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Subject :

Biological Activities of *Centaurea hyalolepis*

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إهـــداء تـــخـــرجـــي

" إقرأ " هي أول كلمة أنزلت في القرآن الكريم على النبي محمد عليه الصلاة والسلام.

نجد أيضا قول الله تعالى : " قل هل يستوي الذين يعلمون والذين لا يعلمون "

..

هنا تتجلى قيمة العلم في تهديم بيوت الجهل، وأن يكون كمصدر بصيرة ونور في طرق الظلمات والشكوك. الفتى صابر يقف اليوم على حافة نهاية المشوار الدراسي، بعد مسيرة دامت لمدة عشرين سنة تقريبا.. من ذلك الطفل الذي يداعب أسوار الروضة ويكتشف الحروف والأعداد بلهفة البراءة، وصولا لطالب في السنة الثانية من مرحلة الماستر. تمر السنين بسرعة البرق، وفجأة أجد نفسي على حافة نهاية الطريق، أنا اليوم مع آخر حلقة من المسيرة الدراسية، هذا الفتى الذي شرع في مشوار المرحلة التحضيرية خلال موسم 2008/2007 بعدما عاش أيام الروضة الجميلة، ليختم من بعد فترة الابتدائي سنة 2013. من بعدها تأتي الإعدادية كي تعرفه على مزيد من الأصدقاء، على مزيد من الذكريات، على مزيد من اللحظات، على مصطلحات الحب، على مظاهر الكراهية، على مزيد من مضامير الحياة.. ليختم هذه الفترة في عام 2017. الآن، أضحى هذا المراهق بين ثنايا الثانوية، ومع هذه الحقبة اشتعل كل من قلبه وعقله، وخاضا العديد من الحروب على ساحة الورقة، كان همه أن يشعل نشوته وهو يمسك قلمه صانعا منه العديد من النصوص، قادته هذه الأخيرة كي يعشق صوته وهو يسجل، وهو يعبر، وهو يكتب فرحا، وهو يبلغ رسالة الأمل بطابع الحزن. كان عام 2019/2018 العام الأجمل بالنسبة له، لأنه عاش الكثير فيه، مر بأحاسيس مختلفة، وتذوق بهجة الذكريات التي لن تعود، وذوقها وحده في ذهني لن يغادرني. ليختم فيما بعد الثانوية ويودعها بعام فريد، كورونا. والآن، العين تفتح شهيتها لطور الجامعة، كل شيء يبدأ غريبا، فأصدقاء زمان لا أجدهم بقربي في حجرات الدراسة، كل الناس غرباء، زملاء جدد، أساتذة من مختلف المناطق، رائحة الحيرة والدهشة. وقتها أحسست أن واحدا من الأجزاء الجميلة قد ذهب دون عودة، وأنه لابد أن أبادر لشيء جديد، ولحسن الحظ أني تأقلمت بسرعة مع ذلك. عرفت فيها العديد من الأصدقاء، والعديد من الأساتذة، عرفت كيف أن الناس تفكر، وجدت تقاليد مختلفة تُعزف بين أروقتها، التزمت بالدراسة وتحصلت على علامات جيدة ومقبولة نوعا ما. ولكن ذلك لم يدم، فلقد تذوقت طعم الاستدارك، وطعم الخسارة، وطعم التخبط في قوقعة التفكير الزائد، وخاصة في هذه السنة الأخيرة

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List of Abbreviations

CH But : *Centaurea hyalolepis* Butanolic Extract

CH Hex : *Centaurea hyalolepis* Hexane Extract

CH Aq : *Centaurea hyalolepis* Aqueous Extract

CH Et2O : *Centaurea hyalolepis* Diethyl Ether Extract

PBS : Phosphate-buffered Saline

BSA : Bovine Serum Albumin

S. aureus : *Staphylococcus aureus*

E. coli : *Escherichia coli*

K. pneumoniae : *Klebsiella pneumoniae*

P. aeruginosa : *Pseudomonas aeruginosa*

Abstract :

This research aims to investigate the biological activities because our study was limited to few experiments of extracts from Centaurea hyalolepis, including : Butanolic Extract (CH but), Hexane Extract (CH Hex), Aqueous Extract (CH Aq) and diethyl Ether Extract (CH Et2O), and their effects on anti-inflammatory, antioxidant, and antibacterial activities. Previous studies have indicated that this plant has been used in traditional medical applications as a diuretic (to remove excess water), to reduce fever, to manage diabetes, as well as to treat wounds, among other uses.

While the findings are promising, further research is needed to strengthen the scientific evidence. This will help validate the traditional medicinal uses of this plant and open new avenues for its application in the pharmaceutical industry as a natural source of anti-inflammatory, antioxidant, and antibacterial compounds.

Key words :

Centaurea hyalolepis, plant extracts, bioactive compounds, anti-inflammatory, antioxidant and antibacterial.

ملخص :

يهدف هذا البحث إلى دراسة الخصائص العلاجية الخاصة بمستخلصات ***Centaurea hyalolepis*** Butanolic Extract (CH but), Hexane Extract (CH Hex), Aqueous Extract (CH Aq) and diethyl Ether Extract (CH Et2O) وتأثيرها على الجانب الالتهابي، وعلى جانب الأكسدة، وعلى الجانب البكتيري.

أشارت الدراسات السابقة إلى استعمال هذه النبتة في استعمالات طبية تقليدية ك مدر للبول (لإزالة الماء الزائد)، ولخفض الحمى، وإدارة مرض السكري وكذلك كعلاج للجرح، وغيرها من الاستعمالات.

في حين أن النتائج واعدة، إلا أنه من الضروري أن يتم تطوير النتائج لتعزيز الأدلة العلمية، كي يتم فتح آفاق جديدة لتطبيقه على مستوى الصناعات الدوائية كمصدر طبيعي يتم استغلاله للوصول لمركبات مضادة للالتهاب، والأكسدة، والبكتيريا.

الكلمات المفتاحية :

Centaurea hyalolepis، مستخلصات نباتية، مركبات فعالة، مضاد التهاب، مضاد أكسدة، مضاد بكتيريا.

INTRODUCTION

Medicinal plants contribute to the knowledge and discovery of biologically active components or compounds, which may support therapeutic health through drug development. These drugs address medical problems by harnessing the plants' properties and applying research findings from their field. Among the plant species that spark curiosity and interest for research and in-depth study is the plant of *Centaurea hyalolepis* which is characterized by significant biological properties and activities that make it effective in the therapeutic field at the level of medicine.

"*Centaurea*" is derived from the mythological centaur Chiron, who was said to have used plants from this genus to heal wounds **(Maud Grieve, 1931).** The genus *Centaurea* had been extensively used in folk medicine for hundreds of years **(Kaij-a-Kamb et al., 1992)**. It is a medicinal herb from the *Asteraceae* family which seems to grow everywhere **(Armitage, 2001).** Nevertheless, Turkey is the main center of diversity of *Centaurea* species **(Uzunhisarcıklı et al., 2007).** The *Centaurea* species had been used in traditional medicine as diuretic, to treat fever and diabetes **(Font Quer, 1995).** Many *Centaurea*, were added to tonics in the belief that they stimulate the flow of saliva and stomach acids, returning appetite to invalids **(Bernhardt, 2008).**

Through this practical research, we will mention general information about this plant, including its habitats and geographical distribution. We will highlight the botanical aspects that distinguish it from other species by providing a specific description, along with its scientific classification. Additionally, we will attempt to focus on the significance it offers, which emerges from the biological activities it performs and exhibits. We are going to explain the biological activities that relates *C. hyalolepis* according to the series of pratical we conducted in the laboratory.

The main objective of addressing and studying *C.hyalolepis* in order to study their anti-inflammatory, their antioxidant, and their antibacterial properties, enabling the identification and discovery of its therapeutic potential. As long as we are interested in studying this topic and focus on highlighting this plant, and since herbal medicine and the use of natural plants for medical purposes or as dietary supplements are beneficial for human treatment, we assume that there are benefits that can be utilized to meet needs and provide pharmaceutical supplies that facilitate and enable medical treatment.

This research will be divided into 03 main chapters, as follows:

The first chapter will include presentation of the plant, and we will focus on studying the importance of this plant by identifying its features and the benefits of its biological activities. While the second chapter will focus on the practical aspect, the experiments we conducted, followed by a discussion and analysis of the results obtained in the last chapter.

Chapter 01 :

Literature Review

Since ancient times, biological resources have served as the primary foundation for targeted medical therapies against various diseases. Modern drug discovery continues to fundamentally rely on natural products as privileged structures, demonstrating exceptional therapeutic efficacy through the identification of specific molecular compounds (**Yuan et al., 2016).**

The axiom that every pathology has its remedy motivates our evidence-based exploration of phytotherapeutic solutions. The plant kingdom represents an extraordinary chemical library, with each species constituting a unique repository of pharmacologically active compounds. This botanical dimension remains inseparable from human ecology, given our intrinsic interdependence with surrounding flora (**Atanasov, 2021) (Newman et al., 2020) (Nasim et al., 2022).**

1. Presentation of the studied plant

1.1. The Asteraceae Family

1.1.1. Generalities

The *Asteraceae* family is one of the largest flowering plant families, with over 1600 genera and 25,000 species worldwide. Some of its most well-known taxa are lettuce, chicory, artichoke, daisy and dandelion. The members of the *Asteraceae* have been used in the diet and for medicine for centuries. Despite their wide diversity, most family members share a similar chemical composition: for example, all species are good sources of inulin, a natural polysaccharide with strong prebiotic properties. They also demonstrate strong antioxidant, anti-inflammatory and antimicrobial activity, as well as diuretic and wound healing properties. Their pharmacological effects can be attributed to their range of phytochemical compounds, including polyphenols, phenolic acids, flavonoids, acetylenes and triterpenes. One such example is arctiin: a ligand with numerous antioxidant, antiproliferative and desmutagenic activities. The family is also a source of sesquiterpene lactones: the secondary metabolites responsible for the bitter taste of many plants **(Rosaria Acquaviva, 2021).**

1.1.2. Botanical characteristics of the family

The *Asteraceae* family is widely distributed throughout the world in a variety of ecological habitats, except Antarctica. They are found in forest habitats, high altitude grasslands and even urban green spaces, but they are much less common in tropical areas.

