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THÈME

Innovations in Nanoparticles: Exploring the Biological Activities of Artemisia Plant Extracts

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بسم الله الرحمن الرحيم

Thanks

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Dedication

Praise be to God, by whose grace good deeds are done, and in remembrance of Him hearts are reassured

To the soul of my dear mother, may God have mercy on you, and make you live in his spacious paradise, and make your grave a kindergarten of paradise

and to my dear father, may God prolong his life and bless him, may God reward you with all the best for me

To everyone who supported me and helped me, from near or far, with a word, a prayer, or a position, I can only pray for you, may God reward you with the best reward, write your reward, and raise your destiny in this world and the hereafter

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"O Allah, make this work purely for your honorable face, and remind us of you at all times, and grant us success for what you love and please ."

HASSANI Saadia

Dedication

In the name of God, the Creator of reason and the enlightener of insight, in his name I begin, and to him praise first and foremost, outwardly and inwardly.

He is the one who shone the light in my heart when it was dark, and opened the doors of knowledge for me when the paths were narrow.

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TAMA Ahmed

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HANI Abderrazak

Abstract

This research aims to study the chemical composition and biological activities of Artemisia herba-alba, a well-known medicinal plant traditionally used for its therapeutic benefits. Both the aqueous extract and essential oil of the plant were examined to assess their bioactive potential. Quantitative analysis was conducted to determine the levels of key phytochemicals such as phenolic compounds, tannins, and flavonoids, which are recognized for their antioxidant, anti-inflammatory, and antimicrobial properties.

Antioxidant activity was evaluated using two methods: DPPH free radical scavenging assay and FRAP (Ferric Reducing Antioxidant Power) assay. The results showed strong radical scavenging and reducing capacities. Anti-inflammatory activity was assessed using the carrageenan-induced paw edema model in rats, where a significant reduction in inflammation was observed. Additionally, both the extract and the essential oil were tested for antibacterial activity against various microbial strains, showing promising inhibitory effects on the growth of certain microorganisms.

In addition to conventional methods, nanotechnology was incorporated into this study to enhance the bioavailability and efficacy of the plant's active compounds. Preliminary results indicated that nanoformulations may improve the stability and delivery of these compounds within the body.

These findings suggest that Artemisia herba-alba, whether in its raw form or formulated using nanotechnology, represents a promising natural source of biologically active compounds. Further research is recommended to better understand the mechanisms of action, develop more efficient nanodelivery systems, and explore its potential applications in pharmaceutical and therapeutic fields.

Keywords: *Artemisia Heba Alba*, DPPH, FRAP, Oxidative stress, Inflammation, nanoparticule.

Resumé

Ce travail vise à étudier la composition chimique et les activités biologiques de l'Artemisia herba-alba, une plante médicinale bien connue, traditionnellement utilisée pour ses vertus thérapeutiques. L'extrait aqueux ainsi que l'huile essentielle de cette plante ont été analysés afin d'évaluer leur potentiel bioactif. Une analyse quantitative a été réalisée pour déterminer les teneurs en composés phénoliques, tanins et flavonoïdes, reconnus pour leurs propriétés antioxydantes, anti-inflammatoires et antimicrobiennes.

L'activité antioxydante a été évaluée à l'aide de deux méthodes : le test de piégeage du radical libre DPPH et le test FRAP (pouvoir antioxydant de réduction du fer). Les résultats ont révélé une forte capacité de piégeage des radicaux libres et de réduction. L'activité anti-inflammatoire a été étudiée par le modèle d'œdème de la patte induit par la carraghénine chez les rats, où une réduction significative de l'inflammation a été observée. Par ailleurs, l'extrait et l'huile essentielle ont montré une activité antibactérienne prometteuse contre plusieurs souches microbiennes.

En complément des méthodes classiques, la nanotechnologie a été intégrée à cette étude dans le but d'améliorer la biodisponibilité et l'efficacité des composés actifs de la plante. Les résultats préliminaires ont indiqué que les nanoformulations peuvent renforcer la stabilité et la diffusion de ces composés dans l'organisme.

Ces résultats suggèrent que l'Artemisia herba-alba, sous forme brute ou formulée par nanotechnologie, constitue une source naturelle prometteuse de composés biologiquement actifs. Des recherches supplémentaires sont recommandées afin de mieux comprendre les mécanismes d'action, développer des systèmes de nano-administration plus efficaces, et explorer ses applications potentielles dans les domaines pharmaceutique et thérapeutique.

Mots clés : *Artemisia Heba Alba*, DPPH, FRAP, Stress oxydatif, Inflammation.

