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Them

In Vitro Study on the Biological Activities of *Ammodaucus leucotrichus* Zn-O nanoparticles, Essential Oil and Hydroethanolic Extract.

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شكر وتقدير

الحمد لله الذي بنعمته تتمّ الصالحات، وبفضله تتحقق الأمنيات، والصلاة والسلام على خير خلق الله، سيدنا محمد، وعلى آله وصحبه أجمعين.

وبعد،

يسرّني أن أتقدّم بخالص الشكر وعظيم الامتنان إلى أستاذي الفاضل **الأستاذ الدكتور شمسة أحمد الخليفة،** لما قدّمه لي من دعم متواصل، وتوجيهات قيّمة، ونصائح ثمينة، أسهمت بشكل كبير في إنجاز هذا العمل الأكاديمي. لقد كان لملاحظاته العلمية، وحرصه على جودة البحث، واهتمامه بأدقّ التفاصيل، الأثر البالغ في إثراء هذا العمل ورفع مستواه. فله مني كل التقدير والاحترام، وأسأل الله أن يجزيه عني خير الجزاء.

كما أتقدم بجزيل الشكر والتقدير إلى **أعضاء لجنة المناقشة الأفاضل،** على قبولهم مناقشة هذا العمل، وعلى ما تفضلوا به من ملاحظات علمية بناءة ساهمت في تقويمه وتحسينه، فكل الامتنان لهم على وقتهم وجهدهم الكريم.

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والله وليّ التوفيق.

إهداء

بسم الله الرحمان الرحيم

﴿وَقُلِ اعْمَلُوا فَسَيَرَى اللهُ عَمَلَكُمْ وَرَسُولُهُ وَالْمُؤْمِنُونَ ۖ وَسَتُرَدُّونَ إِلَىٰ عَالِمِ الْغَيْبِ وَالشَّهَادَةِ فَيُنَبِّئُكُم بِمَا كُنتُمْ تَعْمَلُونَ﴾

- اهدي هذا النجاح الى من أحمل اسمه بكل فخر، الى من حصد الاشواك عن دربي ليمهد لي طريق العلم، الى من كلله الله بالعلم و الوقار . . .الى أبي الحبيب
- الى من أفضلها عن نفسي الى من حاكت سعادتي بخيوط منسوجة بقلبها، الى التي لم أرها يوما تدخر جهدا في سبيل اسعادي، الى بسمة الحياة وست الحبايب امي الغالية
- الى الاكرم منا جميعا الى ابطال العبور المقدس الذين غيروا العالم بخطواتهم الصادقة الى الصامدين على الثغور الى المدافعين عن شرف الامة ولأنني ما نويت عملا فداء للأرض المباركة الا وباركه رب ،فاني اهب ثمرة جهدي كله الى فلسطين الحبيبة ، نفسي الفداء لك
- الى ملاذي ورمز فخري و اعتزازي فانا منهم وهم مني اخوتي الأعزاء . .الى ملهمتي أنيسي ومؤنستي اختي
- الى كل من أضاء بعلمه عقل غيره أو هدى بالجواب الصحيح حيرة سائليه فأظهر بسماحته تواضع العلماء . . .الى اساتذتي الكرام وكل من علمني حرفا خلال مسيرتي الدراسية
- الى الروح البريئة التي اختارت جوار ربها لتشع أنوارها حريتا لهذا الوطن الى روح جدي الشهيد نراوي موسى والى روح عمي الشهيد عمار ريح قطرة الدم التي سقت ارض العزة و الكرامة فأنبتت الامن والسلام لهذا الشعب الأبي
- أهدي ثمرة سنوات من الجد و الاجتهاد الى تلك الفتاة التي قاومت كثيرا وسارت بثبات رغم العثرات وامنت بحلمها حين كاد ان يخبو . .اهديك هذه الثمرة التي نبتت من تعبك ونضجت بصبرك وتفتحت بإصرارك . .الى نفسي

عبير نراوي

إهـــداء

بسم الله الرحمن الرحيم

قال تعالى: ﴿ يَرْفَعِ اللَّهُ الَّذِينَ آمَنُوا مِنكُمْ وَالَّذِينَ أُوتُوا الْعِلْمَ دَرَجَاتٍ ﴾

من قال "أنا لها" نالها، وإن أبت، رغماً عنها أتيتُها .

الحمدلله حباً وشكراً وامتناناً على البدء والختام لم تكن الرحلة قصيرة، ولا الطريق ممهداً بالتسهيلات، لكنني فعلتُها ونلتها .

إلى من زينَ اسمي بأجمل الألقاب،

من دعمني بلا حدود، وأعطاني بلا مقابل،

إلى من علّمني أن الحياة كفاح، وسلاحها العلم والمعرفة،

إلى من غرس في روحي مكارم الأخلاق،

داعمي الأول في مسيرتي، وسندي، وقوتي، وملاذي بعد الله...

إلى فخري واعتزازي (والدي)

إلى من جعل الله الجنة تحت قدميها ،

واحتوائي قلبُها قبل يديها ،

وسهلت عليّ الشدائد بدعواتها ،

إلى القلب الحنون، والشمعة التي أنارت لي ظلمتي،

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إلى عزوتي وسندي ، و مصدر قوتي وفخري

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وإلى كل من ساندني ودعمني بكلمة، أو دعوة، أو حضور،

إلى كل من كان سبباً، ولو خفياً، في أن أواصل . . .

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أنتِ جديرة بهذا الإنجاز . . . وبالفخر الذي يليق بك.

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وأن يعلّمني ما جهلت،

وأن يجعله حُجّةً لي، لا عليّ.

✍ ياسمين زديك

إهـــــداء

﴿وَقُل رَّبِّ زِدْنِي عِلْمًا﴾ –[طه: 114]

بحروف يغمرها الامتنان، وبقلب امتلأ حمدًا وشكرًا لله، أهدي هذا التخرج – الذي لطالما كان حلمًا يسكن روحي – إلى من كانوا لي الدفء

حين بردت الأيام، والسند حين مالت بي الطرقات.

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كل سطر في هذا الإنجاز كُتب على صفحة قلبك، وكل لحظة نجاح، كانت بدعائك الطويل في جوف الليل.

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أهديك هذا النجاح، لأنك كنتَ الداعم الأول، ولأن خطواتي لطالما مشت على أثر حكمتك.

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كنتم دائمًا قريبين من قلبي، بأحاديثكم، بمواقفكم، وبحبّكم الذي لا يشبهه حب...

نجاحي هذا يحمل من روحكم الشيء الكثير، فلكم أزهر هذا اليوم الجميل.

وختامًا، هذا التخرج ليس نهاية، بل بداية لطريقٍ جديد، أسأل الله أن يُبارك فيه، وأن أكون كما تمنيتم دومًا.

وآخر دعوانا أن الحمد لله رب العالمين

✍ هناء

Abstract

This study aimed to evaluate the biological activity of *Ammodaucus leucotrichus* through the analysis of its essential oil, plant extract, and nanoparticles, with a particular focus on the role of flavonoids in biological activities. The evaluations included antioxidant activity, antibacterial effect, anti-inflammatory properties, tyrosinase enzyme inhibition, and flavonoid quantification.

The ethanolic extract showed strong antioxidant activity in the DPPH assay, with of an IC_{50} value of approximately 30 µg/mL. It also demonstrated a significant ability to absorb ultraviolet radiation (SPF =22.5), indicating potential use in sunscreen formulations.

In antibacterial assessments, the nanoparticles exhibited higher efficacy compared to the conventional extract, with inhibition zones ranging from 9–13 mm against Gram-negative bacteria. The nanoparticles also showed anti-inflammatory activity comparable to that of the reference drug.

Regarding tyrosinase inhibition, the extract recorded the highest inhibition rate of 45% at a concentration of 400 µg/mL.

The results indicate that the essential oil, hydro-ethanolic extract, and nanoparticles of *Ammodaucus leucotichus* all show significant biological potential in these areas, opening wide opportunities for further *in vivo* and clinical studies to confirm their safety and effectiveness for future therapeutic use

Keywords: Ammodaucus leucotrichus, flavonoids, the nanoparticles , antioxidants, tyrosinase inhibition

الملخص:

تهدف هذه الدراسة إلى تقييم النشاط البيولوجي لنبات أم دريقة *(Ammodaucus leucotrichus)* من خلال تحليل زيته العطري، مستخلصه النباتي، والجسيمات النانوية، مع التركيز على دور الفلافونويدات في الأنشطة الحيوية. شملت التقييمات الفعالية المضادة للأكسدة، والنشاط المضاد للبكتيريا، والخصائص المضادة للالتهاب، بالإضافة إلى قدرة تثبيط إنزيم التيروزيناز والتقدير الكمي للفلافونويدات.

أظهر المستخلص الايثانولي نشاطًا قويًا مضادًا للأكسدة في اختبارDPPH ، حيث مع قيمة IC_{50} تقارب 30 ميكروغرام/مل. كما أظهر قدرة ملحوظة على امتصاص الأشعة فوق البنفسجية

(SPF =22.5) ، مما يشير إلى إمكانات استخدامه في مستحضرات الوقاية من الشمس.

في التقييمات المضادة للبكتيريا، أبدت الجسيمات النانوية فعالية أعلى مقارنة بالمستخلص التقليدي، حيث تراوحت مناطق التثبيط بين 9–13 مم ضد البكتيريا سالبة الجرام. كما أظهرت الجسيمات النانوية نشاطًا مضادًا للالتهاب يقارب فعالية الدواء المرجعي.

أما فيما يتعلق بتثبيط إنزيم التيروزيناز، فقد سجل المستخلص أعلى نسبة تثبيط بلغت 45% عند تركيز 400 ميكروغرام/مل.

تشير النتائج إلى أن الزيت العطري، المستخلص الهيدروايثانولي، والجسيمات النانوية المستخلصة من *Ammodaucus leucotichus* تظهر جميعها إمكانات بيولوجية كبيرة في المجالات المذكورة، مما يفتح آفاقًا واسعة لمزيد من الدراسات الحية والسريرية التي قد تؤكد فعالية وأمان هذه المستخلصات في التطبيقات العلاجية المستقبلية.

الكلمات المفتاحية: ***Ammodaucus leucotrichus، الجسيمات النانوية ، الفلافونويدات ، مضادات الأكسدة، تثبيط التيروزيناز***

Résumé

Cette étude visait à évaluer l'activité biologique d'*Ammodaucus leucotrichus* en analysant son huile essentielle, son extrait végétal et les nanoparticules, en se concentrant sur le rôle des flavonoïdes dans les activités biologiques, y compris l'activité antioxydante, l'activité antibactérienne, les propriétés anti-inflammatoires, En plus de l'inhibition de la tyrosinase et la quantification des flavonoïdes.

L'extrait éthanolique a montré une forte activité antioxydante dans le test DPPH, avec une valeur IC50 d'environ 30 µg/ml. Il a également montré une remarquable capacité d'absorption des UV (SPF=22,5), ce qui indique son utilisation potentielle dans les produits de protection solaire.

Lors des évaluations antibactériennes, les nanoparticules ont montré une plus grande efficacité par rapport à l'extrait conventionnel avec des zones d'inhibition allant de 9 à 13 mm contre les bactéries Gram négatives. Les nanoparticules ont également montré une activité anti-inflammatoire comparable à celle du médicament de référence.

En termes d'inhibition de la tyrosinase, l'extrait a enregistré la plus forte inhibition de 45 % à une concentration de 400 µg ml.

Les résultats indiquent que l'huile essentielle, l'extrait hydroéthanolique et les nanoparticules extraites d'*Ammodaucus leucotrichus* présentent tous un potentiel biologique significatif dans les domaines mentionnés, ouvrant de larges horizons pour des études in vivo et cliniques supplémentaires qui pourraient confirmer l'efficacité et la sécurité de ces extraits dans de futures applications thérapeutiques.

Mots-clés : *Ammodaucus leucotrichus*, nanoparticules, flavonoïdes, antioxydants, inhibition de la tyrosinase.

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ABBREVIATION LIST

CF	Correction factor (10)
DO(λ)	Spectrophotometric absorbance values at the wavel ength.
Dpph	2,2-Diphenyl-1-picrylhydrazyl
EE(λ)	Erythemogenic effect of radiation at wavelength (λ) nm.
EO	essential oil
EOALF	essential oil *Ammodaucus leucotrichus* flowers
Frap	Ferric Reducing Antioxidant Power
I(λ)	Solar intensity spectrum at wavelength (λ) nm.
IC50	Inhibitory Concentration 50%
SPF	the Sun Protection Factor
UV-Vis	Ultraviolet-Visible Spectroscopy
XRD	X-ray Diffraction
MIC	Minimum Inhibitory Concentration
L-DOPA	(Levodopa)L-3,4-Dihydroxyphenylalanine
NaOH	Sodium Hydroxide
ZnO	Zinc Oxide
HKL	Miller indices
The US	United states
PBS	Phosphate Buffered Saline

Summary

Chapter II : Results & discussion

Introduction

Introduction

Medicinal plants have long played a vital role in traditional medicine systems around the world and are still considered an important source of bioactive compounds used in the development of modern therapies. Among these natural sources, *Ammodaucus leucotrichus*, commonly known as "desert cumin" or "Oum Doreiga", stands out. It is a perennial herb belonging to the Apiaceae family and grows naturally in the arid and semi-arid regions of North Africa and the Middle East. Traditionally, this plant has been used for its medicinal properties, including reducing inflammation, relieving pain, and treating skin disorders, making it a common remedy for a variety of health issues (Bendif *et al*., 2017). It is well known for its small, aromatic seeds that resemble common cumin but have a sweeter scent.

