural alternative to conventional topical treatment

Key words:

Teucrium polium L, antioxidant activity, anti-inflammatory activity, healing activity, stimulating collagen production.

Résumé :

Criblage phytochimique et effet pharmaceutique de Teucrium polium L

Les plantes médicinales représentent l'une des ressources naturelles les plus importantes utilisées par l'homme depuis l'Antiquité pour traiter les maladies et promouvoir la santé, jouant un rôle central dans la médecine traditionnelle à travers différentes cultures. Parmi ces plantes, notre étude s'est portée sur *Teucrium polium L.*, reconnu pour sa diversité morphologique et ses nombreuses propriétés médicinales, notamment ses activités antibactérienne, anti-inflammatoire, antioxydante, cicatrisante, antidiabétique et anticancéreuse. Les analyses chimiques et chromatographiques ont révélé une forte présence de composés à intérêt thérapeutique. Les tests d'activité antioxydante tels que le DPPH et le FRAP, ainsi que le test anti-inflammatoire, ont montré une efficacité significative des extraits de la plante : le test DPPH a donné une valeur de 41,26 mg/mL pour l'extrait aqueux, le FRAP 71,07 mg/mL, et le test anti-inflammatoire 28,10 µg/mL, indiquant une forte bioactivité. Dans ce contexte, une nouvelle crème naturelle à base de *Teucrium polium L.* a été développée, montrant une grande efficacité dans l'accélération de la cicatrisation des plaies, la réduction de l'inflammation, la lutte contre les infections, la stimulation de la régénération tissulaire et l'augmentation de la production de collagène lors des essais cliniques et expérimentaux – le tout sans effets secondaires rapportés – ce qui en fait une alternative naturelle, sûre et efficace aux traitements topiques conventionnels

Mots clés :

Teucrium polium L, Activité antioxydante, Activité anti-inflammatoire, Activité de guérison, Stimulation de la production de collagène.

ملخص

الفحص الكيميائي النباتي والتأثير الصيدلاني لـ.

Teucrium polium L

تُعد النباتات الطبية من أهم الموارد الطبيعية التي استخدمها الإنسان منذ القدم في علاج الأمراض وتعزيز الصحة، وقد أدّت دورًا محوريًا في الطب التقليدي عبر مختلف الثقافات. ومن بين هذه النباتات، ركزت دراستنا على *Teucrium polium L*. الذي يتميز بتنوعه المورفولوجي وخصائصه الطبية المتعددة، مثل النشاط المضاد للبكتيريا، والمضاد للالتهابات، والمضاد للأكسدة، والمُسَرِّع لشفاء الجروح، والمضاد لمرض السكري، والمضاد للسرطان.

أظهرت التحاليل الكيميائية والكروماتوغرافية وجودًا مرتفعًا لمركبات ذات أهمية علاجية. وقد أظهرت اختبارات النشاط المضاد للأكسدة مثل DPPH وFRAP، إلى جانب اختبار النشاط المضاد للالتهاب، فعالية كبيرة لمستخلصات النبات؛

حيث سجّلت نتائج اختبار DPPH قيمة 41.26 ملغ/مل للمستخلص المائي، بينما بلغت قيمة FRAP 71.07 ملغ/مل، وسجل اختبار الالتهاب قيمة 28.10 ميكروغرام/مل، ما يدل على نشاط بيولوجي قوي. وفي هذا السياق، تم تطوير كريم طبيعي جديد يعتمد على *Teucrium polium L*.، وأظهر فعالية عالية في تسريع شفاء الجروح، وتقليل الالتهابات، ومكافحة العدوى، وتحفيز تجديد الأنسجة، وتعزيز إنتاج الكولاجين في التجارب السريرية والمخبرية، دون تسجيل أي آثار جانبية، مما يجعله بديلاً طبيعيًا وآمنًا وفعالًا للعلاجات الموضعية التقليدية

الكلمات المفتاحية:

نبات الجعيدة (الخياطة), النشاط المضاد للأكسدة،النشاط المضاد للالتهابات، نشاط التئام الجروح، تحفيز إنتاجالكولاجين .

Summary

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Introduction

Algeria possesses an extremely rich and diverse flora, represented by aromatic and medicinal plants, most of which grow wild. The valorization of these plants remains a field of great importance for the country **(Amroune, 2018).**

Medicinal plants constitute valuable resources for the majority of rural and urban populations in Africa and represent the main means by which individuals treat themselves **(BADIAGA, 2011).**

Despite advances in pharmacology, the therapeutic use of medicinal plants remains widespread in some parts of the world, particularly in developing countries **(TABUTI et al., 2003).**

The history of phytotherapy is closely linked to that of humanity, as all cultures have always relied on the therapeutic value of plants for healing **(Clément, 2005)**.

Indeed, out of the 300,000 plant species recorded on the planet, more than 200,000 species found in the tropical countries of Africa have medicinal properties **(Millogo et al., 2005).**

The oldest written evidence of the use of medicinal plants for drug preparation was found on a Sumerian clay tablet from Nagpur, dating back approximately 5,000 years. It contained 12 recipes for the preparation of medicines referring to more than 250 different plants, some of which contain alkaloids such as poppy, henbane, and mandrake **(Kelly, 2009).**

The Chinese book on roots and herbs, *Pen T'Sao*, written by Emperor Shen Nung around 2500 BC, discusses 365 medications (dried parts of medicinal plants), many of which are still used today. These include: *Rhei rhizoma*, camphor, *Theae folium*, *Podophyllum*, great yellow gentian, ginseng, *Datura stramonium*, cinnamon bark, and ephedra **(Böttcher, 1965; Wiart, 2006).**

The Indian holy books, the *Vedas*, mention treatment using plants, which are abundant in that country. Many spice plants still in use today originate from India: nutmeg, pepper, clove, and others **(Tucakov, 1971).**

The use of medicinal plants is growing in most countries around the world. This use is mainly based on the idea that plants are a natural means of treatment to alleviate suffering and improve human health. **(Bouacheriene and Benrabia, 2017)**

A plant is considered medicinal when it is listed in the pharmacopeia and its use is exclusively medicinal. This means that it is recognized for its preventive or curative properties with regard to human or animal diseases.

Medicinal plants used in traditional medicine contain at least one part with medicinal properties. Their effect comes from their chemical compounds (primary or secondary metabolites) or from the synergy between the various compounds present **(Moreau, 2003).**

Medicinal plants are important for the research and development of drugs, not only when plant components are used directly as medicines, but also as raw materials for pharmaceutical synthesis or as models for pharmacologically active compounds **(Ameenah, 2006).**

The use of medicinal plants as a fundamental element of the traditional African healthcare system is perhaps the oldest and most diverse of all therapeutic systems.

In many rural areas of Africa, traditional healers who prescribe medicinal plants are the most accessible and affordable health resource for the local community—and sometimes the only existing therapy. Nevertheless, there is still a lack of comprehensive and up-to-date data on promising medicinal plants from the African continent **(Mahomoodally, 2013).**

As these remedies were discovered, humans greatly facilitated the migration of these medicinal plant species and sought to increase their diversity. The extent of these interactions and the many benefits that plants provide to humans illustrate why greater knowledge and conservation of medicinal plant species are essential

The use of plants in phytotherapy is very ancient and currently of public interest. According to the World Health Organization around 65% to 80% of the global population suffers from poverty and lacks access to modern medicine. Traditional medicine is therefore used to meet primary healthcare needs due to this lack of access **(MA et al., 1997).**

According to the WHO, traditional medicine is the sum of knowledge, skills, and practices based on the theories, beliefs, and experiences indigenous to different cultures, used in the maintenance of health, as well as in the prevention, diagnosis, improvement, or treatment of physical and mental illnesses. Traditional medicine that has been adopted by other populations (outside of its indigenous culture) is often referred to as complementary or alternative medicine (CAM) **(Mahomoodally, 2013)**.

The botanical world has greatly contributed to human health at a time when synthetic medicines were not available and medical procedures were not yet conceptualized. It is necessary to regulate these plants associated with traditional knowledge to promote human development and good health. Although synthetic medicines have gained great popularity over natural treatments due to their effectiveness, people are beginning to understand the benefits of traditional remedies **(Sarri D., 2002).**

Medicinal plants are now universally recognized as a foundation **.**There has been a major resurgence of interest in traditionally used medicinal plants, with a number of international and local initiatives actively exploring the botanical resources of Southern Africa with the goal of selecting indigenous plants for pharmacologically active compounds **(Gurib-Fakim et al., 2010; Rybicki et al., 2012).**

Traditional medical systems provide valuable insights into natural remedies. Medicinal plants, as important sources of new chemical compounds with potential therapeutic effects, play a major role in the discovery of new active ingredients. As one of the largest and most distinguished families of flowering plants, the Lamiaceae comprise 236 genera and between 6,900 and 7,200 species worldwide, exhibiting a wide range of biological activities and diverse phytochemicals **(Naghibi et al., 2005; Raja, 2012).**

Wounds remain a major issue in developing countries, often leading to serious complications and high treatment costs. Wound healing is a coordinated response to tissue injury. It is a dynamic physiological process that begins and is influenced by many factors, expressed through the stages of hemostasis, inflammation, proliferation, and remodeling. Wound care is often complex, time-consuming, and at times confusing and expensive. Herbal products are widely used as medicines for wound healing in many clinical cases due to their effectiveness, availability, non-toxicity, ease of use, minimal side effects, and patient compliance. Herbal honey has repeatedly been shown to have potential effects on wound healing. It has long been used for skin wounds and other medical conditions. Moreover, it can be used in non-sterile conditions due to its inherent antibacterial properties **(Medhi B et al., 2008; Robson V et al., 2009).**

Teucrium polium is a wild-flowering herbaceous plant, abundantly found in various regions such as Europe, North Africa, and Southwest Asia **(Nasri et al., 2013).**

It is a plant measuring 10 to 35 cm in height, perennial with robust stems, long woody bases, erect or ascending, branched, with branches covered in cottony hairs. The white flowers, rarely purplish,It blooms in Algeria from April to August, with peak flowering occurring in May and June, This timing corresponds to Algeria's Mediterranean and semi-desert climate, where the plant begins to grow after the spring rains and blooms as temperatures rise.

The leaves, white and cottony on both sides, are narrow or elongated-oval, scalloped in their upper part, and become rolled underneath along their edges. The inflorescences, white in appearance, are dense, globular or ovoid, and at least one centimeter in diameter. **(Bonnier and Douin, 1990)**

Traditionally, in the Mediterranean countries, T. polium has been used against various types of pathological conditions, such as gastrointestinal disorders, inflammations, diabetes and rheumatism **(Abdollahi et al., 2003; Tariq et al., 1989).** It is also used as an antibacterial, antiulcer, hypotensive, antispasmodic, anorexigenic

and antipyretic agent **(Autore et al., 1984; Suleiman et al., 1988; Gharaibeh et al., 1989).** This plant is abundantly found in Iran and names Kalporeh. In traditional Iranian medicine (TIM), its tea is used for treating many diseases such as abdominal pain, indigestion, common cold, and urogenital diseases **(Abdollahi et al., 2003)**., we mentioned many compounds mainly

belonging to terpenes, terpenoids and flavonoids for which, pharmacological activities such as antioxidant, anticancer anti-inflammatory, hypoglycemic, hepatoprotective, hypolipidemic antibacterial and antifungal properties were reported **(Bahramikia and Yazdanparast, 2012).**

It is known by various names:

- **In Arabic:** الخياطة، قطابة الجرح الجعيدة
- **In English:** Mountain germander
- **In French:** Pouliot de montagne, Germandrée tomenteuse, or Germandrée blancgrisâtre
- **In Italian:** Poliot, Camendrio di montagna, Timobianco, Polioprimo
- **In Latin:** *Teucrium polium L.* **(Bonnier, 1990)**

Chemical analyses of *Teucrium polium L*. reveal that its aerial parts (leaves, stems, and flowers) are rich in a large number of active compounds. Polyphenols are the most abundant, comprising 4% to 6% of the plant's content and responsible for most of its antioxidant activity. Flavonoids, such as apigenin, luteolin, rutin, cirsimaritin, salvigenin, eupatorin, and others, are next, comprising 2% to 4% and known for their role in fighting inflammation and free radicals. Phenylethanoid glycosides, such as verbascoside and poliumoside, comprise 0.5% to 1% and have promising biological effects, particularly in combating microbes

(Bahramikia & Yazdanparast, 2012) The plant also contains 0.3% to 0.7% iridoids, bitter compounds used to reduce inflammation and boost immunity. Plant sterols, such as β-sitosterol, are present at 0.2% to 0.5% and contribute to improved heart health and lower cholesterol levels. Essential oils represent 0.5% to 1.5% of the plant's dry weight and contain compounds such as monoterpenes, diterpenes, hydrocarbon sesquiterpenes, and oxygenated sesquiterpenes. In addition, the plant contains significant amounts of tannins, ranging from 3% to 7%, which are useful as astringents in treating diarrhea and some digestive infections. Saponins, at 1% to 2%, are also found, and are known for their antibacterial and immune-boosting effects. Finally, the presence of steroids was detected at a rate of 0.3% to 0.6%, which exhibit anti-inflammatory biological activities. **(Maadi kenza, 2019)**

Les huiles essentielles = essences ou huiles volatiles, sont des produits de composition généralement assez complexe renferment les principes volatils contenus dans les végétaux et plus ou moins modifiés au cours de la préparation. Pour extraire ces principes, il existe divers procédés. Deux seulement sont utilisables pour la préparation des essences officinales: - par distillation entrainer par la vapeur d'eau - par expression. Les H.E. sont généralement associées à la présence des structures histologiques spécialisées telles que les poils sécréteurs, les poches sécrétrices et les canaux sécréteurs, souvent localisées à proximité de la surface de la plante.