الملخص

يهدف هذا البحث إلى دراسة التركيب الكيميائي والأنشطة البيولوجية لنبتة Artemisia herba-alba، وهي نبتة طبية تقليدية معروفة على نطاق واسع بفوائدها العلاجية .تم فحص كل من المستخلص المائي والزيت العطري للنبتة لتقييم إمكانياتهما الحيوية .وقد أُجري تحليل كمي لتحديد مستويات بعض المركبات النباتية الفعالة مثل المركبات الفينولية، التانينات، والفلافونويدات، والمعروفة بخصائصها المضادة للأكسدة والمضادة للالتهاب والمضادة للميكروبات.

تم تقييم النشاط المضاد للأكسدة باستخدام طريقتين هما اختبار التقاط الجذور الحرة DPPHوطريقة FRAPلقياس القدرة الاختزالية، حيث أظهرت النتائج قدرة قوية على معادلة الجذور الحرة والاختزال لل DPPH 0.08 ± 0.78 وكذلكFRAP 0.09 ± 0.718ما تم دراسة الفعالية المضادة للالتهاب باستخدام نموذج تورم القدم المستحث بالكارجينان لدى الجرذان، حيث لوحظ انخفاض كبير في مستوى الالتهاب .بالإضافة إلى ذلك، تم اختبار الفعالية المضادة للبكتيريا لكل من المستخلص والزيت ضد عدة سلالات ميكروبية، وأظهرت نتائج مشجعة في تثبيط نمو بعض الكائنات الدقيقة.

إلى جانب الطرق التقليدية، تم إدماج تقنيات النانو في هذا العمل بهدف تعزيز التوافر الحيوي وفعالية المركبات النشطة في النبتة .وقد أشارت النتائج الأولية إلى أن التركيبات النانوية قد تُحسِّن من استقرار وفعالية هذه المركبات وطرق توصيلها داخل الجسم.

تشير هذه النتائج إلى أن نبتة Artemisia herba-alba، سواء في شكلها الخام أو بعد تحضيرها بتقنيات النانو، تُعد مصدرًا طبيعيًا واعدًا للمركبات الحيوية الفعالة .ويوصى بإجراء دراسات مستقبلية لفهم آليات عمل هذه المركبات، وتطوير نظم توصيل نانوية أكثر كفاءة، واستكشاف تطبيقاتها المحتملة في المجالات الصيدلانية والعلاجية.

الكلمات المفتاحية:DPPHH ، FRAP ، الإجهاد التأكسدي، الالتهاب،. ،AArtemisia Herba Alba

List of abbreviations

Abs: Absorbance

AC: Control absorbance

GA: Gallic acid

AE: Absorbance of the extract

NSAIDs: Nonsteroidal anti-inflammatory drugs

AIS: Steroidal Anti-Inflammatory Drugs

AlCl3: Aluminum Chloride

ATP: Adenosyne-triphosphate

DPPH : Di PhenylPicrylHydrazyl

FeCl3: Trichloride de Fer

FRAP: FerricIon Reducing Antioxidant Power

FV: Flavonoids

G/L: Grams per Liter

M mol/L: Milli mole per liter

H2O2: Hydrogen Peroxide

H2SO4: Sulphuric acid

HO^* : Radical Hydroxyle

$HO2^*$: Radical perhydroxyle

I%: Percentage of inhibition

CI50: 50% percent inhibition

$K3[Fe(CN)_6]$: Potassium ferricyanide

Kg : Kilogram

L : Liters

Mg : Magnesium

Na2CO3: Bicarbonatedesodium

NADPH: Nicotinamide AdenineDiphosphate Reduced

nm: Nanomètre

$O2^{*-}$: Superoxide anion

OH : Hydroxide

WHO: World Health Organization

ONOOH: Triperoxide

EPB: Crude Extract Weight

VMS: Weight of Plant Matter

POD : Peroxidase

PPT : Total Polyphenols

A : Performance

RLO : Oxygen Free Radicals

RO*: Radical alkoxyle

ROO*: Radical peroxyle

ROS: Reactive Oxygyn Species

TCA: Trichloroacetic acid

µl : Microlitre

UV : Ultra-violet

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General Introduction

General Introduction

Medicinal plants have long played a central role in both traditional and modern healthcare systems. Their importance extends beyond being sources of nutrients, as they have been used to treat a wide range of diseases since ancient times, as documented in traditional medical texts of Arabic, Egyptian, Chinese, Hindu, Greek, and Roman civilizations (Kamatou et al., 2017). Currently, statistics show that 70% to 95% of the population in developing countries continue to rely on medicinal plants as a primary source of treatment due to limited access to modern pharmaceuticals or their high cost (WHO, 2013).

The therapeutic efficacy of these plants is primarily attributed to their content of bioactive natural compounds, known as secondary metabolites, such as flavonoids, terpenoids, polyphenols, alkaloids, tannins, and sterols. These substances have demonstrated significant biological activity, acting as antioxidants, anti-inflammatory agents, antimicrobial agents, antitumor compounds, and inhibitors of inflammatory enzymes such as COX and LOX (Hartman, 2007; Khacheba et al., 2014; León-González et al., 2018).

