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In order obtain a diploma of an Academic Master In biological sciences
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THEME

biological activity of *Ammodaucus leucotrichus* Coss. & Dur. collected from Oued Souf region

Presented by: DERKI Manar El Ones
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In front of the jury composed of:

President	Dr TLILI Mohammed Laid		**El-Oued University**
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بسم الله

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الحمد لله حبا وشكراً وامتناناً على البدء والختام لم تكن الرحلة قصيرة ولا ينبغي لها أن تكون ، لم يكن الحلم قريباً ولا الطريق كان محقوقاً بالتسهيلات لكني فعلتها ...

أهدي هذا النجاح لنفسي أولاً ، ثم الى كل من سعى معي لأتمام هذه المسيرة دمتم لي سنداً لا عمر له ...

إلى ركني العظيم في الحياة إلى من كلله الله بالهيبة والوقار إلى من أحمل اسمه بكل فخر إلى ذلك الرجل العظيم الذي بذل كل ما بوسعه مأمني الوحيد وفرحتي الدائمة
والدي الحبيب متعة الله بالصحة والعافية

إلى نبراس أيامي و وهج حياتي إلى معنى الحب والى معنى الحنان والتفاني إلى بسمة الحياة وسر الوجود الى من كان دعائها سر نجاحي وحنانها بلسم جراحي داعمي الأول ووجهتي التي استمد منها القوة والدتي الحبيبه متعها الله بالصحة والعافية.

إلى الأعمدة الثابته في الحياة ، الداعمين الساندين أرضي الصلبة وجداري المتين الى من مدت أياديهم في أوقات الضعف ويؤمنوا بشجاعتي مهما ضعفت وارتخيت واقفين خلفي
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منار الأنس

الاهداء

الذي يسر البدايات واكمل النهايات وبلغنا الغايات

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أهدي عملي هذا الى الذي وهبني كل ما يملك للوصول لبر الأمان لأحقق أحلامه قبل أن تكون أمالي الى الذي قدس العلم ، سهر الليالي وقاسى الجراح لأجلنا، لمن أراد لنا العلى أبي الغالي سندي وحبي الأول أطال الله في عمرك

الى نبع الحنان والعطاء الى صاحبة القلب الطيب من رعتني من نعومة اظافري الى يومنا هذا حق الرعاية ، الى من كانت دافعي الأول للعلم والسعي للعلي ملجئي الأول نبع الحنان أمي أجمل من رأت عيوني وأحب قلبي

جزاكم الله عني خير الجزاء

كما أهدي ثمرة جهدي الى من رافقني في هذا العمل المتواضع الذي ألجأ إليه كلما أضعت طريقي الأستاذ الفاضل " شويخ عاطف " شكرا على كل دقيقة من وقتك وهبتها لي رغم من تعدد مسؤولياتك

أهدي تخرجي الى من كانوا رفاقي طيلة هذه السنوات الى من تمنوا لي النجاح والتوفيق أختي حبيبتي ورفيقتي التي ساهمت معي في هذا العمل أهدي لهم ثمرة نجاحي

ولكل من تمنى لي النجاح والتوفيق في دراستي من عائلتي الكريمة وصديقات دربي لكل من كانوا جزءا لمسح دموعي وتخفيف الامي وفقكم الله

صفاء

Abstract :

This study investigates the effectiveness of flavonoids (Diethyl ether, ethyl acetate, butanol and aqueous fractions) from the Oum Diriga plant (*Ammodaucus leucotrichus* Coss. & Dur.) collected from Oued Souf region as an intidepression, analgesic, antioxidant and anti-inflammatory activities.

In intidepression tests, we studied through In Vivo tests that include two main tests. The first is the tail suspension test, and the second is the forced swim test. The Diethyl ether and butanol extracts (40 mg/mL) showed a more effective than the positive control, where the duration of immobility was estimated at 10 seconds. In addition, diethyl ether extract (40 mg/ml) and in ethyl acetate extract (20 mg/ml) showed effectiveness in relieving pain. Based on the results of the hot plate test and the writhing test, the duration of pain tolerance were estimated as 26 seconds in hot plat test, while the percentage of inhibition was recorded as 84.55% in Writhing test at butanol extracts at a concentration of 40 mg/mL.

In vitro the Antioxidant, anti-inflammatory activities, the ethyl acetate extract showed significant antioxidant activity in the DPPH and FRAP tests, where the results were very close to the ascorbic acid standards, with an IC_{50} value of 0.06 mg/mL and an EC_{50} value of about 0.0352 mg/mL. In the total antioxidant capacity test, the diethyl ether extract showed the highest value with the average estimated at (11 mg E As A\mg Ex)

In the hemolysis test, the best IC_{50} value of the ethyl acetate extract estimated at 0.38 mg/mL. In addition, the Ethyl Acetate extract has proven to be a high sun protection factor estimated 145.

As for the anti-inflammatory activity through egg albumen denaturation test, the Butanol extract it showed an effectiveness of IC_{50} estimate of 0.18 mg/mL.

Key Words: *Ammodaucus leucotrichus* Coss. & Dur., Flavonoids, Intidepression, Analgesic, Antioxidant activity, Anti-inflammatory activity.

الملخص:

تبحث هذه الدراسة في فعالية مركبات مستخلصات أطوار الفلافونويد (ثنائي إيثيل الأثير، أسيتات الإيثيل، البيوتانول والطور المائي) من نبات أم دريقة *Ammodaucus leucotrichus* Coss. & Dur. النامي في منطقة وادي سوف كمضاد للاكتئاب ومسكن للألم ومضاد للأكسدة وللالتهابات.

في اختبارات مضادات الاكتئاب، درسنا من خلال اختبارات *In Vivo* التي تتضمن اختبارين رئيسيين. الأول هو اختبار تعليق الذيل، والثاني هو اختبار السباحة القسرية. أظهرت مستخلصات ثنائي إيثيل إيثر والبيوتانول (40 ملغم/مل) فعالية أكبر من السيطرة الإيجابية حيث قدرت مدة عدم الحركة بـ 10 ثواني. بالإضافة إلى ذلك، أظهر مستخلص ثنائي إيثيل إيثر (40 ملغم/مل) ومستخلص أسيتات الإيثيل (20 ملغم/مل) فعالية في تخفيف الألم. بناءً على نتائج اختبار الصفيحة الساخنة واختبار الالتواء قدرت مدة تحمل الألم بـ 26 ثانية في اختبار اللوح الساخن، بينما سجلت نسبة التثبيط 84.55% في اختبار الالتواء عند مستخلصات البيوتانول بتركيز 40. ملغم/مل.

في المختبر، أظهرت الأنشطة المضادة للأكسدة والمضادة للالتهابات، أن مستخلص أسيتات الإيثيل نشاط مضاد للأكسدة كبير في اختبارات DPPH وFRRAP، حيث كانت النتائج قريبة جدًا من معايير حمض الأسكوربيك، مع قيمة IC_{50} تبلغ 0.06 مجم / مل وقيمة EC_{50} حوالي 0.0352 ملغم / مل. في اختبار القدرة الكلية لمضادات الأكسدة أظهر مستخلص ثنائي إيثيل إيثر أعلى قيمة بمتوسط قدره (11 ملغم E As A\mg Ex)

في اختبار انحلال الدم، أفضل قيمة IC_{50} لمستخلص خلات الإيثيل تقدر بـ 0.38 ملجم/مل. بالإضافة إلى ذلك، أثبت مستخلص إيثيل أسيتات أنه عامل حماية عالي من أشعة الشمس يقدر بـ 145.

أما بالنسبة للنشاط المضاد للالتهابات من خلال اختبار تمسخ زلال البيض، فقد أظهر مستخلص البيوتانول فعالية IC_{50} مقدرة بـ 0.18 ملغم/مل.

الكلمات المفتاحية: *Ammodaucus leucotrichus* Coss. & Dur.، الفلافونويدات، مثبطات الاكتئاب، مسكن، نشاط مضاد للأكسدة، نشاط مضاد للالتهابات.

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Sommaire

Results & Discussion

Abbreviation list

Acetate	Ethyl Acetate
AS	Ascorbic Acid
ASA	Aspirin
A ,leucotrichus	*Ammodaucus Leucotrichus*
BHT	Butylated Hydroxytoluene
Cox-2	Cyclooxygenase-2
DPPH	2,2-Diphenyl-1-Picrylhydrazyl
EC50	Half Maximal Effective Concentration
Ether	Diethyl Ether
FOS	fructooligosaccharides
FRAP assay	Ferric Reducing Antioxidant Power
FST	Forced Swim Test
GOS	galactose
HPLC	High-Performance Liquid Chromatography
IC50	Inhibitory Concentration Of 50%
i.p.	Intraperitoneal
MED	Medicine
NO	Nitric Oxide
SPF	Sun Protection Factor
TAC	Total Antioxidant Capacity
TOS	transgalactose oligosides
Vit	Vitamin

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Introduction

Introduction

The use of medicinal plants and their derivatives for nutritional and therapeutic purposes has been prevalent globally since ancient times. Today, approximately 80% of the world's population, particularly in developing countries, rely on herbal medicines to address their primary healthcare needs and manage various diseases. **(Djahafi et al., 2021)** Herbal medicines are known for their relatively low risk of causing allergic reactions and for providing milder, safer therapeutic effects. The primary criteria for selecting herbal raw materials as sources of pharmacologically active compounds include the abundance of active components, the availability of these materials in nature, and the simplicity of their cultivation methods. **(Viktor et al., 2022).** Plants contain chemicals that target various biological processes, creating complex interactions with organisms. The medicinal properties of plants arise from active substances found in their organs, such as alkaloids, flavonoids, glycosides, vitamins, tannins, and coumarin compounds. These substances exert physiological effects on human and animal bodies or exhibit biological activity against a range of pathogens. **(Ramirez et al., 2020; Shankar et al., 2019).**

There has been increasing interest in research on flavonoids from plant sources. Since flavonoids They are directly related to human dietary components and their health, structural and functional relationships must be evaluated. Flavonoid bioavailability, metabolism, and biological activity, It depends on the composition, total number of hydroxyl groups and substitution of the functional group in relation to its nuclear structure **(Mondal et Rahaman (2020).**

Apiaceae is a large global family of angiosperms with 466 known genera and approximately 3820 species **(Plunkett et al., 2018).** Among these plants is *Ammodaucus leucotrichus*, which is a widespread plant in the Algerian desert (El Oued). **(Alaoui et al., 2014).** In Algerian traditional medicine, *A. leucotrichus* is used to treat colds, fever,**(Velasco-Negueruela et al., 2006)** stomach ailments, vomiting, pain, allergies, etc **(Sadaoui et al., 2018).**

For this reason, this type of plant was chosen in our study, because it contains a high percentage of flavonoids fractions (Diethyl ether, ethyl acetate, butanol and aqueous fractions) for estimate the intidepression, analgesic, antioxidant, anti-inflammatory activities , hemolysis and sun protection factor .

This study diviseded in two parts:

- The first bibliographic section: provides an overview of flavonoids and the specific plant under study.
- The second part: It is divided into two parts: it presents the materials and methods used, highlights the in vitro and in vivo results that we obtained and discussed, and finally we concluded our work with a conclusion appended with recommendations.