Ces substances ont une des fonctions importante dans le domaine écologique pour son rôle de protection contre les prédateurs (insecte et champignon), ou dans les interactions végétales (agents allélopatique), dans le domaine cosmétique comme arome et en médecine pour leurs effets thérapeutiques. **(Bruneton, 2009)**

Djelfa Province is one of the most prominent steppe ecological regions in Algeria. It is located in a transitional zone between the High Plateaus and the Sahara Desert, which gives it unique climatic and topographical characteristics that contribute to its rich plant diversity. The plant *Teucrium polium L*. , grows in several parts of the province such as Aïn Oussera, , at altitudes ranging between 728 and 1320 meters above sea level. These areas are characterized by a semi-arid climate, with hot, dry summers and cold winters.

The average annual rainfall ranges between 200 and 400 mm resulting in vegetation that is well-adapted to drought and strong winds. *Teucrium polium L*. is considered one of the most important medicinal plants in the region's traditional medicine. It is commonly used to treat digestive disorders, reduce blood sugar levels, and it also possesses anti-inflammatory and antimicrobial properties. Recent studies have shown that the plant contains flavonoids and terpenoids with significant biological activity, making the Djelfa region an ideal environment for researching this plant and utilizing it in natural pharmaceutical industries

(Miikaili et al., 2012)

Several studies have demonstrated certain pharmacological effects associated with the use of *Teucrium polium L.*, including antibacterial, anti-inflammatory, antiviral, anti-ulcerogenic, antinociceptive, antispasmodic, antidiabetic, hypolipidemic, antifungal, calcium antagonist, and cytotoxic effects **(Esmaeili & Yazdanparast, 2004).**

It is considered a relatively safe medicinal plant for external use in cancer and diabetes patients. This plant possesses anti-inflammatory, antioxidant, and wound-healing properties, making it a promising natural option for treating chronic wounds and burns in diabetic patients, who often experience delayed wound healing. Its active compounds, such as flavonoids and terpenes, help stimulate tissue regeneration and reduce local infections, making it suitable for cancer patients who suffer from skin sensitivity due to chemotherapy or radiation therapyTeucrium polium L. possesses several recognized medicinal and pharmacological properties:

Antidiabetic activity

Hepatoprotective activity

Antibacterial activity

Anticancer activity

Anti-inflammatory activity

Wound healing activity

Antioxidant activity **(Seifollah Bahramikia, 2020)**

The results of the study on the antibacterial efficacy of *Teucrium polium L*. (known as "Ja'ada") showed that the phenolic extracts of this plant possess effective capabilities in resisting several types of bacteria, particularly pathogenic bacteria that may be resistant to traditional antibiotics. The analysis revealed that phenolic compounds play a significant role in inhibiting bacterial growth, which enhances the potential of using this plant as a promising natural source in developing antibacterial drugs or products. It was also found that the effect of these extracts varies according to their concentration and extraction method, which opens the door for further research to enhance the understanding of the mechanism by which these compounds work and develop their use in medical and pharmaceutical applications **(Hammoudi et al., n.d).**

Despite the widespread traditional use of *Teucrium polium L*. in herbal medicine, especially in regions like Djelfa with unique climatic and environmental characteristics, its biological effectiveness, chemical composition, and potential for pharmaceutical application still require in-depth study and scientific comparison. To what extent can the medicinal and chemical properties of this plant be utilized as a promising natural source in therapeutic and pharmaceutical fields ?

Chaptre 01

Materials and Methods

I- Materials:.

Plant collection and identification

The plant *Teucrium polium L.* is sourced from the deserts and mountains of the Djelfa region in Algeria due to its high quality and abundance of active compounds Between 2° and 5° East and 33° and 35° North. The state covers an area of 32,280 km², with an elevation ranging from 150 meters in the south to 1,613 meters in the east. It has a dry Mediterranean climate, characterized by low rainfall in the summer and cooler, rainy winters.

. The plant material was rinsed and dried in the shade at room temperature to serve as a preferred substance in the comparative study for evaluating wound-healing potential. This region is known for its semi-arid climate and suitable soil, which contribute to the production of medicinal plants with high biological properties. Plant collectors rely on harvesting it during the flowering period to ensure the highest concentration of active constituents, after which it is marketed for use in traditional medicine and the preparation of herbal remedies.

According to **(Quézel & Santa, 1963)** the systematics of *Thymus capitatus* is as follows:

- **Ingdom**: Plantae
- **Phylum**: Spermatophytes
- **Class**: Angiosperms
- **Subclass**: Asteridae
- **Order**: Lamiales
- **Family**: Lamiaceae
- **Genus**: Teucrium
- **Species**: *Teucrium polium L*

Figure 1 Présentation of *Teucrium polium L*(**original photo**)

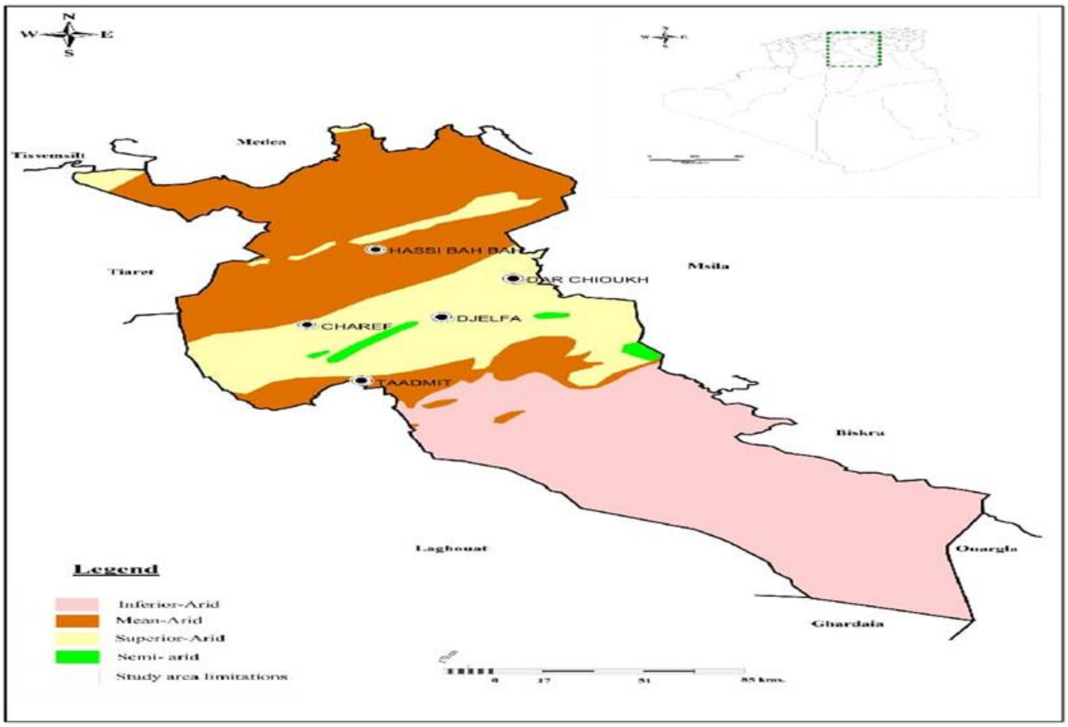

Figure 2 Study area of Djelfa province indicating the bioclimatic stages of the different municipalities **(Benziane et al., 2021)**

The most important Reagent :

The organic solvents used in the various compartments of this study are of analytical grade (methanol and ethanol), provided by the university laboratory.

DPPH, ascorbic acid, potassium ferricyanide, TCA, $FeCl_3$.

albumin, aluminum chloride ($AlCl_3$), and sodium phosphate (Na_3PO_4), as well as Folin–Ciocalteu reagent (FCR).

sodium chloride (NaCl), sodium...

Devices:

Centrifuge (Sigma 3K30)

Spectrophotometer (Perkin Elmer – UV/Vis Lambda 25)

Water bath (MEMMERT)

Oven (MEMMERT)

Precision balance (Explorer OHAUS)

Magnetic stirrer with heating plate (Kika Werk – RCT basic)

Vortex

Pharmaceutical Cream Preparation Protocol

Phase A: Oil Phase

-Salicylic Vaseline 10% (used at 5%): 4%

-Sweet almond oil: 6%

-Beeswax: 3%

-Triethanolamine: 1%

Phase B: Aqueous Phase

-Distilled water: 56%

-Glycerin: 21.5%

Phase C: Final Additives

-Vitamin E: 0.5%

-Preservative: 1%

- Herbal oils (*Teucrium polium L.*): 15%

Application of the Prepared Cream :

The prepared cream based on *Teucrium polium L*. extract was tested on a diverse group of volunteers, encompassing various age groups, including both males and females, young adults, middle-aged individuals, and elderly participants. The sample also included healthy individuals as well as patients with chronic diseases, particularly diabetes. All participants applied the cream according to the prescribed protocol, allowing for the evaluation of its efficacy and safety across different physiological conditions and health statuses.

Excel was used to create graphs and curves for the DPPH ,FRAP,Anti aflamatory date in the note.

II- Methods:

Healing Activity:

Significant advances in cellular and molecular biology in recent years have improved our understanding of the healing process and tissue regeneration. Healing is a natural biological phenomenon that leads to the filling of substance loss and the joining of wound edges. It is a complex, dynamic process that involves blood components, the extracellular matrix (ECM), parenchymal cells, and soluble mediators. The modes of healing depend on the type of tissue and the species involved. There are two healing processes: regeneration and repair **(Pavletic, 2010).** Regeneration allows for the replacement of lost cells or tissues with functional, nearly identical ones. Only tissues that retain a cell population capable of mitotic division can regenerate. For instance, bone can heal through regeneration

of regenerating. Regeneration is the main mode of healing in amphibians. Fetal skin wounds also have the unique ability to regenerate without scar formation, as fetal cells are still undifferentiated and retain the capacity to divide through mitosis. Despite the regenerative potential of certain tissues like the epidermis, most wounds in domestic mammals heal through a repair process that results in a more or less vascularized and fibrous scar connecting the wound edges. The final outcome is a scar that restores the skin's continuity and most of its functions prior to the trauma. Before healing is complete, many complex phenomena occur at clinical, cellular, and molecular levels. The study of the specific roles of different cells during healing began in 1950 and continues to this day.

Technological advances, particularly in molecular biology, have led to significant progress in our molecular and cellular understanding over the past 20 years **(Pavletic, 2010).**

Extract preparation

The aerial parts of Teucrium polium were dried in the shade at room temperature and then stored in paper bags to avoid potential fungal contamination. Afterward, the plant material was ground into a fine powder using an electric blender. The fine powder was then soaked in boiled distilled water for 20 minutes, with a Promagnitic placed in a beaker and the container covered with aluminum foil. It was left to cool for two hours. The resulting infusion was then filtered using a funnel and Whatman No. 1 filter paper. After filtration, the filtrate was placed in a large dish and dried in an étuve (drying oven) for 48 hours until completely dry.(Meguellati, Ouafi et al. 2019)