Among the most widely used medicinal plants in the Maghreb countries (Algeria, Tunisia, and Morocco) is *Artemisia herba-alba*, commonly known as "white wormwood." This plant is traditionally used to treat inflammation, gastrointestinal disorders, and joint diseases, making it a subject of increasing academic and scientific interest (Mostepha et al., 2007; Yangui et al., 2009).

Recent studies have shown that the essential oil of *Artemisia herba-alba* possesses strong biological activity due to its active constituents, particularly monoterpenes such as thujone and cineole, which contribute to its antimicrobial, anti-inflammatory, and natural antioxidant properties (Boukrim et al., 2022; Doctoral Thesis, University of Batna, 2021).

With the advancement of nanotechnology, magnesium oxide nanoparticles (MgO-NPs) have emerged as a promising approach to enhancing the biological effects of plant extracts and essential oils. These nanoparticles, due to their nanoscale size and high reactivity, have demonstrated the ability to improve cellular uptake and increase antioxidant, antimicrobial, and anti-inflammatory activities—thereby reinforcing their potential in pharmaceutical and biomedical applications (El-Sherbiny et al., 2020; Choudhury et al., 2022; Doctoral Thesis, University of Constantine, 2022).

Based on these premises, the present study aims to:

- Evaluate the biological activities (antioxidant, anti-inflammatory, and antibacterial) of the aqueous extract and essential oil of *Artemisia herba-alba*;
- Investigate the effect of environmentally friendly synthesized MgO-NPs on enhancing these biological activities;
- Conduct both in vitro and in vivo experiments, supported by data from published scientific articles and national doctoral research.

This study contributes to the search for safe and natural therapeutic alternatives, whose biological efficacy is enhanced by nanotechnology, thus supporting the global trend toward the development of plant-based pharmaceutical products backed by modern science.

Despite ongoing progress in modern medicine, health challenges such as chronic inflammation, antibiotic-resistant bacteria, and oxidative stress continue to demand the development of effective and natural therapeutic alternatives. Although medicinal plants represent a promising solution, their biological efficacy is sometimes limited due to the poor bioavailability of active compounds.

In light of recent advancements in nanomaterials science, the following key question arises:

To what extent can magnesium oxide nanoparticles (MgO-NPs) enhance the biological activities of the extracts and essential oil of *Artemisia herba-alba*? And can this combination form the basis for developing effective and safe natural therapeutic products?

Part One
Bibliographical summary

Chapter I Generalities about Species Artemisia Herba-Alba

A medicinal plant is a plant used for its therapeutic properties. This means that at least one of its parts (leaf, stem, root, etc.) can be used for the purpose of healing. They have been used for at least 7000 years before our era by men and are the basis of phototherapy.

1. Generalates of the genus Artemisia

The Romans in the first century used dry flower heads obtained from several species of the genus Artemisia [Bora KS and others. 2011], for the treatment of disease caused by roundworm, enterovirus, tapeworm infections and it has become an important part of the pharmacopoeia at the beginning of the 20th century. Artemisia species have been the source of remedies such as Artemisia herba alba, Artemisia Campestris.

The species that belong to the genus Artemisia have therapeutic properties, and not only are they used in traditional medicine [Pandey AK and others .2017], but also in the food and pharmaceutical industry, the parts of the plant used in herbal medicine include the leaves and flowering tops. It has been reported that the genus Artemisia is rich in secondary metabolites such as flavonoids, caffeoylquinic acids, coumarins, essential oils.

2. Artemisia Herba Alba:

Artemisia Herba Alba in French Armoise White Grass is a monthly plant very responsive in arid to semi-arid areas [Pandey AK and others .2017]. It is a species of the genus Artemisia (Artemisia Herba Alba) which belongs to the Asteraceae family. Herbaceous and can measure from 30cm to 50cm high [Sailike B. 2022]. Its stems are floriferous and slender, a little hairy and its leaves are oblong, cut into segments of dark green color on the face and cottony white on their lower part (figure 1), it also has small yellow tubular flowers; it gives off a very strong odor, sometimes unpleasant.

Figure 2 :Photograph of *Artemisia* Herba-Alba

The flowering period of **Artemisia** extends from July to October, producing ovoid achenes as fruits. In herbal medicine, the parts primarily used are the leaves and the flowering tops. Studies have reported that the genus ***Artemisia*** is rich in secondary metabolites, including flavonoids, caffeoylquinic acids, coumarins, and essential oils [Sailike B. 2022].