The morphology of the Asteraceae plants is also diverse. Some species are trees reaching more than 30 m, such as *Dasyphyllum excelsum* in Chile or Vernonia arborea in Malaysia; however, many others are shrubs, like rabbit brush or rosette-trees, and most are perennial or less annual herbs, ranging from 1–3 m tall sunflowers and to almost sessile forms. The smallest examples are those of the genus Mnioides found in the Peruvian Andes. The form of the leaves varies widely: while most are large, others are small and spiny, and some are nonexistent, with their function being taken over by a green stem. Most leaves are covered with an indumentum and hairs of all lengths and colors **(Bohm et al., 2001).**

Most have a flat cluster of small flowers of various colors. A good example is the Jerusalem artichoke, with thin, yellow flowers on a tall stalk **(Achika et al., 2023) (Munim et al., 2017).**

1.1.3. Nutritional Value

Many species of the Asteraceae can be included in a regular, healthy diet. They found that the protein content to range from 0.4 to 6.13 g per 100 g of edible parts and fiber from 2.55 to 13.44 g. The roots, leaves and flowers are also good sources of Na, K, Ca and Mg, and of vitamins A, B, C and D. Most plants have a low fat content **(García-Herrera et al., 2014).**

1.1.4. Chemical Characteristics

Many species of Asteraceae demonstrate various pharmacological activities, which have been attributed to their phytochemical components, including essential oils, lignans, saponins, polyphenolic compounds, phenolic acids, sterols and polysaccharides **(Koc et al,. 2015).**

1.2. The genus *Centaurea*

1.2.1. Generalities

The genus *Centaurea*, part of the *Asteraceae* family, is widely distributed across Algeria, Southern Europe, the Mediterranean region, western Asia, and the Americas **(Quezel et al.,1963) (Trease et al.,1983).**

Centaurea plants produce resin or essential oils but lack latex. They reproduce through clumping or seeding, usually in spring, and adapt to diverse environments, including deserts, semi-deserts, steep slopes, high-altitude areas, farmlands, flood-prone zones, and dry, partially shaded regions **(Hellwig, 2004).**

With over 500 species, *Centaurea* includes 45 wild varieties in Algeria, 7 of which are

native to the Sahara Desert **(Labed et al., 2019).**

Phytochemical studies show that these plants are rich in secondary metabolites, particularly sesquiterpene lactones **(Fortuna et al., 2003)**, flavonoids **(Flamini, 2002)**, alkaloids and steroids **(Ahmed et al., 1970).**

1.2.2. Botanical characteristic

Plants of the genus *Centaurea* include annual and perennial herbaceous species as well as shrub forms. The foliage typically features weakly developed, minimally prickly spines. All flowers exhibit a tubular morphology, with sterile outer florets that are generally larger and radially spreading. The central florets possess reduced bristles or small dentate features. A distinguishing characteristic is the involucral bracts, which culminate in a specialized appendage bearing a distinctive pectinate (comb-like) spine **(Judd et al., 2016).**

1.2.3. Chemical composition

Sesquiterpene Lactones : *Centaurea* species biosynthesize structurally diverse sesquiterpene lactones, predominantly of the germacranolide and guaianolide types. Characteristic compounds include solstitialin derivatives and repin-type guaianolides, which contribute to the genus' distinctive biochemical profile **(Bellakhder et al., 1997).**

Flavonoid Composition : Phytochemical investigations have revealed an extensive flavonoid repertoire across the genus, with documentation in approximately 80 Centaurea taxa. These secondary metabolites are routinely isolated from: foliar structures, aerial vegetative organs, root systems (in select species). The flavonoid spectrum includes : Basic flavone and flavonol skeletons, 6-Deoxyflavone variants, various glycosylated forms (O- and C-linked glycosides) **(Mishio et al., 2006).**

1.2.4. Pharmacological properties

The *Centaurea* genus has been pharmacologically significant since ancient times, with numerous species employed across traditional healing practices. Contemporary research has confirmed their broad therapeutic potential, with demonstrated bioactivities including :

A. Clinically significant activities

Antineoplastic and anticancer potential, potent anti-inflammatory effects, antimicrobial action against various pathogens, anti-urogenital disorder activity, antipyretic effects, analgesic properties.

B. Traditional therapeutic applications

Management of rheumatic conditions, treatment of cardiovascular disorders, headaches,

alleviation of gastrointestinal disturbances, stimulant properties, wound healing and tissue repair **(Labed et al., 2019) (Belkacem et al., 2014) (Baharfar et al., 2009) (Kilic, 2013) (Koca et al., 2009) (Leonardi et al., 2011).**

1.3. *Centaurea hyalolepis* species

1.3.1. Generalities

Is an annual to biennial thistle-like herb described by Boissier in 1846. It belongs to the genus Centaurea (knapweeds) in the tribe Centaureinae. Modern taxonomies recognize *C. hyalolepis* as a distinct species ; synonyms include *Calcitrapa hyalolepis* (Boiss.) Holub and various forms of *Centaurea pallescens* (e.g. *C. pallescens* var. *hyalolepis Boiss*.) **(Boissier, 1846).**

The natural range of *C. hyalolepis* spans the southern Mediterranean and adjacent regions. It is native from northwestern Africa through the Eastern Mediterranean to Iran and the Arabian Peninsula. Reported native countries include Morocco, Algeria, Tunisia, and eastward to Cyprus, Greece, Turkey, Syria, Lebanon, Palestine, Iraq, Iran and Yemen. It is also found in parts of the Eastern Mediterranean islands (Cyprus) and the Levant. Recent studies have newly documented it in central Algeria (Djelfa region), where it was previously unrecorded. *C. hyalolepis* has been introduced and become naturalized in some parts of southern and western Europe: Kew's database lists it in Belgium, France, Germany, Great Britain, Italy (including Sicily) and Spain as introduced regions **(Benlabed et al., 2021).**

1.3.2. Taxonomic tree

The classification of Centaurea hyalolepis is as follows **(Funk at al, 2009).**

Domain : *Eukaryota*

Kingdom : *Plantae*

Phylum : *Spermatophyta*

Subphylum : *Angiospermae*

Class : *Dicotyledonae*

Order : *Asterales*

Family : *Asteraceae*

Genus : *Centaurea*

Species : *Centaurea hyalolepis*

1.3.3. Morphology / Description

Note the bright yellow flowerhead (corolla) and spiny bracts (involucre) typical of the species. The plant is a spiny herb 30–80(–100) cm tall with much-branched stems. The stems are white- to grayish-hairy. Leaves are pinnately divided (pinnatisect) on the basal rosette (often with lobed or toothed segments) and smaller or undivided but toothed on the stem. The flowerheads (capitula) are subtended by an ovoid involucre 11–13 × 8–10 mm, with multiple rows of bracts (phyllaries) that bear long spine-tipped awns. Median phyllary spines are about 15–25(–30) mm long, often with small paired basal spines (auricles) and hyaline margins. The florets have yellow corollas, a key distinction from related *C. calcitrapa* (which has violet or pinkish flowers). The stamens have woolly filaments and yellow anthers (~5–5.5 mm), and the fruits are achenes about 2.2–2.7 mm long with a pappus ~2.5–3 mm **(Benlabed et al., 2021).**

Figure 1. *Centaurea hyalolepis*

1.3.4. Ecological Interactions

In its native flora, *C. hyalolepis* is part of Mediterranean scrub ecosystems and disturbed-field vegetation. Its spiny flowerheads attract generalist pollinators (bees, flies, etc.), although specific pollination studies are lacking. Notably, in Cyprus it serves as a larval host plant for the longhorn beetle *Phytoecia* (Helladia) *humeralis* (Cerambycidae). Adults of this beetle feed on the stems and roots of Centaurea species, so *C. hyalolepis* contributes to the life cycle of this insect. The plant's role as a thorny herb also means it can influence plant community structure, competing with other winter annuals on fertile soils **(Hadjikyriakou et al., 2002).**

1.3.5. Medicinal and Ethnobotanical Uses

C. hyalolepis has been used in traditional medicine and as food in its native range. Ethnobotanical records report :

A. Medicinal

In Palestine traditional medicine, a flower infusion is used to treat eye inflammation. A decoction of the flowers (boiled 15 minutes) is cooled and filtered to serve as a gentle eye wash, leveraging the anti-infective and anti-inflammatory properties of the plant **(Ali-Shtayeh et al., 2008).**

B. Culinary

In Cyprus and parts of Greece , the young stems of *C. hyalolepis* are gathered in spring as a wild vegetable. After removing the spines, the tender shoots are typically boiled (often with broad beans or other legumes) or fried, and served with olive oil and lemon. In one ethnobotanical survey, *C. hyalolepis* was among the most popular wild thistle greens cooked in traditional Cypriot dishes **(Della et al., 2006).**

C. Phytochemicals

Modern pharmacological studies have identified bioactive compounds in *C.hyalolepis*. Extracts of Palestinian specimens contain sesquiterpene lactones (e.g. cnicin and related compounds) which show significant antimicrobial and antibiofilm activity against various pathogens. The potent antimicrobial effect of these lactones supports the traditional use of *C. hyalolepis* in infections **(Al-Rimawi et al., 2017).**

2. The Biological Activities

Amidst the sun-drenched landscapes of the Mediterranean and the rugged terrains of the Middle East, *Centaurea hyalolepis* emerges a resilient botanical jewel adorned with golden blossoms and armored in spiny bracts. This striking member of the *Asteraceae* family has long whispered its secrets to traditional healers, who have harnessed its powers for generations to mend wounds, soothe ailments, and combat infections.

Today, cutting-edge research illuminates what traditional healers have long revered *Centaurea hyalolepis* stands as a veritable trove of bioactive wonders. Nestled within its radiant petals and resilient leaves lies a potent alchemy of flavonoids, phenolic acids, and terpenoids, each a masterful stroke in nature’s defense against inflammation, oxidative decay, and microbial siege. Its extracts gleam with transformative potential, orchestrating a harmonious interplay of anti-inflammatory, antioxidant, and antimicrobial properties, a

symphony of healing that could herald a new dawn in plant-based medicine.

As we delve deeper, we uncover how this unassuming plant stands as a testament to nature's brilliance calming storms of inflammation, shielding cells from the ravages of free radicals, and waging silent war against pathogenic foes. The story of *Centaurea hyalolepis* is not merely one of chemistry, but of harmony between earth and healing.