Phytochemical studies have shown that *Ammodaucus leucotrichus* contains essential oils and extracts rich in active compounds such as thymol and carvacrol, which are responsible for its strong antioxidant, antimicrobial, and anti-inflammatory properties (Hazzit *et al*., 2009). Recent analyses have also identified other compounds like flavonoids, polyphenols, and secondary metabolites that enhance the plant's pharmacological potential. These compounds offer protection against oxidative stress and also show promising cytotoxic activity, suggesting a potential role in cancer research (Ouarghidi *et al*., 2020). In addition to its medicinal uses, the plant has been studied as a natural food preservative and flavoring agent, adding nutritional and economic value.

Based on this evidence, recent studies have provided further insights into the plant's therapeutic potential. For example, Ziane *et al*. (2021) found that the essential oil from *Ammodaucus leucotrichus* seeds had strong antimicrobial effects, especially against Staphylococcus aureus and Escherichia coli, along with high antioxidant activity as shown by DPPH tests. Similarly, Taleb *et al*. (2020) reported that the plant's ethanolic extracts were rich in phenolic compounds linked to antioxidant and anti-inflammatory effects. Boumendjel *et al*. (2018) also analyzed the plant's chemical composition and confirmed the presence of important active compounds that support its medicinal use.

Therefore, this study aims to provide a comprehensive evaluation of the biological activities of *Ammodaucus leucotrichus* by examining its essential oil, hydroethanolic extract, and newly developed nanoproducts through a series of in vitro laboratory tests. These evaluations will include antioxidant and anti-inflammatory activity, enzyme inhibition, antibacterial activity,

and measurement of sun protection factor (SPF), which is important for potential cosmetic and dermatological applications. Although previous studies have offered valuable insights, there is still a lack of research combining traditional phytochemical analysis with advanced nanotechnology. This study aims to fill that gap by enhancing the bioavailability and effectiveness of the plant's active compounds through the development of nanostructured formulations, which may contribute to the creation of new, effective, and safe natural therapies based on this traditional medicinal plant.

First part

Bibliographic synthesis

Chapter I: medicinal plant"*Ammodocus lactrichous* "

1-Definition of *Ammodaucus leucotrichus*

Ammodaucus leucotrichus, commonly known as "Um Driqa," is an annual herbaceous plant from the Apiaceae (Umbelliferae) family. It typically grows to a height of 10 to 12 centimeters. The plant has somewhat fleshy leaves that are divided into fine green lobes, and its stems are striated with fine grooves. The flowers are grouped in umbel-shaped inflorescences, and the fruits are small, oval to elongated in shape. This plant is known for its strong aromatic scent, which is due to the essential oils produced in its vegetative organs—a characteristic feature of many plants in the Apiaceae family. The taste of the plant is slightly bitter (Hleis .Y, 2007). Figure (01)

Figure (01): *Ammodaucus leucotrichus* Plant (Origine Zaoui Abir, Zedik Yasmine and Miloudi Hana 2025)

2-Alternative Names for the *Ammodaucus leucotrichus* Plant:

Scientific Name: *Ammodaucus leucotrichus*

English Common Name: Hairy Cumin

French Name: Cumin cheveluArabic Names: Um Driqa أم دريقة، الكمون الصوفي، المصوفة (RAHMA BELAMIN، 2007)

3- Morphological description of *Ammodaucus leucotrichus*:

Ammodaucus leucotrichus is a small annual plant, either wild or cultivated, with a height ranging from 10 to 12 cm. It features delicate, slightly fleshy leaves and white flowers arranged in umbels composed of 2 to 4 branches, each bearing five free petals. The fruit is a diachene, measuring between 6 and 10 mm in length, and is covered with dense, soft, white hairs. Due to this characteristic, it is commonly referred to in some regions as "hairy cumin" **(Abderrezag *et al*., 2021).**

4- Classification scientific

Table (01): botanical taxonomy of *Ammodaucus leucotrichus***(GOUNNI Ahlem et al … 2024)**

Species	*Leucotrichus*
Genus	*Ammodaucus*
Family	Apiaceae
Order	Araliales
Class	Rosopsida
Branch	Magnoliophyta
Kingdom	Plants

5- *Ammodaucus leucotrichus* – Naming of Plant Parts

Figure (02): diagram showing the parts of *Ammodaucus leucotrichus* Plant (Hleis Y. 2007)

1. The stem is striped with fine lines.

2. The leaves are somewhat fleshy and divided into thin lobes.

3. The umbel inflorescence contains a limited number of flowers.

4. The fruits are small, oval (8–10 mm), and covered with long, dense, woolly hairs. Figure (02)

6- Geographical Distribution of *Ammodaucus leucotrichus*:

Ammodaucus, which belongs to this family, is an endemic genus found in North African Saharan and Sub-Saharan countries, including Algeria, Morocco, Tunisia, and Egypt. The only species of this genus that grows wild in Algeria is leucotrichus Coss & Dur subspleucotrichus.

Another subspecies, leucotrichus Coss & Dur subsp. nanocarpus, was described in the Canary Islands by Bultrán. (Mohammedi *et al*., 2).

7- Traditional uses of *Ammodaucus leucotrichus*:

Ammodaucus leucotrichus, locally known in Algeria as "Moudrayga" or "El Massoufa," is a medicinal plant commonly sold by herbalists in traditional markets, especially in the southern regions of the Algerian Sahara. Nomadic communities typically gather its seeds and leaves during spring, when the fruits are mature. In these regions, the leaves are often used to flavor herbal teas, while the fruits serve as a spice in cooking. Both the leaves and seeds are traditionally consumed as decoctions or infusions to treat various health conditions, including high blood pressure, chest pain, liver and digestive disorders, gastroenteritis, and even diabetes (Idm'hand *et al*., 2020).

8- Medical Uses:

The plant plays an important role in alternative medicine due to its effectiveness in treating many diseases. Its seeds are used to treat digestive system disorders and to relieve stomach and liver pain. The leaves are used to treat chest diseases. The fruits are prepared as a powder or decoction to relieve stomach pain, intestinal problems, and indigestion. They are also used to treat digestive issues in children such as diarrhea, nausea, and vomiting. Previous studies have shown that some extracts of the plant have the ability to prevent the formation of kidney stones. (RAHMA BELAMIN , 2007)

9-Growth and flowering:

In the Souf region, the *Ammodaucus leucotrichus* plant grows early compared to neighboring geographical areas, such as the northern regions. Generally, these plants appear in late winter, grow and develop for a short period, followed by the production of flowers and fruits. (Hleis .Y, 2007)

10- Previous Studies on *Ammodaucus leucotrichus*:

A. leucotrichus was collected from the Algerian Sahara Desert, a region known for its unique climate. This plant exhibited a high essential oil (EO) yield, exceeding 2.5%. Perillaldehyde was identified as the dominant compound in the oil's chemical composition, accounting for approximately 85%. Analyses revealed that essential oil *Ammodaucus leucotrichus* flowers (EOALF) possesses multiple biological activities, including antioxidant,

antibacterial, and anti-inflammatory properties. These bioactivities are associated with the presence of perillaldehyde and limonene—the latter being the second most abundant compound in the EO.

Given the cosmetic industry's growing interest in natural bioactive ingredients, the performance of EOALF was tested when incorporated into a base cream. The results showed that the biological activity of the essential oil persisted for 28 days, although a gradual decrease in effectiveness was observed over time. This decline became noticeable immediately after incorporation, suggesting possible interactions with the cream matrix. In this context, the need to enhance the oil's stability and extend its activity was highlighted, which could be achieved through microencapsulation. Nevertheless, the individual bioactive properties of EOALF indicate its promising potential as a natural cosmeceutical ingredient. (Halla *et al*., 2018)

A comparative analysis was conducted on the content and chemical composition of essential oils (EOs) extracted from the roots, stems/leaves, flowers, and fruits of A. leucotrichus subsp. leucotrichus, naturally growing in the Algerian desert. The results demonstrated significant variation in the pattern of specialized metabolites among the different plant organs. The EO content and composition also varied considerably, with oil yields ranging from 0.52% to 3.04% (w/w, based on dry weight). The oils contained complex mixtures comprising up to 89 different compounds.

The major chemical constituents identified in the extracts of various plant parts included: (Z)-falcarinol, β-bisabolene, myristicin, methyl eugenol, α-pinene, muurola-4,10(14)-dien-1-β-ol, geranyl acetate, as well as perillaldehyde and limonene.

The analysis highlighted a wide chemical diversity in both major and minor components, underscoring the potential of this plant as a model for studying the biosynthetic regulation of specialized compounds, particularly in response to biotic and/or abiotic constraints. Based on the rich and diverse chemical composition, different parts of A. leucotrichus represent a valuable source of essential oils and biologically active volatiles (bio-volatiles), with promising applications in the pharmaceutical sector. (Neghliz-Benabdelkader *et al*., 2021)

Chapter II :

Nanoparticles and

DRX staduy

1-Nanoscience

Nanoscience is the field that focuses on studying materials at the nanoscale, emphasizing the description of their chemical, physical, and mechanical properties, as well as exploring the phenomena arising from reducing their sizes. The purpose of reducing materials to the nanometer scale is not merely to minimize size, but rather to represent a qualitative shift in traditional physical and chemical concepts. This aims to develop nanomaterials with unique properties that align with the requirements of advanced technological applications in this century, enhancing performance in innovative and unprecedented ways (MOHAMMED cherif eleskendri, 2010)

2-The term Nano

The term Nano is derived from the ancient Greek word meaning "dwarf." It is used in science and technology to refer to an extremely small scale, representing one billionth of a basic unit such as a meter or a gram. (MOHAMMED cherif eleskendri, 2010)

3-Definition of Nanometer

A nanometer is a unit of measurement used to measure extremely small lengths that can only be observed using an electron microscope. This unit is utilized to express the dimensions of atoms, molecules, cells, and microscopic particles such as bacteria. One nanometer is equal to one-billionth of a meter, or in other words, one meter contains one billion nanometers (nm). For comparison, one nanometer is approximately the length of a row of 13 hydrogen atoms placed side by side (Figure 03). (TOUAHER Fatima and sabrina, 2022)

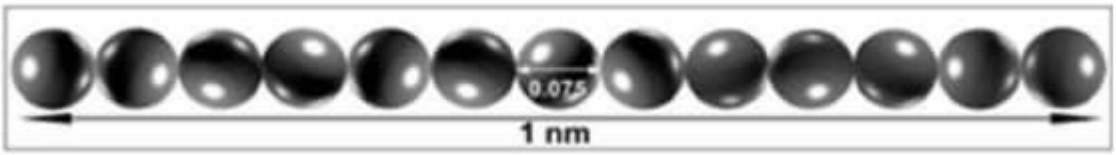


Figure (03): A horizontal row consisting of 13 hydrogen atoms is approximately equal to one nanometer. (MOHAMMED BEN SALEH SALHI, 2007)

4-The concept of nanotechnology

Nanotechnology is the science and engineering that aim to apply scientific principles to produce materials and devices at the nanoscale. It is defined as the ability to control matter at the atomic and molecular levels, allowing the design and manufacture of materials, structures,

and devices with dimensions less than 100 nanometers. This involves studying and manipulating their physical, chemical, magnetic, mechanical, and electrical properties, enabling various scientific and practical applications in everyday life. (FOUAD NEMR, 2015)

5-Benefits of Nanotechnology

Nanotechnology offers many important benefits that help improve human life, industry, and the environment. Some of the key benefits include:

-Providing eco-friendly materials used for water purification and resource protection (Khan et al., 2019)

-Increasing agricultural production through genetically modified crops and foods, with less labor and fewer resources. (Mahmoud Mohammed Salim Saleh et al., 2015)

-Improving smart nutrition by developing low-cost, nutrient-rich, and responsive food products. (Roco & Bainbridge, 2005)

- Enhancing manufacturing efficiency with clean and high-performance production methods. Khan et al., 2019)

- Creating smart interactive devices that improve human performance using converging technologies. (Roco & Bainbridge, 2005)

_Upgrading energy and manufacturing systems with lower costs and higher efficiency (Bhushan, 2017).

_Boosting large-scale food production using advanced nano-based techniques. (Mahmoud Mohammed Salim Saleh et al., 2015)

_Advancing public health and nanomedicine by enabling precise drug delivery and better disease treatment (Khan et al., 2019).