Generality about Flavonoids

1. Definition

Flavonoids are a group of natural polyphenols found in vegetables, fruits, cereals, and teas. Their molecular structure is similar to that of flavone (from the Latin flavus; yellow) hencethe name "flavonoids". They were first discovered in 1936 by Albert Szent-Györgyi, who isolated a substance from lemon that had the power to cure diseases caused by the fragility of capillary blood vessels, called "vitamin P". This name was quickly replaced by "flavonoids" because they are not essential for life **(Heut, 1962).**

As secondary plant metabolites, they play an important role in many biological processes and in plant responses to environmental factors. Flavonoids, which are common in the human diet, have antioxidant effects as well as other biological activities, including antibacterial and anti-inflammatory effects, which can reduce the risk of disease. The biologicalfunctions of flavonoids are related to their potential cytotoxicity and their ability to interact withenzymes. This ability depends on the structural substitution patterns in their C6-C3-C6 rings **(Shen *et al*.,2022).**

2. Structure

Flavonoids are derivatives of the flavone or 2-phenyl chromone (Milane,2004) nucleuswith 15 carbon atoms (C6-C3-C6), consisting of two aromatic rings, designated by the letters A and B, linked by an oxygenated heterocycle, designated by the letter C (Dacosta., 2003), bearing free phenol, ether or glycoside functions. It is noted that the flavone nucleus itself is a derivative of the basic flavan nucleus.

The distribution of the different flavonoid families in the plant kingdom has been evaluated byseveral authors. (Tab. I.1).

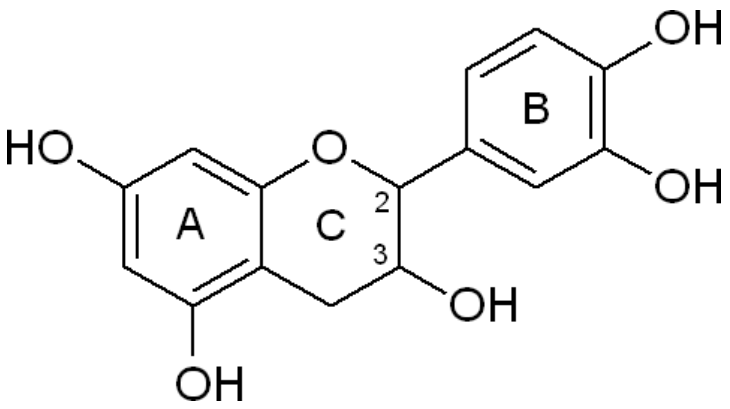

Figure 1. Structure of flavonoids (Coste, 2015).

Table 1. The distribution and Sources of the different flavonoid families in the plant kingdom

Family	**Main molecules**	**Distribution**	**References**
Flavone	Luteolin, Apigenin	Parsley, celery, rosemary, thyme (content = 5-100 mg/kg) Fruit skin (traces)	**Di Carlo et al., 1999; Fargeix, 2000; Heller and Forkmann, 1986; Hollman and Katan, 1999; Rice-Evans et al., 1996; Sampson et al., 2002)**
Flavanone	Hesperidin, Naringenin, Taxifolin	Citrus fruits (especially citrus) (content = 250-6000 mg/kg)	
Flavonol	Kaempferol, Quercetin, Myricetin	Radish, broccoli, black tea Onion, apple, olive, tomato (content = 56-250 mg/kg)	
Isoflavones	Genistein, Daidzein	Legumes (soybeans, black beans and green chickpeas), alfalfa and clover sprouts and sunflower seeds (content = 150-1500 mg/kg)	
Flavan-3-ols	Catechin and Epicatechin Epigallocatechin and their esters	Green and black tea mainly in white and green tea and to a lesser extent in black tea (content = 5-250 mg/kg)	
Anthocyanes	Cyanidin, Pelargonidin	Red and purple fruits and vegetables (apples, grapes, strawberries, raspberries, blackcurrants...) (100-4000 mg/kg)	

3. Flavonoid Biosynthesis

Several scientific studies have been conducted to elucidate the flavonoid biosynthetic pathways from a genetic point of view. Scientists have chosen to work on different plants such as corn (Zea mays), snapdragon (Antirrhinum majus), petunia (Petunia hybrida) and Arabidopsis (Arabidopsis thaliana), focusing on the isolation of genes involved in flavonoid biosynthesis.

Flavonoids are synthesized via the phenylpropanoid pathway, which converts phenylalanine into 4-coumaroyl-CoA. In the flavonoid biosynthetic pathway, 4-coumaroyl-CoA reacts with 3-malonyl-CoA in the presence of a specific enzyme, chalcone synthase (CHS). This enzyme triggers an enzymatic chain reaction that produces several chalcones, fromwhich all flavonoids are derived (Figure 2) **(Falcone Ferreyra et al., 2012).**

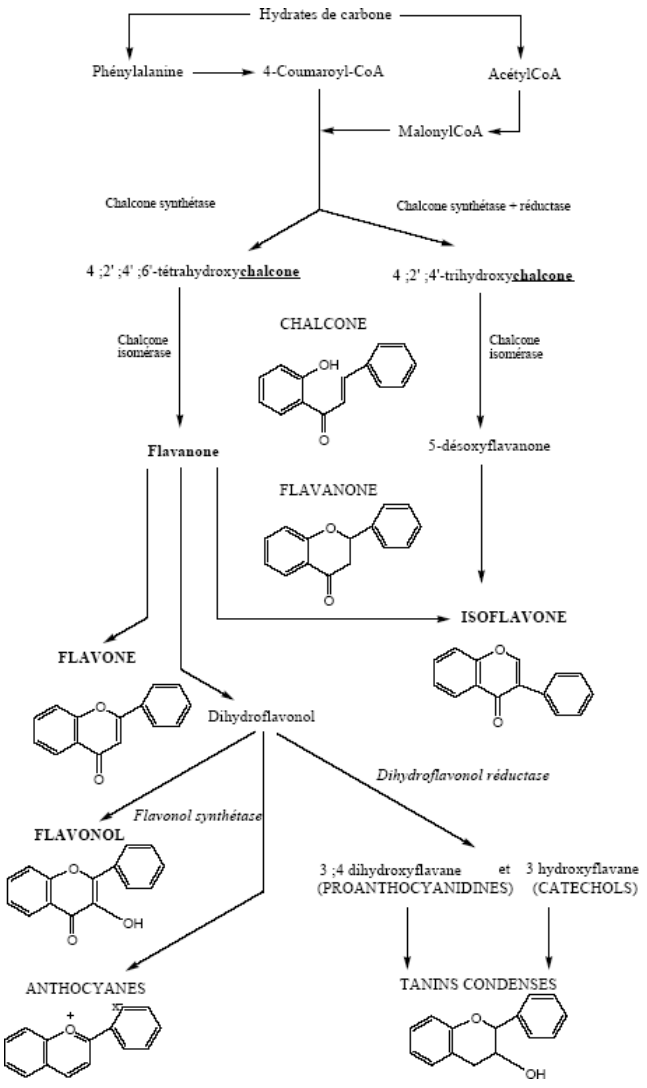

Figure 2. Biosynthesis of Flavonoids (Kebieche, 2009).

4. Classification

All flavonoids have a common biosynthetic origin and therefore have the same basic structural element. They can be grouped into different classes according to the degree of oxidation of the central pyran ring, the B ring linked to the heterocycle C in positions 2, 3 (SeeFigure 03).

- In position 2:The flavonoid is called Flavane.
- If the 4 position of the flavane bears a carbonyl group, the flavane is called Flavanone.
- If the C2-C3 bond in the flavanone skeleton is unsaturated, the compound is namedFlavone.
- If the skeleton is substituted in position 3 by a hydroxyl group, it is designated by thename Flavonol.
- In position 3: The flavonoid is designated by the term Isoflavane **(Athamena, 2009).**

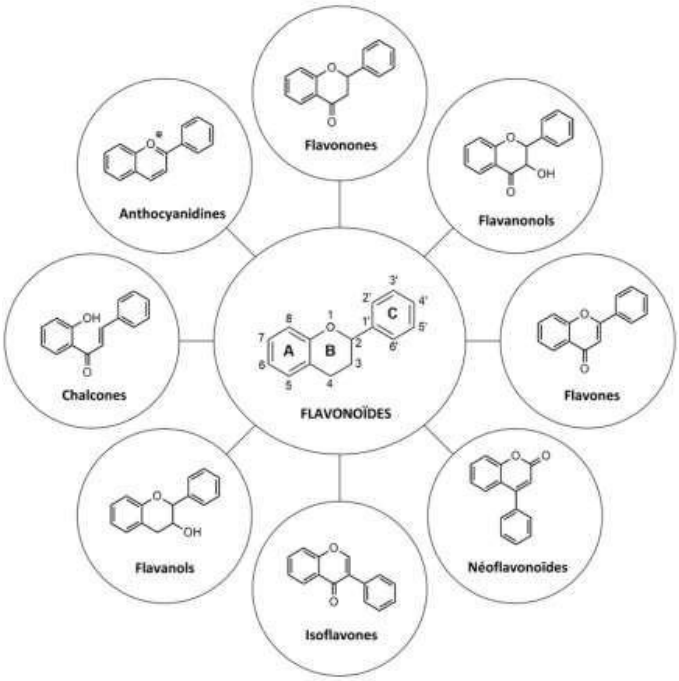

Figure 3. Different classes of flavonoids (Rousserie, 2019).

5. Biological Activity

Flavonoids have many activities: antioxidants, anti-inflammatories, enzyme inhibitors, and prevention of cardiovascular diseases. **(Hadj, 2009)** A synthesis of some

studies concerning the action of flavonoids for the prevention of diseases is presented in Table 02.

Table 2. Biological Activity of Flavonoids

References	**Flavonoids**	**Activities**
Mercader et al 2008	Flavonoids	Inhibition of aldolase reductase: prevention of catarate formation in diabitics
Cushnie and Lamb 2005	Different types of flavonoids (flavones, isoflavones, flavonols)	Antifungal, antiviral, antibacterial activity
Ziaee et al 2009	Rutin	Inhibition of hypercholesterolemia in mice on a hypercholesterolemic diet
Hooper et al 2008	Different subclasses of flavonoids and flavonoid-rich foods (chocolate, black tea, green tea, soy, cocoa)	Decrease in LDL level, decrease in blood pressure
Arts et al 2001	Catechin	Prevention of death from ischemic heart disease
Nakagawa et al 2000	Quercetin, rutin	Limitation of lipid peroxidation in lysosomal fractions. Antioxidant activity due to its localization in membranes.
Hurano et al 2001	Catechin, epicatechin, epigallocatechin, epicatechin gallate, epigallocatechin gallate, myricetin, quercetin, apigenin, kaempferol, and luteolin	DPPH radical inhibition, LDL oxidation inhibition.
Kosmider et al 2004	Different classes of flavonoids	Antitumor activity

Ammodaucus leucotrichus Coss. & Dur.

1. Distribution, Habitat and Botanical Description

Ammodaucus leucotrichus (*A. leucotrichus*) is a medicinal plant belonging to the Apiaceae family, formerly known as Umbelliferae (**Velasco-Negueruela *et al*., 2006).**

It is a glabrous annual plant with erect, branched and finely striated stems. The leaves aremuch divided into narrow, slightly fleshy strips with umbels of 2-4 rays, involucres with much divided bracts. The flowers are white, equal. The mericarps are elongated with secondary ribs covered with very dense, curly, yellow-red silky hairs at the base and then white and 8 to 10 mm long. This plant has a strong smell of aniseed. It is quite common throughout the Saharawhere it is highly appreciated and gathered, which tends to make it rare (Figure 4) **(Ozenda, 1991).**

The systematic classification of A. leucotrichus is as follows **(Quezel and Santa, 1963):**

- **Division:** Spermatophytes
- **Subdivision:** Angiosperms
- **Class:** Eudicots
- **Subclass:** Asterids
- **Order:** Apiales
- **Family:** Apiaceae
- **Genus:** Ammodaucus
- **Species:** *Ammodaucus leucotrichus* Coss. & Dur.