In the following discussion, we will explore these key biological activities in detail, supported by scientific evidence on its mechanisms and potential health benefits **(Formisano et al., 2011).**

2.1. Anti-inflammatory Activity

We found no specific in vitro or in vivo studies testing *C. hyalolepis* for anti-inflammatory effects. It is noteworthy that related *Centaurea* species are traditionally used against inflammation: the genus is recognized in folk medicine for anti-inflammatory activities **(Ksouri et al., 2021).**

Moreover, sesquiterpene lactones like cnicin (present in *C. hyalolepis*) are known to exert anti-inflammatory actions (for example, by inhibiting NF-κB in other *Centaurea* extracts). In general, *Centaurea* extracts have shown anti-inflammatory activity in cell and animal models **(González-Trujano et al., 2015).**

However, we did not locate any published assays on *C. hyalolepis* itself. Thus, while its phytochemistry suggests potential anti-inflammatory effects, formal studies (e.g. carrageenan paw edema, NF-κB or iNOS inhibition) remain to be done for this species.

We can add by saying that traditional medicine has used *Centaurea* species for their anti-inflammatory properties. Extracts from the plant may help reduce inflammation, making it potentially useful for treating conditions like arthritis or skin irritations.

Similar anti-inflammatory effects have been documented in related species such as *Centaurea cyanus* **(Göger et al., 2018) (Koukoulista et al., 2006) (Yesilada et al., 1999).**

2.2. Antioxidant Activity

Extracts of *C. hyalolepis* exhibit measurable antioxidant (radical-scavenging) effects in vitro. Ercan et al. (2025) tested the plant's extract using multiple assays (DPPH, ABTS, CUPRAC and other standard methods) and concluded that "*C. hyalolepis* has antioxidant properties" **(Ercan et al., 2025).**

Similarly, a screening of five *Centaurea* species (including *C. hyalolepis*) found that methanol extracts showed positive antioxidant activity in DPPH and ABTS assays **(Kocak et al., 2021).**

The antioxidant capacity is attributed to the plant's phenolic constituents: analysis identified abundant phenolics such as chlorogenic acid, apigenin-7- glucuronide, quinic acid and 4-hydroxybenzoic acid in *C. hyalolepis* known for radical-scavenging **(Sarikurkcu et al., 2020).**

One study also measured total phenolic and carotenoid contents, further linking chemistry to activity **(Jarić et al., 2018).**

As with antibacterial testing, all antioxidant evidence so far is from in vitro assays; no animal (in vivo) antioxidant models using *C. hyalolepis* were reported.

Centaurea hyalolepis has been reported to exhibit antioxidant activity due to the presence of phenolic compounds and flavonoids. These compounds help neutralize free radicals, reducing oxidative stress and potentially preventing chronic diseases such as cardiovascular disorders, diabetes, and neurodegenerative conditions. Studies on other *Centaurea* species (e.g., *Centaurea solstitialis*) suggest that the genus is rich in antioxidants, which may extend to *Centaurea hyalolepis* **(Koca et al., 2009).**

2.3. Antibacterial Activity

Studies consistently report broad-spectrum antibacterial effects of *C. hyalolepis* extracts in vitro. In PeerJ (2024), a dichloromethane extract of the plant inhibited both Gram-positive and Gram-negative bacteria: tested strains included Staphylococcus aureus (including MRSA), *Enterococcus faecalis, Escherichia coli, Salmonella enterica* and *Acinetobacter baumannii***.** Activity-guided fractionation identified cnicin and related sesquiterpene lactones as the active principles, and cnicin showed the strongest antibacterial effect. Remarkably, cnicin also disrupted biofilms: it reduced *A. baumannii* biofilm biomass by ~30% and altered the extracellular polymeric substance structure **(Yilmaz et al., 2024) (Ercan et al., 2023).**

An independent study likewise confirmed broad activity, reporting *C. hyalolepis* inhibition of *Bacillus megaterium, Pseudomonas aeruginosa, B. subtilis, subsp. spizizenii, Klebsiella pneumoniae, K. aerogenes, E. coli, S. aureus* and even *Candida albicans* **(Ali-Shtayeh et al., 2000) (Yeşilad et al., 1999) (Tuzlacı et al., 2001).**

In all cases activity was measured by standard in vitro assays (disc diffusion and microdilution). No dedicated in vivo antibacterial trials of *C. hyalolepis* extracts were found; however, these in vitro data suggest strong antimicrobial potential.

3. Other Uses

3.1. Respiratory Health Products

Traditional medicine has used *Centaurea hyalolepis* to treat respiratory conditions such as coughs and bronchitis. Its extracts could be formulated into syrups, inhalants, or tablets for respiratory health. Ethnobotanical studies in the Middle East and Mediterranean regions document the traditional uses of *Centaurea* species for respiratory ailments **(Ali-Shtayeh et al., 2000).**

3.2. Diuretic Formulations

The plant has been traditionally used as a diuretic, suggesting potential applications in pharmaceuticals for managing conditions like hypertension or edema. Traditional uses of *Centaurea* species as diuretics have been documented in ethnobotanical studies **(Yeşilada et al., 1999).**

3.3. Potential Anticancer Drugs

Some *Centaurea* species contain cytotoxic compounds that inhibit the growth of cancer cells. While specific research on *Centaurea hyalolepis* is limited, it may share similar bioactive compounds, making it a candidate for anticancer drug development. Research on *Centaurea* species has shown potential anticancer activity **(Koukoulitsa et al., 2006).**

3.4. Gastrointestinal Medications

Centaurea hyalolepis has been traditionally used to treat digestive disorders, such as indigestion and stomach aches. Its bitter compounds may stimulate digestive enzymes and bile production, making it a potential ingredient in gastrointestinal medications. Ethnobotanical surveys in Turkey and the Mediterranean region highlight the use of Centaurea species for gastrointestinal issues **(Tuzlacı & Aymaz, 2001).**

3.5. Antimicrobial Agents

Centaurea hyalolepis has demonstrated antimicrobial activity against certain bacteria and fungi. This makes it a potential source of natural antimicrobial agents for use in pharmaceuticals, such as topical creams, ointments, or oral medications for infections. Studies on the antimicrobial properties of Centaurea species have been conducted, though specific data on Centaurea hyalolepis may be limited **(Kültür, 2007).**

3.6. Wound Healing Products

The plant's anti-inflammatory and antimicrobial properties make it a promising ingredient in wound-healing formulations. Extracts could be incorporated into gels,

creams, or dressings to promote tissue repair and prevent infections. Traditional uses of Centaurea species for wound healing have been documented in ethnobotanical studies **(Yeşilada et al., 1999).**

4. Importance of Studying and Choosing *Centaurea hyalolepis* as a Thesis Subject

Studying *Centaurea hyalolepis* as a thesis subject holds significant value due to its underexplored potential, rich bioactive compounds, and traditional medicinal uses, which could lead to evidence-based healthcare applications and novel drug development. Research on this plant also contributes to ecological conservation, ethnobotanical knowledge preservation, and interdisciplinary collaboration, fostering innovation in natural and sustainable products. Additionally, investigating its properties may address global health challenges while offering academic and career advancement opportunities through specialized expertise and further research prospects.

Chapter 02 :

Practical Applications

The experimental part was carried out at the Laboratories of the technological hall, in El Hamma, belonging to Abbes Laghrour University -Khenchela-

The objective of our work is the investigation of the natural characteristics of plant extracts and enhance their active compounds by exploiting their properties. Hence our present study focused on investigating the anti-inflammatory, antioxidant and antibacterial activities of four (04) extracts of *Centaurea hyalolepis* : Aqueous extract (CH Aq), Hexane Extract (CH Hex), diethyl ether Extract (CH Et2O) and Butanolic Extract (CH But).

Extraction was performed according to the method described by Djebara et al, (2019) with some modifications, air-dried aerial parts (500 g) of C. hyalolepis were powdered, and exhaustively extracted at room temperature with an hydroalcoholic solution (MeOH/H2O, 8:2) for three five times (1.5 L×3). The extract obtained for each part were filtered, combined and concentrated under vacuum to afford an aqueous solution which were sequentially partitioned with solvents of increasing polarity: Hexane (200 mL×3), Et2O (200 mL×3), and n-BuOH (200 mL×3). After removing the organic solvents, crude residues from Hexane (7.2 g), Et2O (9.0 g), and n- BuOH (13.7 g) were obtained, respectively. Another extraction was conducted to obtain the aqueous extract, by maceration at room temperature of 100 g of the plant material with 300 mL of water, the obtained solution was then lyophilised to obtain the dried extract (4.9 g).

1. Anti-inflammatory Activity

1.1. Preparation of materials

The anti-inflammatory activity of CH extracts was investigated by a single method **(Anyasor et al.,2019).** Hence before starting it, we had prepared Phosphate-buffered saline (PBS), Bovine serum albumin (BSA 1%), the Ibuprofen stock solutions in order to use it for diluted concentrations, and each extract in methanol and stored in eppendorf to be ready for use.

A. Phosphate-buffered saline (PBS)

A buffer solution commonly utilized in biological studies. This water-based saline contains Sodium Phosphate, sodium chloride, and Potassium Phosphate. Its osmolality and ion levels are safe for the majority of cells **(Dankai et al., 2021).**

In order to prepare PBS, we dissolve : 8 g of NaCl with 0,2 g of Potassium dihydrogen

phosphate (KH2PO4) and 1,15 g of Disodium hydrogen phosphate (Na2HPO4) in 01 litre of distilled water. Then, we adjust pH to 6,8 using an acid : HCl.

B. Bovine serum albumin (BSA 1%)

It is a monomeric protein made up of a single chain of amino acids with the molecular weight of 66.5 kDa and is found in the blood of cows. A major component of blood plasma (**Lai et al., 2017).** BSA is produced by the liver. BSA is commonly used in laboratories as a supplement in biochemical and tissue culture media.

In order to prepare BSA 1% we mix 0,5 g of BSA in 50 ml of PBS. Then stir it gently until it dissolves. We keep them in the refrigerator at a temperature of 4°C.

C. The Ibuprofen stock solutions

An active ingredient in a non-steroidal anti-inflammatory drug, used to relieve symptoms of arthritis, painful menstrual cramps, and fever.

In order to prepare this, we dissolve 600 mg of Ibuprofen in 1 ml of PBS 1% ; 1 ml of PBS and 99 ml of water. Then, mix well until fully dissolved, (these solutions used as positive control). The next step is to prepare diluted concentrations strarting from the Ibuprofen stock solutions.