3. Systematic classification:

Table 1 Systematic classification(Al-Snafi, A. E. 2015. EL Rhaffari L. (2008)

Classification	Specificname
Reign	Plantae
Phylum	Spermaphytes
Subphylum	Angiosperms
Class	Dicotyledons
Subclass	Asteridae
Order	Asterales

Family	Asteraceae or compound
Tribe	Anthemideae
Sub-tribe	Artemisiinae

4. Phytogeography

Artemisia herba-alba is a wild plant widely distributed across North Africa and the Middle East. It thrives in dry and hot climates and often forms large populations in desert regions. **Artemisia herba-alba** is classified as an Irano-Turanian species. However, the presence of Irano-Turanian flora in North Africa remains a subject of considerable debate. [abad m j, **etal 2012**].

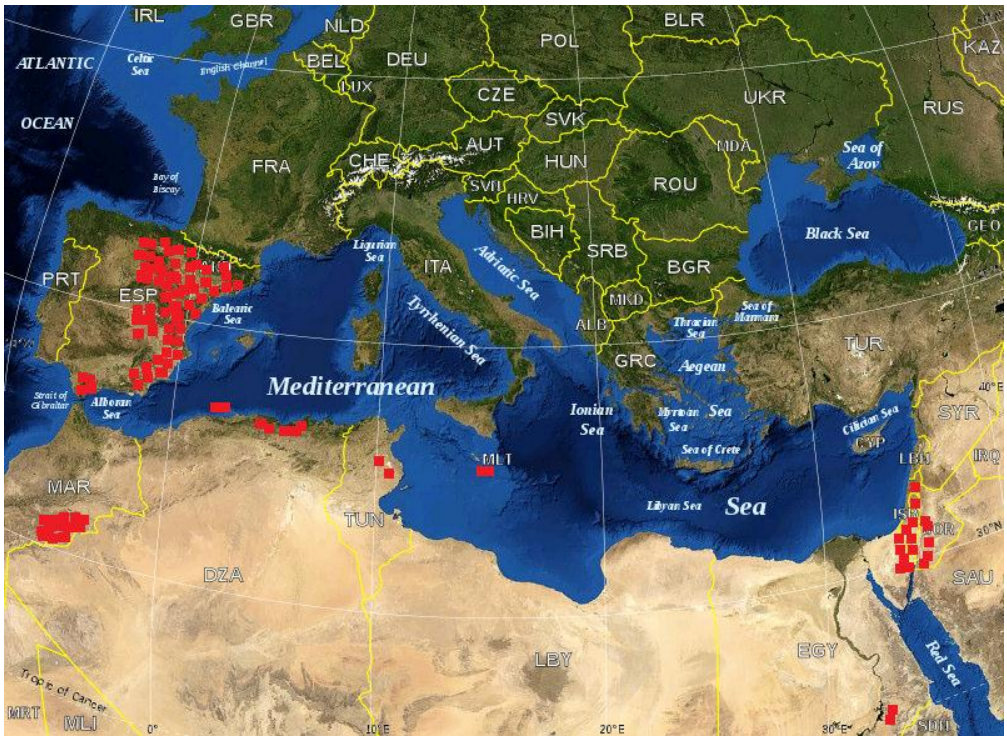

Figure 3 Global distribution of White Artemisia determined(bakkali f, etal 2008)

5. Plant ecology

Artemisia has a wide geographical distribution covering, in Algeria, about 4 million hectares; Mediterranean shrub that abounds in the Middle East, in the South of Algeria and in Morocco on deep sands [al-harbi, l. m etal 2024]. Artemisia herba-alba exists in bioclimates ranging from semi- arid to Saharan. It seems indifferent to altitudes and can live in warm to cool winter regions. In the south, this plant grows on brown steppe soils of medium texture

and in the extreme south on sandy soils. It is drought resistant, tolerates gypsum and moderately high salinity levels. It grows in clay steppes where rainfall is around 200mm / year. Its development is linked to the nature of the soil. Indeed, it must be low permeability, compacted and plugged. Accompanied by the alfa "stipatenacissima", it often covers very large areas in the highlands. Its presence is more frequent on the edges of the Wadis in the day as (Depression of the steppe with impermeable soil which are more or less humid sectors). It constitutes a means of combating erosion and desertification [Mandru, A., Mane etal 2023].

6. Botanical description

White Wormwood is a herbaceous plant with woody and branched stems, 30 to 50cm, very leafy with a thick stump. The leaves are small, sessile, pubescent and silvery in appearance. The flowers are grouped in clusters, with very small (3 / l.5 mm), ovoid heads. The involucre has imbricated bracts, the outer ones orbicular and pubescent. The floral receptacle is bear with 2 to 5 yellowish flowers per capitulum, all hermaphroditic[14].