Figure 4. ***Ammodaucus leucotrichus*** **in Natural habitat (Idm'hand** ***et al*****., 2020).**

A. leucotrichus is known under the name "Kûmun-Sûfi or akâman" in most Arab-African countries, while in Algeria, it is generally called "Moudrayga" and in French it is called "Cumin chevelu" **(Idm'hand** ***et al*****., 2020).** Its English name is "Hairy Cumin" **(Velasco- Negueruela** ***et al*****., 2006).**

The geographical area of this so-called Saharan species extends from the Atlantic to Egypt. Its entire distribution is based in North Africa (Algeria, Morocco, Tunisia, Libya) and extends to Egypt and tropical Africa **(Manssouri** ***et al*****., 2020).**

2. Traditional Therapeutic Uses of *Ammodaucus leucotrichus* Coss. & Dur.

A. leucotrichus is a plant widely used in traditional medicine in North African countries, particularly in the southern Sahara of Algeria. The seeds of this plant are used to treat diseases related to the digestive system and to relieve stomach and liver pain. The leaves are also used to relieve chest pain. In the Tassili region of Algeria, the plant studied is mainly used in the form of powder or infusion to treat the symptoms mentioned above. In the same region, a mixture of seeds of the plant A. leucotrichus with milk or millet is used to regain appetite or avoid indigestion. The leaves are used to flavor tea. In powder form, it is considered a highly prized spice in the Djanet region **(Louail et al., 2016).**

Ammodaucus leucotrichus is a medicinal plant used by Saharan populations. The

main uses are against: stomach aches, indigestion, diarrhea, vomiting, spasms and colic, intestinal worms and constipation. The plant is also used to treat allergy symptoms (DIDI et al., 2003; HAMMICHE and MAIZA, 2006). In North Africa, the fruits are used as a condiment and in traditional medicine. They are used to treat colds, fever and digestive disorders, particularly for children (ADAMS, 1995). A recent study by Kabbaj et al. (2012) reports that some indigenous populations in Morocco use the fruits of *Ammodaucus leucotrichus* to treat lung cancer in the form of a powder mixed with honey and administered orally. It is also used against cough, as an emmenagogue and against anorexia (HAMMICHE and MAIZA, 2006). *Ammodaucus leucotrichus* are used to treat heart palpitations (JOUAD et al, 2001). The genus Ammodaucus is among the most used genera in traditional pharmacopoeia (FLEURNTIN et al., 2002). And it is considered a spice or condiment (BELLAKHDAR and YOUNOS, 1993). Thus, different parts of the crushed leaves, flowers and fruits are added to meals for flavoring (HELLIS, 2005). The fruits are used in infusion or in powder form against vomiting. This plant has aphrodisiac properties (HAMMICHE and MAIZA, 2006).

3. Isolated secondary metabolites of *Ammodaucus leucotrichus* Coss. & Dur.

Despite the importance of the species *Ammodaucus leucotrichus* L. in traditional medicine, very few phytochemical studies have been reported on this species. Gas chromatography analysis of the essential oil extracted from the fruits of *Ammodaucus leucotrichus* L., showed the presence of two major compounds, D-limonene and perillaldehyde **(Abu Zarga et al., 2013 ; Halla et al., 2018; Idm'hand et al., 2020; Telli et al., 2016).**

Most phytochemical research on A. leucotrichus has focused on essential oils. Few scientificstudies have been conducted on non-volatile compounds. Recently, **Ziani et al., (2019)** described by HPLC-DAD-ESI/MSn, the phenolic compounds of decoctions and hydroethanolic fruit extracts of A. Leucotrichus L. Soluble sugars, fatty acids, organic acids and tocopherols were also characterized. The results of this study showed that malic acid was the main organic acid. Analysis of the tocopherol composition showed a dominance of α- tocopherol. The study also revealed the presence of 7 phenolic compounds; 2 phenolic acid derivatives and 5 flavones which are derivatives of apigenin and luteolin. These are apigenin- 6,8-C-diglucoside, luteolin-7-O-glucoside, di-O-caffeoylmalonylquinic acid, two isomers of luteolin-O-(malonyl-hexoside), di-O-caffeoyldimalonylquinic acid and apigenin-O-(acetyl- hexoside).

Muckensturm et al., (1997) reported the isolation of ammlactone-A as a new guaianolide lactone from the ethereal extract of fruits of the species *Ammodaucus leucotrichus* L. This in addition to some common monoterpenoids ((+) limonene, (+) perillaldehyde, (+) 3- hydroxypiperillaldehyde), (-) methyl perillate, (+) borneol angelate and (+)-7-decalactone).

4. Ethnopharmacological aspects of *Ammodaucus leucotrichus* Coss. & Dur.

4.1.Anticoagulant Activity

Anticoagulants are used to prevent and treat serious thrombotic events. The most commonly used anticoagulants to date are heparin and its derivatives and vitamin K antagonists (VKAs). Numerous clinical studies have demonstrated their action in the prevention and treatment of thromboembolic complications **(Kortchinsky *et al*., 2013).**

The anticoagulant effect is defined as the inhibition of the formation of active thrombin in plasma, while the antithrombotic effect is defined as the inhibition of the formation of thrombus and/or its growth **(Colliec et al., 1990).**

Thrombosis (the most common vascular diseases that result from a complex interaction between circulating coagulation proteins, platelets, and the vascular wall) is a major cause of thromboembolic disorders affecting thousands of people worldwide **(MANSOOR, 2013).** It also causes ischemic heart disease, stroke, and traumatic injuries **(LANCE, 2015).**

Venous thrombosis occurs worldwide with an annual incidence of 1 per 1000 adults. The treatment of thrombosis uses thrombolytic agents with anticoagulant and antiplatelet activities**(SOUZA et al., 2015).**

Four distinct classes of sulfated polysaccharides (heparin, Dermatan sulfate, fucosylated chondroitin sulfate, and algal fucoidan) all have anticoagulant activity, due to their interactionwith enzymes and inhibitors of the coagulation system.

Their anticoagulant effects depend on an exact pattern of sulfate substitution. A small change in the structure leads to an almost complete loss of anticoagulant activity (**Mulloy *et al*., 2000)**.

Polysaccharides from higher plants also have heterogeneous chemical structures, but their actions in coagulation and thrombosis are poorly explored **(Souza *et al*., 2015)**.

MANSOOR,**(2013)** reports that the consumption of anticoagulants or food phytochemicals with anticoagulant properties can reduce or eliminate the risk of thromboembolic diseases. Anticoagulant activity of plant polysaccharides, associated with the presence of uronic acid residues, are reported **(Souza *et al.*, 2015)**. Heparin is a highly sulfated linear polysaccharide containing mainly a repeating disaccharide unit (α-D-glucosamine alternating with α-L- iduronic acid).

It is the most widely used polysaccharide clinically as an anticoagulant and antithrombotic, and sulfated polysaccharides isolated from marine algae and invertebrates constitute a complex group of macromolecules that are widely studied as anticoagulants and antithrombotics **(Souza *et al.*, 2015)**.

4.2.Prebiotic Activity

Prebiotics are non-digestible food ingredients that selectively stimulate the growth or activity of one or a limited number of bacterial groups in the colon that are likely to improve the host's physiology.

To date, the bacterial groups concerned are mainly bifidobacteria (in which case we speakof a bifidogenic effect) and other lactic acid bacteria (RAMBAUD et al., 2004).

Prebiotics exert several biological activities. Their effects are a change in the composition of SCFAs (short chain fatty acids) produced in the intestine, an increase in fecal weight, a reduction in pH and a modulation of the host's immune system (SAAD et al., 2013).

According to the Food and Agriculture Organization of the United Nations (FAO) and the recommendations of the World Health Organization (WHO) (FAO/WHO, 2002), probiotics are microorganisms provided by food that must survive the passage of the digestive tract whichthey colonize.

This means that they must have the ability to resist gastric juice and exposure to bile. Probiotics are stimulated in terms of growth by prebiotics which they catabolize into SCFAs.

The most commonly used microorganisms as probiotics belong to the heterogeneous group of lactic acid bacteria (Lactobacillus, Enterococcus,…) and to the genus Bifidobacterium(HOLZAPFEL et al., 2001 ;FAO/WHO, 2001).

Synbiotics are defined as the association of a probiotic and a prebiotic whose concomitantingestion could favor the multiplication of the former in the digestive tract (for example, an association of bifidobacterium or lactobacillus and fructooligosaccharides) (RAMBAUD et al.,2004).

The best known and already used prebiotics are fructans – fructooligosaccharides (FOS), oligofructose, inulin – and other galactose and transgalactose oligosides (GOS and TOS).

Many other carbohydrates could claim the name of prebiotics (xylooligosaccharides, isomaltooligosaccharides, gluco-oligosaccharides, etc.). Sugar alcohols could also have prebiotic properties (RAMBAUD et al., 2004).

4.3.Antidiabetic Activity

Yassir et al., (2016) evaluated the antidiabetic activity of the aqueous extract of *Ammodaucus leucotrichus* L. fruits in diabetic rats. Blood glucose levels were significantly reduced after 15 days of oral administration of the plant extract at a dose of 10 mg/kg. In addition, the aqueous extract of *Ammodaucus leucotrichus* L. fruit was able to show a beneficial effect on the histological structure of the liver and a remarkable influence on glucose tolerance.

4.4.Cytotoxic Activity

Ziani et al., (2018) tested the cytotoxicity of the aqueous and hydroethanolic extracts of the aerial part of the *Ammodaucus leucotrichus* L. species. The growth inhibitory activity of four human tumor cell lines (NCI-H460 (lung cancer), HeLa (cervical cancer), HepG2 (hepatocellular carcinoma) and MCF-7 (breast cancer)) was determined by the sulforhodamine B colorimetric assay. The results obtained showed a more interesting sensitivity of tumor cellsto the hydroethanolic extract than the aqueous extract.

4.5.Antimicrobial activity

The essential oil of the fruits of *Ammodaucus leucotrichus* L. was tested for its antimicrobial activity against various microorganisms, including Gram-positive and Gram-negative bacteria. The oil showed a strong inhibitory action against most of the tested organismswith minimum inhibitory concentrations ranging from 0.37 to 0.92 mg/ml (Louail et al., 2016). According to Dahmane et al., (2017), the essential oil of *Ammodaucus leucotrichus* L. exerted a broad antibacterial spectrum with a diameter of inhibition zones

ranging from 9.66 to 52.66 mm, while it was from 10.66 to 39.00 mm for the positive control.

4.6.Anticancer activity

The study of Lenzi, et al (2022). investigated the effects of a standardized ethanolic extract of *Ammodaucus leucotrichus* fruits and R-perillaldehyde (a compound found in the plant) on human cancer cell lines. The results showed that the extract and perillaldehyde could induce apoptosis (programmed cell death) in human lymphoblast cells (TK6). Apoptosis is a natural process that helps get rid of damaged or unwanted cells in the body. By inducing

apoptosis in cancer cells, *Ammodaucus leucotrichus* extract and perillaldehyde could potentially help to eliminate cancer cells.

Additionally, the study found that the extract and perillaldehyde seemed to protect thesecells from DNA damage caused by other compounds. DNA damage is a major factor in cancer development. By protecting cells from DNA damage, *Ammodaucus leucotrichus* might have genoprotective properties, helping to prevent the initiation and progression of cancer.