_Developing infrastructure and industries such as the automotive sector, with high-quality, low-cost, and energy-saving products. **(mahmoud mohammed salim saleh *et al.*, 2015)**

6-Current and future applicatione of nanotechnology

-6-1-Medical Nanotechnology

Smart nanomaterials can enter the body to detect diseases, deliver drugs, repair tissues, and control hormones, while implanted nanosensors in the brain may restore movement in paralyzed patients. Nano-scale silicon “teeth” (cell-sized devices) enable precise drug or gene delivery for advanced cell therapy. **(Mahmoud Mohammed salim saleh *et al*., 2015)**

6-2-Environmental Nanotechnology fild

Nanotechnology is a promising tool for addressing environmental pollution, one of the most serious human-made problems, especially since it is hard to detect with human senses and involves complex, interconnected factors. (TOUAHER Fatima and sabrina, 2022)

6-3-Industrial Nanotechnology fild

Nanotechnology opens wide possibilities for various industrial applications, as nanomaterials have new properties due to their high surface-to-volume ratio, improving quality and precision in many industries. (TOUAHER Fatima and sabrina, 2022)

6-4- Electronics Nanotechnology fild

Electronics are essential in our daily lives, and we can't imagine life without them. Nanotechnology has become crucial in developing the electronics industry, known as nanoelectronics. (ALI Yousef el al., 2014)

6-5-Nanotechnology in Space Field

Making rockets from plastic reinforced with nanoparticles is cheaper and easier than using metal structures. This hybrid plastic can withstand the cold of space and the heat from friction with Earth’s atmosphere. (NOUHA aloui habchi, 2009)

6-6-Nanotechnology in the Military Field

Nanotechnology is used to develop a robotic wasp with a nano-engine for surveillance, attack, and communication jamming. The U.S. military also uses nanofibers to create combat uniforms that allow airflow while blocking toxic gases. (Ahmed mohammed sabri, 2020)

7-Advantages and Disadvantages of Nanotechnology:

Nanotechnology has witnessed remarkable progress in recent years, offering numerous advantages across various sectors. In industry and electronics, it has enabled the development of smaller, faster, and more efficient devices. In medicine, it has contributed to targeted drug delivery, early disease detection, and advanced diagnostic tools. Moreover, in the environmental field, nanotechnology has shown potential in water purification and pollution control. However, despite these significant benefits, nanotechnology also raises several concerns and criticisms, such as potential health and environmental risks, ethical issues regarding human enhancement, and the lack of clear regulatory frameworks to manage its widespread use :

7-1- Penetration of Biological Systems:

Nanoparticles are extremely small, allowing them to bypass biological barriers like the immune system or enter through skin and lung cells. This could cause severe damage to human tissues or unknown diseases. (Adel sobhi bacha, 2019)

7-2- Nano-Weapons Threat:

Some countries are exploring "smart" nano-weapons that can identify targets using genetic material. This could transform future warfare, replacing traditional armies with nano-soldiers capable of mass destruction within hours.(Ahmed mohammed sabri, 2020)

7-3- Hidden Toxicity Risks:

The long-term health effects of nanomaterials remain unclear. Some studies suggest they might interact with human cells in toxic or cancer-causing ways, especially due to insufficient safety research. (Ahmed mohammed sabri, 2020)

7-4- Invisible Nature of Nanomaterials:

Their microscopic size makes nanomaterials hard to detect with the naked eye or standard tools. This complicates efforts to control or neutralize them in case of leaks or misuse. (WALED Youcef attou, 2017)

7-5- Unregulated Production:

Rapid manufacturing of new nanomaterials—often without strict safety checks—raises concerns about releasing untested products that could harm humans or the environment. (WALED Youcef attou, 2017)

7-6- Global Technology Gap:

Advances in nanotechnology widen the gap between developed nations (Global North) and developing countries (Global South). This imbalance risks creating scientific dependency and global inequality. (WALED Youcef attou, 2017)

7-7- Self-Replicating Nanobots:

Scientists warn of a hypothetical "grey goo" scenario, where self-replicating nanobots escape human control, multiply endlessly, and threaten Earth's ecosystems. (Adel sobhi bacha, 2019)

Table (02): Future Goals and Applications of Nanotechnology (Mahmoud Mohammed Salim Saleh et al., 2015)

Field	Future Goals and Applications
Industry	Manufacture of materials and tools with higher precision, efficiency, and special properties.
Electronics	Reducing the size of electronic chips, increasing their speed, and reducing their energy consumption.
Medicine	Developing diagnostic and therapeutic devices with higher precision, speed, and efficiency.
Transportation	Reducing the weight of transportation means to lower energy consumption and increase efficiency.
Energy	Discovering new sources and renewable energy and reducing the use of traditional fuel.
Environment	Manufacturing environmentally friendly materials, recycling waste, and reducing pollution.
Agriculture	Producing nanomaterials for plant nutrition, improving soil fertility, and modifying plant genes.

Table (03) : Principles and Means of Nanotechnology (Mahmoud Mohammed Salim Saleh et al., 2015)

Principles	Advantages
Ability to control atoms and individual particles directly and rearrange them.	Unique physical and chemical properties of materials at the nanoscale, which differ from their properties at the natural scale.
Depends on the principles of physics, chemistry, biology, electrical and electronic engineering.	Discovering special properties in materials that help in many innovations and practical applications.
Ability to build any material because atoms are the building blocks of all materials.	Stronger connection between sciences and encourages collaboration among scientists from different fields.
Allows control over the smallest particles when making materials and devices, making them better.	Produces materials and devices that are smaller, stronger, faster, cheaper, and more energy-efficient.
Relies on scientific research that was once just imagination.	Turns scientific imagination into real inventions and useful applications.

8-Definition of X-rays (XRD):

X-rays are a type of electromagnetic wave with high frequency and high energy. Their wavelength ranges from 0.01 to 10 nanometers, placing them between ultraviolet rays and gamma rays in the electromagnetic spectrum.

X-rays can penetrate various materials, including human tissues and bones. Because of this penetrating ability, each material shows a unique pattern of how it diffracts X-rays.

In X-ray Diffraction (XRD), the diffraction pattern depends on the crystalline structure of the material. Every crystal has a specific way of scattering X-rays, which can be described using Miller indices (HKL). By applying Bragg's Law, we can calculate the interplanar spacing between crystal planes and understand the material's atomic structure. **(Mohcen Taiti, 2022)**

9-Basics of XRD Technic :

X-rays are produced in a vacuum tube called an X-ray tube. Inside, accelerated electrons are directed at a positively charged metal target. When these electrons hit the inner shells of the metal atoms, they excite the atoms. As the atoms return to their stable state, they emit X-ray radiation.

However, only a small part of the energy becomes X-rays; most of it is released as heat. That's why cooling systems are important in XRD equipment to keep it working properly. **(Mohcen Taiti, 2022)**

10-Bragg's Law

X-ray diffraction in a material (sample) is described by Bragg's Law. When X-rays interact with the crystal structure of the sample, they undergo interference—either constructive or destructive—depending on the arrangement of atomic planes within the crystal.

Constructive interference occurs when X-rays are reflected from parallel crystal planes in such a way that their paths differ by an integer multiple of the wavelength. This is described by the following equation:

$$\mathbf{2}\boldsymbol{d_{hkl}} = \mathbf{\sin}\,\boldsymbol{\theta} = \boldsymbol{\lambda n}$$

Where:

$\boldsymbol{n}$**:** an integer representing the order of reflection

$\boldsymbol{\theta}$**:** the angle of incidence (the angle at which X-rays hit the sample)

$\boldsymbol{\lambda}$: the wavelength of the X-rays

$\boldsymbol{d_{hkl}}$**:** the distance between crystal planes (Miller indices hkl)

For diffraction to occur and satisfy Bragg's condition, the following must also be true: **(Mohcen Taiti, 2022)**

$$\mathbf{2}\boldsymbol{d_{hkl}} \geq \boldsymbol{\lambda}$$

Second part:

Experimental part

Chapter I:

Materials & methods

1- Materials

-Plant sample preparation

The plant *Ammodaucus leucotrichus* was purchased from the herbalist at the market in Wilaya Oued Souf.

The sample was then cleaned, washed with water, and dried in the shade over a cloth, turning it twice a day to prevent spoilage and avoiding direct sunlight. The drying process was completed after ensuring that no moisture remained in the plant.

Set of equipment ,and solutions used in laboratory experiments		
Tools	Solusions and materials	Equipment
Beaker;Filter paper Aluminum paper;Funnel Weighing bottle;Heating ovens (1000 .500.100 .50 ml) Graduated tube;Spoon Erlenmeyer flask Micropipette; Etuve Microplates 96	Plant material Distilled water Ethanol;Glass volumetric flasks calibrated; Albumin Drog biofinac;Methylene blue (C16H18CIN3); Tampon buffer solution(6.8);L-DOPA -sodium hydroxide(NaOH) -Zinc acetate (ZnC4H6O4) Plant Extract; salin solution 0.9%;DPPH solution;Ascorbic Acid Trichloroacetic Acid Solution ;Nano Solution E.O Solution; Enzyme Tyrosinase;Phosphate Buffer Solution (PH 6.6)	Spectrophotometer;Centerifuge Etuve;Rotary evaporator Clivengre;Magnetic stirrer X-ray diffraction(DRX); -Water Bath Ultrason device

3-Methods

3-1 Extaction

3-1-1 Preparation of Plant Extract

The plant extract was prepared using the maceration method. A quantity of 100 g of the powdered plant material was placed in a beaker, and 350 ml of ethanol (70%) and 150 ml of distilled water (30%) were added. The mixture was left to macerate in the dark for 24 hours. After that, the mixture was filtered using filter paper to collect the filtrate.

The plant extract was then transferred to a round-bottom flask of a rotary evaporator and heated at the appropriate temperature to evaporate the solvent (ethanol). Subsequently, the plant extract was separated from the solvent, which was reused for further maceration of the remaining filtrate.

This process was repeated for three consecutive days. Finally, the resulting plant extract was placed in a glass dish and dried in a Etuve at 70°C until a dry extract was obtained. **Figures (04)**

The production yield of plant extracts is calculated using the following relationship:

R (%)= (P1/P2) x 100

Where:

(%): Production yield of the extracts in %.

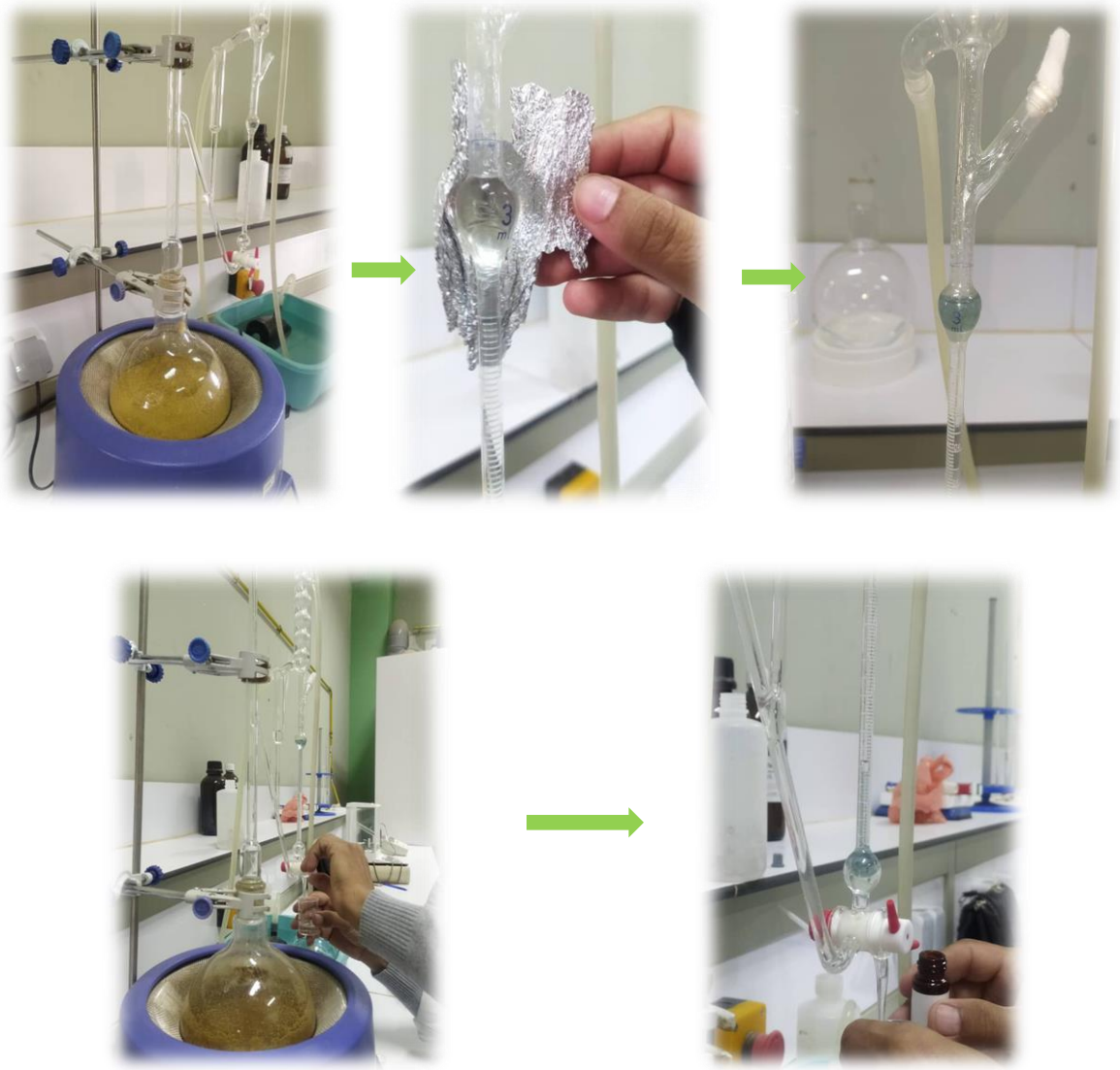
P1: Weight of the extract after evaporation of the solvent.