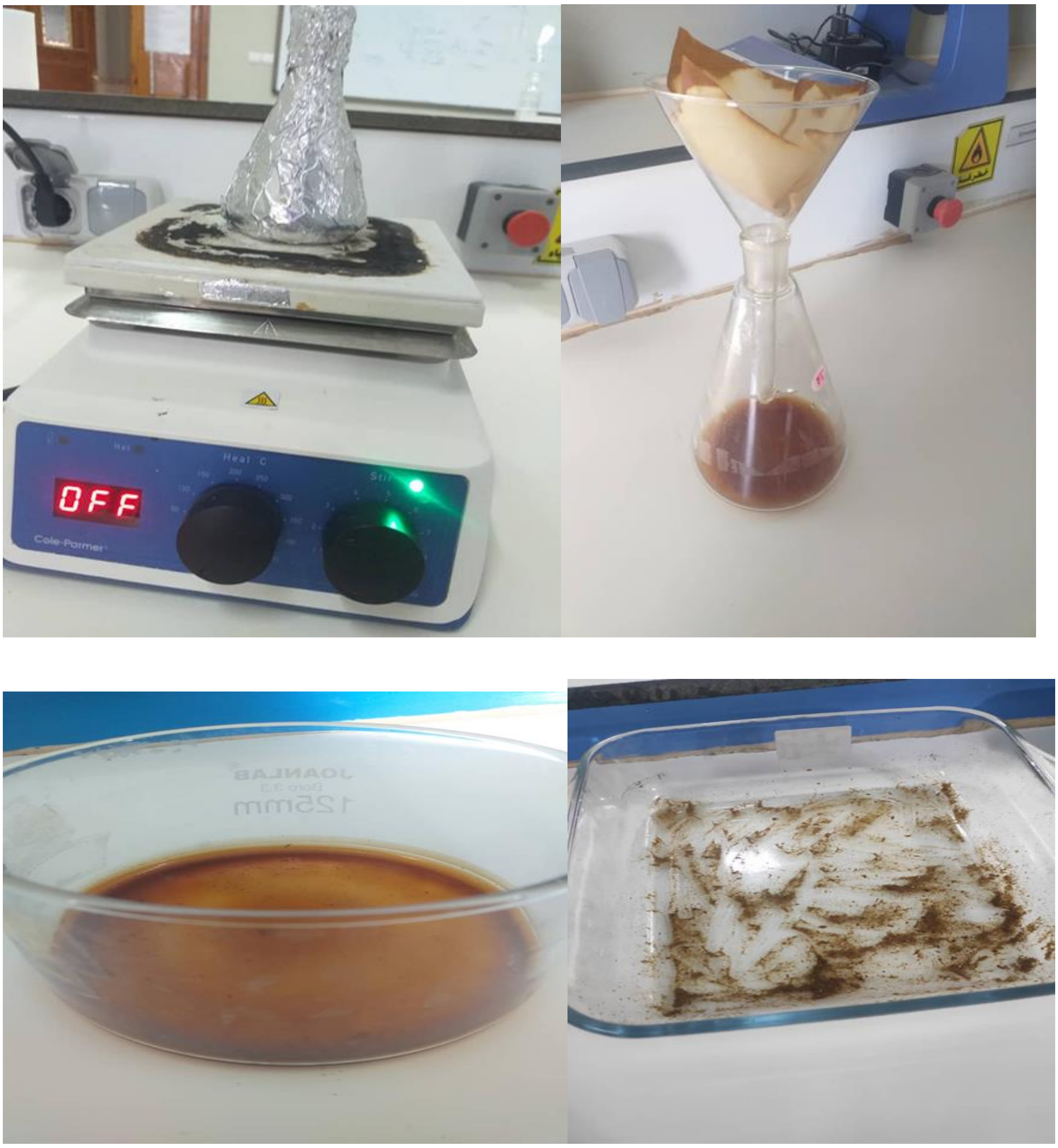

Figure 3 Preparing the mother solution(**original photo**)

Extraction of Essential Oils

The essential oils from the aerial parts of Teucrium polium were Clevenger extracted using the hydrodistillation technique with a modified Clevenger-type apparatus **(Jalal et al., 2015).**

This technique involves boiling, for 3 hours, 800 ml of distilled water and 200 g of the studied plant in a 2-liter flask. Under the action of heat, the plant cells burst and release the essential oils. These oils are then carried away by the steam produced. They pass through a water-cooled condenser where they are condensed and then collected in a container. The obtained essential oils were stored in small airtight vials in a refrigerator at 4–5 °C for chemical analysis and biological testing. .**(Yassine 2020)**

Prodution of 50 ml oil of Teucrium polium.

Figure 4Extraction of *Teucrium polium L*. plant oil(original photo)

Preparation Method:

1-Preparation of Phases A and B:

- Place the ingredients of Phase A in a heat-resistant container.
- Place the ingredients of Phase B in a separate container.
- Heat both containers in a bain-marie until the temperature reaches 70°C.

2-Emulsification:

- Gradually add the oil phase (A) to the aqueous phase (B) while stirring continuously using an appropriate mixer.

- Continue stirring until the temperature decreases to 40°C.

3-Addition of Final Additives (Phase C):

- Add the ingredients of Phase C to the mixture once it has cooled to 40°C.

- Mix thoroughly until a homogeneous cream is obtained.

4-Packaging and Storage:

- Fill the cream into appropriate sterile containers and store in a cool, dry place (**Ikram jaber**

)

Figure 5 healing cream based on -*Teucrium polium L.*-(original photo)

Evaluation of Biological Activity of *Teucrium polium* :

Antioxidant Capacity :

In this study, the in vitro antioxidant capacity of all Teucrium polium plant extracts was evaluated using DPPH, FRAP:

Antioxidant Activity using DPPH The DPPH test (2,2-diphenyl-1-picrylhydrazyl) is a widely used method for analyzing the antioxidant activity of various extracts due to its ability to generate stable free radicals and the simplicity of the analysis **(Benhammou et al., 2007)**

The presence of DPPH• free radicals in the solution results in a dark purple coloration at a wavelength of 515 to 520 nm Antioxidants reduce DPPH• (2,2-diphenyl-1-picrylhydrazyl), which causes the color to shift from purple to yellow **(Thomas, 2011).** Therefore, the intensity of the purple color is inversely proportional to the antioxidant capacity present in the medium.

FRAP Assay (Ferric Reducing Antioxidant Power)

The FRAP assay (Ferric Reducing Antioxidant Power) is a colorimetric test based on an electron transfer mechanism, used to evaluate the ability of antioxidants present in extracts to reduce ferric ions (Fe^{3+}) to ferrous ions (Fe^{2+}), resulting in the formation of a blue-colored complex that can be measured spectrophotometrically The significance of this assay lies in the role of ferric ions (Fe^{3+}) in promoting the formation of hydroxyl radicals through the Fenton reaction, highlighting the importance of antioxidants in inhibiting this process by preventing the transformation into more reactive compounds. **(Pellegrini et al., 2003).**

Anti-inflammatory Capacity :

Anti-inflammatory assays are among the most important biological evaluations used to study the ability of plant extracts or chemical compounds to reduce the inflammatory response. These assays rely on a range of in vitro and in vivo models. Anti-inflammatory activity is typically measured by comparing the results with a control group and another treated with a known anti-inflammatory drug, and the percentage of inhibition is calculated to assess efficacy. These models reflect the interactive dynamics of inflammatory systems, making them vital tools in the discovery and development of anti-inflammatory treatments. **(Olajide et al., 1999)**

Chaptre 02

Discussion

Discussion

1-Antioxidant Capacity

In this study, the in vitro antioxidant capacity of all *Teucrium polium* plant extracts was evaluated using DPPH, FRAP:

1-1-DPPH Assay

The DPPH assay was the most commonly used method to determine antioxidant scavenging activity .It was conducted with the aim of assessing the effectiveness of *Teucrium polium* as a natural source of bioactive compounds. **(Ait Chaouche et al., 2017).**

The study reveals a difference in the antioxidant effectiveness of the aqueous and methanolic extracts of Teucrium polium in the DPPH assay. The methanolic extract demonstrated significantly stronger activity(lowest IC50)., largely attributed to methanol's ability to extract higher quantities of phenolic and flavonoid compounds, known for their antioxidant properties. Conversely, the aqueous extract may be less effective in extracting these compounds, indicating that solvent selection plays a crucial role in determining the type and amount of active compounds extracted and, consequently, the biological activity of the extract. **. (El Atki et al., 2020).**

Antioxidant activity is stronger when using essential oils because they contain non-polar volatile compounds like terpenes, which are known for their high effectiveness as antioxidants. In contrast, methanol extracts polar compounds like flavonoids, but they are often less effective compared to concentrated oils.

When discussing the antioxidant activity results of *Teucrium polium* using the DPPH assay, the superiority of using the whole plant in achieving an inhibition rate exceeding 75% is evident. This rate surpasses the values recorded when testing the separated plant parts, where the study **(Sharifi-Rad, Pohl et al., 2022)** showed that the aerial parts during the vegetative stage recorded inhibition rates ranging between 65% and 70%, while the roots were less active at approximately 50%. This notable variation is attributed to the potential synergistic effect among the various chemical classes present in the whole plant, including phenolics, flavonoids, alkaloids, and saponins. The combination of these compounds from the aerial parts and roots may significantly enhance the overall free radical scavenging ability. Furthermore, differences in growth stages and the diversity of the chemical composition between the different plant parts can contribute to this variation in activity. Consequently, these results suggest that using the whole plant of *T. polium L.* represents a promising strategy for maximizing its antioxidant properties, underscoring the importance of studying extracts derived from the entire plant in the context of the search for compounds with high biological efficacy.

The discussion of the DPPH assay results from this study (IC50 = 41.26) compared with the data from (**Goulas et al.2012)** using the β-carotene bleaching assay (IC50 = 25.8) reveals a discrepancy in the assessment of the antioxidant activity of the *Teucrium polium* extract. This difference reflects the distinct mechanisms upon which each technique relies to evaluate the compounds' ability to resist oxidation. While DPPH measures the extract's capacity to reduce a free radical, β-carotene bleaching assesses its ability to protect an unsaturated molecule from oxidation. This variation suggests that the extract's effectiveness may differ depending on the antioxidant mechanism and the reaction environment.

When discussing the DPPH assay results for the methanolic extract of *Teucrium polium* in this study (IC50 = 41.26 µg/ml) and comparing them to the IC50 value for BHT (22 ± 0.1 µg/ml) as reported in the study by **(Sharififar et al., 2009)**, a clear discrepancy in antioxidant effectiveness is evident. The higher IC50 value of the extract indicates that a greater concentration is required to achieve the same level of DPPH radical scavenging compared to BHT. This difference reflects the fact that BHT, a pure synthetic antioxidant, may possess a more efficient mechanism of action or an optimized molecular structure for reducing DPPH radicals compared to the complex mixture of compounds present in the methanolic extract. Nevertheless, this does not diminish the potential of the extract as a natural source of antioxidants, but it highlights that its effectiveness in this specific assay is lower than that of pure BHT

In this study, it was indicated that the hydroalcoholic extract of *Teucrium polium L.* of Iranian origin exhibited significant antioxidant activity **(Tungmunnithum et al.,2018)**, with a reported DPPH IC_{50} value of 20.1 ± 1.7 µg/mL, reflecting a strong free radical scavenging ability. In comparison, our results using the DPPH assay on the aqueous extract of the same plant showed an IC_{50} value of 41.26 µg/mL with a determination coefficient (R^2) of 0.95, indicating good antioxidant activity but lower than that reported for the Iranian species. Additionally, the positive control (ascorbic acid) recorded a superior IC_{50} value of 6.91 µg/mL, highlighting the difference in efficacy between the standard and the plant extract. This variation in antioxidant potential can be attributed to several factors, primarily the geographic origin of the plant (Algeria vs. Iran), which significantly influences the content and composition of secondary metabolites such as flavonoids and phenolic compounds. Furthermore, the study emphasized the role of flavonoids in combating oxidative stress through various mechanisms, including the stimulation of antioxidant enzymes and inhibition of nitric oxide-induced oxidative stress, which could also explain the differences in activity based on flavonoid content both studies confirm that *Teucrium polium L*. .

possesses real and exploitable antioxidant capacities, with a clear influence of environmental and geographical factors on its biological activity, thus encouraging further research to enhance the extraction of active compounds and improve the plant extract's bioefficacy.

In this study, it was emphasized that the antioxidant mechanism of flavonoids mainly relies on free radical scavenging, where polyphenol groups interact with and terminate the free radical chain reaction. Structural features critical for effective radical scavenging include the presence of an ortho-dihydroxy structure in the B ring, a 2,3-double bond conjugated with a 4-keto group enabling electron transfer from the B ring, and hydroxyl groups at positions 3 and 5 forming hydrogen bonds with the keto groups, as described in Compared to our results in the DPPH assay for the *Teucrium polium L*. aqueous extract, which recorded an IC_{50} value of 41.26 µg/mL with a determination coefficient (R^2) of 0.95, the extract exhibited good free radical scavenging activity, although it was less potent than the standard ascorbic acid (IC_{50} = 6.91 µg/mL) **(Croft, 1998)**

.These findings support the hypothesis that the antioxidant capacity of *Teucrium polium L*argely depends on its flavonoid content and the distribution of active functional groups within its molecular structure. Thus, the diversity and specific configuration of phenolic compounds may explain the variability in antioxidant activity observed across different samples .

In summary, this comparison confirms that the *Teucrium polium L*. extract possesses genuine free radical scavenging activity, but its efficacy is directly linked to its specific phenolic composition, highlighting the need for further studies to identify and enhance the bioactive compounds responsible for its antioxidant properties.

The antioxidant activity of phenolic and flavonoid compounds is significantly influenced by the arrangement of functional groups, the type of substitution, the stereochemistry, and the number of hydroxyl groups, which influence their ability to chelate metals and scavenge free radicals **(Heim et al., 2002)**Comparing the results of the DPPH test for the *Teucrium polium L*. extract, which showed an IC_{50} = 41.26 µg/ml and a correlation coefficient R^2 = 0.95, it is clear that the extract has good antioxidant activity, but the reference substance (ascorbic acid IC_{50} = 6.91 µg/ml) is better. This is due to the fact that the phenolic and flavonoid compositions in the extract do not have the ideal structure that enhances activity, such as the presence of an ortho-dihydroxy structure or suitable double bonds. Thus, the effectiveness of the Teucrium polium extract is real, but it clearly depends on the molecular structure of the active compounds.