4.7.Anticholinesterase activities

The anticholinesterase activity of the Ammodaucus leucotrichus and the individual main compounds of the essential oil were compared to Donepezil, used as a standard drug against Alzheimer's disease. Essential oil and perillaldehyde have not exhibited an activity against the acetylcholinesterase. In contrast, limonene induced high acetylcholinesterase inhibitory activity with an IC50 of about 51.6 μg/ mL. Essential oil appeared to be effective compared to inhibition results of Donepezil. For anti-butyrylcholinesterase activity, all samples have a high inhibitory activity and the lowest value of IC50 was obtained with perillaldehyde followed by limonene and essential oil (Sadaoui, 2018)

4.8.Antioxidant activity

Louail et al., (2016) evaluated the antioxidant activity of the essential oil extracted from the fruits of *Ammodaucus leucotrichus* L. by measuring the inhibition of β-carotene bleaching, resulting from the oxidation of linoleic acid. The essential oil showed more interesting antioxidant activity than that shown by ascorbic acid. In another study, Dahmane et al., (2017) evaluated the antioxidant power of the essential oil of *Ammodaucus*

leucotrichus L. using the 2, 2-diphenyl-1- picrylhydrazyl (DPPH) test. The results obtained indicated a low antioxidant capacity to reduce DPPH radicals.

4.9.Anti-inflammatory activity

This study provides strong evidence that *Ammodaucus leucotrichus* has anti-inflammatory properties, supported by both in vitro and in vivo experiments. The extract seems to work by targeting various mechanisms involved in inflammation. In vitro studies suggest the extract can neutralize free radicals that contribute to tissue damage during inflammation. It may also inhibit enzymes that play a role in the inflammatory cascade, further reducing inflammatory processes. Additionally, the extract's ability to protect proteins from damage suggests it may help stabilize cells and tissues during inflammation.

The in vivo studies support these findings by demonstrating the extract's effectiveness inreducing inflammation in animal models. The extract's ability to reduce edema (swelling) caused by inflammatory agents and modulate inflammatory markers like nitrite and malondialdehyde suggests it has a multifaceted approach to inflammation. Furthermore, the modulation of catalase activity, an antioxidant enzyme, indicates the extract may also enhancethe body's natural anti-inflammatory defenses.

Materials & Methods

1. Materials

1.1.Plant materials

The plant (*Ammodaucus leucotrichus Coss. & Dur.)* and its seeds originated from the Oued Souf region in the Southeast of the Algerian Sahara. In this investigation, the plant material underwent specific preparation methods to achieve dryness. Initially, samples were collected and purchased when fresh from stores. Subsequently, the leaves were dried in shaded areas away from sunlight, allowing them to air dry thoroughly. The dried material was then stored in a sealed container until needed. As for the seeds, they were partially ground before direct utilization.

Figure 5. Location of *Ammodaucus leucotrichus Coss. & Dur.* on Google Map (34°10'55.2"N 7°18'14.5"E)

1.2.Animal materials

1.2.1. Animal and husbandry condition

-In this investigation, a group of male Wistar rats, aged 10 weeks and weighing between 120-190 grams, were obtained from the animal service of the Pasteur Institute in Algeria. They were housed in the animal facility of the Faculty of Natural and Life Sciences, University of Oued, Algeria. Rats underwent an adaptation period in a laboratory environment under controlled conditions of temperature (22.27 ± 0.52 °C), humidity (72 ± 2.01%), and a 12-h light/12-h dark photoperiod. Throughout the experiment, rats had access to standard diet and water. The study extended for 20 days. After the adaptation period, the rats were

divided into six experimental groups.

- Group 1 (Control -): consisted of therr normal rats that received 1mL of saline water via intraperitoneal (i.p.) injections.

- Group 2 (MED treatment, as control +): comprised three rats administered 10 mg/ml dose of MED solution prepared in saline water via intraperitoneal (i.p.) injections.

- Group 3 (ACETATE treatment, two different doses 20-40 mg/ml): included (3*2) rats receiving dose of ACETATE extract (1mL) via intraperitoneal (i.p.) injections.

- Group 4 (BUTANOL treatment, two different doses 20-40 mg/ml): consisted (3*2) of rats receiving dose of BUTANOL extract (1mL) via intraperitoneal (i.p.) injections.

- Group 5 (Diethyl ether treatment, two different doses 20-40 mg/ml): involved (3*2) rats receiving dose of Diethyl ether extract (1mL) via intraperitoneal (i.p.) injections.

- Group 6 (Aqueous treatment, two different doses 20-40 mg/ml): comprised (3*2) rats receiving dose of aqueous extract (1mL) via intraperitoneal (i.p.) injections.

Standards used in the study included:

- MED (Medicine)

- Sulpiride , Aspegic (Acetylsalicylic acid)

2. Methods

2.1.Extraction of flavonoids

Flavonoid extraction follows the protocol outlined by Markham (1982), with adjustments influenced by Bruneton's method (1993). The technique relies on the solubility of flavonoids in organic solvents (Figure 6). This extraction process involves four primary stages.

2.1.1. Methanolique extract preparation

Flavonoids extraction begins by utilizing finely ground dry matter with methanol solution of 85% purity (10% w/v). The first step involves preparing the 85% methanol solution by adding 90ml of distilled water to 510ml of 90% methanol. The extraction solution, methanolic, is then prepared by combining 600ml of 90% methanol with 60g of the dry plant

powder. Following a 72-hour maceration period at 4°C, the mixture is filtered using Whatman paper.

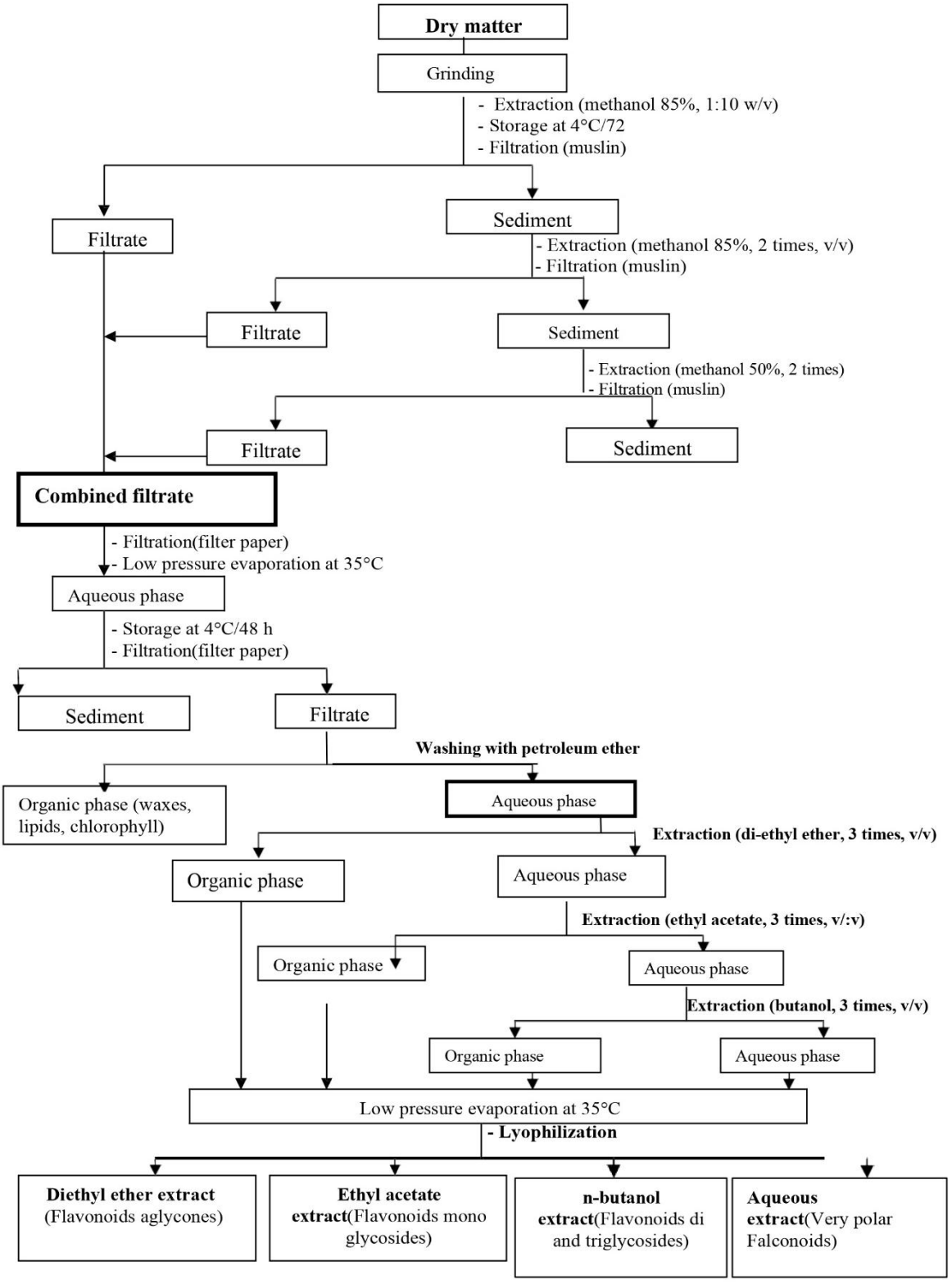

Figure 6. Protocol for Flavonoid Extraction. by Markham (1982), with adjustments influenced by Bruneton's method (1993).

2.2.In Vitro

2.2.1. Antioxidant activity

2.2.1.1. Free radical scavenging activity, DPPH assay

According to Chouikh & Alia (2021), a mixture containing 0.5 mL of various concentrations of plant extracts dissolved in methanol is combined with 0.1 mM DPPH solution after thorough mixing. These solutions are then incubated in the dark at room temperature for 15 minutes. Subsequently, the optical absorbance intensity is measured at a wavelength of 517 nm to assess the effectiveness of the studied extracts. Ascorbic acid serves as a reference compound. The inhibition rate is estimated following the method outlined by Chaouche and colleagues (2013) using the following relationship:

$$\text{DPPH scavenging-radical (\%)} = ((A0 - As) / A0) \times 100$$

while As: denotes the absorbance of the sample solution containing the test compound (Alia, 2022) .

2.2.1.2. Ferric Reducing Antioxidant Power assay (FRAP)

This test relies on the capacity of phenolic compounds to donate electrons, facilitating the reduction of ferric (Fe^{3+}) to ferrous (Fe^{2+}) ions. This interaction occurs through the following mechanism .

$$K_3[Fe(CN)_6] + FeCl_3 \xrightarrow{\text{Phenolic Compounds}} K_4[Fe(CN)_6] + FeCl_2$$

As per Ben Ali et al.(2023) the iron reductive capacity is assessed by combining 250µL of various concentrations of plant extracts with 625µL of phosphate buffer solution (pH: 6.6, 0.2M) and 625µL of K3[Fe(CN)6] (1%) following thorough mixing. The resulting mixture is then incubated in a water bath at 50°C for 20 minutes. Subsequently, 625µL of TCA (10%) is added, and the mixture is centrifuged at 3000 rpm for 10 minutes. Following centrifugation, 625µL of the supernatant is extracted and combined with 625µL of distilled water and 125µL

of FeCl3 (0.1%). The optical absorbance intensity of the mixture is measured at a wavelength of $\lambda = 700$ nm.

For comparison purposes, ascorbic acid served as a positive control, where an elevation in the optical absorbance intensity of the reaction mixture signifies an enhancement in the reductive potency. The recuperative potential of both the extracts and ascorbic acid was evaluated by computing the EC_{50} coefficient, which represents the concentration value corresponding to an absorbance intensity of 0.5 (Alia et al., 2021).

2.2.1.3. Determination of total antioxidant capacity (TAC)

The total antioxidant activity of different fractions of the extracts was assessed using the phosphomolybdate assay system, following the procedure outlined by Bousbia Brahim et al. (2022). Specifically, 150 µl of the extracts solution was combined with 1.5 ml of reagent solution containing sulfuric acid (6 M), sodium phosphate (28×10^{-3}M), and ammonium molybdate (4×10^{-3}M). The mixture was thoroughly mixed and then incubated for 1 hour in a water bath at 95°C. After cooling to room temperature, the absorbance values were measured at 695 nm to determine the mean absorbance.