P2: Weight of the plant materials used in the extraction.

Figure (04): Pictures showing the stages of preparing the plant extract (Origine Zaoui Abir, Zedik Yasmine and Miloudi Hana 2025)

3-1-2 The essential oil extraction

The essential oil was extracted from *Ammodaucus leucotrichus* using a Clevenger apparatus. A quantity of 100 g of the powdered plant material was placed with 500 ml of distilled water in the extraction device. The extraction process lasted for 6 hours, resulting in the collection of a light blue essential oil. Figures (05)



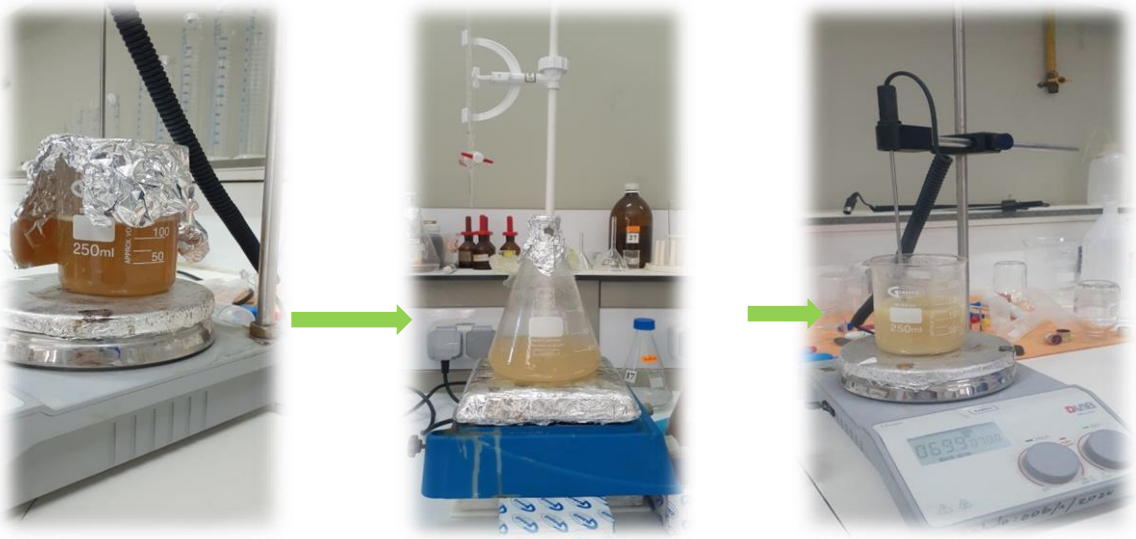
Figures (05) : Pictures showing the stages of preparing the essential oil (Origine, Zedik Yasmine Miloudi Hana and Zaoui Abir 2025)

3-1-3 Preparation of ZnO Nanoparticles Using Plant Leaf Extract:

A volume of 50 mL of plant leaf extract was prepared and mixed with 100 mL of 0.1 M zinc acetate solution (Zn(CH3COO)2). The mixture was stirred continuously at 70°C for 2 hours. Subsequently, a 2 M NaOH solution was slowly added to the mixture, leading to a color change in the solution, indicating the formation of ZnO nanoparticles

The precipitate was separated from the solution using centrifugation at 3000 rpm for 10 minutes to remove impurities. The collected precipitate was then dried at 80°C for 24 hours and further calcined at 600°C for 5 hours to obtain the final ZnO nanoparticles.

After synthesizing zinc oxide nanoparticles (ZnO), the success of the preparation process is confirmed by characterizing the samples using spectroscopic techniques, including X-ray Diffraction (XRD), and Ultraviolet-Visible Spectroscopy (UV-Vis). **Figures (06)**



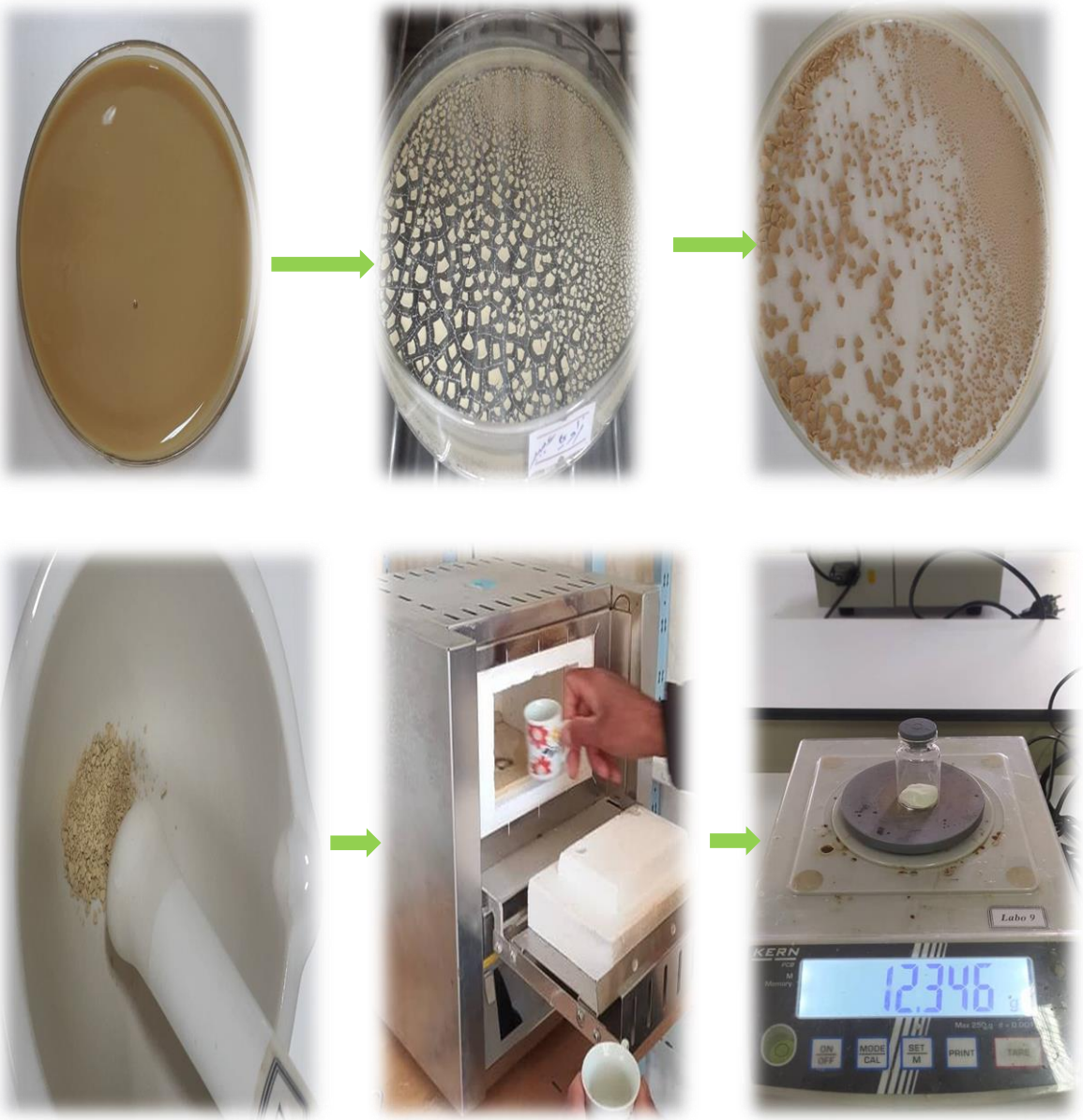


Figure (06): Pictures showing the stages of preparing nanoparticles. (Origine, Miloudi Hana Zaoui Abir and Zedik Yasmine 2025)

1-PROTOCOLS:

1-1 THE DPPH assay "Evaluation of the Antioxidant Activity of Vitamin C"

The DPPH test is used to evaluate the antioxidant activity based on the ability of plant extracts to scavenge DPPH free radicals. This depends on the extracts' ability to reduce the purple-colored DPPH compound, which can be measured spectrophotometrically. The reduction of DPPH is expressed as a decrease in absorbance, which generally varies depending on the type and quantity of the plant extracts.

Different concentrations of the extract, ranging from 30 µg to 4000 µg, are mixed with the DPPH solution, and the antioxidant activity is determined by comparing the results with a reference sample (Vitamin C).

-Preparation of Ascorbic Acid (Vitamin C) Concentration:

10 mg of pure ascorbic acid is dissolved in a specific volume (usually distilled water) to obtain a final concentration of 4000 µg/mL.

-Preparation of DPPH Solution:

DPPH solution is prepared at a concentration of 0.1 mM by dissolving 3.94 mg of DPPH powder in 100 mL of ethanol.

-Experimental Steps**:**

200 µL of each concentration is mixed with 800 µL of DPPH solution (in triplicate), and absorbance is measured at 517 nm after 30 minutes of keeping the mixture in the dark.

-Definition of IC_{50} Value:

IC_{50} is the concentration of the extract (as an antioxidant) required to inhibit (reduce) 50% of the DPPH free radicals. This value is calculated from the inhibition percentage curve of the DPPH radical as a function of the extract concentration . figure (07)

The percentage of DPPH inhibition is expressed using the following formula:

$$I\% = \frac{A_0 - A_i}{A_0} \times 100$$

Where:

Absorbance of DPPH at 517 nm

Absorbance of DPPH in the presence of the extract (measured at the same wavelength) after 30 minutes of reaction. **(Samarth et *al.*, 2008)**.

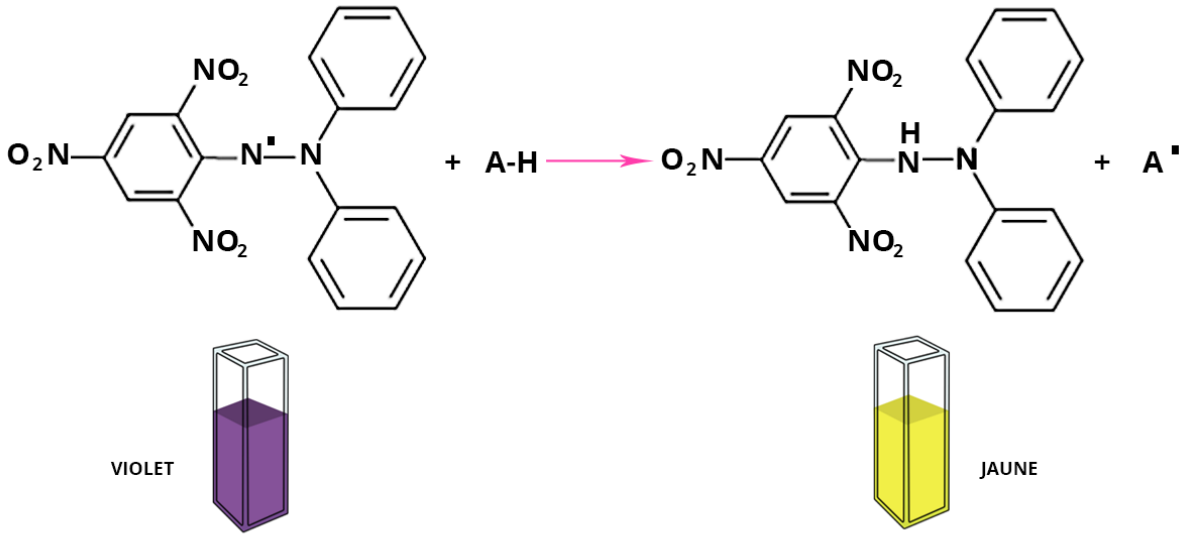


Figure (07): Reaction mechanism of 2,2-diphenyl-1-picrylhydrazyl (DPPH) with antioxidant (Boligon et *al.*, 2014)

1-2-FRAP Test (Ferric Reducing Antioxidant Power)

The FRAP test is used to evaluate the antioxidant activity of compounds by measuring their ability to reduce oxidation through a color change reaction. It shows how well plant extracts can act as antioxidants. Potassium ferricyanide reacts with Fe ions to form a complex with a very high extinction coefficient at a wavelength of 700 nm. Through this test, the reduction of potassium ferricyanide by the antioxidants present in the studied extract is monitored (Pisoschi and al., 2016). The reducing power of the extract was evaluated using the potassium ferricyanide method (Muthukrishnan and al., 2018).

.Stage 1: Reduction Reaction

1. Dissolve 2 mg of the plant sample in 2 ml of distilled water, then take 0.5 ml of this extract and place it in a test tube.