Scientific research indicates that the antioxidant activity of flavonoids and phenols is significantly influenced by the structural composition of the compounds **(Njoya, 2021)**, including the arrangement of functional groups, the number of hydroxyl groups, and the nature of the chemical substitutions, which determine their ability to chelate metal ions and scavenge free radicals **(Rahman et al., 2015).** Comparing these concepts with the results of the DPPH assay for the *Teucrium polium L*. extract, which recorded an IC_{50} value of 41.26 µg/mL and an R^2 of 0.95, reveals that the extract possesses good antioxidant activity. The effectiveness of the extract is likely influenced by the composition of the phenols and flavonoids present, which may not possess the ideal structures necessary to promote free radical scavenging, such as the presence of ortho-dihydroxy groups or active double bonds. Thus, the effectiveness of the Teucrium polium extract demonstrates that chemical composition plays a fundamental role in determining the strength of natural antioxidants.

The results indicate that the aerial parts extract of T. polium collected during the flowering stage exhibited the highest antioxidant activity in the DPPH test, reaching 83%, while the root extract showed the lowest activity at 26%. These findings align with previous studies highlighting variations in antioxidant activity among different plant parts and phenological stages. On the other hand, the data analysis in the image reveals strong linear relationships between concentration and inhibition (R^2 = 0.93 and 0.95), with IC50 values of 6.91 and 41.26 mg/ml for the extract and the reference standard (ascorbic acid), respectively, confirming the extract's efficacy compared to the control. These results support the hypothesis that the phenological stage and plant part play a crucial role in determining antioxidant effectiveness

The antioxidant activities and free radical scavenging capacities of several medicinal plants have been studied. Different parts of *Tabebuia pallida* cultivated in Bangladesh demonstrated high efficacy **(Rahman et al., 2015).** Additionally, saponin extracts from *Dianthus basuticus* showed antioxidant and inhibitory effects on enzymes associated with type 2 diabetes **(Nafiu & Ashafa, 2017).** In other studies, the total phenolic and flavonoid contents, along with the antioxidant potential, were analyzed in wild vegetables from western Nepal **(Aryal et al., 2019).** Similarly, the leaf extract of *Erythroxylum cuneatum* exhibited antioxidant and anti-inflammatory properties **(Li et al., 2020)**. Finally, it was observed that the fruits of medlar (*Mespilus germanica*) contain phenolic acids with strong radical scavenging activity at different stages of ripening **(Gruz et al., 2011).**

1-2- Frap Assay:

FRAP was evaluated for electron transfer, based on the ability of the testes to reduce ferrous iron (Fe^{+3}) from the yellow color of ferrous iron (Fe^{+2}). Here, we return.

In this recent study, the antioxidant activity of *Teucrium polium L*. It was determined that the essential oils of *T. polium* subsp. *aureum* possess a higher antioxidant power than *T. polium* subsp. *polium*, in both the DPPH radical scavenging and FRAP assays, as reported by **(El Atki et al., 2020)**When comparing these results to an aqueous extract of *Teucrium polium* in a FRAP assay conducted by El **(El Atki et al., 2020)**, which yielded an IC50 value of 71.07 µg/mL (R^2 = 0.96), indicating moderate antioxidant activity, it becomes evident that the methanolic extract was more effective (lower IC50) compared to the aqueous one, consistent with the higher IC50 value in your test. In a subsequent study by **(El Atki et al., 2020),** the essential oils of the *T. polium* subsp. *aureum* strain exhibited higher antioxidant capacity than the *polium* strain in FRAP and DPPH assays, with a total capacity of 3308.27 mg ascorbic acid equivalent/g of oil, demonstrating a strong effect attributed to components such as tocopherols and phenolic compounds. In comparison, your aqueous extract shows antioxidant activity but is less potent than the essential oils and organic extracts, underscoring that the type of extract and the plant subspecies significantly influence the antioxidant efficacy.

The study conducted by **(Boghrati et al., 2016)**demonstrated that *Teucrium polium L.* possesses high antioxidant efficacy, with an IC_{50} value of 5.90 ± 0.12 µg/mL in the FRAP assay, while the FRAP value for some isolated compounds such as poliumoside reached 14.32 mmol/g, reflecting strong reducing activity compared to other plants of the same genus. In contrast, my test results showed that the *T. polium* extract exhibited lower efficacy in the FRAP assay, recording an IC_{50} = 71.07 µg/mL with an R^2 = 0.96, indicating moderate activity compared to the aforementioned studies. This discrepancy can be attributed to several factors, including the difference in the type of extract (alcoholic vs. aqueous), the plant's source, the phenological stage, or the concentration of active compounds such as phenolics and flavonoids, which were high in the samples tested in previous studies. The study also indicates that specific compounds like poliumoside possess high antioxidant activity, which was not identified in my extract. This comparison confirms that *Teucrium polium* has high potential as an antioxidant, but its efficacy varies depending on the type of extract and its chemical composition, highlighting the importance of analyzing the active components and selecting appropriate extraction methods to enhance biological performance.

The reducing power of the studied extracts was assessed using the FRAP assay. Reducing power is generally associated with the presence of antioxidants that exert their effect by breaking free radical chains through the donation of hydrogen atoms **(Brantner, 2010).** Therefore, the reducing power assay is often used to evaluate the ability of extracts to reduce Fe3+ to Fe2+, and this ability is compared to that of quercetin. Comparing the study results with our findings indicates moderate antioxidant activity. When compared to a FRAP study that reported the methanolic extract of *T. polium* subsp. *aureum* showed the highest reducing power (EC50 = 0.193 ± 0.006 mg/mL), it is evident that the extract from our experiment possesses better reducing power, despite being aqueous rather than methanolic. On the other hand, the ethyl acetate extract from the same subspecies showed the least effectiveness (EC50 = 3.947 mg/mL), which reinforces the hypothesis that the type of solvent plays a significant role in the extraction of active compounds. However, the superiority of the aqueous extract of *T. polium* (without subspecies specification) might be attributed to differences in environmental conditions or phytochemical content. Compared to quercetin (EC50 = 0.033 mg/mL), our extract shows less potent reducing power, but it remains promising as a natural source of antioxidants.

The study by **(Sadeghi et al., 2015)** demonstrated the effectiveness of methanol in extracting antioxidants using the ABTS assay, where the methanolic extract was the most potent (IC50 = 2.79 mg/L), outperforming other extracts such as aqueous (which ranked after ethanolic). Your aqueous extract showed an IC50 value of 71.07 µg/mL (0.07107 mg/mL) with a high correlation coefficient (R^2 = 0.96), indicating moderate antioxidant activity. The ethereal and chloroform extracts in Sadeghi et al.'s study exhibited lower performance than the synthetic antioxidant Trolox (IC50 = 0.82 mg/L). These results highlight the crucial role of the solvent type in extracting antioxidant compounds, with methanol's ability to extract larger quantities of phenolic compounds explaining its high efficacy compared to water. Although your aqueous extract showed acceptable activity, it does not reach the efficiency of organic extracts like methanol, which aligns with the literature indicating a direct relationship between phenolic content and antioxidant capacity. Recent studies indicate a strong linear relationship between total phenolic content and antioxidant activity, where phenolics can be considered a crucial primary indicator for evaluating the effectiveness of plant extracts as natural antioxidants, as pointed out by **(Viuda-Martos et al., 2011)** and others. This is attributed to the chemical structure of phenolics, which includes aromatic rings and hydroxyl groups capable of reacting with free radicals and donating electrons or hydrogen atoms, thus conferring them with an effective ability to inhibit oxidation.

Comparing this to the FRAP assay results for your aqueous extract of *Teucrium polium*, which showed an IC50 value of 71.07 µg/mL and a good correlation coefficient $R^2 = 0.96$, in addition to an inhibition percentage of 70%, we observe moderate antioxidant activity. This result supports the hypothesis that antioxidant efficacy largely depends on the phenolic content. Given that the extract is aqueous, it likely has a lower phenolic content compared to organic extracts, which explains the moderate activity. Consequently, these findings underscore the importance of measuring total phenolics as a fundamental factor in interpreting and predicting antioxidant activity.

The study indicates that the *Teucrium polium* extract exhibited a high reducing power in the FRAP assay, with a value of 6.41 µg TEAC/g of extract, demonstrating strong antioxidant activity. The study attributes this effectiveness to the high content of phenolic and flavonoid compounds in the extract, which are known for their potent ability to inhibit and neutralize free radicals **(Kallassy et al., 2017).**

In comparison to your FRAP assay results, which showed an IC50 = 71.07 µg/mL, an inhibition rate of 70%, and a good correlation coefficient $R^2 = 0.90$, your aqueous extract demonstrated good antioxidant activity but not as high as reported in the study. This difference could be attributed to the type of extract (aqueous versus methanolic or ethanolic), as organic extracts often extract a larger quantity of phenolic compounds compared to water, which is reflected in the antioxidant effectiveness.

This comparison supports the hypothesis that the concentration of phenolics and flavonoids is the crucial factor in determining the antioxidant potency of plant extracts. Furthermore, your results still reflect a considerable ability to inhibit oxidation, making them of biological and applicative significance.

The provided study indicates the use of the FRAP assay to evaluate the ability of plant extracts to reduce ferric ions (Fe^{3+}) to ferrous ions (Fe^{2+}), using the TPTZ reagent. Antioxidant activity was determined by measuring absorbance at 593 nm after 8 minutes of reaction, and the result is calculated in µg of Trolox equivalents per gram of extract. This method directly measures the sample's ability to donate electrons, thus reflecting the reducing power of the antioxidant compounds **(Ahmadi et al., 2011).**

In comparison to your FRAP assay results on the aqueous *Teucrium polium* extract, which showed an IC50 = 71.07 µg/mL with an inhibition rate of 70% and a good correlation coefficient R^2 = 0.96, the extract demonstrated moderate antioxidant activity. Despite the difference in measurement units between the two studies (Trolox equivalents versus IC50), both methods measure the same principle: the ability of active compounds to reduce iron. This suggests that your aqueous extract possesses a considerable capacity to react with free radicals, although it is less potent than highly active extracts or extracts using organic solvents. This activity is likely due to the presence of phenolic or flavonoid compounds, even if they are in lower concentrations.

2- The Anti-inflammatory Activity of *Teucrium polium*:

Regarding the anti-inflammatory activity of the aerial parts of Teucrium polium, its pronounced activity, demonstrated by an estimated 97.53% inhibition of BSA degradation at 2 mg/ml, is primarily due to its richness in flavonoids**.(Iloki-Assanga, Lewis-Luján et al. 2015)**It should be noted that water/ethanol is an effective solvent for extracting phytochemicals from the plant because it combines polar and medium polar properties. However, the efficacy of the decanophenol extract is significantly better, achieving 80% inhibition at only 120 µg/mL, while the water/ethanol extract required 2,000 µg/mL to achieve 97.5%. This is because extraction methods using organic solvents such as decanophenol allow for the concentration of active compounds with high anti-inflammatory efficacy, compared to water/ethanol extraction, which extracts less potent compounds or at weaker concentrations

The anti-inflammatory effects of TP extract have a clear ability to inhibit inflammation via two main mechanisms: inhibiting histamine signaling in the first phase, and increasing the expression of the anti-inflammatory IL-10 in the second phase.**(Rajput, Zehra et al. 2021)**

This study confirms that the results of our biochemical study indirectly support this mechanism through the observation of proven biological activity.

The high stimulation of IL-10 and the marked inhibition of inflammatory factors are consistent with achieving high inhibition rates in our biological tests.

T. polium extract has remarkable antioxidant activity in the body, and strong antioxidant properties thanks to its phenols and flavonoids. The hydroalcoholic extract of T. polium has the highest antioxidant and, consequently, antimicrobial properties**, (Fazeli-Nasab, Rahnama et al. 2017)**The results of *Teucrium polium L*. extract using the diceno-phenol solvent showed a more effective anti-inflammatory effect than the alcoholic extract, achieving 80% inhibition at a low concentration (120 µg/mL) with an IC_{50} value of 28.10 µg/mL, demonstrating the strength and high efficiency of the extract compared to the hydroalcoholic extracts used in other studies. Although the hydroalcoholic extracts have proven to be potent antioxidants, the anti-inflammatory efficacy of the diceno-phenol extract was more pronounced due to its higher concentration of less polar bioactive compounds. These results indicate that the extraction method and solvent type play a crucial role in determining the biological efficacy of plant extracts

The results of a clinical study on the effect of Teucrium polium extract indicate that the hydroalcoholic extract can reduce blood levels of inflammatory factors such as IL-6, CRP, fibrinogen, and TNF-α, demonstrating its effectiveness in inhibiting inflammatory pathways associated . These results demonstrate that T. polium extract , also modulates systemic inflammatory biomarkers **(Bell and Bloomer 2010)**. Comparing the results of our study with a clinical study examining the effect of Teucrium polium extract on inflammatory biomarkers such as IL-6, CRP, and TNF-α, our study demonstrated a high and precise anti-inflammatory efficacy, demonstrated by specific quantitative values such as IC_{50} (28.10 µg/ml), achieving 80% inhibition at a concentration of 120 µg/ml, with a strong correlation coefficient ($R^2 = 0.95$), reflecting the precise and clear relationship between concentration and biological activity. In contrast, the clinical study provided strong evidence of the extract's real-world efficacy in vivo by reducing levels of inflammatory factors associated with chronic diseases, giving it significant clinical and practical relevance. In summary, our study demonstrated the efficacy of the extract in a scientifically rigorous laboratory setting, while the clinical study supported this efficacy in a real biological setting, indicating the complementarity of the results and enhancing the therapeutic value of T. polium extract as a promising anti-inflammatory.