The antioxidant capacity of the extracts was assessed and expressed as milligrams of ascorbic acid equivalents per gram of extracts (mg Ascrobic Acid E/g extract), as described by Khelef et al. (2019).

2.2.2. Hemolysis assay

This assay is employed to evaluate the capability of plant extracts in safeguarding erythrocytes from oxidative stress and free radical-induced damage or membrane disruption. It quantifies the percentage of dissolved erythrocytes following exposure to such stressors (Dolci et al., 2014).

According to Chouikh et al. (2018), 40 µL of human erythrocytes was combined with 2 mL of varying concentrations (ranging from 0.25 to 1 mg/mL) of the extract and incubated for 5 minutes at 37°C. Subsequently, 40 µL each of H_2O_2 (30×10^{-3}M), FeCl3 (80×10^{-3}M), and ascorbic acid solution (50×10^{-3} M) were added. Following a 1-hour incubation period at 37°C, the mixture underwent centrifugation at 700 rpm for 10 minutes. The absorbance of the supernatant was measured at $\lambda = 540$ nm. The percentage of hemolysis was determined using the following formula:

$$\text{Hemolysis}\% = (\text{Abs control}/\text{Abs sample}) \times 100$$

2.2.3. Sun Protection Factor SPF assay

The effectiveness of protection against UV rays is evaluated in vitro by determining the Sun Protection Factor (SPF) value through UV-Vis spectrophotometry, as outlined in Ben Ali et al. (2023). This factor is calculated by assessing the variance in spectroscopic readings of an alcohol solution (0.5 mg/ml) within the spectral range of 290 nm to 320 nm, with spectral transitions measured at 5 nm intervals.

$$\text{SPF Spectrophotometric} = CF \times \sum_{290}^{320} EE(\lambda) \times I(\lambda) \times Abs(\lambda)$$

Where:

- EE: represents the erythemal effect spectrum,

- I: denotes the solar intensity spectrum,

- Abs: signifies the absorbance of the sunscreen product, and

- CF: indicates the correction factor, which is equal to 10 (Alia et al., 2021).

2.2.4. Determination of Anti –inflammatory activity (Albumin denaturation test)

The anti-inflammatory properties of crude extracts from *Ammodaucus leucotrichus* Coss. & Dur. were assessed in vitro using the albumin denaturation model. This method involves the utilization of the egg albumen protein denaturation assay, as described by Chouikh et al. (2020). Specifically, 0.05 mL of egg albumen from fresh chicken eggs was combined with 0.7 mL of phosphate-buffered saline (pH: 6.4, concentration: 0.1 M), along with 0.5 mL of various concentrations of plant extracts .

The mixture was subjected to incubation at 37°C for 15 minutes followed by immediate placement in a water bath at 70°C for 5 minutes. After cooling, centrifugation was carried out at 3000 rpm for 10 minutes for both the plant extracts and ASPEGIC®. Subsequently, absorbance was measured at 660 nm.

ASPEGIC® was utilized as a positive control (reference drug). The percentage inhibition of protein denaturation was determined using the following formula:

Inhibition (%) = [(Abs control – Abs sample)/Abs control] × 100

Where in:

- Abs control represents the absorbance in the absence of the extract, and

- Abs sample denotes the absorbance in the presence of the plant extracts or the standard.

2.3.In vivo:

2.3.1. Antidepressant Activity

2.3.1.1. Forced Swim Test

In the Forced Swim Test (FST), male rats were individually placed in an open cylindrical container with dimensions of 10 cm in diameter and 25 cm in height, filled with water to a depth of 19 cm at a temperature of 25±1°C. Treatments were administered 60 minutes prior to the commencement of the test, according to the study design. During the test, all animals were compelled to swim for a duration of 6 minutes, and the period of immobility was observed and recorded during the final 4-minute interval. Immobility was defined as the cessation of struggling and remaining motionless in the water, with movements limited to keeping the head above water. A reduction in the duration of immobility suggests an antidepressant-like effect, following the methodology outlined by Porsolt *et al*. (1978).

2.3.1.2. Tail Suspension Test

The tail suspension method utilized in this study closely followed the protocol outlined by Cryan *et al*. (2005) Treatments were administered 60 minutes prior to the initiation of the study, as per the study design. Rats were suspended on the edge of a table, positioned 50 cm above the floor, using adhesive tape applied approximately 1 cm from the tail tip. The total duration of immobility induced by tail suspension was recorded over a 6-minute period within a total test duration of 10 minutes. Immobility was defined as the absence of body movement, with the animal hanging passively and entirely motionless.

2.3.2. Evaluation of the analgesic power

2.3.2.1. Hot-plate test

The hot-plate test was conducted according to the method described by Ferreira et al. (2002). On the experimental day, animals (n = 3) received intraperitoneal (i.p.) injections of 0.2 ml of normal saline (control group), paracetamol (10 mg/kg) as a positive control, and test samples (20 and 40 mg/kg) of the plant extracts for the experimental groups. After 30 minutes, the animals were placed onto a heated plate maintained at 55°C, and the time interval (in seconds) between placement and their hind paws' licking or jumping response was recorded as the latency period. The maximum allowable time for a response was set at 30 seconds. This reaction time was initially measured and then again at 60 and 90 minutes after injection.

2.3.2.2. Writhing test

The acetic-acid writhing test was utilized to evaluate the analgesic effects of the Flavonoid extract. Four sets of rat groups, each consisting of three rats (n = 3 per group), were subjected to acetic acid injections at a dose of 10 mL/kg body weight. Nociception levels were assessed by counting the total instances of writhing observed over a 30-minute period. Prior to the acetic acid injection, animals were administered two different doses of the plant extract (20 and 40 mg/kg body weight) or sterile saline (control group, 0.9%, w/v) with a 30-minute interval. Indomethacin (10 mg/kg body weight) served as a reference substance (positive control). Writhing episodes were recorded 5 minutes post-acetic acid injection and continued for 30 minutes, following the methodology outlined by Koster et al. (1959).

The degree of writhing inhibition was calculated using the following formula:

Percentage inhibition of writhing = [(Mean number of control - Mean number of test) / Mean number of control] × 100 (Chouikh, 2020).

Results & Discussion

1. Results

1.1.In Vitro

1.1.1. Antioxidant activity

1.1.1.1. Free radical scavenging activity, DPPH assay

The antioxidant potency of *Ammodaucus leucotrichus* was assessed using a DPPH• radical scavenging assay, with results indicating the following sequence of effectiveness: Aqueous extract > butanol extract > diethyl ether extract > acetate extract. Interestingly, the acetate extract exhibited the highest activity in scavenging DPPH radicals, showing a potency comparable to that of the standard Ascorbic acid (Vitamin C).

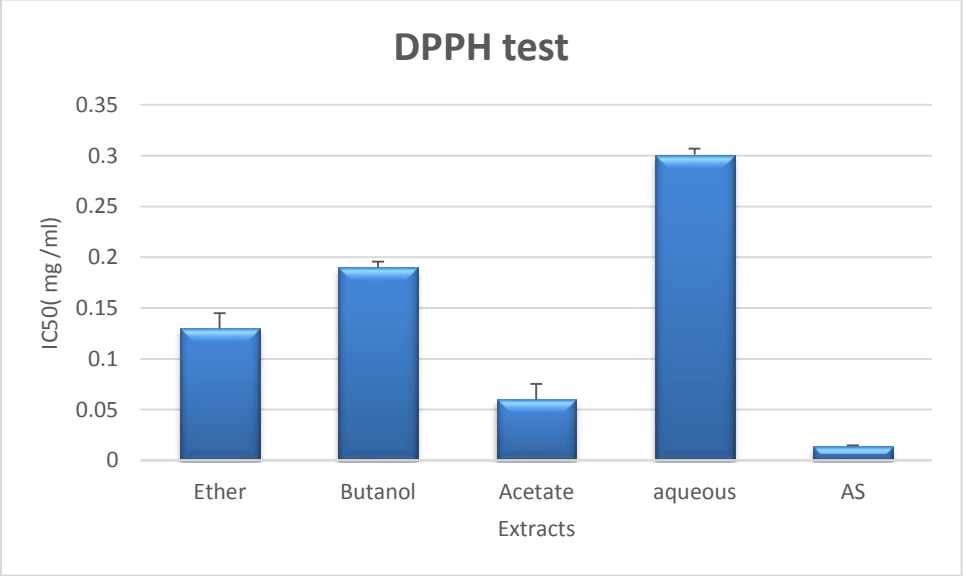

Figure 7. IC_{50} mg/ml value of DPPH radical scavenging activity

1.1.1.2. Ferric Reducing Antioxidant Power assay (FRAP)

The FRAP test assesses antioxidant activity, with the figure illustrating the antioxidant potency of the extracts through their EC50 levels. The aqueous extract exhibited a relatively higher EC50 value compared to the other extracts, suggesting lower efficacy in terms of antioxidant effects..

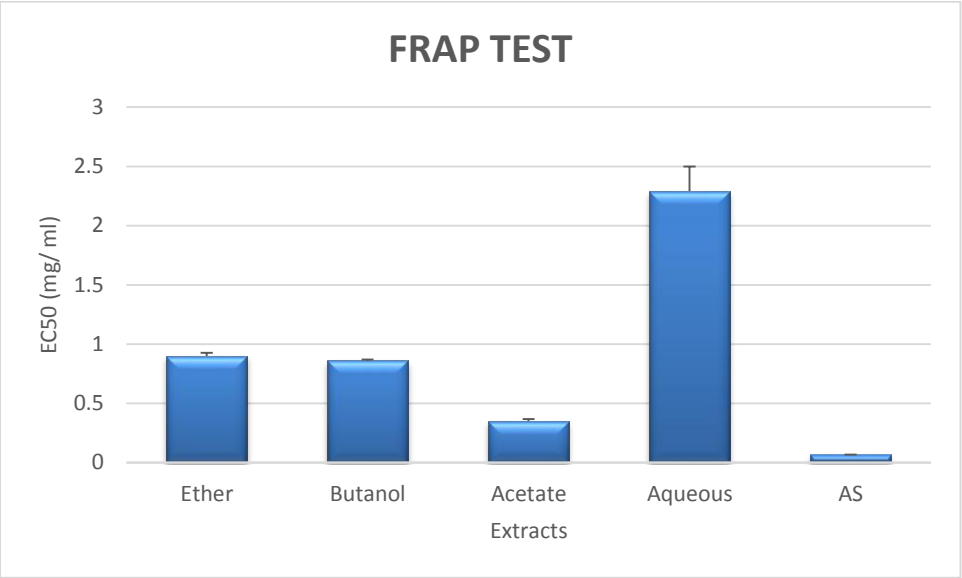

Figure 8. EC_{50} mg/ml values in FRAP assay

1.1.1.3. Determination of total antioxidant capacity (TAC)

The total antioxidant activity of the extracts was assessed to gauge their antioxidant capacities. The findings highlighted remarkably robust antioxidant capacity in the ether extract, in contrast to the weaker antioxidant capacities observed in the other extracts.