2. Add 1.25 ml of phosphate buffer solution to each tube.

3. Add 1.25 ml of 1% potassium ferricyanide solution to each tube.

4. Mix well, then incubate the tubes in a water bath at 50°C for 20 minutes.

Stage 2: Precipitation and Centrifugation

1. After incubation, add 1.25 ml of trichloroacetic acid to each tube.
2. Mix thoroughly, then centrifuge at 3000 rpm for 10 minutes.

Stage 3: Analysis and Absorbance Measurement

1. After centrifugation, take 650 µl (microliters) from the upper layer (supernatant).
2. Add 650 µl of distilled water and 0.25 ml of 0.1% ferric chloride ($FeCl_3$) solution.
3. Mix well and let the mixture stand at room temperature for 10 minutes.
4. Measure the absorbance at a wavelength of 700 nanometers.

1-3-Quantitative Determination of Flavonoids:

Flavonoids are phytochemical substances that exist either as free aglycones or as glycoside conjugates (Middleton *et al*., 2000). They play different roles in plants as secondary metabolites, being involved in defense against UV rays, pigmentation, stimulation of nitrogen-fixing nodules, and resistance to diseases (Crozier, 2003).

The flavonoids, which are considered phenolic compounds, were chemically determined by their reaction with aluminum nitrate, according to the method mentioned in(Chouikh *et al*., 2018)

1 mL of the extracts and different concentrations of the quercetin solution were taken, then 1 mL of $AlCl_3$ solution at a concentration of 2% was added. A yellow-green color appeared, and after waiting for 30 minutes, absorbance was measured using a spectrophotometer at a wavelength of 420 nm. The absorbance results were obtained in relation to the concentration

1-4-Protocol for Enzyme Preparation and L-DOPA Analysis

For enzyme preparation, 1 mg of the enzyme is dissolved in 250 µl of Tampom solution (pH 6.8). The resulting solution is divided into five Eppendorf tubes, each containing 50 µl. The tubes are stored in the freezer until needed.

Upon use, if the solvent used is alcoholic (control), 2000 µl of Tampom solution (pH 6.8) is added to 50 µl of the enzyme solution. If the solvent used is water (control), 3000 µl of Tampom solution (pH 6.8) is added to 50 µl of the enzyme solution.

To prepare L-DOPA, 5 mg of L-DOPA is dissolved in 5 ml of distilled water and mixed thoroughly until fully dissolved.

In the analytical procedure, 150 µl of Tampom solution (pH 6.8) is added to a test tube, followed by 10 µl of the sample (extract or control) and 20 µl of the enzyme solution. The mixture is incubated for 3 minutes with shaking in a spectrophotometer, then incubated for 10 minutes at 37°C. After that, 20 µl of the L-DOPA solution is added, and the kinetic reading is taken using a spectrophotometer at a wavelength of 475 nm.

1-5-Protocol of Antimicrobial Activity: (Gheraissa Noura, ,2023)

Bacteria are microscopic organisms that can only be seen through a microscope. They exist everywhere — in the air, water, on the human body, and inside the digestive and respiratory systems. Some bacterial germs can survive for long periods under harsh environmental conditions, such as extreme heat or cold, by forming a thick protective layer. When the environmental conditions improve, the bacteria shed this layer and return to their normal active state.

-Activation of Bacteria

The bacteria used in the antimicrobial activity study are stored at a low temperature of about 4°C in a liquid nutrient broth medium. To activate the bacteria, the test tube containing the bacterial suspension in nutrient broth is placed in an incubator for 30 minutes at 37°C. Then, 1 ml of this suspension is taken and placed into a new test tube containing only nutrient broth medium and incubated for 18 to 24 hours at 37°C, depending on the type of bacteria. After incubation, to prepare active bacteria for biological activity testing, 1 ml of the active bacterial suspension is transferred into 7 ml of nutrient broth in a sterile test tube. To ensure the bacterial concentration is about 10^6 cells per ml, the optical density of the suspension is measured at 600 nm wavelength, which should be between 0.8 and 1 (or 0.4-0.5 in a spectrophotometer with a 1/2 cm path length), or by using the McFarland standard.

-Preparation of Mueller-Hinton Agar Medium

Seventeen grams of Mueller-Hinton agar are weighed and dissolved in 500 ml of distilled water. The medium is heated and stirred using a magnetic stirrer until it boils. Then, the medium is distributed into test tubes each containing 20 ml, which will later be poured into Petri dishes. The tubes containing the medium are sterilized in an autoclave at 121°C for 15 minutes (or at 100°C for 45 minutes).

-Practical Steps for the Antimicrobial Activity Study

Five hundred microliters of the active bacterial suspension are placed into a Petri dish after labeling it with the required information. Twenty milliliters of Mueller-Hinton medium are added to a test tube containing 500 microliters of the bacterial suspension. The Petri dishes are gently swirled in a figure-eight motion to mix the bacteria with the medium and then left to solidify for about 10 minutes. Using sterile forceps near a Bunsen burner flame, sterile discs of 6 mm diameter are placed on the surface of the solidified medium. Then, 20 microliters of the extract to be tested for biological activity are added onto each disc using a micropipette. After placing all extracts carefully and following the recorded data, the plates containing the bacteria and the discs with extracts are incubated at 37°C for 24 hours (depending on the type of bacteria studied). Finally, the zones of inhibition around the discs are measured in millimeters.

1-6-Protocole of the Sun Protection Factor (SPF)

The in vitro method measures the reduction of the irradiation by measuring transmittance after passing through a film of product. As in the operative conditions of the transmission measurement are correct, this to be a very precise and single value, always reproducible for the same product and expressed as a single UV curve, in the absorbance scale**. (Osterwalder and Herzog,2009)**

The photoprotective activity of the extract was evaluated by calculating the Sun Protection Factor (SPF) using the following equation, after measuring the absorbance of a sample dissolved in ethanol (1 mg/mL) at seven different wavelengths (290-320 nm) with intervals of 5 nm **(Mansur *et al*., 1986)**:

$$\textbf{Spectrophotometric SPF} = \textbf{CF} \times \sum \textbf{(EE}(\lambda) \times \textbf{I}(\lambda) \times \textbf{DO}(\lambda)\textbf{)}$$

Where:

CF: Correction factor (10).

EE(λ): Erythemogenic effect of radiation at wavelength (λ) nm.

I(λ): Solar intensity spectrum at wavelength (λ) nm.

DO(λ): Spectrophotometric absorbance values at the wavel ength.

The constant values of EE(λ) × I(λ) are indicated.

1-7 Protocole of the Anti-inflammatory activity test

Scientific Principle of the Egg Albumin Denaturation Assay for Evaluating Anti-inflammatory Activity

This test is based on the ability of plant extracts to inhibit the denaturation of egg albumin when exposed to heat. It is used as a preliminary test to evaluate anti-inflammatory activity. During inflammation, plasma proteins like albumin leak out and get denatured when heated. Plant extracts with anti-inflammatory properties can prevent this denaturation, indicating their inflammation-inhibiting effect (Sakat et al., 2010).

Preparation of Egg Albumin Solution

Dilute the white of one egg in 100 ml of saline solution (0.9%) or PBS (Phosphate Buffered Saline).

Filter the solution using filter paper or centrifuge it (3000 rpm for 10 minutes) to remove impurities (Oyedepo & Femurewa, 1995).

Preparation of Different Concentrations of the Plant Extract

Prepare several concentrations of *Ammodaucus leucotrichus* extract (e.g., 100, 200, 500 µg/ml) using a suitable solvent (water).

Performing the Albumin Denaturation Inhibition Test

In test tubes, add the following:

Positive Control:

0.5 ml egg albumin + 0.5 ml saline solution.

Negative Control:

0.5 ml egg albumin + 0.5 ml of a known anti-inflammatory drug (e.g., diclofenac at a specific concentration).

Experimental Group:

_ 0.5 ml egg albumin + 0.5 ml of the plant extract (for each concentration).

_ Heat all tubes in a water bath at 70°C for 5 minutes (Mizushima & Kobayashi, 1968).

_ Let them cool at room temperature.

_ Measure the absorbance at 660 nm using a spectrophotometer.

4. Calculating the Inhibition Percentage

The percentage of inhibition of albumin denaturation can be calculated using the formula:

$$\text{Inhibition (\%)} = \left(1 - \frac{\text{Absorbance of Sample}}{\text{Absorbance of Control}}\right) \times 100$$

2- In vitro biological activities

2-1 Antioxidant activity

Both the essential oil and the aqueous ethanolic extract showed strong antioxidant activity when tested using several techniques:

_ In the DPPH free radical scavenging assay, the aqueous ethanolic extract showed higher activity (IC_{50} =25 µg/mL) compared to the essential oil.

_ In the FRAP assay, a high reduction capacity was observed, especially in the extract, due to the presence of phenolic compounds.

2-2 The antibacterial activity

The antibacterial activity was evaluated using agar diffusion and broth dilution methods, and the results showed inhibitory effects against both Gram-positive and Gram-negative bacteria, including:

Staphylococcus aureus, Escherichia coli, Salmonella typhi, and Pseudomonas aeruginosa.

The essential oil showed higher antibacterial activity, likely due to its lipophilic nature that enables it to penetrate the cellular membranes of bacteria.

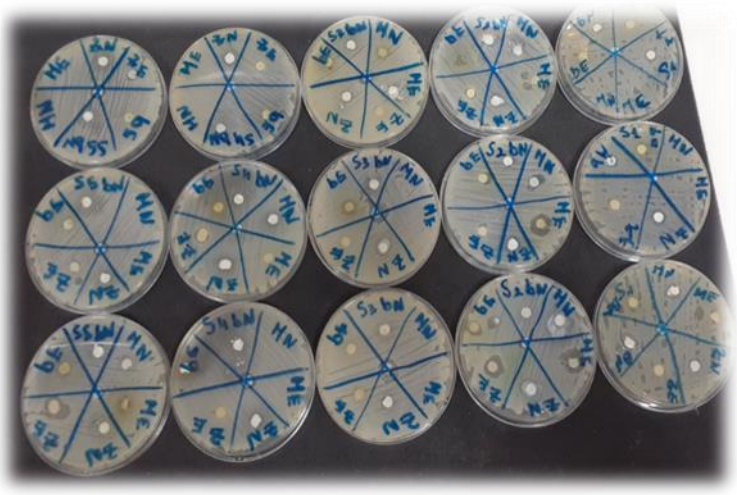


Figure (08): investigation of antimicrobial properties

2-3 Enzyme Inhibitory Capacity

Tyrosinase is a copper-containing enzyme that helps start the process of melanin production. It changes L-tyrosine into L-DOPA, and then into dopaquinone. These steps are important for making melanin, the pigment that gives color to skin, hair, and eyes in living organisms. **(Chang, T. S. 2009)**

Both preparations were tested for the inhibition of key enzymes related to metabolic disorders, and the results were as follows:

- Inhibition of α-amylase and α-glucosidase, suggesting potential use as antidiabetic agents.
- Inhibition of the enzyme tyrosinase, which is associated with skin whitening and skin cancer prevention.
- The essential oil showed greater efficacy in inhibiting tyrosinase.
- The percentage inhibition was calculated using the following Equation:

$$\textbf{Percentage inhibition (I\%)} = \left(1 - \frac{A}{B}\right) \times 100$$

Chapter II :

Results & discussion

Results

1-Average DPPH Activity

The scavenging activity of the ethanolic extract of the plant *Ammodaucus leucotrichus* on the roots of dpph at different concentrations. The results showed a high ethanolic scavenging activity of the plant *Ammodaucus leucotrichus*, where the extraction rate from the extract reached 85.72 at a concentration of 500, which indicates a high capacity for free radicals using low concentrations of the plant extract. figure (09)