It is distinguished by a characteristic odor of thymol oil and a bitter taste hence its astringent character. Artemisia herba-alba flowers in October - November, fruits and disseminates these seeds in December [Gatou, M. A etal 2024]. This fall bloom is an adaptive strategy adopted by many species in arid regions. It would allow the seeds to escape predation during the summer period. In its annual cycle, the leaves of white sagebrush show a seasonal polymorphism. The first that develop in winter in general are large and jagged [Abdel-Aziz, M. M. etal 2020]. The following ones are becoming smaller and smaller and less and less cut up, which allows the reduction of the transpiring surface because this polymorphism constitutes one of the adaptive characters of the species to drought. Artemisia herba-alba can reduce its weight during the dry season by more than 70%. This decrease considerably lowers the transpiration of the plant. For the underground part, it takes the form of a main root, woody and thick, distinct from the secondary roots and which sinks into the ground like a pivot. The root penetrates deeply up to 40 to 50 centimeters and branches out only at this depth [Abinaya, S.etal 2021].

That the density and depth of root branches vary with the aridity of the climate. In a desert region, this species has a shallow root system with a large number of particularly abundant lateral branches between 2 and 5 cm deep [afnor. **e**tal 2000]. This shape and method of lateral extension of the roots allows, when the aridity increases, better interception of infiltrated water.

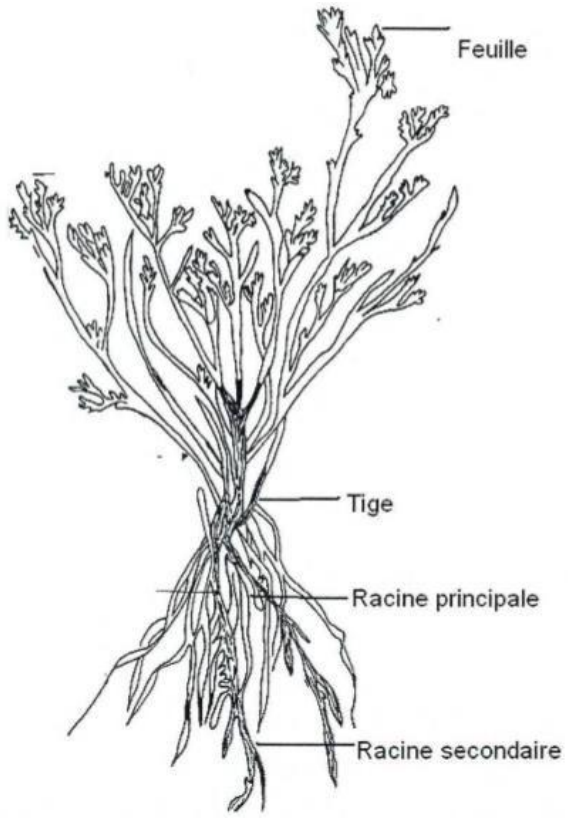

Figure 4 General morphology of the plant Artemisia herba-alba

Figure 5 Morphology of the leaf of Artemisia herba Alba

7. Use of Artemisia herba-alba

White *Artemisia* is a medicinal and aromatic plant used for a long time in traditional medicine, it is considered as a material full of medicinal and nutritional substances (fodder plant), it is also a source of substances (essential oil) which have remarkable effects biologically [aidoud a., etal 1989].

7.1. Medicinal us

It is the most famous ***Artemisia*** in Algeria. CHIH is a very popular remedy that is often used: to facilitate digestion, calm abdominal pain and certain liver ailments and antidiabetic. Its roots are indicated against certain nervous disorders. Artemisia herba alba is widely used in traditional medicine for gastric disorders such as diarrhea and abdominal pain. It is also used as a remedy for inflammation of the gastrointestinal tract [akrout a., etal 2004]. By far the most frequently cited is the use of Artemisia herba Alba As so in the treatment of diabetes mellitus. Several scientific studies have also proven the effectiveness of white ***Artemisia*** as an anti- diabetic, anti-parasitic, antibacterial, antiviral, antioxidant, anti-malarial, antipyretic, antispasmodic and antihemorrhagic. This species has been used as a decoction for fever, menstrual and nervous problems.

On the other hand, wormwood has been used since ancient times for the treatment of digestive disorders [Al Alawi, A. M etal 2018]. The active parts of the plant are all very bitter. They are used internally, either pure or in mixtures, to stimulate appetite, the secretion of digestive juice and bile, against intestinal colic as well as against intestinal parasites. It can be used in particular to treat arterial hypertension, heart failure, certain edemas, portal hypertension or hypokalaemia [athamena s etal 2010].

7.2. In pastoralism

- **Food use:** In food, white ***Artemisia*** is considered the aroma of certain drinks such as tea or coffee. Nevertheless, its use in the food industry remains very limited because of the toxicity of beta-thujone, the level of which must not exceed 5 mg / kg .(Choudhury, et al, 2022).
- **Forage use:** Artemisia herba-Alba represents an important forage resource, it is the main plant species grazed especially in spring and summer. It constitutes a very important source for the livestock. The biomass of this steppe plant constitutes a substitute food for raising livestock in times of scarcity. (Joudeh, , & Linke. 2022).