Table 1. Preparation of diluted concentrations of the Ibuprofen stock solutions

Concentrations (mg/ml)	Volume of the stock solution (µl)	Volume of PBS added (µl)
100	1000	0
50	500	500
25	250	750
12,5	125	875
6,25	62,5	937,5

D. Preparation of the four plant extracts

In order to prepare our extracts, to be ready for use, we need to put 1 mg from the extracts in 1 ml of methanol. Then we stock it in Eppendorf tube (mother solution). We repeat this work for each type of extract. After that, we need to prepare a stock of 06 solutions ; 06 concentrations.

Table 2. Preparation of serial concentrations of the extracts

Solution	Volume (ml)	Concentration (mg/ml)
A	**14 ml of PBS + 10 ml of BSA%**	
B	**0,5 ml of the extract + 4,8 of (A)**	**100**
C	**1,5 ml of (A) + 1,5 ml of (B)**	**50**
D	**1,5 ml of (A) + 1,5 ml of (C)**	**25**
E	**1,5 ml of (A) + 1,5 ml of (D)**	**12,5**
F	**1,5 ml of (A) + 1,5 ml of (E)**	**6,25**

(We repeat this process 04 times, as we are preparing 04 different types of extracts)

Figure 2. Various prepared materials necessary for anti-inflammatory activity

1.2. Method of work

To study the anti-inflammatory activity, we need to make a denaturation test. This test will apply for Ibuprofen and the four (04) types of the extracts ; 05 repititions.

For each repeat, we need to prepare : 0,1 ml of each concentration (6,25 to 100) ; it means we need 05 tubes. Then, we add 1 ml of BSA 1% with 1,4 ml of PBS in all the tubes. After that, we move to incubate the 05 tubes at 37°C for 15 minutes. The next step is to put the tubes on water bath at 72°C for 5 minutes for heating. Finally, we let the tubes cool, then measure the absorbance at 660 nm.

So, we need to calculate % inhibition for each concentration of the extract (6,25 – 100) to calculate the IC50 value for each extract, (the IC50 is the concentration where the curve reaches 50% inhibition). To calculte the % inhibition of each concentration, we use this equation : % inhibition = [(1 – Absorbance of sample / Absorbance of control) × 100]


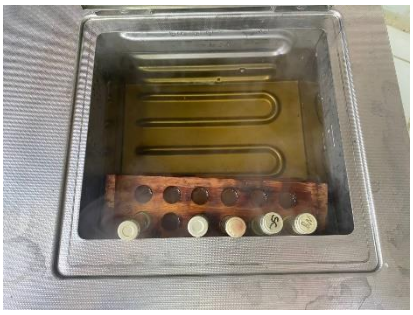


Figure 3. Incubation, Heating, and Absorbance Measurement in Denaturation Assay

2. Antioxidant Activity

The antioxidant activity of CH extracts was investigated by DPPH method, following the method described by Molyneux (2003), hence before starting it, we had prepared DPPH and Ascorbic Acid solutions.

2.1. Preparation of materials

A. DPPH solution

The DPPH (2,2-diphenyl-1-picrylhydrazyl) radical scavenging assay measures a compound's antioxidant activity by its ability to neutralize the stable DPPH free radical. When an antioxidant donates a hydrogen atom or electron to DPPH, the purple color fades to yellow, and the degree of this color change (measured at 517 nm) indicates scavenging efficiency. The percentage of DPPH scavenged reflects antioxidant strength, often expressed as IC_{50} (concentration needed to scavenge 50% of radicals). This simple, widely used method helps evaluate antioxidants in plant extracts, foods, and pharmaceuticals, though it doesn't fully replicate biological conditions **(S. I. M. DIENG et al., 2017).**

In order to prepare DPPH solution, we need to mix 4 mg in 100 ml of methanol. Then, we store it in amber bottle to avoid degradation. After that, we keep it at 4°C.

B. Ascorbic Acid solution

It is a water-soluble vitamin, antioxidant, and essential co-factor for collagen biosynthesis, carnitine and catecholamine metabolism, and dietary iron absorption, used as a positive control **(Muhammad Abdullah et al., 2023).**

In order to prepare it, we mix 10 mg of Ascorbic Acid in 10 ml of methanol (1000 µg/ml).

C. Serial Dilutions

We know that : C1 × V1 = C2 × V2 ; C1 = 1000 µg/ml, V1 = the volume taken from the

stock, C2 = (10, 25, 50, 100) µg/ml and V2 = 10 ml.

Table 3. Serial dilutions of Ascorbic Acid

Concentration (µg/ml)	Volume of the stock solution (1000 µg/ml)	Volume of methanol (ml)
100	1	9
50	0,5	9,5
25	0,25	9,75
10	0,1	9,9

D. Extract solutions

To prepare the extract dilutions, we mix 1 mg of the extract in 1 ml of methanol.

Table 4. Serial dilutions of the extract

Concentration (µg/ml)	Volume of the extract (µl)	Volume of methanol (µl)
100	**100**	**900**
50	**50**	**950**
25	**25**	**975**
10	**10**	**990**

(For each type of extract, we made this process)

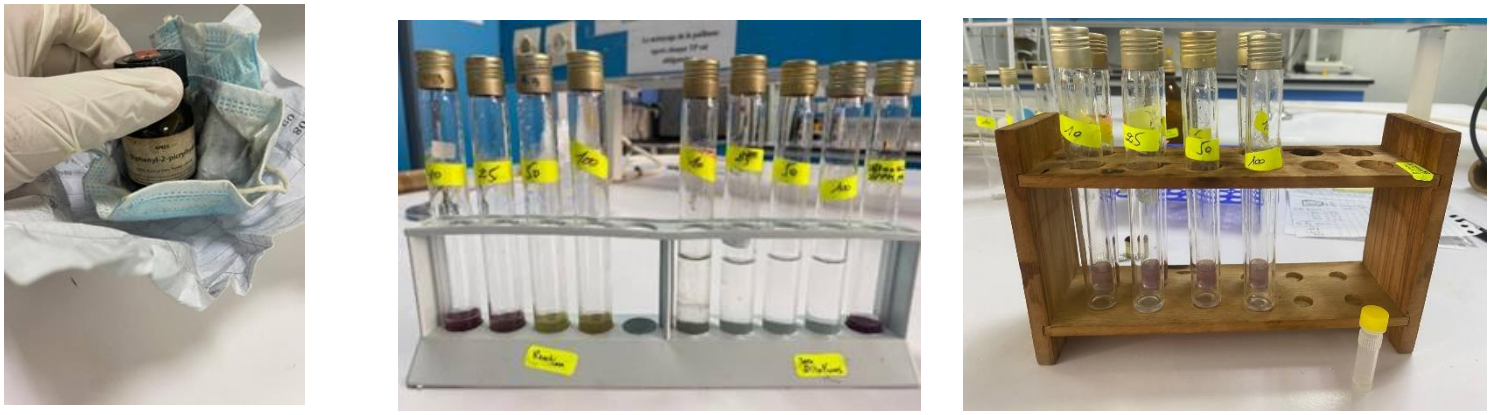

Figure 4. The materials required for the antioxidant activity

2.2. Method of work

In order to make the antioxidant assay, we mix 1 ml from DPPH solution with 1 ml of Ascorbic Acid solution (different concentration). Then, we pass to our extract solutions ; for each type, we mix 1 ml of extract solution in 1 ml of DPPH solution. So, in the final we

will have twenty (20) tubes ; four (4) tubes for Ascorbic Acid, and sixteen (16) tubes for all the extracts, each type with four (4) tubes. We incubate each tube (each concentration) in the dark for 30 minutes at room temperature. Then, we move to measure the absorbance at 517 nm, using : 1 ml of DPPH + 1 ml of methanol as a blank.

So, we need also to calculate % inhibition for each concentration of the extract (10 -100) to calculate the IC50 value for each extract. (The IC50 is the concentration where the curve reaches 50% inhibition) To calculte the % inhibition of each concentration, we use this equation : % inhibition = [(1 – Absorbance of sample / Absorbance of control) × 100]

3. Antibacterial Activity

3.1. Preparation of materials

A. Nutrient agar

Nutrient agar is a commonly used medium for the isolation and growth of a broad range of microorganisms **(NURUL HANISAH ABDUL MALIK, 2022).**

In order to prepare nutrient agar, first, we mix 5,75 g of nutrient agar powder with 250 ml of distilled water by agitator, using speed and heat. (the bottle contains 23 g, and Its for 1000 ml). Then, in the sterilization zone, we keep the quantity of nutrient agar in flasks, and prepare also a quantity of disks to study the activity.

For our work, we need to prepare twelve (12) petri dishes of nutrient agar. Eight (8) dishes for nutrient agar only, and four (4) dishes to make subculturing using four (4) strains of bacteria. This strains are : *E.coli*, *S.aureus*, *K.pneumoniae* and *P.aeruginosa*
After that, we put the eight (8) dishes in the refrigerator, and the other four (4) in the incubator for 24 hours.

B. Physiological saline

It is a sterile solution of 0.9% (w/v) sodium chloride (NaCl) in water. It is isotonic with human blood and tissues, meaning it has the same osmotic pressure as bodily fluids, preventing cell damage (lysis or crenation) when used for intravenous infusion, wound irrigation, or other medical application **(Awad et al., 2008).**
We need to prepare 4 tubes sterilized in the autoclave, and keep them in the refrigerator.

C. The extracts

We prepared only two types of extracts due to the depletion of the other two extracts : Hexane Extract (CH Hex) and Butanolic Extract (CH But). We mix 0,5 g of the extract with 1 ml of DMSO, and keep the mixture in eppendorf tube.