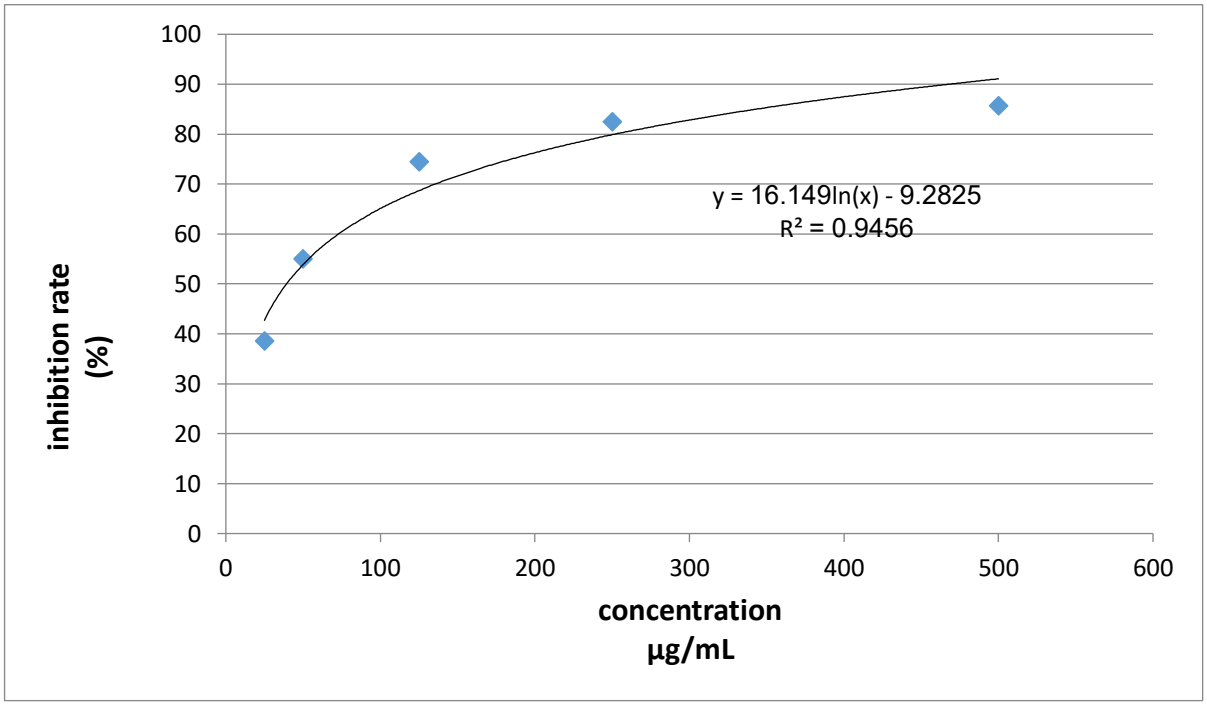


Figure (09) : DPPH radical scavenging activity of different concentration of the extract vegetal

2-FLAVONOID

From the quercetin calibration curve, the flavonoid content in the extract was determined and expressed as milligrams of quercetin per gram of dry extract, as shown in the curve. Figure (10)

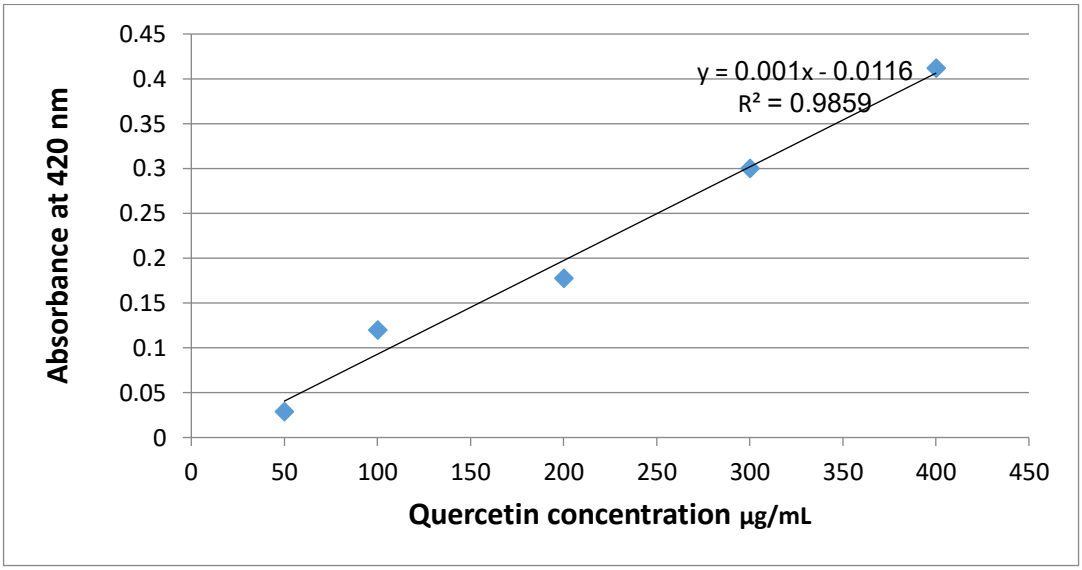


Figure (10): Total flavonoid contents of the extract vegetal

3- Ferric Reducing Antioxidant Power (Frap)

The results of the frap test are shown in Figure (11). The greatest inhibitory activity was observed in the case of vitamin C, reaching 88% at a concentration of 500 µg/mL, while the greatest inhibitory activity of the *Ammodaucus leucotrichus* was achieved at a concentration of 4000 µg/mL (96%). The concentration of the ethanolic extract of the *Ammodaucus leucotrichus* that produced 50% free radical inhibition (63 µg/mL) with regression coefficients (R 2=0.9791 and Y 2=0.9791 and Y=0.026x+0.0783) while the IC50 values of standard vitamin C (16.5 µmol/mL) with regression coefficients (R2=0.9748 and Y=0.0066x+0.0757)

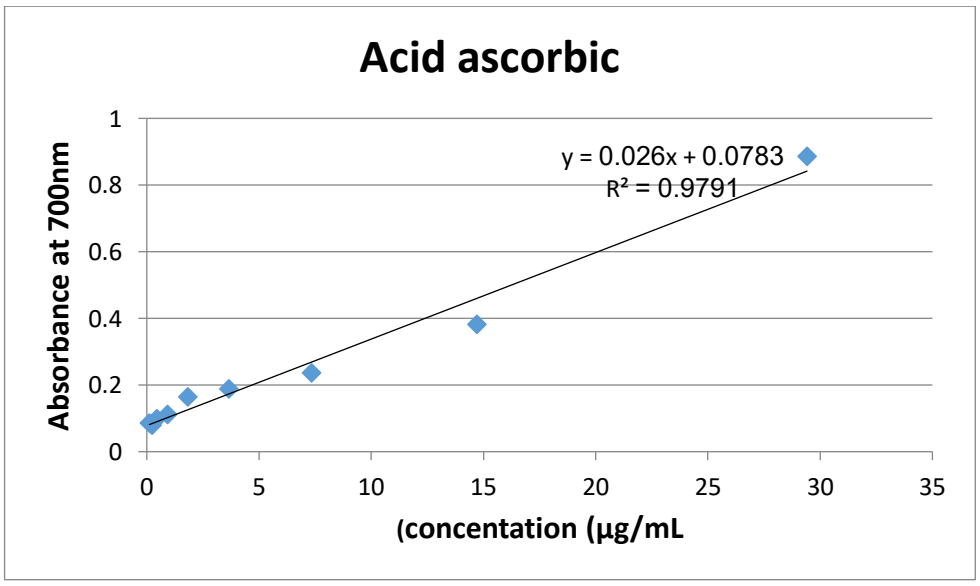


Figure (11): standard curve of ascorbic acid as a function of the constraction of the Frap

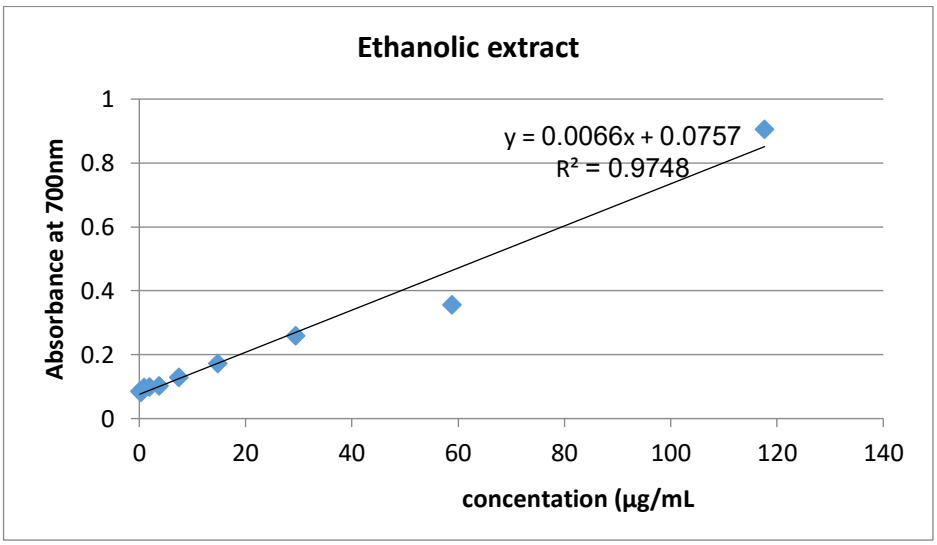


Figure (12): Ferric Reducing Antioxidant Power (Frap) activity of different concentration of the extract vegetal

4-Tyrosinase inhibition test

The bar chart shows a clear increase in tyrosinase inhibition percentage as the concentration of the tested material increases from 50 to 400 µg/mL. The inhibition starts at about 18% at 50 µg/mL and reaches around 37% at 400 µg/mL, indicating a direct relationship between concentration and tyrosinase inhibitory activity. This pattern suggests that the tested compound (such as nano ZnO or a plant extract) is effective in inhibiting tyrosinase, a key enzyme in melanin synthesis, which supports its potential use in cosmetic or therapeutic applications related to skin lightening or hyperpigmentation reduction. figure (13 & 14)

4-1-Ethanolic extract

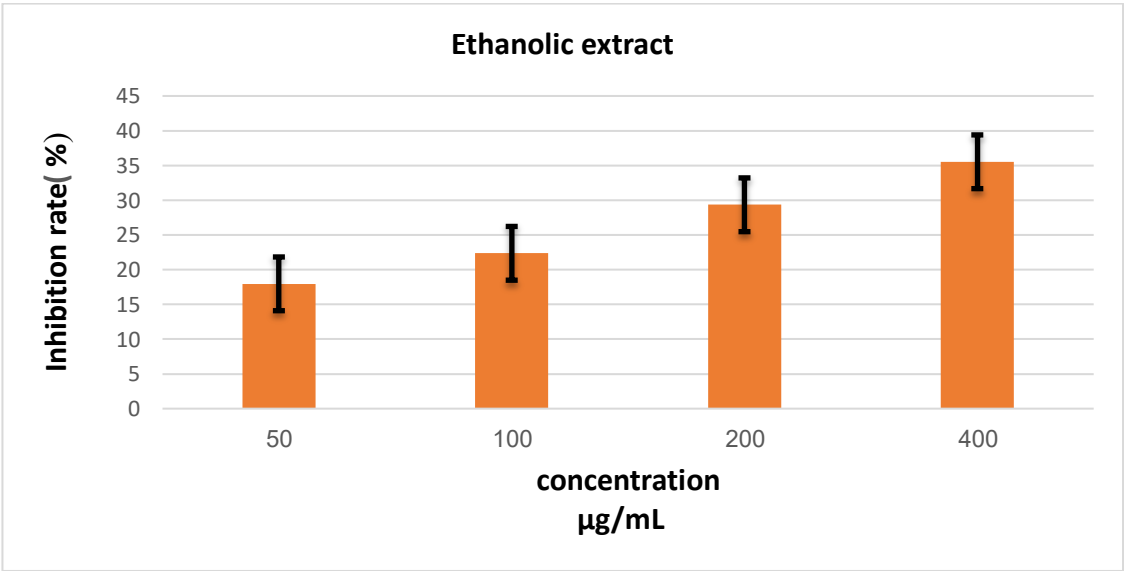


Figure (13): Tyrosinase inhibition rate of ethanolic extract

4-2- Essential Oil

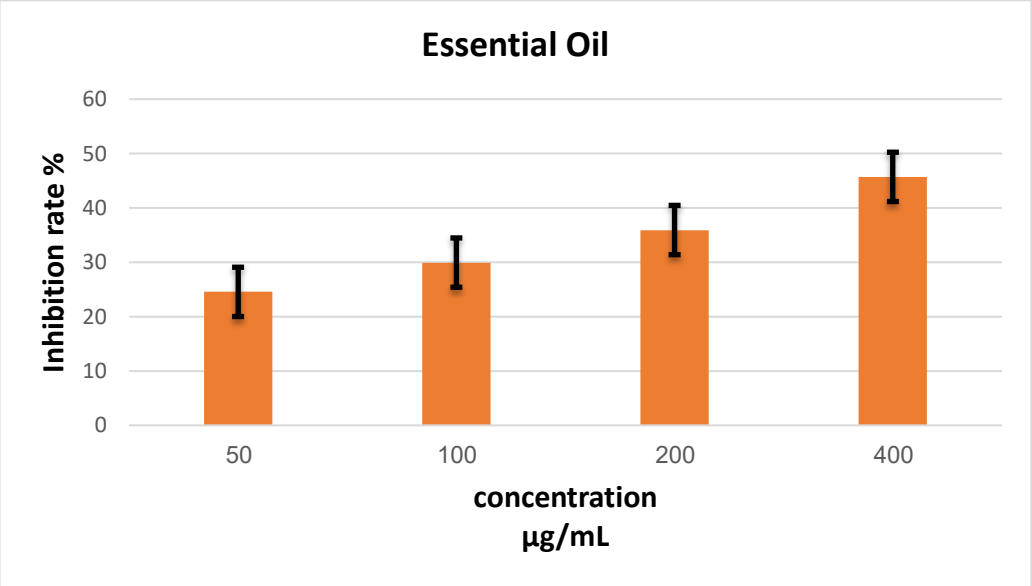


Figure (14): Tyrosinase inhibition rate of Essential Oil

5-SPF

Figure (15) shows the SPF values of the ethanolic extract of *Ammodaucus leucotrichus*, ZnO nano-extract, EO and ACM sunscreem, where the ethanolic extract of *Ammodaucus leucotrichus* and the sunscreen had better protection among the extracts while EO and ZnO had weaker sun protection.