For a long time, white ***Artemisia*** has been recognized by pastoral and nomadic populations for its purgative virtues. It is used in particular as an anthelmintic in sheep. It is used by the people of Nague to relieve gastrointestinal complaints. In Tunisia, it is used for digestive diseases and for anti-diabetic treatment. Artemisia herba-alha is used as an anti- diarrhea, against abdominal cramps, and for healing external injuries. It is used against

diabetes and jaundice. It is recommended for neurological disorders .*(* Kamatou,.et al, 2017)

It is also used in traditional medicine to aid digestion, soothe abdominal and liver pain, in

the treatment of diabetes and as an anthelmintic. The roots are effective against convulsions. In high doses, *Artemisia* is abortive, neurotoxic and hemorrhagic. Thujone is the toxic and bioactive substance in *Artemisia* and the most toxic form is alpha-thujone. It has convulsive effects and also heavy consumption of white *Artemisia* has a purgative effect, especially on sheep, and can cause death in young lambs .(Joudeh, & Linke,. 2022).

8. Chemical composition of essential oils

GC/MS was used to analyze the chemical composition of essential oil extracted from the aerial parts of A. herbaalba via hydrodistillation. 38 constituents were identified, accounting for 69.37% of the oil, with the major ones being thujone (9.875%), camphor (3.762%), cis-p-menthadien-1- ol (3.572%), and isoborneol (2.334%) (Table 1) According to Belhattab et al. (2014), the major compounds are composed of thujone (28.4%) and camphor (22.8%). Zaim et al. (2012) also concluded that the major compound is Chrysanthenone (28.10%). Camphor (49.3%), according to Dahmani-Hamzaoui and Baaliouamer (2010). The major compounds are composed of Cischrysanthenyl acetate (25.12%) (Bezza et al. 2010)

Table 2 The composition of Artemisia herba-alba aerial part's essential oil

RT (min)	Compound	%
5,346	β-Pinene	0.329
5,452	1-Hexen-3-yne	0.373
5,604	Benzene	1.100
5,777	α -Phellandrene	0.219
6,269	cis-p-Menthadien-1-ol	3.572
6,794	Eucalyptol (1.8-Cineol)	0.012
7,127	1-Octene	0.06
7,343	3-Octyne	0.166
8,269	Thujone	9.875
8,934	Isocyclocitral	0.162
9,128	trans-Pinocarveol	0.24
9,218	Camphor	3.762
9,950	Isoborneol	2.334
11,383	D-Verbenone	0.434
11,825	Lenacil	5.355
12,275	Bornyl acetate	0.386

12,362	(-)-Myrtenyl acetate	0.202
12,542	Carveol (fr.1)	0.146
13,003	Thymol	0.179
14,078	2-Cyclopenten-1-one	0.267
14,211	Copaene	0.179
15,197	Humulen- (v1)	0.004
16,092	Isoaromadendrene epoxide	0.22
16,349	Azulene	0.025
16,401	Farnesene epoxide	0.022
16,806	ɣ -Elemene	0.036
16,933	Davana ether	0.053
17,965	Caryophyllene oxide	0.065
18,595	(-)-Spathulenol	0.482
18,830	Aristolene epoxide	0.098
18,918	1H-Cycloprop[e]azulen-4-ol,	0.096
19,656	Longipinocarvone	0.043
19,698	Androstan-3-one	0.036
19,813	ɣ-Gurjunenepoxide	0.057
19,925	Ledene alcohol	0.027
20,096	2-Naphthalenemethanol	0.072
25,293	Limonen-6-ol, pivalate	0.005
25,624	Phosphinous chloride	0.017
NI	38.102	

Note: a RT: Retention time of the compound in minutes

8.1. Phytochemical analysis

During the phytochemical screening of A. herba-alba plant extracts, the presence of alkaloids, saponin, flavonoids, steroid and triterpene, tannins, and reducing sugars was discovered (Table 2). These chemicals have been shown to have physiological activity. Our findings are very similar to those of Mouhamed et al. (2010).

Table 3 Phytochemicals found in methanolic extract of Artemisia herba-alba

Phytochemicals	Aerial part
Flavonoids	+
Saponin	+
Steroids	+
Reducing sugars	+
Tannins	++
Alkaloids-wagner's reagents	+
Alkaloids-Draghandroff-reagents	+
Volatile oils	+++

Note: a Key: + = present, - = absent

8.2. Biological uses

- Antioxidant activity

Many medicinal plants contain large amounts of antioxidant compounds, which could be isolated and then used as antioxidants for the prevention and treatment of free radical- related disorders. In a study by Djeridane (Kamatou,.et al,2017) , the purpose was the evaluation by a chemical method of the antioxidant capacity of phenolic compounds in some Algerian medicinal plants, including A. herba-alba. These medicinal plants showed stronger antioxidant activity and content in phenolics than the common nutritional plants. It has been also noted in this study that these Algerian plants are strong radical scavengers and can be considered as good sources of natural antioxidants for medicinal and commercial uses .