Regarding the anti-inflammatory activity of the aerial parts of Teucrium polium, its activity is clearly demonstrated by its ability to inhibit the degradation of phosphoric acid (BSA) **(Iloki-Assanga et al., 2015).** The study demonstrated that the aerial extract of Teucrium polium exhibits strong anti-inflammatory activity (97.53% inhibition of BSA degradation at 2 mg/ml), primarily due to its rich flavonoid content. Water/ethanol proved to be an effective solvent for extracting these bioactive compounds.

These results are qualitatively consistent with the anti-inflammatory test results in our study, where extract (B) showed higher efficacy (IC_{50} = 11.44 µg/ml) compared to the reference extract (A) (IC_{50} = 28.10 µg/ml). The linear regression equations (Y=0.4233X+45.1548 for the extract versus Y=0.3787X+39.13 for the reference source) and high R^2 values (0.95-0.96) indicate a strong concentration-response relationship. reflects differences in bioanalytical models (protein denaturation versus other assays), but collectively confirms that the bioactivity of the extract depends on the flavonoid concentration and the analytical method used. This consistency across different experimental approaches underscores the potent anti-inflammatory properties of *T. polium*, which are mediated by its flavonoid components.

The anti-inflammatory effect of the extract appears to be due to the presence of flavonoids and phenolic compounds in the plant. Sericilol has been reported to have a potent and selective inhibition of 5-lipoxygenase, further supported by computational studies**. (Amraei et al., 2018; Kohno et al., 1985)** Scientific evidence suggests that the anti-inflammatory effect of Teucrium polium extract is primarily due to its content of flavonoids and phenolic compounds, which inhibit inflammatory pathways through multiple mechanisms. These findings gain further credibility from computational studies that confirmed the ability of sericilol (a plant component) to selectively inhibit 5-lipoxygenase (5-LOX), a key enzyme in the formation of inflammatory leukotrienes.

Comparing these results with the accompanying anti-inflammatory assay data, we note a concordance in the combination effect in which ourcontrol (a) showed a higher efficacy (IC_{50} = 11.44µg/ml) compared to the extract (b) (IC_{50} = 28.10 µg/ml), supporting the hypothesis that phenolic compounds (e.g., flavonoids) are responsible for this activity. The inhibition mechanism, as indicated by linear regression equations (inhibition efficiency ~96% for the extract), indicates a concentration-dependent inhibition, similar to the effect of sericilol on 5-LOX in computational studies. The high values of the coefficient of determination ($R^2 \geq 0.95$) in our assay reflect the reliability of the results and are consistent with the accuracy demonstrated by computational studies. This comparison confirms that the anti-inflammatory activity of the extract results from a synergistic interaction between its phenolic compounds, with the possible contribution of enzyme inhibitors (similar to sericilol) to this mechanism. Computational studies also play a key role in explaining the molecular mechanisms underlying laboratory findings

Conclusion

This work aims to conduct a comprehensive evaluation of the plant *Teucrium polium L.*, known locally as "Kh'yata" or "Ja'ada," a medicinal plant endemic to regions of Algeria that enjoys widespread traditional use for treating various health conditions. This study focused on the phytochemical and pharmacobiological assessment of this plant, with the primary objective of identifying the active constituents responsible for its therapeutic properties and evaluating its pharmacological potential.

In the phytochemical aspect, qualitative analyses revealed that extracts of *Teucrium polium L.* contain significant concentrations of phenolic and flavonoid compounds, two classes of plant secondary metabolites known for their important biological properties, particularly antioxidant and anti-inflammatory activities. Quantitative studies showed high levels of:

- **Total Phenolics:** Their levels were measured using the Folin-Ciocalteu reagent, and the methanol-water extract exhibited high proportions reflecting its significant capacity to scavenge free radicals and thus contribute to protection against oxidative stress.
- **Total Flavonoids:** Their concentrations were determined using the aluminum chloride ($AlCl_3$) assay, and considerable levels were observed, potentially explaining the plant's anti-inflammatory effects.
- **Flavonols:** A subclass of flavonoids known for their protective role at the cellular and tissue levels through various mechanisms, their presence was reported at a notable percentage in the extract under study.

In the experimental phase of this study, a series of *in vitro* and *in vivo* tests were conducted to evaluate the biological activities of the methanol-water extract:

- **Antioxidant Activity:** Evaluated using two established assays, DPPH (2,2-diphenyl-1-picrylhydrazyl) and FRAP (Ferric Reducing Antioxidant Power). The results demonstrated a remarkable ability of the extract to inhibit free radicals, indicating its effectiveness in mitigating harmful oxidative reactions, in addition to its capacity to restore iron-reducing power, thereby enhancing its efficacy in combating oxidative stress.
- **Anti-inflammatory Activity:** Tested using the human serum albumin inhibition assay *in vitro*, which serves as a model for assessing the ability to inhibit protein denaturation associated with inflammation. The results showed that the extract possesses a potent capacity to reduce potential inflammatory responses.

- **Topical Efficacy in Wound Healing:** The extract was tested topically on a group of individuals with wounds and burns of varying degrees in a field study. The experiment showed promising positive results characterized by:
    - A significant reduction in the duration and intensity of the inflammatory phase associated with the injury.
    - A notable acceleration in the rate of wound contraction, an indicator of rapid tissue healing.
    - A substantial shortening of the time required for the regeneration of the damaged skin layer (epithelialization), reflecting an accelerated complete healing process.

Based on these encouraging results, a preliminary formulation of a topical ointment (pomade) based on the *Teucrium polium L.* extract was developed. This preparation demonstrated promising efficacy in initial field trials, reflecting the real potential for transforming this traditional plant into a safe and effective natural product for treating wounds and burns.

This development represents a significant step towards providing an effective and safe therapeutic alternative, capable of contributing significantly to improving the treatment of skin injuries, especially in areas that may lack access to specialized healthcare.

In light of the above, the results of this study unequivocally confirm that the plant *Teucrium polium L.* is an important and rich source of biologically active compounds with significant pharmacological value.

It exhibits potent antioxidant and anti-inflammatory properties, as well as a remarkable effectiveness in accelerating the healing process of wounds and burns. These findings strongly support the traditional uses of this plant in folk medicine and scientifically justify the efforts to develop topical therapeutic preparations based on it, thus opening promising avenues for the innovation of natural and effective solutions in the field of wound care and the treatment of damaged skin.

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- ombinées avec certains antibiotiques ou molécules volatiles."

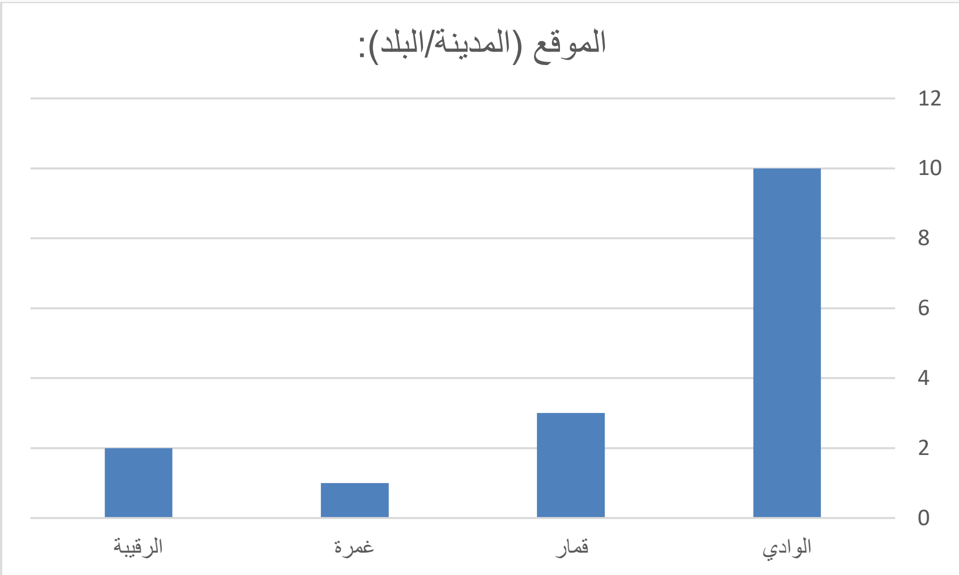
APPENDICES

استبيان لتجار الأعشاب والمعالجين بالأعشاب حول النباتات الطبية لتندب الجروح

القسم 1: المعلومات المهنية

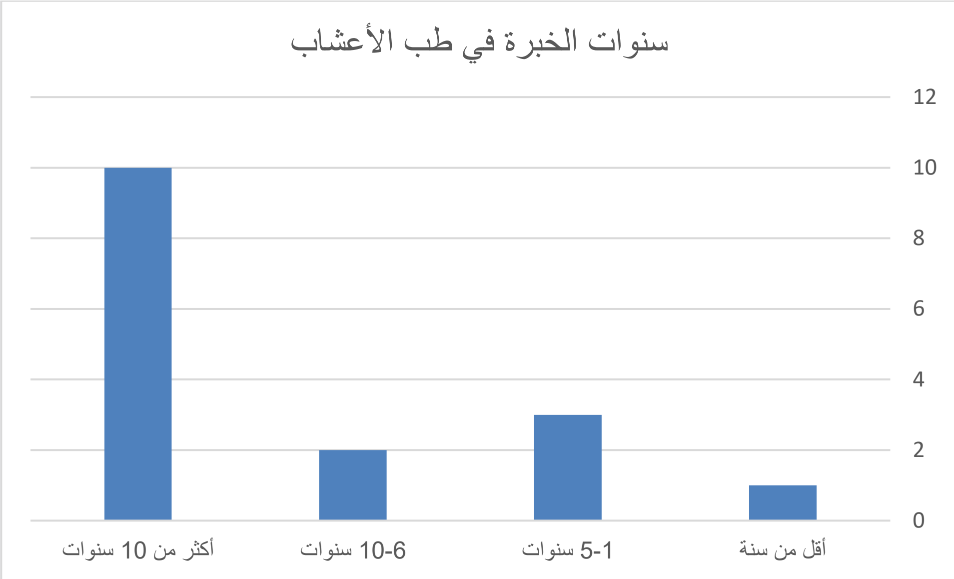
1. الاسم:
2. الاسم التجاري:
3. الموقع (المدينة/البلد):

	الوادي	قمار	غمرة	الرقيبة
التكرار	10	3	1	2
النسبة %	62.5	18.8	6.3	12.5



4. سنوات الخبرة في طب الأعشاب:

سنوات الخبرة	**أقل من سنة**	**5-1 سنوات**	**10-6 سنوات**	**أكثر من 10 سنوات**
التكرار	1	3	2	10
النسبة %	6.3	18.8	12.5	62.5



القسم 2: معرفة النباتات الطبية

5. ما هي النباتات الطبية التي تنصح بها لندب الجروح؟

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-

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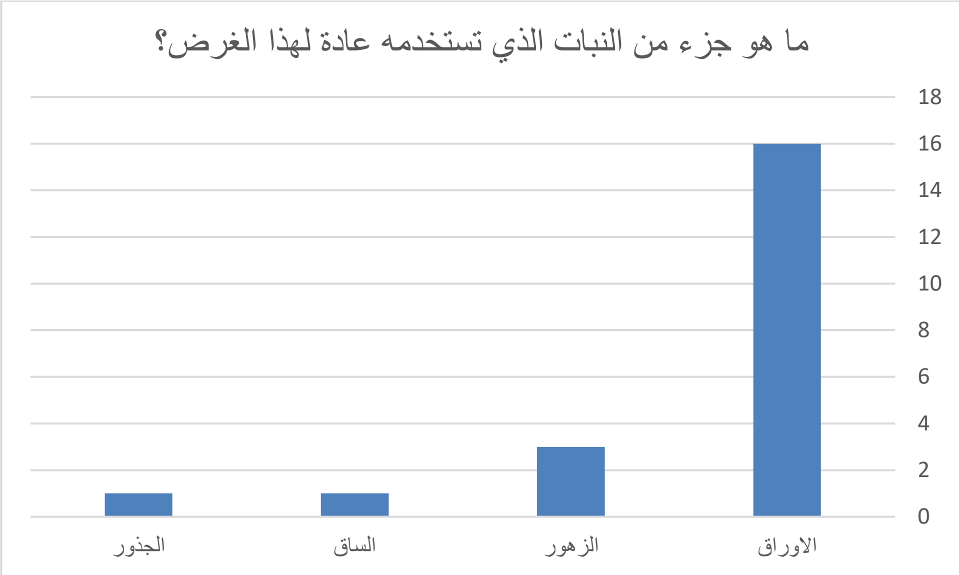
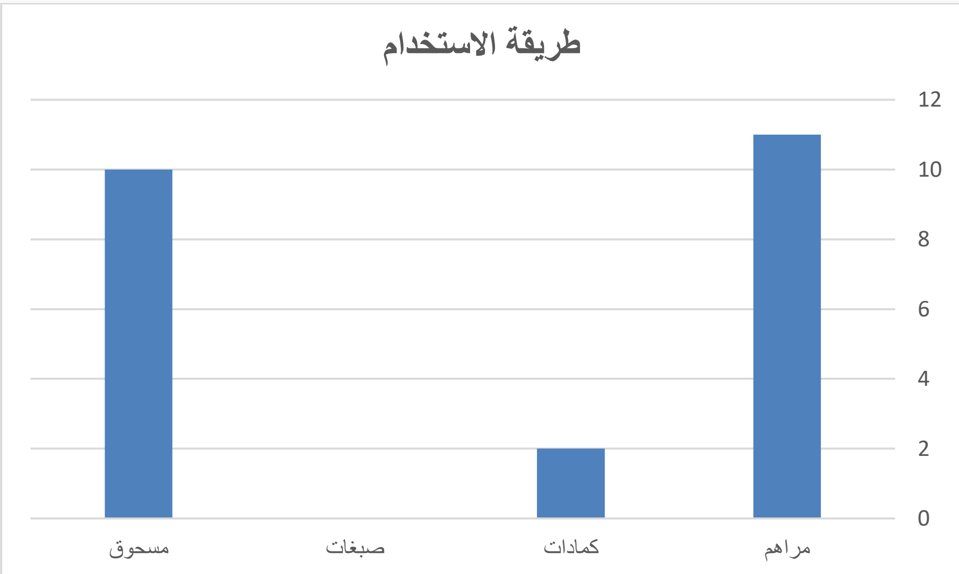
6. ما هو جزء من النبات الذي تستخدمه عادة لهذا الغرض