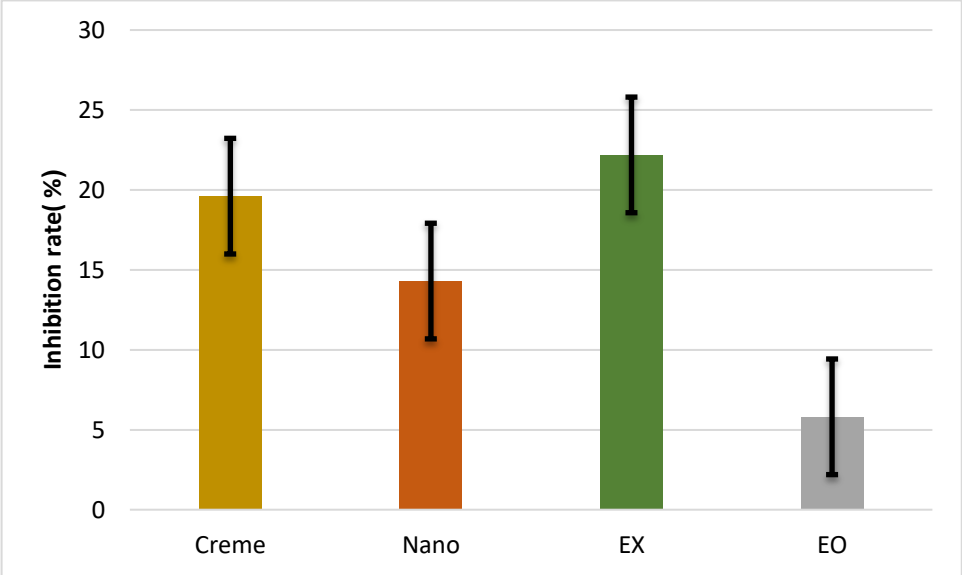


Figure (15): values sun protection factor of Ethanolic extracts, Essential Oil, Zno nanoparticles, and ACM Crème

6- Antibacterial activity test

The nano extract demonstrates superior antibacterial activity compared to the plant extract across all four tested bacterial strains:

- Pseudomonas aeruginosa (S1): The nano extract exhibits a stronger effect (9-11 mm inhibition zone) than the plant extract (6-7 mm).

- Bacillus subtilis (S2): Both extracts show effectiveness, but the nano extract (13-15 mm) outperforms the plant extract (9-12 mm).- Klebsiella pneumoniae (S3): The nano extract (10-13 mm) displays significantly higher antibacterial activity compared to the plant extract (6-7 mm).table (04)

- Escherichia coli (S4):While the nano extract (10-13 mm) is more effective, the plant extract (8-10 mm) still shows notable antibacterial action.Overall, the nano-formulated extract consistently exhibits enhanced antibacterial performance against all tested strains The effects of extracts (EXTRACT) and nanoparticles (NANO) were analyzed on four different bacterial strains**:** Pseudomonas aeruginosa (S1), Bacillus subtilis (S2), Klebsiella pneumoniae (S3), and Escherichia coli (S4). The results showed that nanoparticles were more effective than extracts in most strains, especially S1 and S3, while the effect was less pronounced in S4. Additionally, Bacillus subtilis (S2) appeared to be less affected by the treatments, which may be attributed to structural differences between Gram-positive and Gram-negative bacteria. The higher efficacy of nanoparticles could be related to their physicochemical properties, which enhance bacterial membrane penetration and interaction with cells.

Table (04): MIC value (mg/ml) for plant extract against for (04) bacteria strains

Result (mm)						
	NANO	EXTRACT	NANO	EXTRACT	NANO	**EXTRACT**
S1(Pseudom Aeruginosa)	10	6	11	9	12	**7**
S2(Pacillus Subtilis) Ps	12	10	15	14	13	**9**
S3(Klebsiella Pneimoniae)	10	6	13	6	10	**6**
S4(Escherichia coli)	10	8	13	10	10	**8**
Reputation	R1		R2		**R3**	

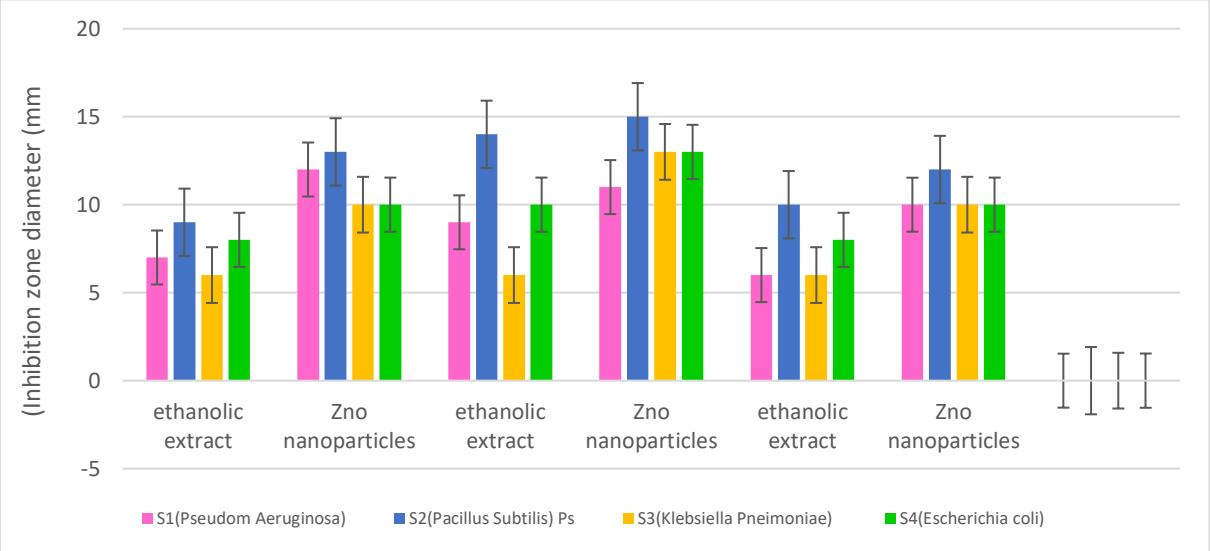


Figure (16): Antibacterial activity of plant extract and Zno nanoparticles

7-Anti-inflammatory activity:

The results were summarized in this study. The effectiveness of the nano and the essential oil in heat-induced albumin denaturation was good and nearly similar to the reference drug Biofenac, while the extract was less effective than the reference drug Biofenac. figures (17,18 ,19 and 20)

7-1 The essential oil

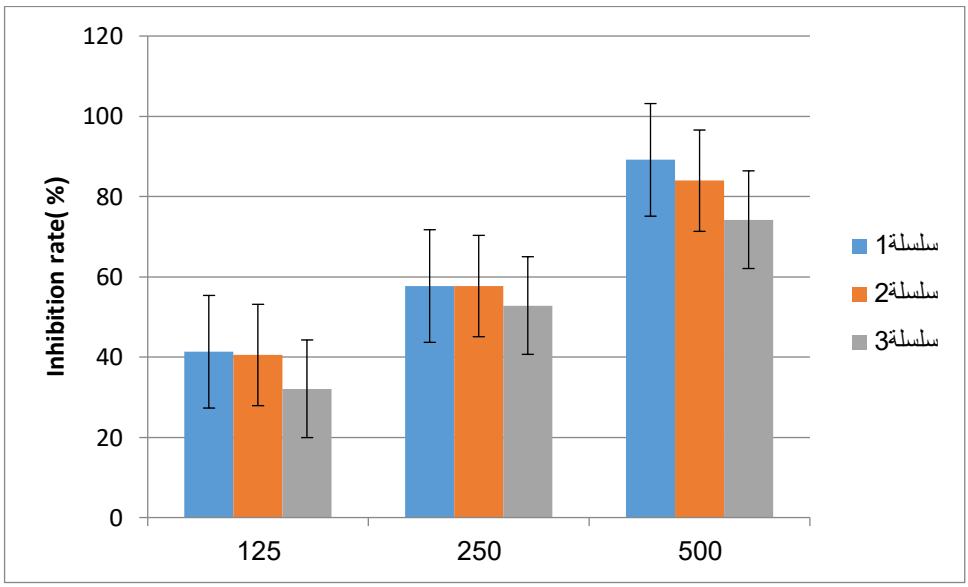


Figure (17): The essential oil

7-2 Zno nanoparticles

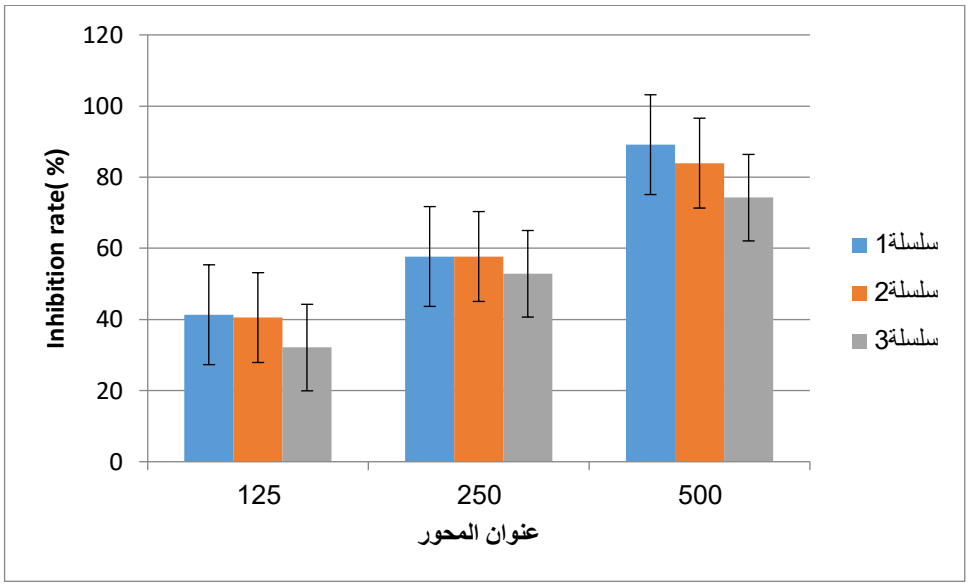


Figure (18): Zno nanoparticles

7-3 EXTRACT

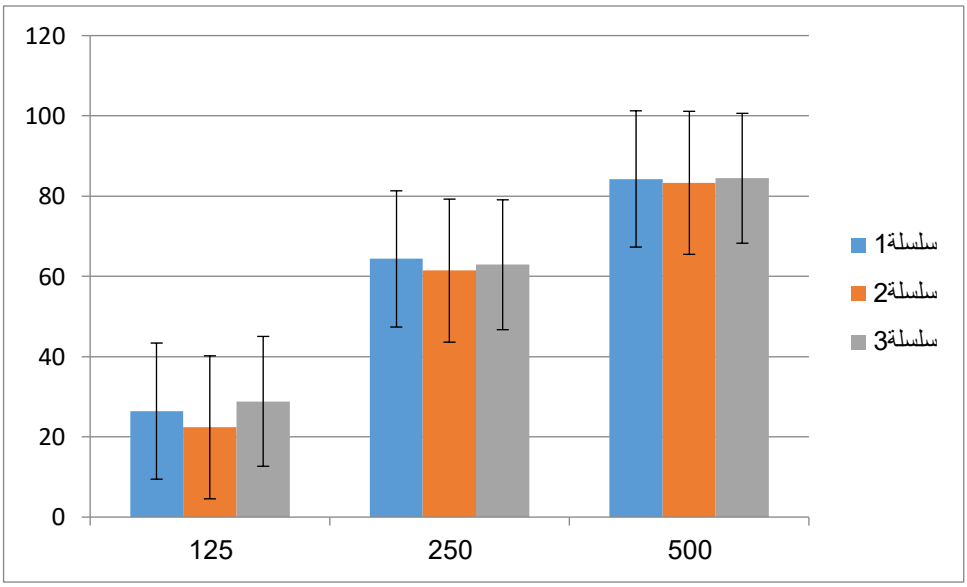


Figure (19): EXTRACT

7-4 Drog

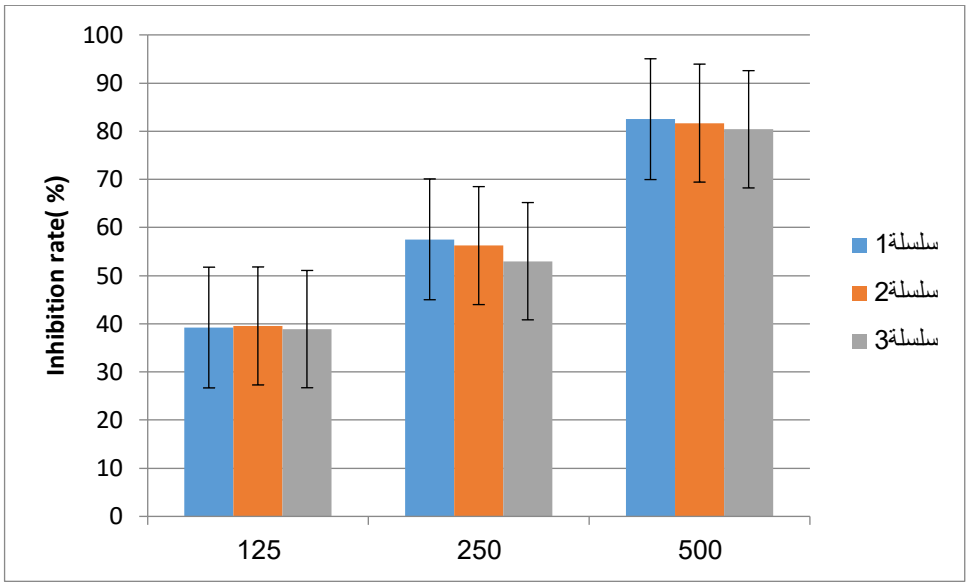


Figure (20): Drog

8-DRX

The X-ray diffraction (XRD) analysis of the zinc oxide (ZnO) nanoparticles shows a series of peaks at the following 2Theta positions:

31.770 – 34.422 – 36.253 – 47.539 – 56.603 – 62.864 – 66.380 – 67.963 – 69.100 –

72.762 – 76.955.