(Khacheba.et al,2014). Raw and cooked ground beef patties were treated with an aqueous extract of A. herba-alba, rosemary, fennel and rue at levels of 5 mm of 10% (w/w vegetable matter to water) extract for every 100 g of meat. Patties were kept under refrigeration (4º) for a period of 16 days, and samples were drawn at 4-day intervals. Results showed that cooked meat was more susceptible to oxidative deterioration than raw meat. In addition, A. herba-alba had a somewhat less effective role than the other herbs .(Khacheba, H.,et al.2014) . In another study, 21 plant samples were collected from different Jordanian locations and used for antioxidant evaluation. The level of antioxidant activity, determined by DPPH and ABTS assays, showed that Artemisia herba- alba has a moderate antioxidant activity compared to the other plants (Khan, I.et al, 2019).

Abid compare the long-term effects of Artemisia herba-alba decoction with a green or black tea decoction, prepared without sugar, on the antioxidant processes in rats. The conclusion of this study showed that Artemisia, as well as green tea decoctions, increased the total antioxidant status, whole blood glutathione peroxidase activity and zinc and copper status, and prevented weight gains and increased conjugated dienes, plasma glucose, lipids and iron status. The beneficial antioxidant effects were in descending order: Artemisia decoction ≥ green tea decoction > black tea decoction. So, Artemisia could constitute a good adjuvant to combat obesity, hyperglycemia, hyper-triglyceridemia, hyper-cholesterolemia and particularly oxidative stress (León-González.et al,2018)

The effects of seven medicinal plants including Artemisia herba-alba on protein degradation, lipid peroxidation, erythrocyte deformability and osmotic fragility of erythrocytes exposed in vitro to 10 mM H_2O_2 for 60 min at 37°C have been examined. The result was that Artemisia herba-alba did not protect erythrocytes against lipid peroxidation (Moreira. 2022).

- **The antimicrobial activity**

The antimicrobial activity of A. herba-alba has been evaluated in many studies of experimental nature. In studies examined the essential oils of A. herba-alba reported that it had a great potential on microbial activity against strains of Staphylococcus aureus,Micrococcus luteus, Escherichia coli, Salmonella typhimurium, Bacillus cereus, and Entrococcus faecalis. Furthermore, antimicrobial activity of A. herba-alba have been confirmed in some yeast strains of Candida

- **Anti-inflammatory**

An anti-inflammatory drug is a drug that acts on the pathophysiological consequences of the inflammatory reaction, regardless of its origin. (Muster, 2005). There are two main groups: non-steroidal anti-inflammatory drugs (NSAIDs) and steroidal anti-inflammatory drugs (AIS), which have different pharmacodynamic targets. (Adepo Apie, 2018).

Non-steroidal anti-inflammatory drugs (NSAIDs) are one of the most popular treatment classes used worldwide for anti-inflammatory and antipyretic and analgesic properties. There are currently more than 50 different NSAIDs on the market worldwide. The mechanism of action of NSAIDs was clarified by Vane's work in 1971, based largely on the competitive inhibition, reversible or not, of cyclooxygenase, an enzyme that allows the production of prostaglandins from arachidonic acid. Amazingly, the common properties of all NSAIDs lead to a decrease in the production of prostaglandins (including PGE2 and PGI2), important mediators of inflammation.

Steroidal anti-inflammatory drugs (AIS) are a large family of drugs derived from cortisol, the main adrenal glucocorticoid. Glucocorticoids are substances derived from cholesterol whose production is stimulated by ACTH released according to the nycthemeral cycle by the anterior lobe of the pituitary gland.

In target tissues, glucocorticoids bind to glucocorticoid (GR) receptors cellular cytoplasm. Subsequently, it enters the receptor complex formed in the cell nucleus where it binds to many elements of the glucocorticoid response in the promoter region of the target gene. The receptor, which is attached to a DNA molecule, interacts with essential transcription factors, resulting in an increase in gene expression of selected target genes. This process is referred to as transactions and terms of most glucocorticoid metabolic and cardiovascular side effects.

Secondary metabolites

Secondary metabolites have a limited distribution in different plant species. They have very important functions for the survival and propagation of the plants that produce them, as chemical signals, to defend their producer against herbivores and pathogens. They participate in allopathic responses (competition between plants for germination and growth) (Larbi and Amrous, 2018).