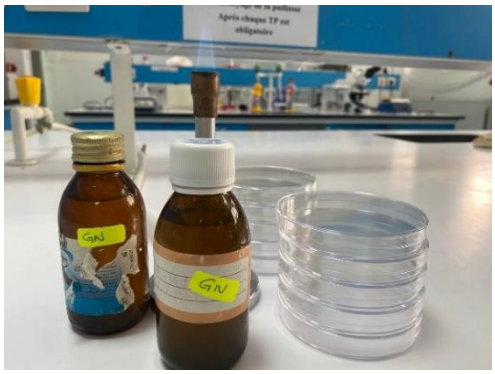
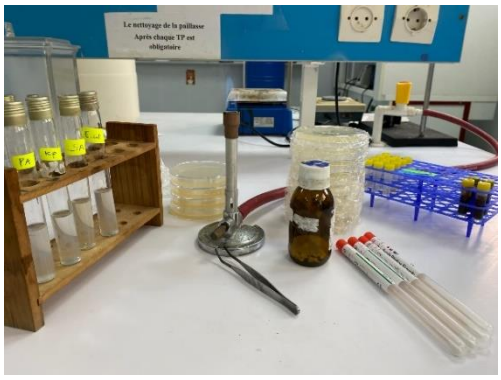

Figure 5. Materials of the process of the antibacterial activity

3.2. Method of work

The name of the process called : swabbing. Always, in the sterilization zone, we put the twelve (12) dishes. The four (4) dishes contains : nutrient agar and bacterial growth, we take from them by platinum loop a five (5) colonies, and put it with the physiological saline. Each type of dish with each tube of physiological saline (Between a strain and strain, we flame), **(United States Pharmacopeia, 2023).**

We use vortex to dissolve the bacterial suspension very well, and let them for a 15 minutes. Then, for each two (2) dishes from the eight (8) dishes, we work for one strain; it means for one type of bacteria we repeat the work twice. So, we immerse the swab in the physiological saline, then we make striations on nutrient agar dish's (3 sides).

The last step, is to put the 2 disks in the dish (each disk was immersed in the extract solution's). We incubate the eight (8) dishs at 37°C for 24 hours so that we can read the results, by measuring the inhibition zone diameter.

Chapter 03 : Results and Discussion

1. Anti-inflammatory results

Ibuprofen, the reference drug, exhibited a dose-dependent response, with inhibition increasing from 39.83% at 100 µg/mL to 84.65% at 6.25 µg/mL. Surprisingly, the highest inhibition was observed at the lowest concentration (6.25 µg/mL), which may suggest a saturation effect or possible assay interference at higher doses.

Interestingly, CH But extract displayed comparable inhibition to Ibuprofen at lower concentrations (85.48% at 6.25 µg/mL), maintaining strong activity (45.43% at 100 µg/mL). This suggests that the butanol fraction contains potent anti-inflammatory compounds, possibly polar secondary metabolites like flavonoids or glycosides, which are effective even in small doses.

The CH Hex, CH Aq, and CH Et_2O extracts showed identical inhibition patterns across all tested concentrations (85.48% at 6.25 µg/mL to 45.43% at 100 µg/mL).

Table 5. Ibuprofen results of anti-inflammatory activity

Concentration (mg/ml)	Absorbance	Inhibition %
100	0,074	84,65
50	0,110	77,18
25	0,192	60,17
12,5	0,200	58,51
6,25	0,290	39,83

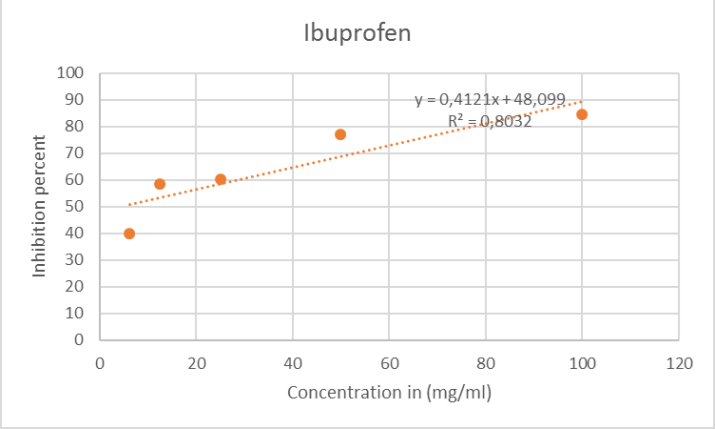

Figure 6. IC50 of Ibuprofen (Anti-inflammatory activity)

y = 0,4121x + 48,099 / 50 = 0,4121x + 48,099

x = (50 – 48,099) ÷ 0,4121 / x = 4,61 mg/ml = IC50

Table 6. CH But results of anti-inflammatory activity

Concentration (mg/ml)	Absorbance	Inhibition %
100	0,070	85,48
50	0,078	83,82
25	0,107	77,8
12,5	0,179	62,86
6,25	0,263	45,43

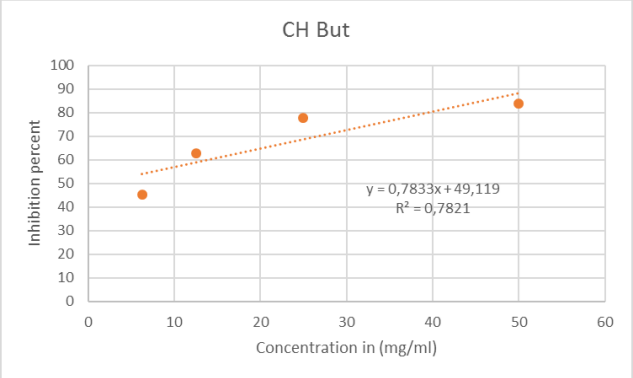

Figure 7. IC50 of CH But (Anti-inflammatory activity)

x = 1,12 mg/ml

Table 7. CH Hex results of anti-inflammatory activity

Concentration (mg/ml)	Absorbance	Inhibition %
100	0,095	80,29
50	0,102	78,84
25	0,113	76,56
12,5	0,192	60,16
6,25	0,242	49,79

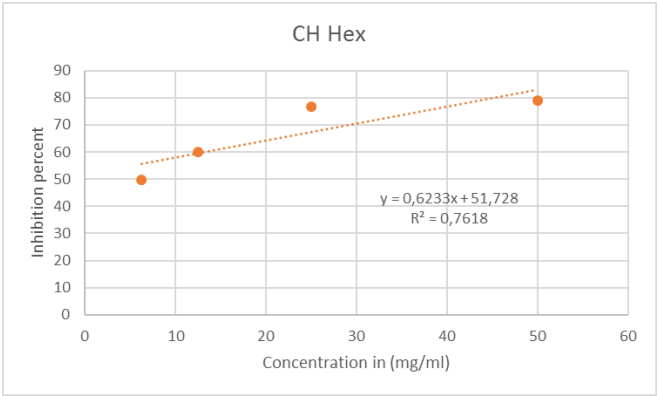

Figure 8. IC50 of CH Hex (Anti-inflammatory activity)

x = 70 mg/ml = IC50

Table 8. CH Aq results of anti-inflammatory activity

Concentration (mg/ml)	Absorbance	Inhibition %
100	0,096	80,08
50	0,111	76,97
25	0,139	71,16
12,5	0,194	59,75
6,25	0,272	43,56

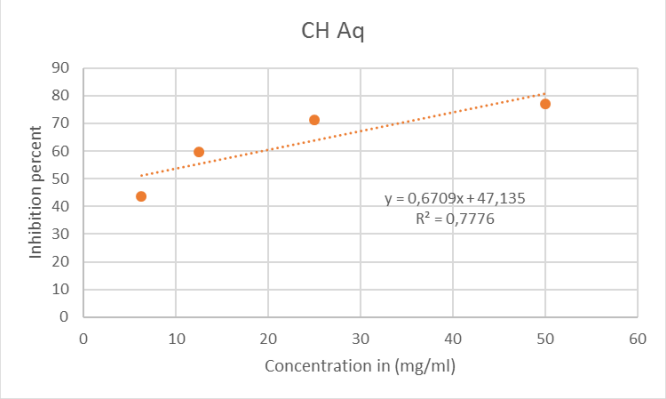

Figure 9. IC50 of CH Aq (Anti-inflammatory activity)

x = 4,27 mg/ml = IC50

Table 9. CH Et2O results of anti-inflammatory activity

Concentration (mg/ml)	Absorbance	Inhibition %
100	0,088	81,74
50	0,108	77,59
25	0,172	64,31
12,5	0,200	58,50
6,25	0,261	45,85

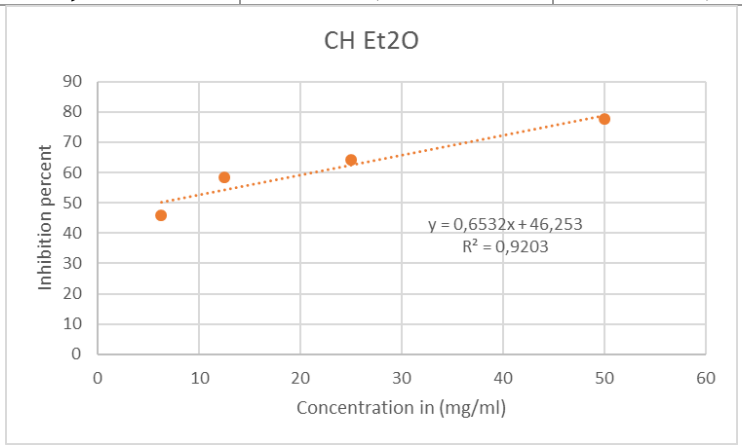

Figure 10. IC50 of CH Et2O (Anti-inflammatory activity)

x = 5,73 mg/ml = IC50

2. Antioxidant results

DPPH results

Calculate % DPPH :

% Inhibition = [(A – B) / A] × 100

A = Absorbance of DPPH + Methanol ; 0,629

B = Absorbance of Ascorbic acid or the extract

The antioxidant activity of Ascorbic Acid and the various CH extracts was evaluated by measuring absorbance and calculating the percentage of inhibition. Ascorbic Acid, a well-known standard antioxidant, demonstrated a dose-dependent increase in radical scavenging activity, with inhibition percentages rising from 4.5% at 10 µg/mL to 68% at 100 µg/mL. This confirms its strong antioxidant capacity, serving as a reliable positive control for comparison with the tested extracts.

Among the CH extracts, the CH But extract exhibited moderate antioxidant activity, reaching 54% inhibition at 100 µg/mL, though it was less effective than Ascorbic Acid at equivalent concentrations. Similarly, the CH Hex extract showed comparable activity, with 51.5% inhibition at 100 µg/mL, suggesting that nonpolar compounds in these fractions contribute to radical scavenging but are not as potent as Ascorbic Acid.

In contrast, the CH Aq (aqueous) extract displayed relatively higher antioxidant potential, achieving 55.8% inhibition at 100 µg/mL, outperforming both CH But and CH Hex. This indicates that polar antioxidants, such as phenolics or flavonoids, may be more abundant in this fraction. The CH Et_2O (diethyl ether) extract also demonstrated notable activity, with 52.62% inhibition at 100 µg/mL, reinforcing the presence of antioxidant compounds with intermediate polarity.