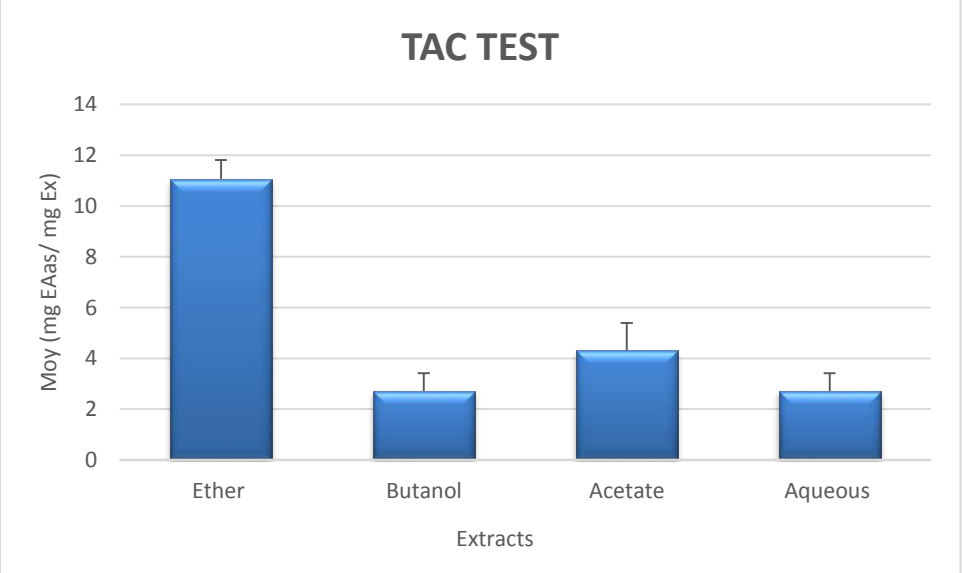

Figure 9. value of total antioxidant capacity test

1.1.2. Hemolysis assay

The results depicted in the figure illustrate the outcomes of the anti-hemolysis assay conducted on red blood cells. We observed anti-hemolytic activity in the diethyl ether extract (IC50: 0.5 mg/ml), aqueous extract (IC50: 0.8 mg/ml), and acetate extract (IC50: 0.4 mg/ml).

noteworthy , the butanol extract (IC50: 0.85 mg/ml) exhibited the highest effectiveness compared to the other.

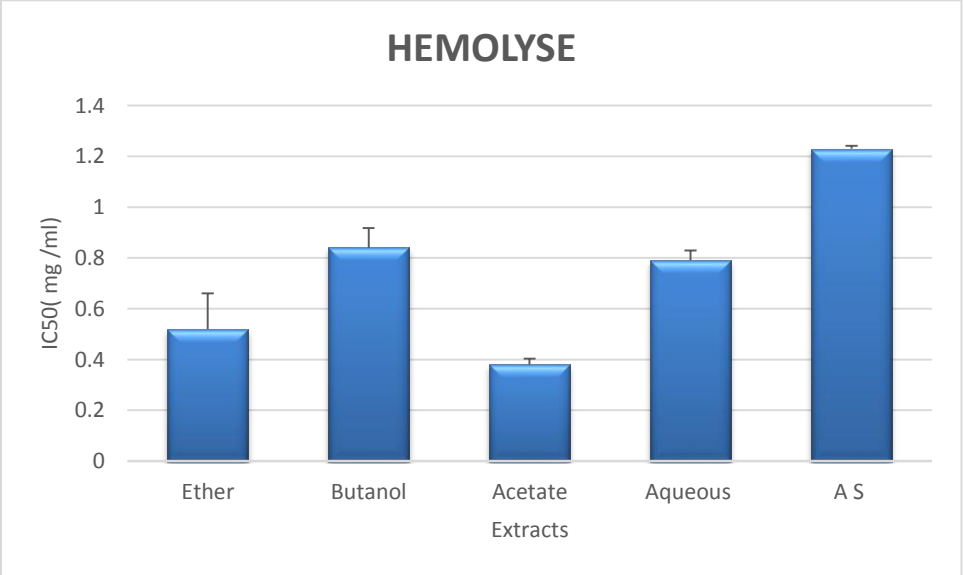

Figure 10. IC_{50} mg/ml values in Hemolysis assay

1.1.3. Sun Protection Factor (SPF) assay

Using the UV spectrophotometric method, SPF values for four extracts were determined. Analysis of the results depicted in the figure reveals that the acetate extract exhibited noticeably high protection coefficient compared to the other extracts.

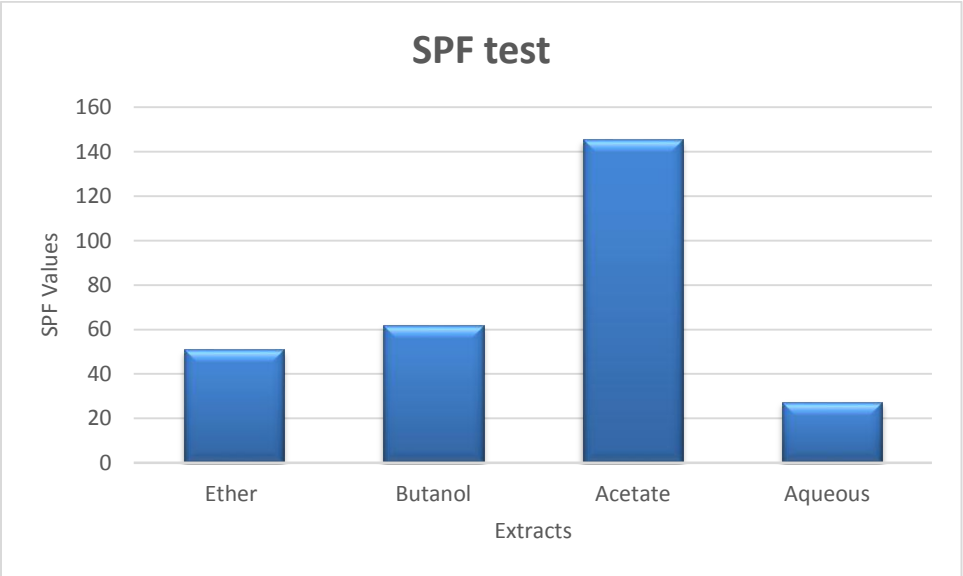

Figure 11. Sun protection factor values

1.1.4. Determination of Anti –inflammatory activity (Albumin denaturation test)

The results obtained in Figure (12) indicate that the butanol extract exhibited significantly higher anti-inflammatory efficacy compared to the other extracts, nearly reaching the effectiveness of diclofenac as indicated by its level.

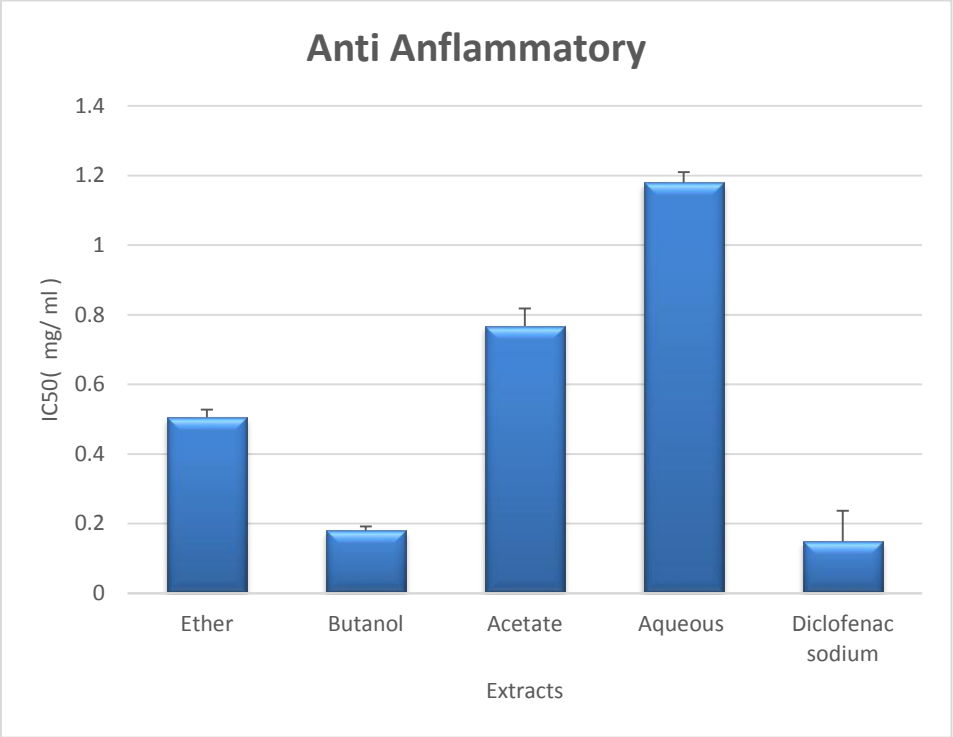

Figure 12. IC50mg/ml values in Anti –inflammatory activity

1.2.In vivo Tests:

1.2.1. Antidepressant Activity

1.2.1.1. Forced Swim Test

The findings from the forced swim test indicate promising antidepressant potential, notable in the significantly reduced immobility time. Concentration 40 mg/ ml consistently yielded superior results across all four extracts tested. Particulary the diethyl ether extract at concentration 40 mg/ ml exhibited the most robust effect, with an immobility duration as short as 10(S). This duration is notably three times shorter than the positive control, which lasted 30 (S) , underscoring a substantial decrease in immobility.

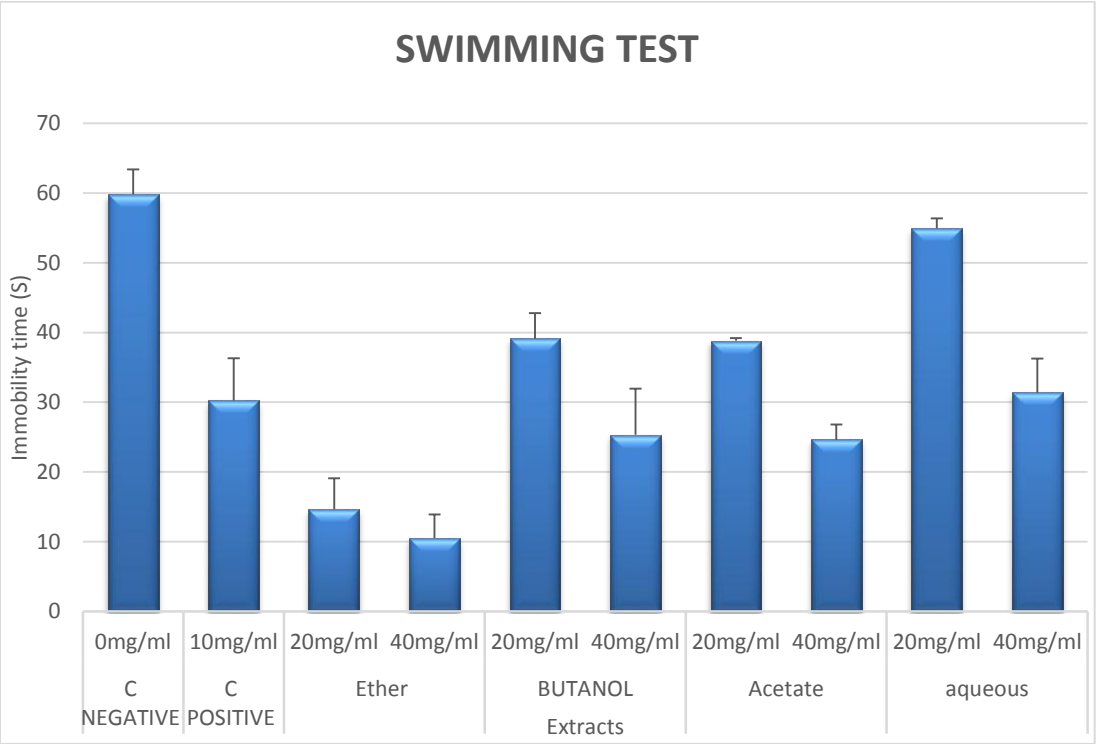

Figure 13. Duration of immobility (S) in the forced swim test

Table 3. P values of forced swim test

Extracts	C.POSITIVE	Ether diethyl		BUTANOL		Acetate		Aqueous	
consontration mg /ml	10	20	40	20	40	20	40	20	40
P value	0.00096043	8.334E-05	3.36E-05	0.00112199	0.0006896	0.0002791	6.69E-05	0.04780385	0.000628
	***	***	***	**	***	***	***	*	***

The table shows the results of the p values for the different extracts, as all extracts showed very differences significant in concentrations 20 and 40 ($P<0.001$). With the exception in the aqueous extract at a concentration 20 ($P\ 0.04<0,05$) there were less differences significant

1.2.1.2. Tail Suspension Test

The tail suspension test, which measures antidepressant activity by assessing the duration of immobility in animals, decreased immobility time indicates potential antidepressant effects. Across all four extracts, concentration 40 mg /ml proved effective in the four extracts. It is worth noting that the butanol extract at concentrations 40 and 20 mg /ml recorded the lowest value in immobility, which indicates the extent of its effectiveness.