الجزء المستخدم	الاوراق	الزهور	الساق	الجذور
التكرار	**16**	**3**	**1**	**1**
النسبة %	**100**	**18.8**	**6.3**	**6.3**



7. كيف يتم تحضير هذه النباتات للاستخدام؟

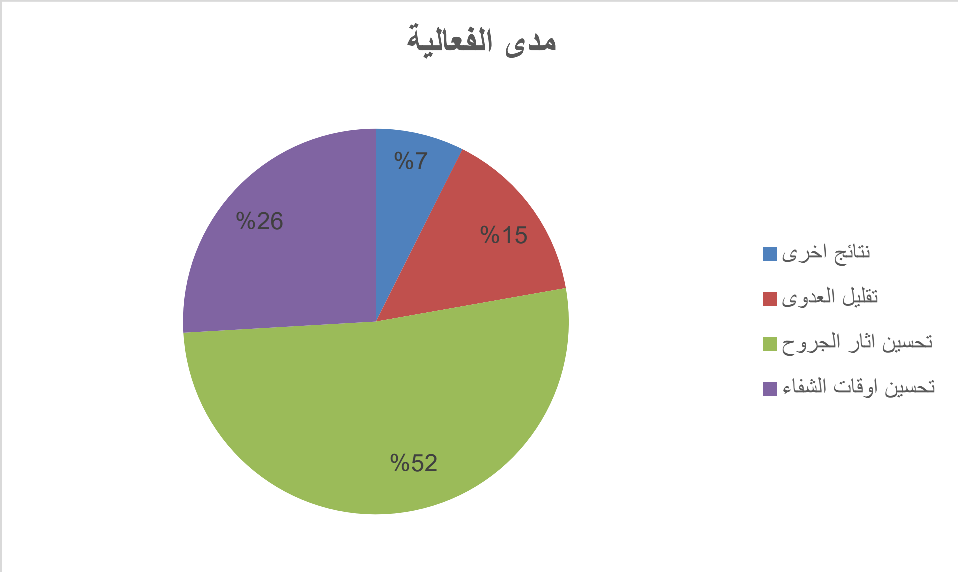
طريقة الاستخدام	مراهم	كمادات	صبغات	مسحوق	طريقة اخرى
التكرار	11	2	0	10	1
النسبة %	68.8	12.5	0	62.5	6.3



القسم 3: الفعالية وملاحظات العملاء

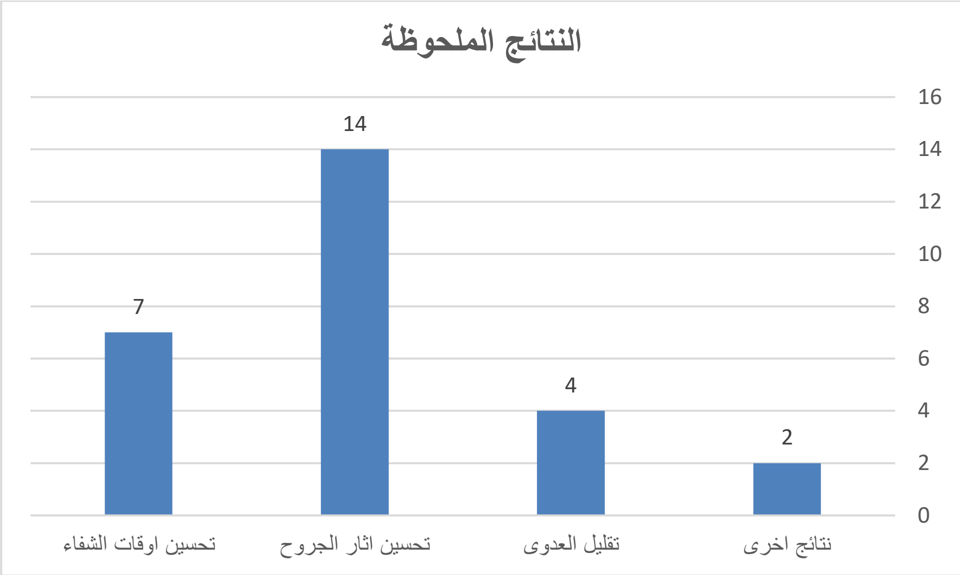
8. من خلال تجربتك، ما مدى فعالية هذه النباتات في شفاء الجروح؟

مدى الفعالية	غير فعال	فعال الى حدا ما	فعال	فعال جدا
التكرار	0	2	9	5
النسبة %	0	12.5	56.3	31.3



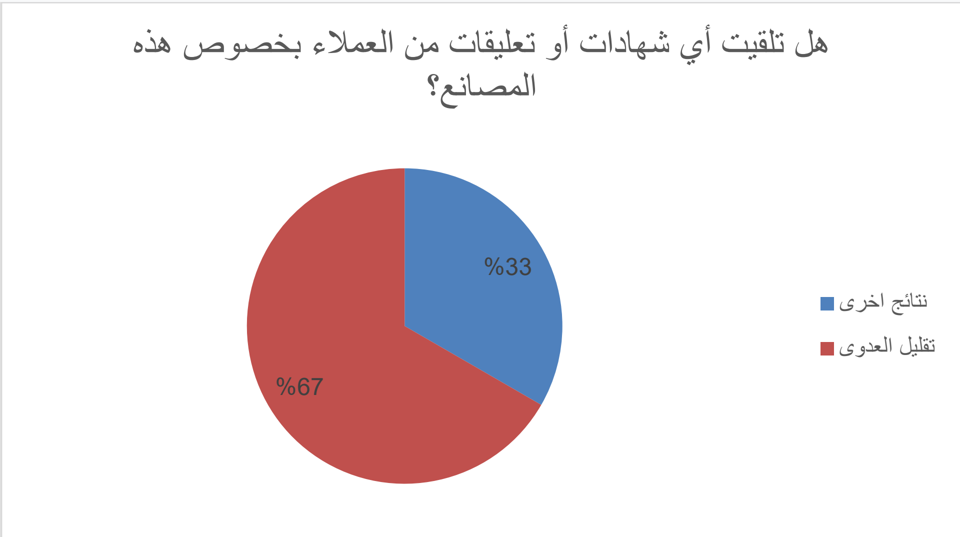
9. ما هي النتائج المحددة التي لاحظتها من عملائك الذين يستخدمون هذه النباتات

النتائج الملحوظة	تحسين اوقات الشفاء	تحسين اثار الجروح	تقليل العدوى	نتائج اخرى
التكرار	7	14	4	2
النسبة %	43.8	87.5	25	12.5



10. هل تلقيت أي شهادات أو تعليقات من العملاء بخصوص هذه المصانع؟

	نعم	لا
التكرار	10	6
النسبة %	62.5	37.5



- إذا كانت الإجابة بنعم، يرجى تلخيص الملاحظات:

-

القسم 4: الاتجاهات والممارسات

11. هل لاحظت أي اتجاهات في استخدام النباتات الطبية لشفاء الجروح في السنوات الأخيرة؟ (على سبيل المثال، زيادة الشعبية، واكتشافات النباتات الجديدة، وما إلى ذلك)

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-

12. ما هي التحديات التي تواجهها عند البحث عن النباتات الطبية أو التوصية بها من أجل التندب؟

-

-

القسم 5: تعليقات إضافية

13. هل لديك أي أفكار أو تجارب إضافية تتعلق باستخدام النباتات الطبية لشفاء الجروح؟

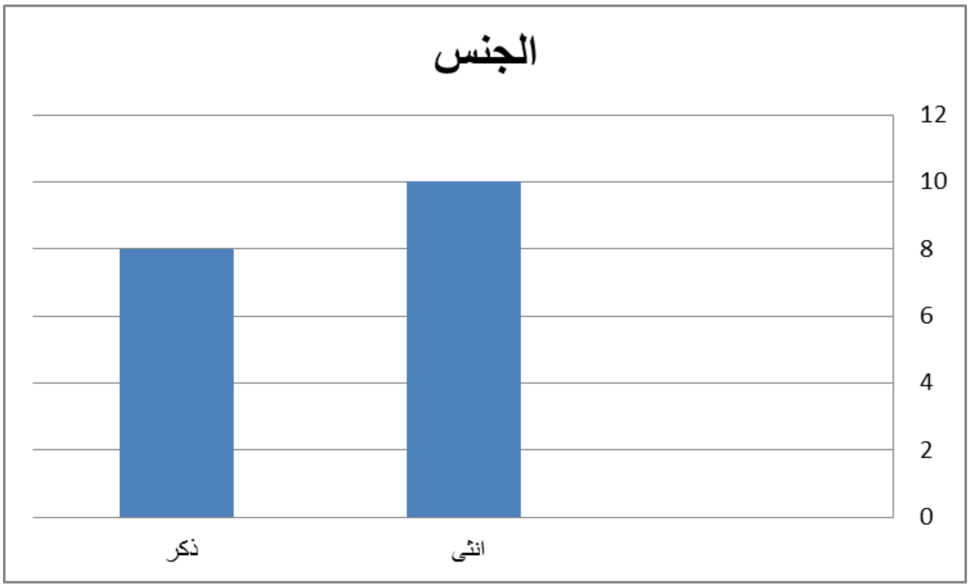
-

إحصائيات حول استخدام المرهم لنبات الخياطة

القسم 01:

- **الجنس :**

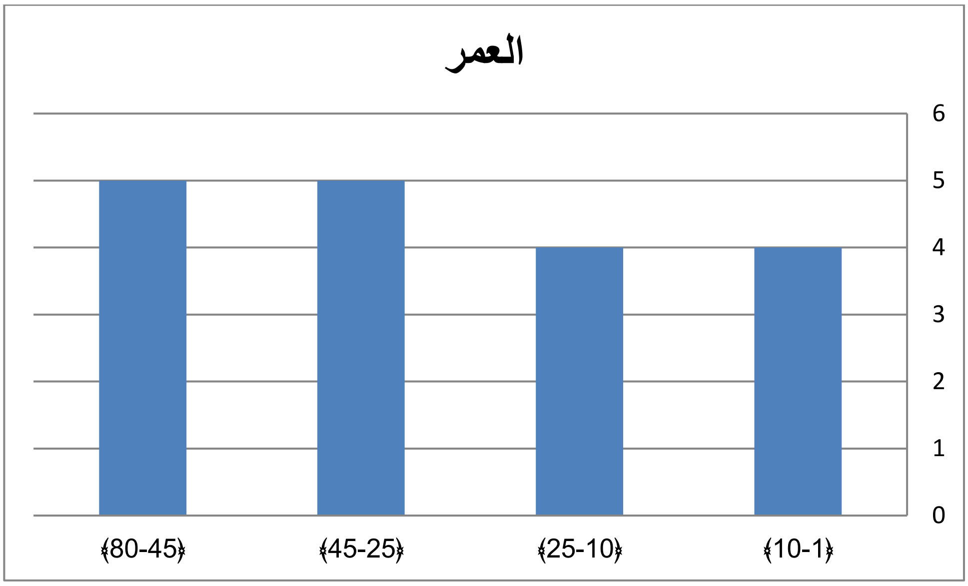
ذكر	انثى
08	**10**



القسم 02:

- العمر :