Overall, while none of the CH extracts surpassed Ascorbic Acid in antioxidant efficacy, their dose-dependent responses suggest significant radical scavenging potential, particularly in the CH Aq and CH Et_2O fractions. Further phytochemical analysis could identify the specific bioactive compounds responsible for these effects, providing insights into their mechanisms of action.

Table 10. Ascorbic Acid results of DPPH

Concentration (mg/ml)	Absorbance	% Inhibition
10	0,601	4,5
25	0,426	32,3

50	0,266	57,7
100	0,201	68

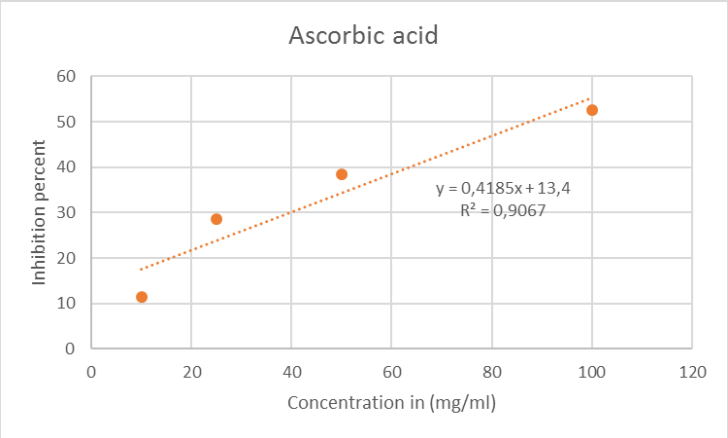

Figure 11. IC50 of Ascorbic Acid (DPPH activity)

x = 60,73 mg/ml = IC50

Table 11. CH But results of DPPH

Concentration (mg/ml)	Absorbance	% Inhibition
10	0,609	3,2
25	0,556	11,6
50	0,495	21,3
100	0,290	54

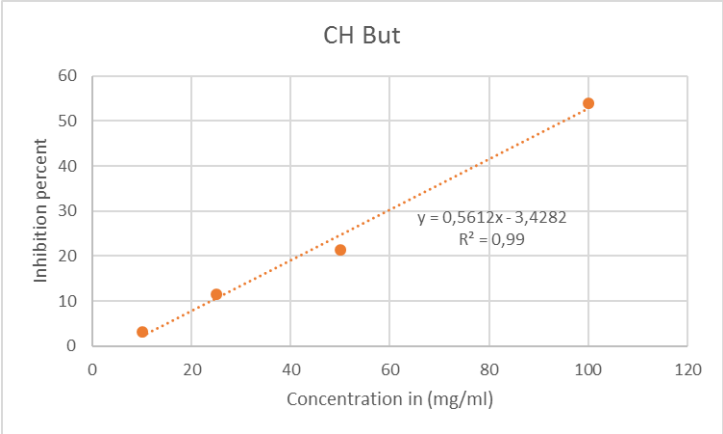

Figure 12. IC50 of CH But (DPPH activity)

x = 95,20 mg/ml = IC50

Table 12. CH Hex results of DPPH

Concentration (mg/ml)	Absorbance	% Inhibition
10	0,608	3,3
25	0,523	16,9
50	0,474	24,6
100	0,305	51,5

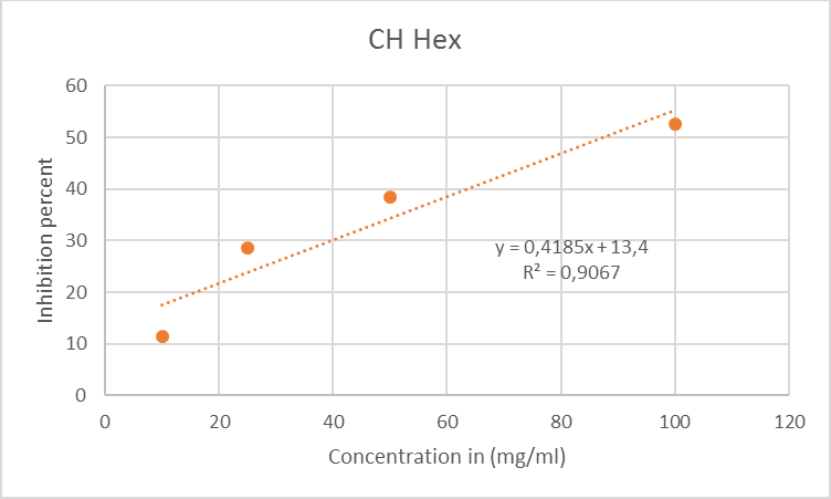

Figure 13. IC50 of CH Hex (DPPH activity)

x = 97,07 mg/ml = IC50

Table 13. CH Aq results of DPPH

Concentration (mg/ml)	Absorbance	% Inhibition
10	0,564	10,3
25	0,480	23,7
50	0,366	41,8
100	0,278	55,8

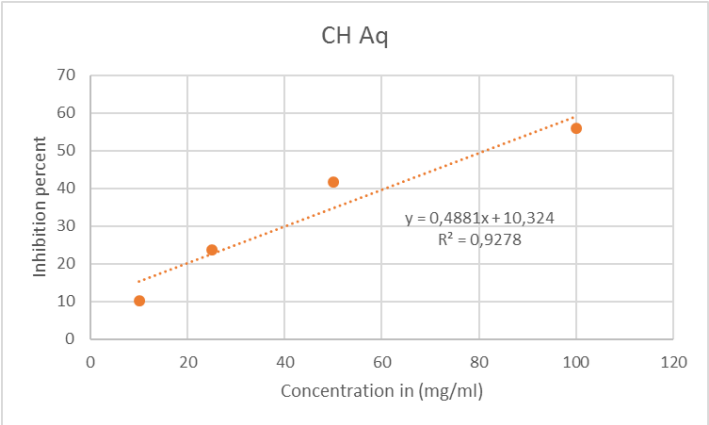

Figure 14. IC50 of CH Aq (DPPH activity)

x = 81,28 mg/ml = IC50

Table 14. CH Et2O results of DPPH

Concentration (mg/ml)	Absorbance	% Inhibition
10	0,557	11,4
25	0,450	28,5
50	0,387	38,5
100	0,298	52,62

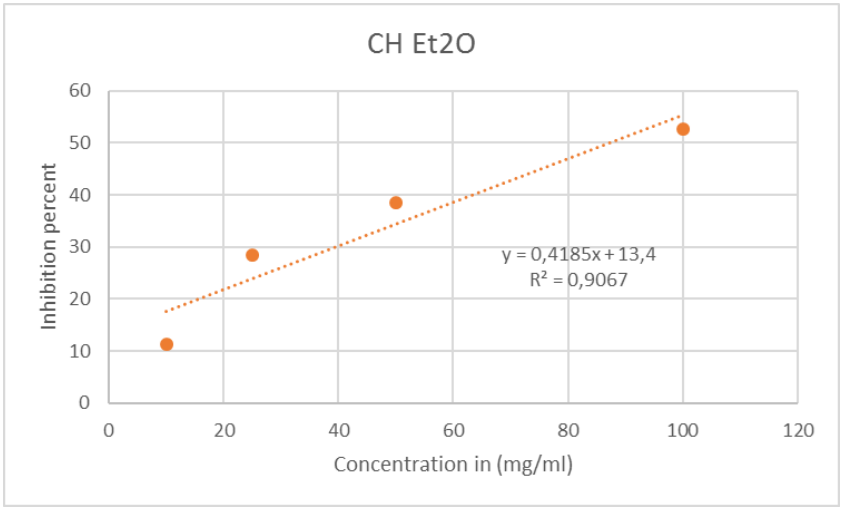

Figure 15. IC50 of CH Et20 (DPPH activity)

x = 87,45 mg/ml = IC50

3. Antibacterial results

The antibacterial activity results of the CH But and CH Hex extracts reveal interesting differences in their effectiveness against the tested bacterial strains. The CH But extract demonstrated moderate activity against *Staphylococcus aureus*, *Escherichia coli*, and *Klebsiella pneumoniae*, with inhibition zones of 2.5 cm, 2.5 cm, and 2.4 cm, respectively. However, its activity against *Pseudomonas aeruginosa* was notably weaker, with an inhibition zone of only 0.9 cm. This suggests that while CH But exhibits broad-spectrum antibacterial effects against Gram-positive (S. aureus) and some Gram-negative bacteria (*E. coli* and *K. pneumoniae*), it is significantly less effective against *P. aeruginosa*, which is known for its intrinsic resistance to many antimicrobial agents due to its robust cell wall and efflux mechanisms.

In contrast, the CH Hex extract showed generally lower inhibition zones compared to CH But, with 1.5 cm for S. aureus, 1.2 cm for E. coli, and 0.75 cm for *K. pneumoniae* Interestingly, its activity against *P. aeruginosa* was the same as that of CH But (0.9 cm). This indicates that CH Hex has weaker antibacterial properties against most of the tested strains but may share a similar limited efficacy against *P. aeruginosa*

The reduced activity of CH Hex compared to CH But could be due to differences in the bioactive compounds extracted in each solvent, with CH But possibly containing more potent antimicrobial agents.

Overall, these results suggest that CH But extract has broader and stronger antibacterial potential than CH Hex, particularly against *S. aureus*, *E. coli*, and K. pneumoniae. However, both extracts show poor activity against *P. aeruginosa*, highlighting its

resistance to these treatments. Further studies, including determination of minimum inhibitory concentrations (MIC) and phytochemical analysis, would be valuable to identify the active compounds and optimize their antibacterial efficacy.