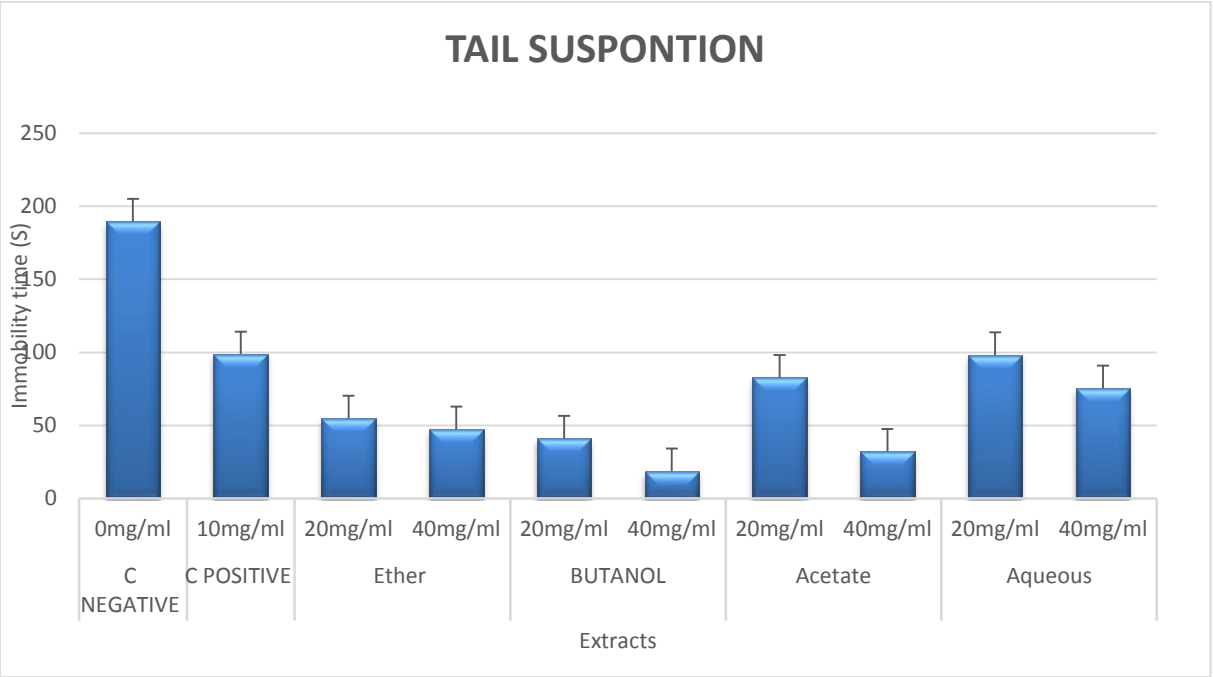

Figure 14. Duration of immobility (S)in the Tail Suspension Test

Table 4. P values of tail suspension test

Extracts	C.POSITIVE	Ether diethyl		BUTANOL		Acetate		Aqueous	
consontration mg/ml	10	20	40	20	40	20	40	20	40
P value	4.8326E-05	1.648E-05	1.118E-05	4.48E-06	4.84E-06	2.312E-05	8.136E-06	0.000129	5.31E-05
	***	***	***	***	***	***	***	***	***

The table displays the p-values for various extracts, with all extracts demonstrating highly significant differences at both 20 and 40 concentrations ($P < 0.001$).

1.2.2. Evaluation of the analgesic power

1.2.2.1. Hot-plate test

This test aims to measure the effects of pain relief. Thirty minutes after injection with different extracts, the rats reaction times to the painful hot plate were recorded. Interestingly, the diethyl ether dose at a concentration of 40 mg/ml gave the highest value for the pain relieving effect, with the endurance duration estimated at approximately 27(s)

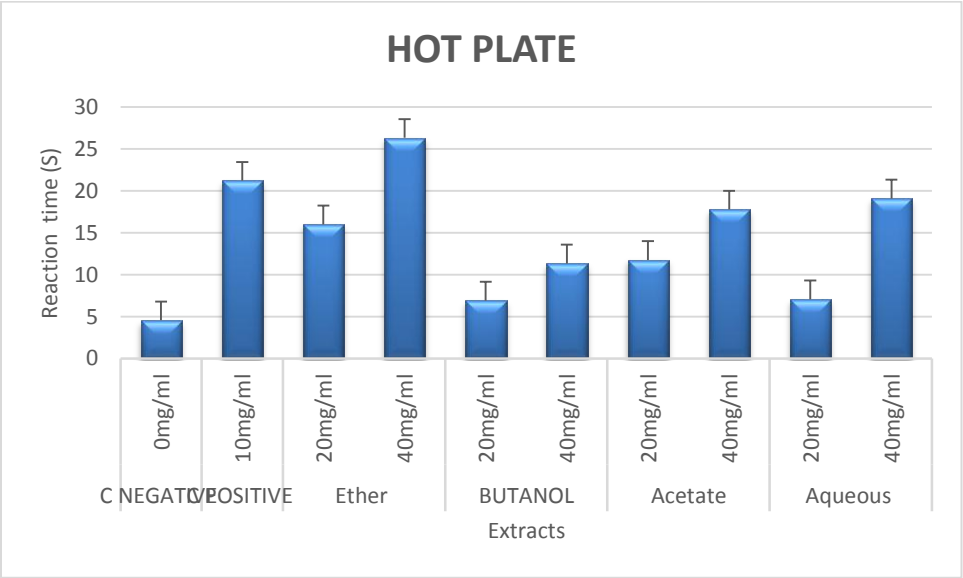

Figure 15. Reaction time (S) in the Hot-plate test

Table 5. P values of Hot plate test

Extracts	C.POSITIVE	Ether diethyl		BUTANOL		Acetate		Aqueous	
consontration mg/ml	10	20	40	20	40	20	40	20	40
P value	1.835E-05	0.000279	0.000147	0.030322	2.94E-05	0.00023	0.002526	0.03653	0.00035
	***	***	***	*	***	***	**	*	***

The table presents the p-values for different extracts. All extracts exhibited highly significant differences at both 20 and 40 concentrations ($P < 0.001$), except for the aqueous and butanol extracts at a concentration of 20, which showed less significant differences ($P < 0.05$).

1.2.2.2. Writhing test

The writhing test, a behavioral assessment used to measure pain sensitivity, revealed a high rate of inhibition in butanol, aqueous, and ether extracts at concentrations of 20 and 40 mg/mL, indicating their effectiveness. In contrast, the 20 mg/mL ethyl acetate extract achieved the lowest rate of inhibition, indicating that it is less effective in this test..

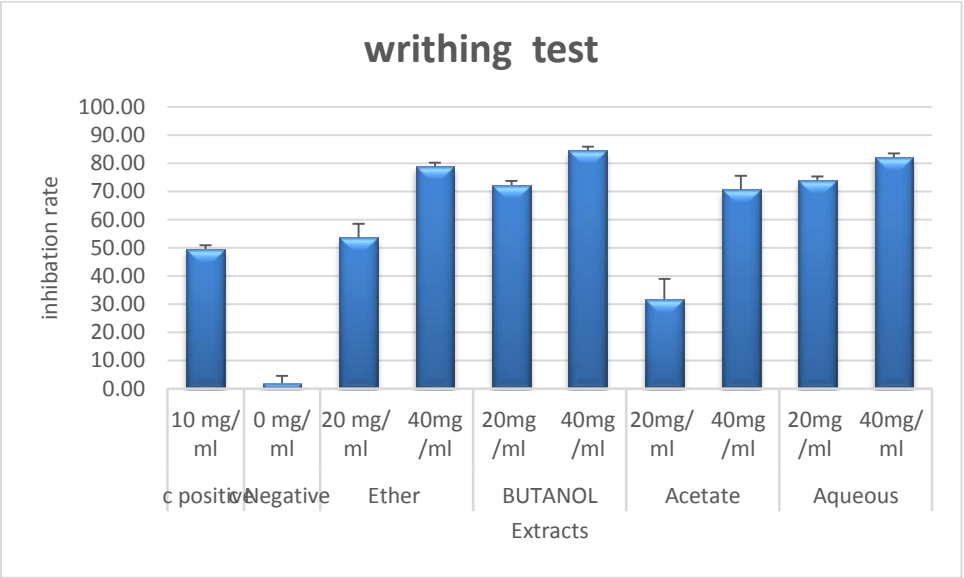

Figure 16. Inhibition rate (S) in the Writhing test

Table 6. P values of writhing test

Extracts	C.POSITIV	Ether diethyl		BUTANOL		Acetate		Aqueous	
consontration mg/ml	10	20	40	20	40	20	40	20	40
p value		4.59E-06	3.44E-06	9.64E-07	2.79E-06	0.001338	0.000156	8.8E-07	3.05E-06
	***	***	***	***	***	**	***	***	***

The table shows the p-values for different extracts, indicating that all extracts exhibited highly significant differences at both 20 and 40 concentrations ($P < 0.001$).

2. Discussion:

2.1.In vivo Tests

2.1.1. Antidepressant Effect

These findings highlight the importance of exploring natural compounds as potential antidepressants, providing new avenues for treatment development of the antidepressant effect of *Ammodicus leucotricus* on animal models, specifically using male rats in the forced swim test (FST) and tail suspension test (TST). The results indicate that the diethyl ether extract, at concentrations of 40 and 20 mg/ml, decreased the duration of immobilization in the FST. Additionally, other extracts such as acetate, butanol, and aqueous, at a concentration of 40 mg/ml, exhibited a duration of immobilization similar to the positive control (30 seconds).In the tail suspension test (TST), the butanol extract at a concentration of 40 mg/ml exhibited the shortest duration of movement, followed by acetate and then diethyl ether extracts. This suggests that these extracts have a stronger effect compared to the control.

Depression is a prevalent mood disorder that profoundly impacts individuals, influencing their mood, thoughts, and behavior (**Dhingra et al., 2018**) . Various theories attempt to elucidate its origins, including imbalances in neurotransmitters like serotonin, norepinephrine, and dopamine, as well as structural alterations in the brain and the hypothalamus-pituitary-adrenal axis (**Haque et al., 2018 ; Belujon et al., 2017**). Current treatments prompt exploration into novel natural compounds for more effective therapeutic approaches.