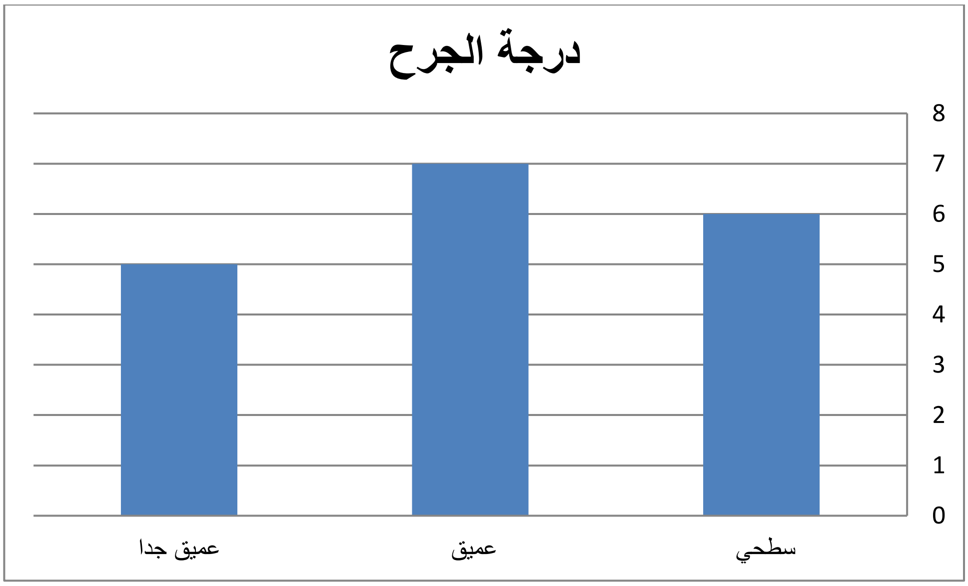
)45-80(	)25-45(	)10-25(	)1-10(
5	5	4	4



القسم 03:

- درجة الجرح :

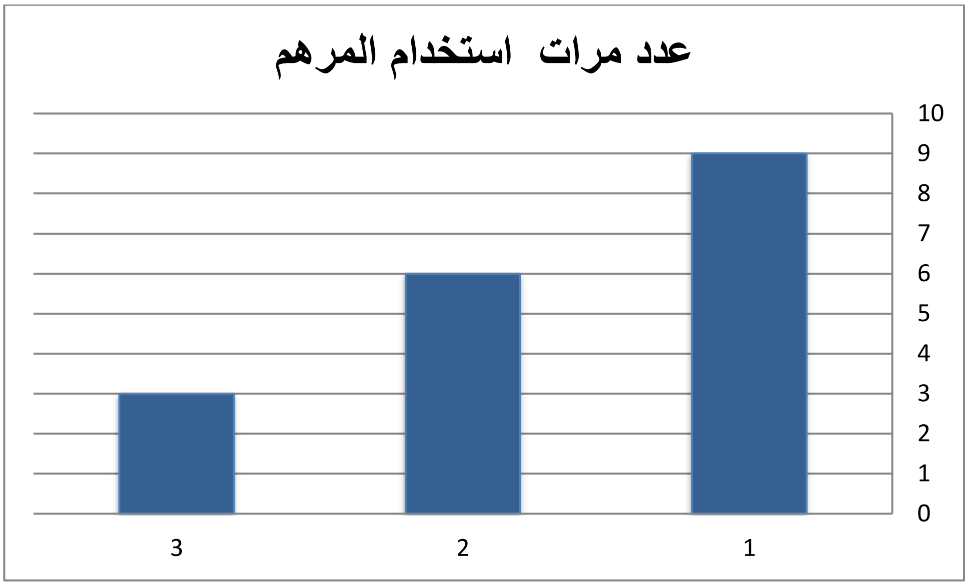
عميق جدا	عميق	سطحي
5	7	6



القسم 04:

- عدد مرات استخدامالمرهم:

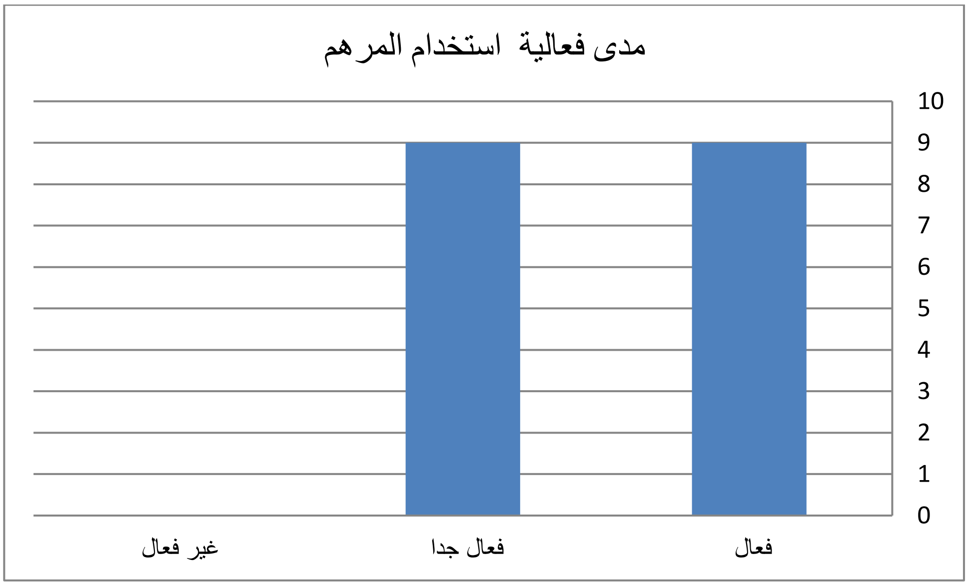
3	2	1
3	6	9



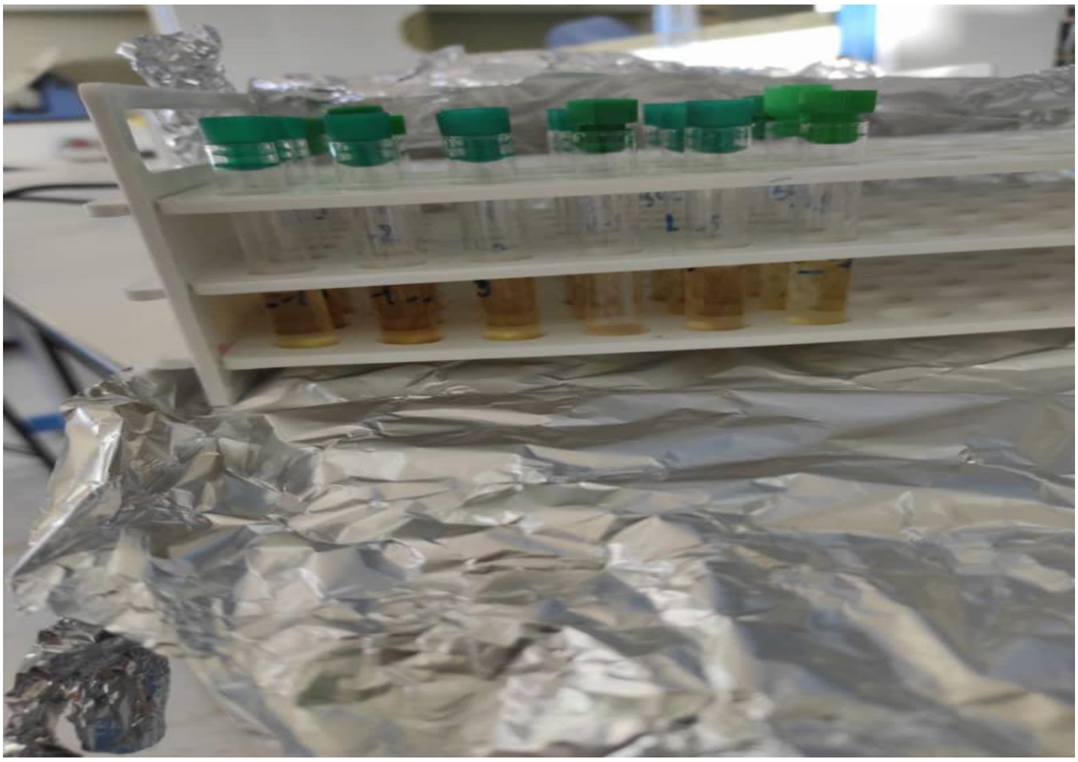
القسم 05:

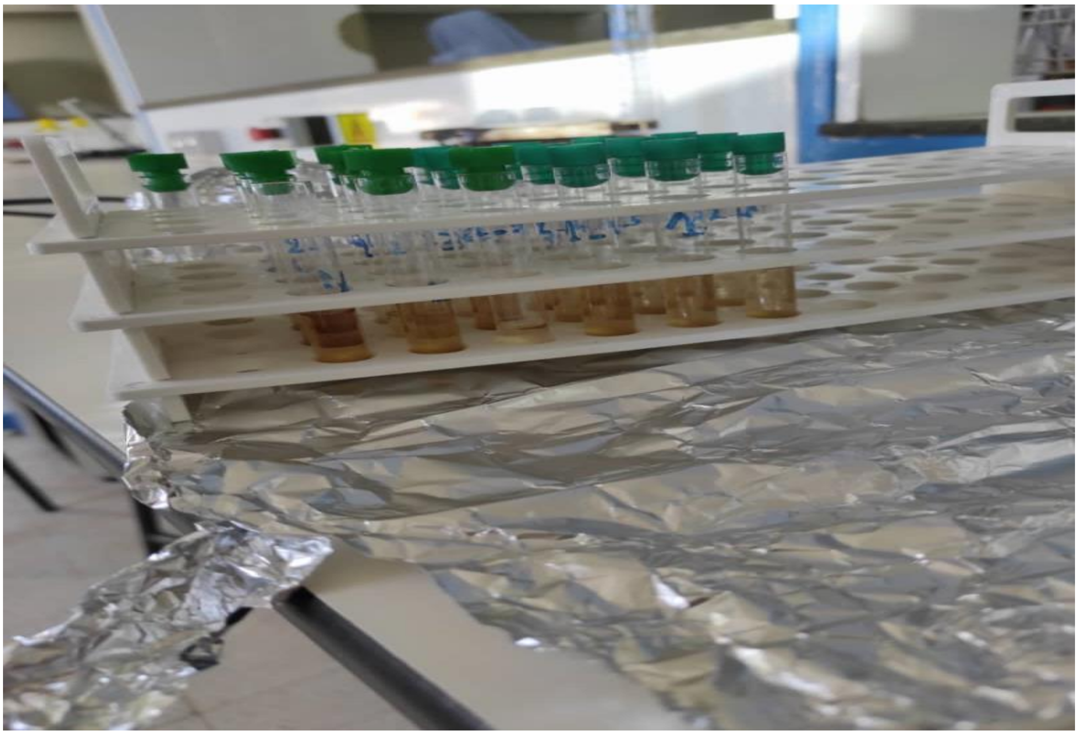
- ما مدى فعالية استخدام المرهم على الجروح