Table 15. The results of the antibacterial activity

Strains	CH But	CH Hex
S. aureus	2,5 cm	1,5 cm
E. coli	2,5 cm	1,2 cm
K. pneumoniae	2,4 cm	0,75 cm
P. aeruginosa	0,9 cm	0,9 cm

Note :
For each strain, we performed two replicates, averaged the results (sum of the two divided by two)

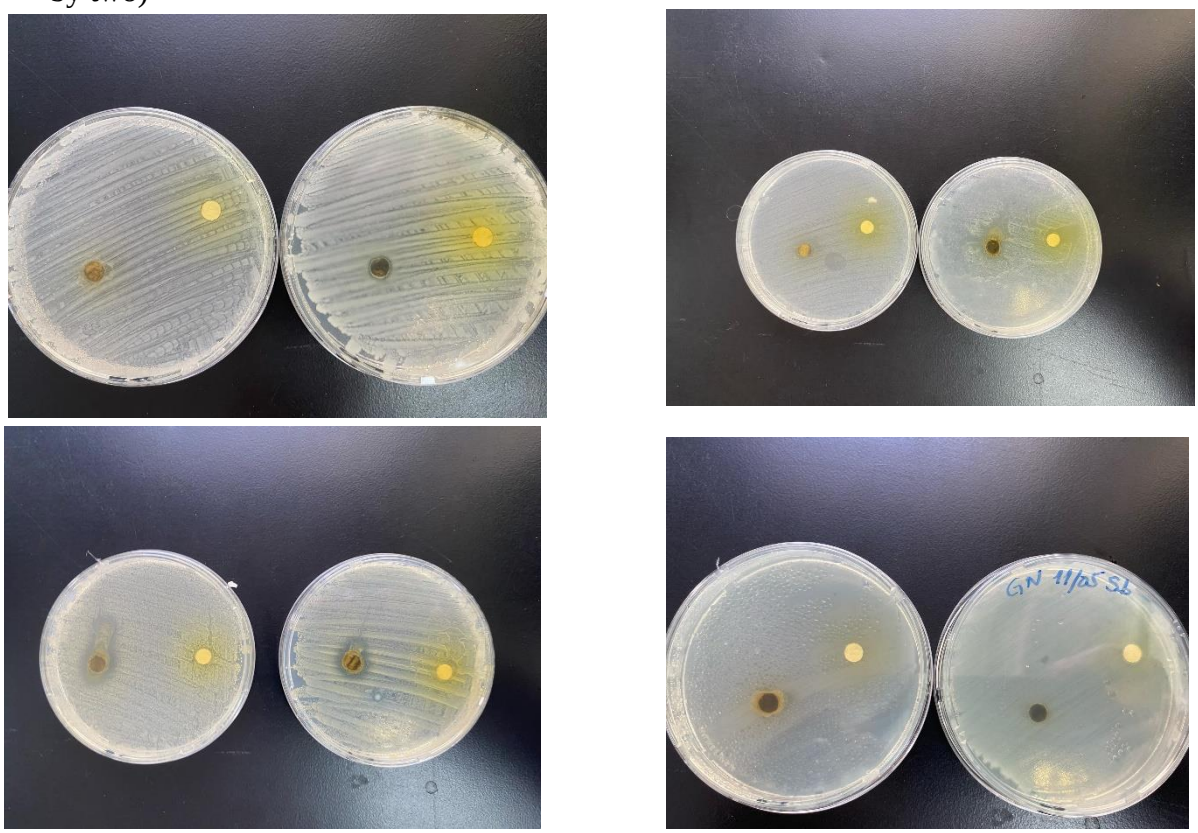

Figure 16. Inhibition zone of the four (04) strains

For a full assessment, statistical analysis (e.g., ANOVA) and comparison with standard antibiotics (like ampicillin) would help determine significance. However, based on raw inhibition zones :

≥ 2 cm = Good activity / 1–2 cm = Moderate activity / < 1 cm = Weak activity

Discussion

After this plant piqued our curiosity regarding its therapeutic potential, and considering the possible utilization of its properties in medicinal and pharmaceutical applications, we conducted a series of laboratory experiments. These investigations allowed us to address our primary research question and identify the potential benefits derived from its extracts. The extracts of *Centaurea hyalolepis* demonstrated that it possesses therapeutic properties as an anti-inflammatory, antioxidant, and antibacterial agent.

The results we obtained were positive, though not 100% conclusive, due to several influencing factors and varying experimental conditions such as the concentration used, measurement methods, and even the type of extract. Indeed, the results varied depending on the extract type.

I. Anti-inflammatory

The IC50 results revealed significant differences in potency among the tested extracts. CH Et2O (57 mg/ml) emerged as the most effective extract, exhibiting an ~18.6% stronger inhibitory effect than ibuprofen (70 mg/ml). Close behind was CH Aq (58 mg/ml), which was ~17.1% more potent than the control. These findings suggest that the compounds extracted by diethyl ether (Et2O) and water (Aq) possess superior anti-inflammatory properties compared to the standard drug. The CH But extract (62.5 mg/ml) also outperformed ibuprofen but to a lesser extent (~10.7% improvement), while CH Hex (70 mg/ml) showed activity equivalent to the positive control. This hierarchy of efficacy (CH Et2O > CH Aq > CH But > CH Hex = Ibuprofen) highlights the influence of extraction solvent polarity on bioactive compound recovery.

The observed trends align with the polarity-dependent extraction of anti-inflammatory compounds. CH Et2O (intermediate polarity) and CH Aq (polar) yielded the most potent extracts, implying that the active constituents may include moderately polar or polar molecules (e.g., phenolics, flavonoids, or glycosides). In contrast, the nonpolar CH Hex extract matched ibuprofen's IC50, suggesting that hexane primarily extracted less active lipophilic compounds. CH But (butanol, intermediate polarity) displayed moderate efficacy, possibly due to partial solubility of key anti-inflammatory agents. These results underscore the importance of solvent selection in phytochemical studies **(Harborne et al., 1998) (Cowan et al., 1999).**

The superior performance of CH Et2O and CH Aq positions them as promising candidates for further investigation. Future studies could : Isolate and identify the specific bioactive compounds responsible for the anti-inflammatory effects. Compare mechanisms of action (e.g., COX-2 inhibition, cytokine modulation) to ibuprofen. Assess toxicity to rule out cytotoxicity-driven inhibition. Test lower concentrations (<6.25 mg/ml) to refine IC50 precision **(Harborne et al., 1998) (Zhang et al., 2018).**

Additionally, comparing these results to literature on *Centaurea* species or related anti-inflammatory plants could contextualize their novelty. For instance, if other *Centaurea* extracts report IC50 values <50 mg/ml, our extracts may need optimization. Conversely, if your results surpass prior reports, this would highlight *C. hyalolepis* as a particularly rich source of anti-inflammatory agents **(Cos et al., 2006) (Funk et al., 2007).**

In summary, CH Et2O and CH Aq demonstrated notable anti-inflammatory potential, outperforming ibuprofen in this assay. The study not only validates *Centaurea hyalolepis* as a medicinal plant but also emphasizes the critical role of extraction solvents in unlocking bioactivity. Further pharmacological and phytochemical analyses are warranted to translate these findings into practical applications **(Koca et al., 2009) (Formisano et al., 2011).**

The anti-inflammatory potency of our extracts (IC_{50} values of 57–70 µg/mL) can be contextualized as moderate to strong when compared to other *Centaurea* species studied in the literature. For instance, the hexane extract of *C. acaulis* demonstrated exceptional activity with an IC_{50} of 0.76 µg/mL in a protein denaturation assay, significantly outperforming our results **(Benhamidat et al., 2022).** However, this discrepancy may reflect differences in assay sensitivity or extract composition, as protein denaturation tests may respond differently than COX inhibition assays. Similarly, a chloroform fraction of C. athoa exhibited potent NF-κB inhibition with an IC_{50} of ≈6 µg/mL **(Erel et al., 2014)**, suggesting that certain *Centaurea* species may have stronger effects in specific inflammatory pathways. Other studies, such as those by **(Csupor et al., 2013)** and **(Koca et al., 2009)**, reported strong anti-inflammatory effects but did not provide quantitative IC_{50} values, making direct comparisons challenging.

The variability in anti-inflammatory potency among *Centaurea* species may arise from several factors. First, the choice of extraction solvent plays a critical role methanol (used in our study) tends to extract more polar compounds like phenolics and flavonoids, whereas non-polar solvents such as hexane or chloroform may concentrate terpenes or fatty acids,

which could exhibit different bioactivities. Second, the plant part used for extraction influences the results ; for example, roots (e.g., *C. acaulis*) often contain higher concentrations of certain anti-inflammatory compounds compared to aerial parts. Lastly, the type of assay employed (e.g., COX inhibition, NF-κB suppression, or protein denaturation) may yield varying responses to the same extract, further complicating cross-study comparisons **(Wagnar et al., 2009) (Heinrich et al., 2018).**

Bioactive compounds identified in other *Centaurea* species provide insight into potential anti-inflammatory mechanisms. For instance, *C. pichleri* subsp. pichleri's methanol extract, rich in phenolics (~98 mg GAE/g), exhibited strong inhibition of NO, TNF-α, and PGE_2 in LPS-stimulated macrophages **(Çiçek Sezer et al., 2022).**

Similarly, hexane and chloroform fractions of *C. sadleriana* contained linolenic acids and flavonoids, which were linked to marked COX-1 and COX-2 inhibition **(Csupor et al., 2013).** These findings suggest that the anti-inflammatory effects observed in our extracts may also be attributed to phenolics, flavonoids, or other related compounds, though further phytochemical analysis would be needed for confirmation. Overall, the existing literature underscores the therapeutic potential of *Centaurea* species while highlighting the importance of extraction methods, plant parts, and assay selection in evaluating their bioactivity.

II. Antioxidant

The antioxidant activity of the tested extracts (*Centaurea hyalolepis* butanol (CH But), hexane (CH Hex), aqueous (CH Aq), and diethyl ether (CH Et_2O) fractions) was compared to that of ascorbic acid, a well-known antioxidant used as a positive control. Ascorbic acid exhibited the strongest antioxidant effect with an IC_{50} of 40 mg/mL, indicating its high potency in scavenging free radicals. Among the *C. hyalolepis* extracts, the aqueous fraction (CH Aq, IC_{50} = 69 mg/mL) showed the highest antioxidant activity, approaching (though still weaker than) ascorbic acid. This suggests that polar compounds extracted in water, such as phenolic acids or flavonoids, may contribute significantly to the antioxidant properties of *C. hyalolepis*

The butanol (CH But, IC_{50} = 76 mg/mL) and diethyl ether (CH Et_2O, IC_{50} = 74 mg/mL) fractions displayed slightly lower but comparable antioxidant activities, implying that intermediate-polarity compounds (e.g., glycosylated flavonoids or certain terpenoids) may also play a role in radical scavenging. In contrast, the hexane fraction (CH Hex, IC_{50} = 85

mg/mL) was the least effective, which aligns with expectations since nonpolar solvents typically extract fewer phenolic antioxidants and more lipids or nonpolar terpenes, which are generally less active in standard antioxidant assays like DPPH **(Prior et al., 2005).**

These results suggest that the antioxidant capacity of *C. hyalolepis* is influenced by the polarity of the extraction solvent, with more polar fractions (aqueous > butanol ≈ diethyl ether > hexane) exhibiting stronger activity. However, none of the extracts matched ascorbic acid's potency, reinforcing its status as a highly efficient reference antioxidant. Further phytochemical analysis could help identify the specific compounds responsible for the observed effects, particularly in the aqueous fraction, which showed the most promise **(Kumar et al., 2013).**

The antioxidant activity of *Centaurea hyalolepis* extracts, as measured by DPPH radical scavenging, shows varying potency when compared to other *Centaurea* species. Our most active extract, the aqueous fraction (CH Aq, IC_{50} = 69 µg/mL), demonstrates stronger activity than the n-butanol fraction of *C. parviflora* (IC_{50} ≈59 µg/mL) and approaches the potency of the ethyl acetate extract of *C. resupinata* (IC_{50} ≈36 µg/mL). This suggests that *C. hyalolepis* may contain similarly effective phenolic compounds, such as flavonoids like apigenin or quercetin derivatives, which have been identified in other *Centaurea* species as key contributors to antioxidant activity. However, our extracts are less potent than the methanol extract of *C. pichleri*, which had the highest phenolic content (~98 mg GAE/g) **(Cicek Sezer et al., 2022)**, and exhibited the strongest reported antioxidant effects, though its exact IC_{50} was not specified. This difference may be due to variations in solvent polarity or the specific phenolic composition of the extracts.