Studies often employ behavioral tests like the forced swim test (FST) and tail suspension test (TST) in animal models to assess antidepressant effects. Reduction in immobility time during these tests is indicative of potential antidepressant activity. The inertia you're describing is often termed "behavioral despair" in animals, which is suggested to resemble human depression **(Tabssum et al., 2010).** Numerous studies have demonstrated that antidepressant medications like venlafaxine, sulpiride, and imipramine stimulate serotonin activity. Serotonin functions by inhibiting the reuptake of biogenic amines in the central nervous system. These drugs, widely used as antidepressants have been approved for testing in animal models, including the forced swim test (**Porsolt et al., 1978).** Furthermore, selective noradrenaline uptake inhibitors, such as sulpiride, have been found to decrease immobility time in these tests, potentially through their action on dopamine and serotonin receptors in the brain **(Kim et al., 2016 & Nakazato et al., 1998).**

Over the past decades, a significant number of studies have focused on investigating the

antidepressant properties of natural chemical compounds, particularly flavonoids. Flavonoids are known to have various effects on the brain, as documented in multiple studies.(29)Several preclinical studies have demonstrated that certain flavonoids possess antidepressant potential and can reverse symptoms of depression. In these studies, flavonoids extracted from natural plant sources have been reported to exhibit antidepressant-like effects in both cell cultures and animal models, as documented by**(Khan et al. (2018) and Lan et al. (2008).**

2.1.2. Analgesic effect

The results of this study at a concentration (40mg/ ml) in extracts of diethyl ether, Aqueous, acetate and in butanol (20 mg/ml) we recorded longest duration was recorded for diethyl ether compared to other extracts. We conclude that diethyl ether recorded the longest duration of pain tolerance at both concentrations compared to other extracts, which indicates the extent of its effectiveness (27 s), which was stronger than the positive control Aspirin (10 mg/kg), which had an analgesic effect in the hot plate test but its value does not exceed (21s).

Pain is a discomforting feeling that serves as an alert for potential injury or harm within the body's physiological processes, often prompting individuals to seek medical attention. (**Kennedy et al., 2007**)**.** In general, primary afferent nociceptors found in both the peripheral and central nervous systems are crucial in transmitting diverse stimuli that trigger sensations of pain by relaying signals to the brain. (**Kulkarni et al., 2015**)**.** Anti-nociceptive or analgesics are substances that decrease the sensation of pain by raising the threshold for pain in response to external stimuli, a process often mediated through the prostaglandin pathway. (**Kumar et al., 2009**)**.** Despite the development of newer synthetic agents, there is a growing popularity for analgesics derived from natural sources, with expectations of reduced side effects, availability, and lower cost (**Bhuiyan et al., 2020**)**.**

The objective of this research is to evaluate the efficacy of flavonoid extracts obtained from the *Ammodaucus leucotrichus*, The hot plate test was conducted to assess the pain-relieving effects. Aspirin (ASA) was employed as a positive control in this experiment (**Ezeja et al.,2011**)**.**

The analgesic activity is a primarily known for its ability to inhibit COX-2 enzymes, thus achieving analgesic effects**.** In the human body, COX-2 plays a significant role in the process of pain perception. COX-2 interacts with the substances these have a analgesic effect to produce prostaglandins **,** By inhibiting COX-2, thus reducing prostaglandin levels **(Mirshafiey et al.,2017).**

To validate previous results, we carried out a writhing test to verify the pain-relieving

efficacy of these extracts. The writhing test has been used to evaluate the antinociceptive and anti-inflammatory efficacy of extracts in acetic acid-induced abdominal sprains, and the acetic acid-induced writhing response technique is often used to evaluate the peripheral analgesic effects of plant components. Here, acetic acid acts as the main nociceptive stimulus in animal models, causing characteristic writhing responses. This method is valuable for evaluating the ability of plant extracts to relieve pain, providing a direct and reliable means of examining analgesic activity. (**De Souza et al., 2009**)**.** The response is thought to be induced by peritoneal mast cells and prostaglandin pathways (**Ribeiro et al. 2000, & Vogel et al., 1997**)**.** When acetic acid is administered intraperitoneally, it enhances the release of certain inflammatory mediators, including bradykinin, serotonin, histamine, and prostaglandins. The release of these inflammatory mediators is responsible for further abdominal cramps or pain (**Ikeda et al.,2001**) Derrett et al. 1980 showed elevated quantification of prostaglandins in peritoneal secretions after intraperitoneal injection of acetic acid **(Derrett et al.,1980).** An increased amount of prostaglandins PGE2α e PGF2α is observed immediately 30 minutes after acetic acid injection. Moreover, intraperitoneal administration of acetic acid has also been found to release some sympathetic nervous system mediators **(Borsato et al., 2000 & Duarte et al., 1988).** Jiang et al. In this study, it was found that butanol extract at a concentration of 40 mg ml had a pain suppressive effect estimated at 84.55% and analgesic effect more than aspirin as a peripheral analgesic, and this is evidence of the effectiveness of this extract. In a similar study with the essential oil extract of the plant Bunium persicum showed a significant reduction in pain induced by acetic acid in a torsion test using percentages (0.001, 0.01, 0.05, 0.1, 0.5, and 1%).

2.2.In Vitro

2.2.1. Antioxidant Activity

The results show that aqueous extract have the strongest antioxidant activity, followed by butanol, ether, and then acetate extract. while the aqueous extract might be generally more potent, the acetate extract contains specific compounds with exceptional antioxidant abilities, as strong as Ascorbic acid.

Ammodaucus leucotrichus from different regions has been studied regarding their bioactive properties **Dahmane et al. (2017)** studied the antioxidant of *A. leucotrichus* essential oils from Algeria. That study reported that the oils are tested for their antioxidant activity by using the DDPH and β-carotene/linoleic acid assays. Both of these in vitro methods showed that the essential oils have a less powerful reducing than the well-known

synthetic antioxidants, butylated hydroxytoluene (BHT) and E-Vitamin. The DPPH method showed a significant activity for both oils with IC50 values of 6.5 $mg.mL^{-1}$ for sample from Debdeb and 9.35 $mg.mL^{-1}$ for sample from Messaad.

In addition several studies have been conducted to clarify the possible substances involved in antioxidant properties of the essential oil. Among the identified compounds in the essential oil from *Ammodaucus leucotrichus* monoterpene hydrocarbons and oxygenated monoterpenes may be considered the main contributors to the antioxidant activity (**Louail, 2016 ;Yanishlieva, 1999**).

According to the results shown in Ferric Reducing Antioxidant Power assay (FRAP), The aqueous extract exhibited a relatively higher EC_{50} value compared to the other extracts, suggesting lower efficacy in terms of antioxidant effects.

The reducing power of the extracts is probably due to the presence of hydroxyl groups in the phenolic compounds, which act as electron donors. Consequently, antioxidants are considered to be reducers and inactivators of oxidants (Makkar et al., 2007).

The reducing power of a compound can also serve as a significant indicator of its potential antioxidant activity **(Jeong et al., 2004 ; Kumaran and Karunakaran, 2007).**

Furthermore, most non-enzymatic antioxidant activities, such as free radical free radical scavenging and peroxidation inhibition implemented by the redox reaction (Zhu et al., 2002).

The results indicate that all extracts – diethyl ether, aqueous, acetate, and butanol possess anti-hemolytic properties, meaning they can inhibit the hemolysis of red blood cells. Among these, the butanol extract demonstrated the strongest activity, with an IC_{50} value 0.85 mg/ml. The lower of the IC_{50} value have a best of inhibits of hemolysis. The butanol extract exhibited the highest effectiveness compared to the other.

The results of SPF assay suggest that the acetate extract displayed the highest sun protection factor (SPF) among the four extracts tested. A higher SPF value signifies greater protection against UV radiation, which can cause sunburn and contribute to skin cancer development.

The results of Laroui et al. (2023) showed that all extracts from *A. leucotrichus* significantly protected red blood cells from hemolysis in a concentration-dependent manner. This means the higher the concentration of the extract, the greater the protection against hemolysis. At low concentrations (0.05 and 0.025 mg/mL), all extracts performed similarly and offered protection comparable to Ascorbic acid. This suggests that all extracts have potential anti-hemolytic activity, and their effectiveness is comparable to Ascorbic acid at low

doses.

The antihemolytic , sun protection factor (SPF) and antioxidative properties of this plant extract may be due to the presence and synergistic role of polyphenols such as flavonoids. The high antihemolytic activity observed at different concentrations could be attributed to their high lipophilicity, which, leads to a decrease in fluidity of the membrane, hindering the diffusion of free radicals **(Costa et al., 2009)**

The observed sedimentation of erythrocytes in microplates may suggest that some phenolic compounds bind to erythrocyte membranes and exert their antioxidative properties **(Costa et al., 2009)**

2.2.2. Anti-inflammatory Activity

Research suggests that the essential oil extracted from *Ammodaucus leucotrichus* fruits exhibits anti-inflammatory properties. **Mohammedi et al., (2018)** study investigating this effect used carrageenan-induced paw edema in rats as a model. The paw edema weight in the rats treated with the essential oil of *Ammodaucus leucotrichus* was comparable to that observed in the group treated with diclofenac, a standard anti-inflammatory drug. This indicates that the essential oil may be effective in reducing inflammation. In anti-inflammatory activity results the butanol extract's efficacy was comparable to diclofenac, a reference anti-inflammatory drug, indicating its potential anti-inflammatory properties

Another study by **Ziani et al. (2019)** investigated the anti-inflammatory properties of *Ammodaucus leucotrichus* using a different approach. They examined the effect of a hydroethanolic extract from the aerial parts of the plant on nitric oxide (NO) production by immune cells stimulated with a substance that triggers inflammation. The plant extract reduced the production of NO, indicating its potential to reduce inflammation.

Conclusion

Conclusion

Traditional medicine has significantly contributed to the treatment of various diseases due to the increasing interest in medicinal plants. Our study explored the therapeutic value of active substances in the *Ammodaucus leucotrichus* plant. After soaking the ground plant with methanol 85% for 72 hours at 4 degrees Celsius, we obtained four flavonoid extracts: diethyl ether, butanol, ethyl acetate, and aqueous

Various tests performed to examine the chemical and biological activities of *Ammodaucus leucotrichus*, including antioxidant, anti-inflammatory, hemolytic, sun protection factor, and tests for pain and depression.

The results demonstrated the biological activity of the flavonoids extracts:

- ✓ In the DPPH and FRAP tests, the ethyl acetate extract showing the highest inhibitory activity.
- ✓ The diethyl ether extract exhibited the highest value in the total antioxidant capacity test.
- ✓ The hemolysis test indicated that the ethyl acetate extract had the best IC_{50} value.
- ✓ The butanol extract demonstrated the most effectiveness in anti-inflammatory activity.
- ✓ The ethyl acetate extract proved to have a high sun protection factor (SPF).

Regarding the plant's effectiveness against depression, it was validated through the

- ✓ Forced swimming test and the tail suspension test, with diethyl ether and butanol extracts showing significant effects.

Additionally, the plant's pain-relieving properties were confirmed via:

- ✓ Hot plate test and the writhing test, where both the diethyl ether and butanol extracts were effective.

Finally, based on all of the above, we can conclude that this plant has proven to be highly effective, because it contains various medicinal properties, which opens new hopes for treatment with medicinal plants for many diseases

Given the importance of these results, they open experimental perspectives and other indepth studies that should allow us to clearly identify:

- ✓ Advanced Analytical Techniques:

Utilize advanced techniques like HPLC, LC-MS or NMR for detailed profiling of the chemical constituents in each extract. This can help in identifying specific compounds

responsible for the observed activities

- ✓ Antimicrobial Studies:

Expand the scope to include antimicrobial activity testing against various bacterial, viral, and fungal pathogens to explore additional therapeutic uses.

- ✓ Expand Sample Size and Diversity:

Increase the number of samples and diversify the geographical locations from which the plant collected to ensure the results are robust and generalizable.

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