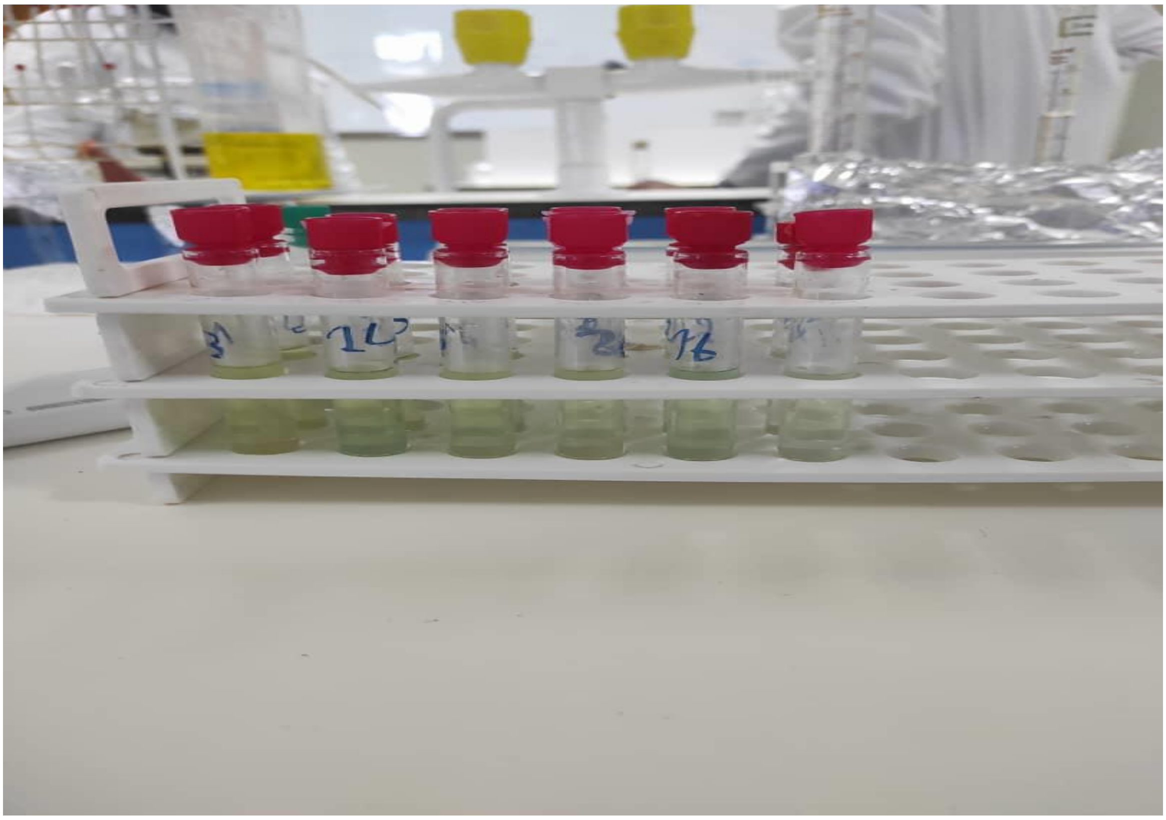
فعال	فعال جدا	غير فعال
9	9	0

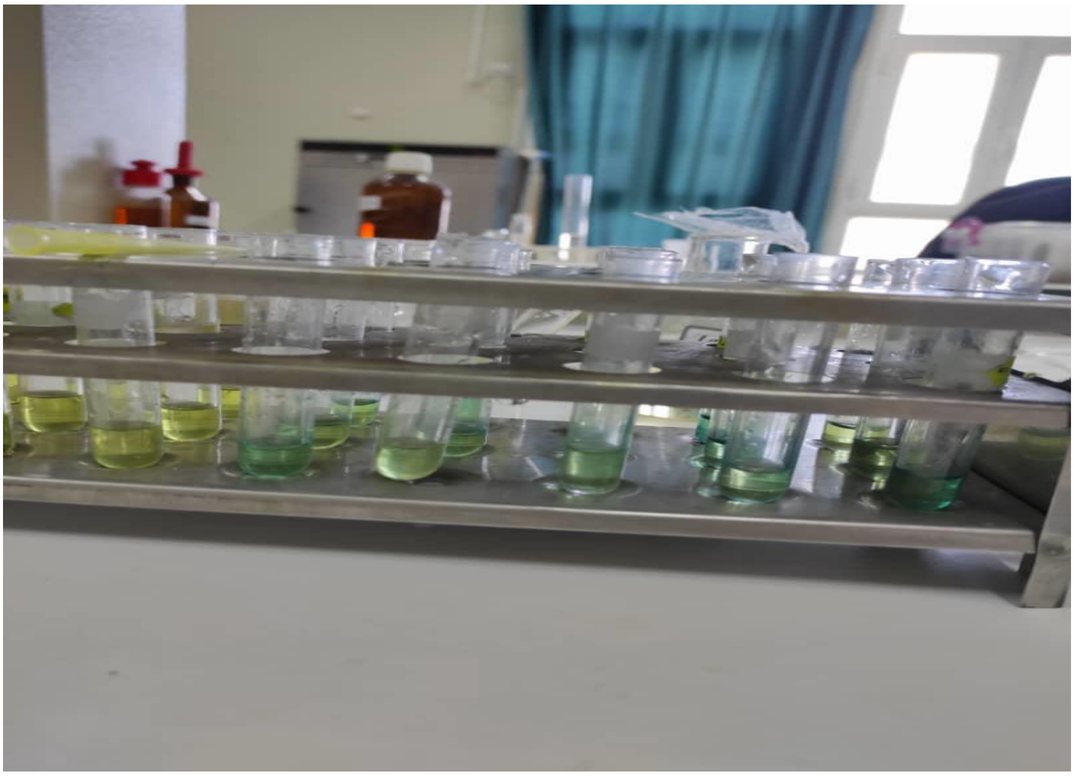


Test antioxydant (Dpph and Frap):

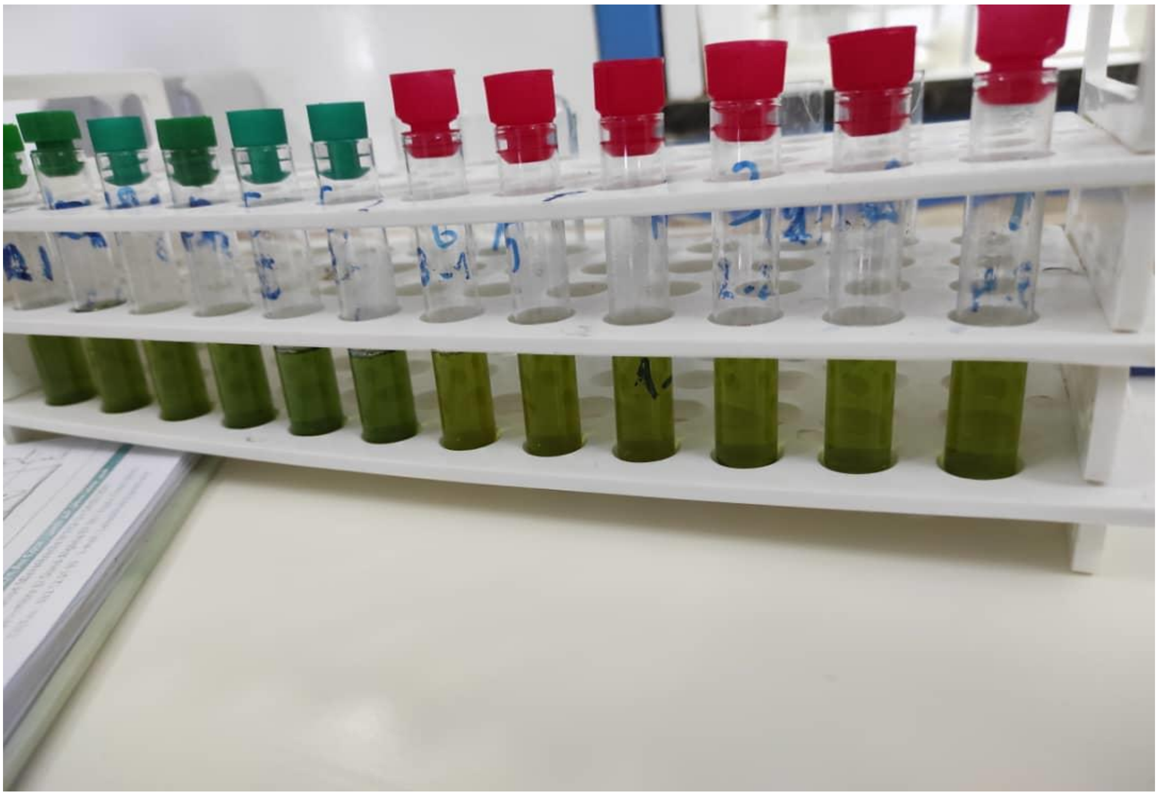


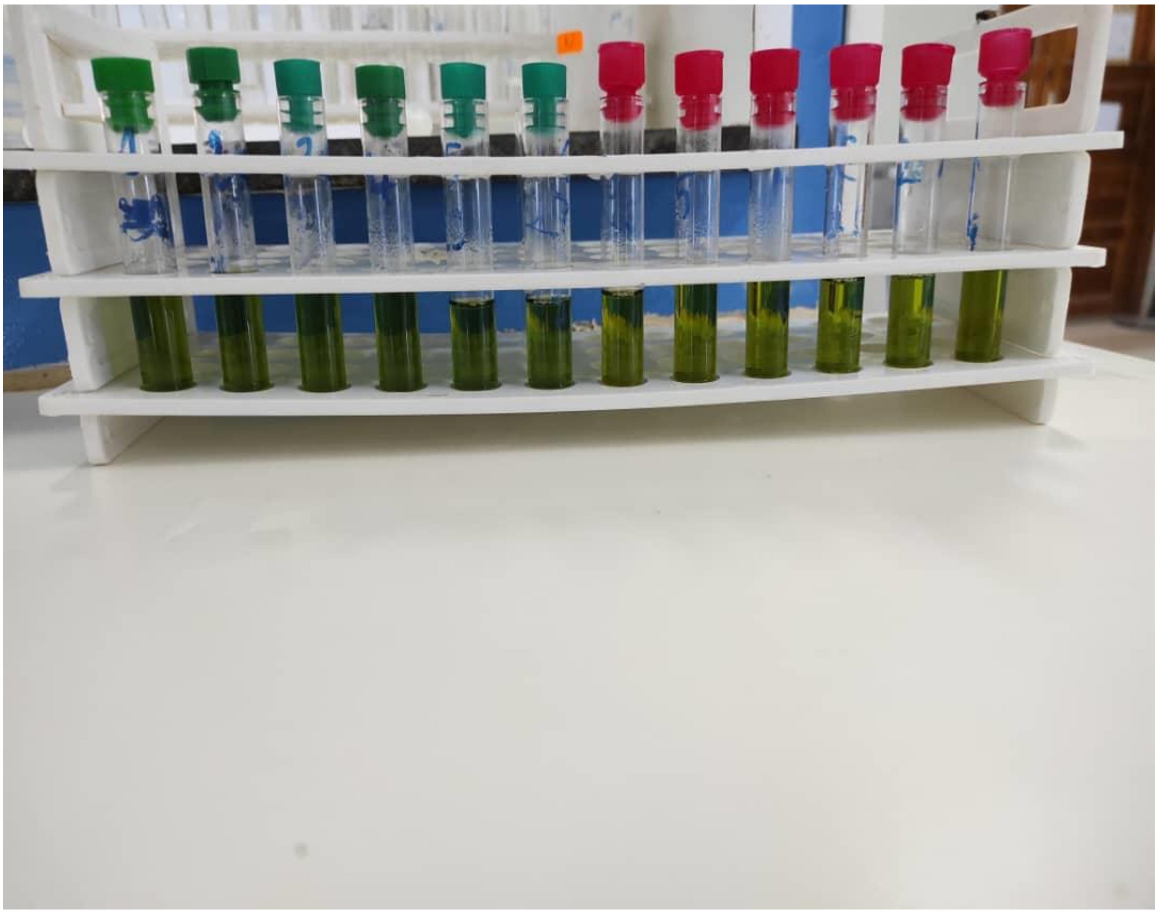


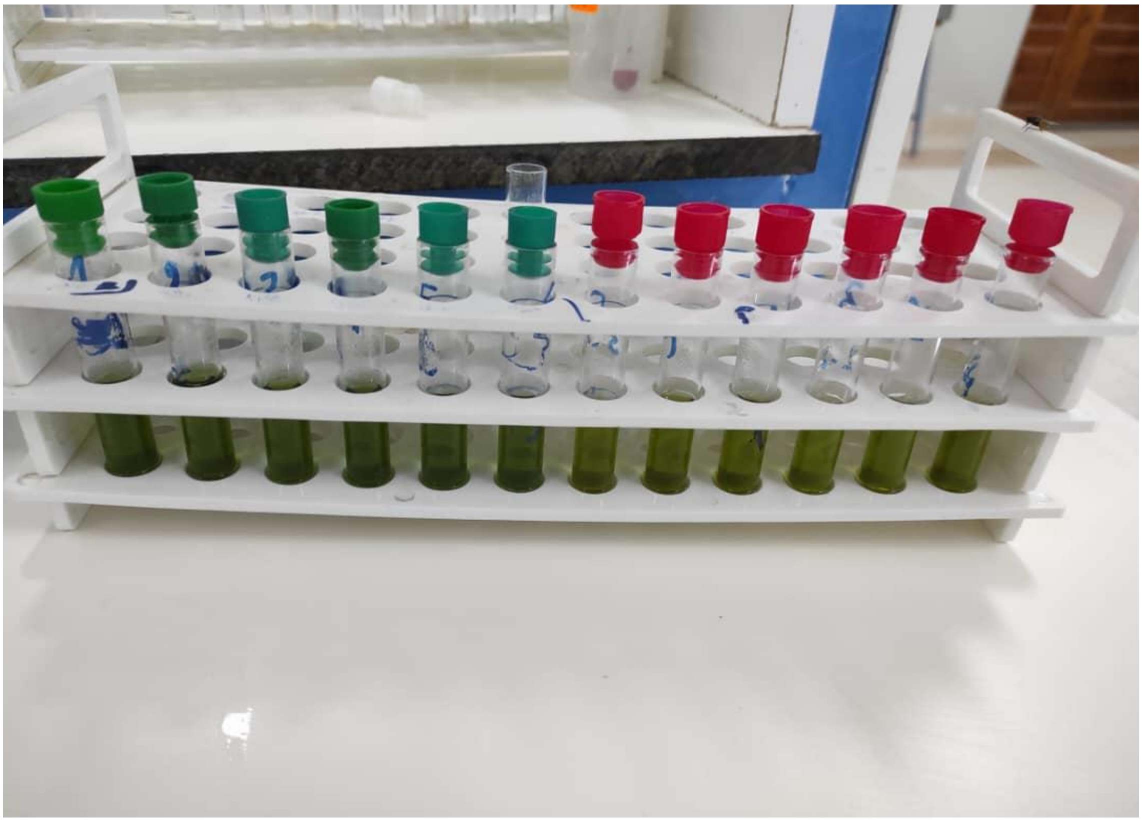




Test anti-inflammatory:







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