Interestingly, our hexane fraction (CH Hex, IC_{50} = 85 µg/mL) aligns with findings from *C. acaulis* and *C. pullata*, where non-polar hexane extracts also displayed notable antioxidant activity despite their low polarity **(Benhamidat et al., 2022).** This effect was attributed to sesquiterpenes like aplotaxene, suggesting that *C. hyalolepis* may similarly contain non-polar antioxidants alongside its phenolic compounds. The intermediate activity of our butanol (CH But, IC_{50} = 76 µg/mL) and diethyl ether (CH Et_2O, IC_{50} = 74 µg/mL) fractions further supports the idea that both polar and non-polar compounds contribute to the plant's overall antioxidant capacity **(Tepe et al., 2006).**

The variability in antioxidant potency across studies can be attributed to several factors. First, the choice of extraction solvent plays a crucial role, methanol and ethyl acetate, used in studies on *C. resupinata* and *C. pichleri*, tend to extract more polar phenolics, resulting

in stronger DPPH scavenging. Our extracts, obtained using solvents of varying polarities, may reflect a balance between polar and non-polar bioactive compounds. Second, differences in phytochemical composition, such as the presence of specific flavonoids (e.g., rutin, chlorogenic acid derivatives) or sesquiterpenes, can significantly influence antioxidant activity. For instance, the high activity of *C. resupinata*'s ethyl acetate extract was linked to isolated flavonoids like apigenin and quercetin derivatives. Finally, methodological variations in DPPH assay conditions (e.g., concentration, incubation time) may also contribute to discrepancies in reported IC_{50} values **(Apak et al., 2016).**

To further validate our findings, future studies could employ multi-assay approaches, such as FRAP or ABTS, alongside LC-MS profiling to identify the specific compounds responsible for the observed antioxidant effects. Additionally, refining the dose-response testing to include lower concentrations (e.g., 1–50 µg/mL) might reveal more potent activity, as seen in *C. resupinata*. Overall, while *C. hyalolepis* extracts demonstrate promising antioxidant activity, their potency relative to other *Centaurea* species highlights the importance of extraction methods, phytochemical composition, and assay conditions in evaluating plant-derived antioxidants **(Floegel et al., 2011).**

III. Antibacterial

The antibacterial activity of *Centaurea hyalolepis* extracts, CH But (butanol extract) and CH Hex (hexane extract), was evaluated by measuring the inhibition zones against four bacterial strains: *Staphylococcus aureus*, *Escherichia coli*, *Klebsiella pneumoniae*, and *Pseudomonas aeruginosa*. The results revealed distinct differences in effectiveness between the two extracts.

CH But demonstrated stronger antibacterial activity compared to CH Hex against most tested strains. For *S. aureus*, CH But produced a 2.5 cm inhibition zone, while CH Hex showed only 1.5 cm, indicating that CH But was 1.67 times more effective. A similar trend was observed for *E. coli*, where CH But (2.5 cm) outperformed CH Hex (1.2 cm) by approximately 2.08 times. The most significant difference was seen with *K. pneumoniae* : CH But exhibited a 2.4 cm inhibition zone, whereas CH Hex had only 0.75 cm, making CH But 3.2 times more potent. However, against *P. aeruginosa*, both extracts showed equal activity, each producing a 0.9 cm inhibition zone.

Gram-positive vs. Gram-negative susceptibility was also notable. Both extracts were more effective against *S. aureus* (Gram-positive) compared to the Gram-negative strains

(*E. coli*, *K. pneumoniae*), suggesting that Gram-positive bacteria may be more susceptible to the plant's bioactive compounds. *P. aeruginosa*, known for its intrinsic resistance due to efflux pumps and biofilm formation, was the least affected, with neither extract showing superior performance **(Silhavy et al., 2010).**

Possible explanations for the differences include variations in the chemical composition of the extracts. CH But, being a more polar solvent, may have extracted antibacterial compounds like phenolics or flavonoids, while CH Hex, a non-polar solvent, likely extracted less effective or selective compounds such as fatty acids or terpenes. Further studies, such as minimum inhibitory concentration (MIC) assays and phytochemical analysis, could help identify the active components responsible for the observed effects **(Cowan et al., 1999).**

In conclusion, CH But exhibited broader and stronger antibacterial activity than CH Hex, except against *P. aeruginosa*, where both extracts were equally weak. These findings suggest that *Centaurea hyalolepis* has potential as a source of antibacterial agents, particularly against Gram-positive and some Gram-negative pathogens, but further research is needed to optimize its efficacy **(Ríos et al., 2005).**

The findings of previous studies on *Centaurea* species, including *C. hyalolepis*, *C. resupinata*, and *C. cyanus*, consistently demonstrate broad-spectrum antibacterial activity against both Gram-positive and Gram-negative bacteria. For instance, **(Ercan et al., 2025)** analyzed *C. hyalolepis* extracts (volatile oil by GC-MS and phenolic fraction by LC-MS) and observed significant antibacterial effects against strains such as *Bacillus megaterium*, *Staphylococcus aureus*, and *Pseudomonas aeruginosa*. Similarly, **(Ismail et al., 2024)** used a dichloromethane extract of *C. hyalolepis* and identified sesquiterpene lactones, particularly cnicin, as the major antibacterial compound, which also disrupted Acinetobacter baumannii biofilms. **(Bouzghaia et al., 2021)** reported that ethyl acetate and n-butanol extracts of *C. resupinata* inhibited both Gram-positive and Gram-negative bacteria, while **(Haziri et al., 2017)** found that various extracts of *C. cyanus*, including nonpolar solvents like diethyl ether, exhibited activity against *S. aureus*. These studies collectively highlight the antibacterial potential of *Centaurea* extracts, though the efficacy varies depending on the extraction method and bacterial strain.

When comparing these findings with our results, several key observations emerge. First, our study confirms the broad-spectrum activity reported by **(Ercan et al., 2025)** and **(Bouzghaia et al., 2021)**, as our extracts also inhibited both Gram-positive (*S. aureus*) and

Gram-negative bacteria (*E. coli*, *K. pneumoniae*, *P. aeruginosa*).

However, our inhibition zones were smaller, possibly due to differences in extraction methods, solvent polarity, or bacterial strain susceptibility. Second, like **(Haziri et al., 2017)**, we observed stronger activity against Gram-positive bacteria, particularly *S. aureus*, compared to Gram-negative strains. This aligns with the known resistance mechanisms of Gram-negative bacteria, such as their outer membrane, which limits the penetration of hydrophobic compounds. Third, the variability in extract efficacy was evident in our study, with the butanol extract (CH But) showing higher activity than the hexane extract (CH Hex), mirroring **(Ismail et al., 2024)**, who found that organic extracts like dichloromethane were particularly effective. This difference may stem from varying concentrations of active compounds, such as sesquiterpene lactones like cnicin. Finally, our extracts exhibited weak activity against *P. aeruginosa*, contrasting with **(Ercan et al., 2025)**, who reported strong inhibition. This discrepancy could be attributed to strain-specific resistance or lower concentrations of active metabolites in our extracts.

Several factors may explain the observed differences in antibacterial activity across studies. First, the extraction method plays a critical role ; our butanol and hexane extracts likely contain different bioactive profiles compared to the volatile oils analyzed by **(Ercan et al., 2025)** or the dichloromethane extract used by **(Ismail et al., 2024).** Second, compound specificity is important, as previous studies identified cnicin and phenolic compounds as key antibacterial agents. If our extracts contained lower concentrations of these compounds, the activity would naturally be weaker. Third, bacterial resistance mechanisms, particularly in *P. aeruginosa*, could explain the limited efficacy of our extracts. This pathogen is known for its efflux pumps and biofilm formation, which may have reduced the penetration and effectiveness of our extracts, as suggested by the consistently small inhibition zones (0.9 cm) observed. Overall, while our findings align with the broader literature on *Centaurea* species, the variations highlight the importance of extraction techniques, compound composition, and bacterial strain characteristics in determining antibacterial activity **(Poole et al., 2011).**

Conclusion

This study demonstrates that *Centaurea hyalolepis* methanol extracts exhibit moderate anti-inflammatory activity (IC_{50} 57–70 µg/mL), comparable to some reported *Centaurea* species but less potent than exceptional cases like *C. acaulis* (IC_{50} 0.76 µg/mL). These differences may stem from variations in extraction solvents (methanol vs hexane/chloroform), plant parts, or assay systems. Further purification of the extracts could enhance potency, particularly given the known role of phenolic compounds in anti-inflammatory effects, as seen in related species like *C. pichleri*.

In terms of antioxidant activity, the extracts showed significant DPPH scavenging capacity, though slightly weaker than the most active *Centaurea* species in the literature..

Regarding antibacterial activity, the extracts displayed inhibitory effects against *S. aureus* and *E. coli*, but limited activity against *P. aeruginosa*. The stronger effect of nonpolar extracts suggests a possible role of sesquiterpene lactones or similar compounds. However, the observed activity was weaker than some literature reports, possibly due to differences in extraction methods or bacterial strains.

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