These peaks correspond to the following crystallographic planes:

(100 – 002 – 101 – 102 – 110 – 103 – 200 – 112 – 201 – 004 – 202), respectively, of hexagonal wurtzite ZnO with lattice parameters a = b = 3.2498 Å and c = 5.2066 Å, and space group $P6_3m$.

According to the JCPDS card No. 00-036-1451, the crystallite size of the synthesized nanoparticles was calculated using the Scherrer equation:

$$DP = \frac{(K\lambda)}{(\beta \cos \theta)}$$

The calculated crystallite size was 56.31569 nm

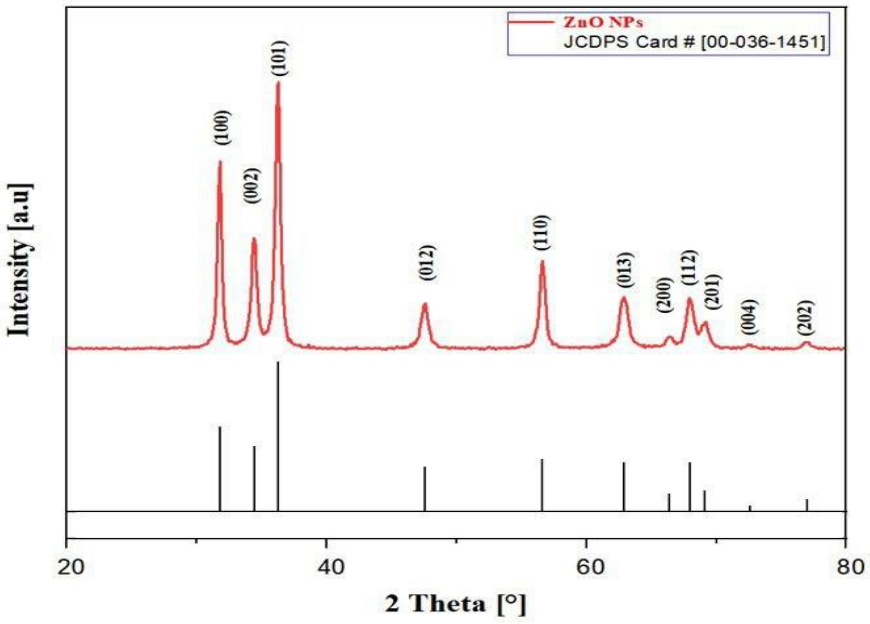


Figure (21): DRX Curve for ZnO Nanoparticles

9-NANO Zn-O

The FTIR spectrum in the image shows a scan range from 4000 to 400 cm^{-1}, with no clear absorption peaks in the higher regions of the spectrum. This suggests a low presence or weak activity of organic functional groups. However, a sharp drop in transmittance appears below 500 cm^{-1}, which is characteristic of Zn–O bond vibrations. This pattern supports the presence of zinc oxide (ZnO) in the sample and is commonly used in FTIR analysis to confirm the nanostructure of metal oxides like ZnO. Figure (22)

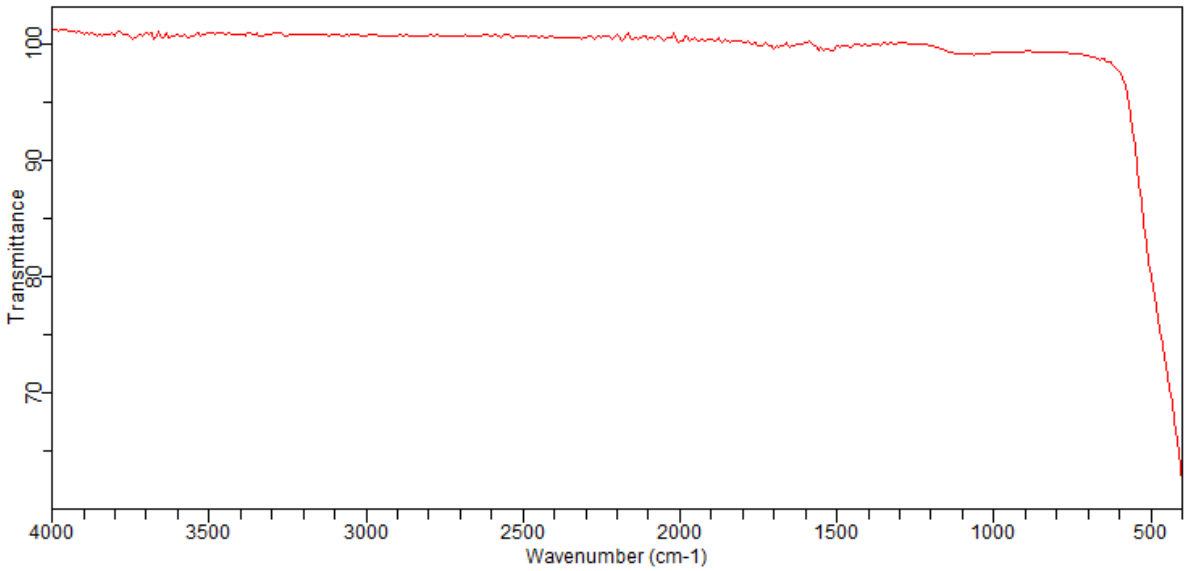


Figure (22):FTIR Curve for Zn-O Nanoparticles

Discussion

The results showed that the ethanolic extract of *Ammodaucus leucotrichus* possesses strong antioxidant activity and natural sun-protective potential, making it a promising candidate for pharmaceutical and cosmetic applications. In the DPPH assay, the extract exhibited potent free radical scavenging activity with an inhibition rate of 86% at a concentration of 500 µg/mL, and an IC_{50} value of approximately 30 µg/mL, although it was less effective than ascorbic acid (vitamin C), which recorded an IC_{50} of 5 µg/mL. However, this value was less effective compared to a previous study conducted by Fatima Zohra Belbachir *et al*. (2024), in which the methanolic extract of the same plant showed a lower IC_{50} of 0.86 µg/mL.

In the FRAP assay, the extract demonstrated increased reducing power with increasing concentration, with absorbance values ranging from 0.086 at 1.945 µg/mL to 0.962 at 4000 µg/mL. At 500 µg/mL, the absorbance value was 0.26, falling within the typical range for medicinal plants (0.2–0.8) according to Zengin *et al*. (2018). These antioxidant activities are mainly attributed to the high content of phenolic and flavonoid compounds, which neutralize free radicals by donating electrons, as explained by Pulido *et al*. (2000). Nevertheless, the need for higher concentrations compared to vitamin C may be due to the presence of inhibitory compounds or a lower concentration of active molecules.

Regarding sun protection activity, the ethanolic extract recorded the highest sun protection factor (SPF = 22.5), outperforming the commercial cream (ACM), which recorded an SPF = 19.5. This is likely due to its richness in phenolic and flavonoid compounds that absorb ultraviolet rays and reduce oxidative stress on the skin. Zinc oxide (ZnO) nanoparticles showed moderate protection (SPF = 14), possibly due to uneven particle size distribution. On the other hand, the essential oil provided weak protection (SPF =7.5), indicating the need to combine it with other active ingredients to enhance its efficacy.

In the tyrosinase inhibition assay, a notable increase in inhibition percentage was observed with increasing concentrations in both the general extract and the essential oil, with inhibition rates ranging from 38% to 45% at the highest concentration (400 µg/mL). This effect is attributed to active compounds that inhibit the tyrosinase enzyme responsible for melanin production, suggesting its potential use in skin-lightening products or treatments for hyperpigmentation. Interestingly, the essential oil showed slightly higher inhibition at certain concentrations, indicating a higher content of active phenolics or terpenoids.

Regarding anti-inflammatory activity, the heat-induced albumin denaturation inhibition assay revealed that the crude extract exhibited moderate activity that increased with concentration but remained lower than the reference drug Biofenac. This is consistent with previous studies indicating that crude plant extracts generally contain lower proportions of active compounds (Kumar *et al*., 2017). In another study, it was found that both plant extracts and sodium diclofenac prevent protein denaturation and maintain its function. The methanolic extracts exhibited the highest inhibition of protein denaturation (IC_{50} = 35.87 µg/mL) according to Fatima Zohra Belbachir *et al*. (2024). On the other hand, the essential oil showed high efficacy at the highest concentration (500 µg/mL), comparable to Biofenac, supporting the findings of Miguel (2010) regarding essential oils rich in phenolic compounds.

The most remarkable results were obtained from the nano-extract, which outperformed the crude extract and approached the efficacy of the reference drug, highlighting the role of nanotechnology in enhancing the absorption and bioavailability of active compounds, as noted by Patra *et al*. (2018). In antibacterial tests, the nano-extract was more effective than the conventional extract, especially against Gram-negative bacteria such as Pseudomonas aeruginosa and Klebsiella pneumoniae, showing inhibition zones ranging from 9–11 mm and 10–13 mm, respectively, compared to 6–7 mm for the plant extract. This supports the findings of Sharma *et al*. (2020) and Ahmed *et al*. (2019), who noted that nanoparticles can penetrate bacterial cell walls due to their small size and surface properties. For Gram-positive bacteria such as Bacillus subtilis, Zhou *et al*. (2018) reported their relative resistance to nanoparticles, which was reflected in our results, with smaller differences between the extracts (13–15 mm for the nano-extract vs. 9–12 mm for the plant extract). The extracts also showed notable activity against Escherichia coli (10–13 mm for the nano-extract vs. 8–10 mm for the plant extract), consistent with the findings of Kumar *et al*. (2021).

Regarding chemical composition, the extract was found to be rich in flavonoids according to Fatima Zohra Belbachir *et al*. (2024)-natural compounds well-known for their strong antioxidant properties and protective effects against oxidative cell damage. Ghasemzadeh *et al*. (2010) confirmed that plant extracts rich in flavonoids exhibit high free radical scavenging capacity. Bouayed *et al*. (2007) also emphasized the fundamental role of flavonoids in enhancing the antioxidant activity of extracts, underscoring their value in pharmaceutical and food industries. Singleton *et al*. (1999) explained that measuring these compounds is a key indicator in evaluating the biologicaAl activity of plants.

The graph shows X-ray diffraction (XRD) results for a solid sample. The sharp peaks at specific angles indicate a high-quality crystalline structure. Each peak represents X-rays reflecting off ordered atomic planes within the crystal, following Bragg's law. The peak height reveals high abundance of those atomic planes, while the sharpness and narrowness of the peaks confirm large crystal size. The absence of a broad background or scattered peaks in other regions proves no significant amorphous material (randomly arranged atoms) is present. This confirms sample purity and enables identification of its crystal structure by comparing results to standard XRD databases.

These comprehensive results indicate that the extract of *Ammodaucus leucotrichus*, especially in its nano-form and essential oil, shows great potential as a natural source of biologically active compounds with antioxidant, anti-inflammatory, antibacterial, and tyrosinase-inhibitory properties. This supports its use in pharmaceutical and cosmetic applications, although further studies are needed to precisely identify the active compounds and understand their mechanisms of action.

Conclusion

Conclusion

Ammodaucus leucotichus shows great potential as a rich source of bioactive compounds with various important therapeutic applications. Studies have shown that both the essential oil and the hydroethanolic extract, including the use of nanotechnology in preparing and delivering these compounds, exhibit strong and noticeable biological activities in laboratory experiments. These properties help improve the treatment effects and reduce possible side effects. Therefore, future research should focus on isolating the active individual compounds and studying their effects carefully, as well as evaluating possible synergistic interactions between them to increase drug effectiveness. It is also recommended to expand studies to include nanotechnology applications more deeply, as they play an important role in improving drug absorption and targeting.

Moreover, preclinical studies should be developed and accelerated to assess safety and effectiveness in different biological models, preparing for the practical and safe use of these compounds in healthcare. This way, *Ammodaucus leucotichus* can be a strong basis for developing new, effective natural treatments that help manage many health conditions

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