Of extraordinary structural diversity but produced in small quantities, these molecules determine the mode of origin of a species, a family or a genus of a plant and sometimes make it possible to establish a chemical taxonomy (Boussouf and Menaa, 2020).

Phenolic compounds, terpenes, and alkaloids. Each of these classes contains a very diverse group of compounds that have a very wide range of activity in human biology and are "examples of secondary metabolites" (Larbi and Amrous, 2018; Boussouf and Menaa, 2020) .

9. Polyphenols

9.1. Definition of polyphenols

Polyphenols are molecules that plants make during their secondary metabolism to defend themselves against environmental aggressions. Phenolic compounds also play an important role in human health due to their various pharmacological activities such as antiviral, anti-inflammatory, anticancer, antiallergic, antimicrobial, cardioprotective, and vasodilator.

In addition, they can prevent oxidative modification by neutralizing and removing free radicals, or reduce metals through their antioxidant activities (Ladoh *et al.*, 2015). They are located in different parts of plants (roots, stems, leaves, flowers, pollen, fruits, seeds and wood) depending on the type of plant and the polyphenolic group considered (Hadri, 2015).

These compounds include many molecules and represent one of the most important groups in the plant kingdom.

As a definition, polyphenols are water-soluble phenolic compounds, with a molecular weight between 500 and 3000 Dalton, they result biogenetically from two main synthetic pathways, the shikimate and acetate pathway. The fundamental structural element that characterizes them is the presence of an aromatic ring (benzoic) carrying at least one free hydroxyl group or one hydroxyl group engaged in another chemical function (ether, methyl, ester, sugar, etc.). The structure of these compounds varies, from simple molecules (simple phenolic acids) to highly polymerized molecules (condensed tannins) (Gobbi and Khebbaz, 2014).

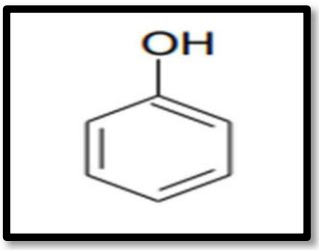

Figure 6 Structure of the phenolic nucleus (Cheynier, 2005).

9.2. Classification of polyphenols

The different classes of polyphenols can be distinguished based on the number of constituent atoms on the one hand and on the basic backbone structure on the other hand (Behih and Ben Amrouvhe, 2017).

Two main classes are widespread:

1. Flavonoids.
2. Tannins.

10. Flavonoids

10.1. Definition

The term flavonoid is well known, and it is derived from the Greek word Flavus, which means yellow color (Zaatir,2006). They are compounds of phenolic origin that are very widespread in the plant kingdom (Bouakaz, 2006), More than 6000 natural flavonoids have been extracted (Alaoui, 2015). Plant pigments distributed over all parts of the plant, and more so in the above-ground part of the plant (Lghroun 2016), are generally responsible for the colours of flowers, fruits and sometimes leaves (Yezza and Bouchama 2014; Elalaoui, 2015), It is found in most varieties of plants, especially the most high-end, and almost non-existent in

algae, These

compounds may be found in their free form (aglycones) or as bound sugar (glycosides) (Erlund 2004; Zerrouki, 2009).

10.2. Chemical structure and classification

All flavonoids have a 15-carbon structure with an arrangement C6-C3-C6.Il consists of two aromatic units, one known as the A ring and the other as the B ring, linked by a side chain. It is composed of 3 carbons that can be opened and can be cyclic to form the C ring (Figure 4), which represents the Chromane ring (central pyran ring) and gives the basic structure to the flavonoids from which the basic unit called 2-phenylchromane is derived (Azri, 2013; Ghayaba, 2015).

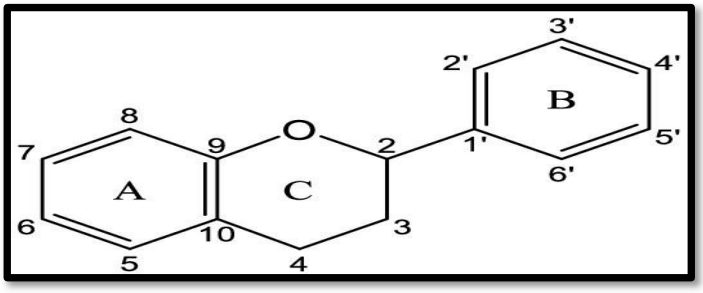

Figure 7 Basic structure of flavonoids (Azzi, 2016).

This group of compounds has been divided into different categories according to: the number, position and nature of the substituents, which are most often hydroxyl or methoxyl groups, or the level of oxidation of the heterocyclic ring (Figure 5) (Tamma, 2018; Alaoui, 2015).

They are generally divided into five classes: flavonols, flavones, anthocyanidins, flavonones and chalcones (Guerrah, 2015).

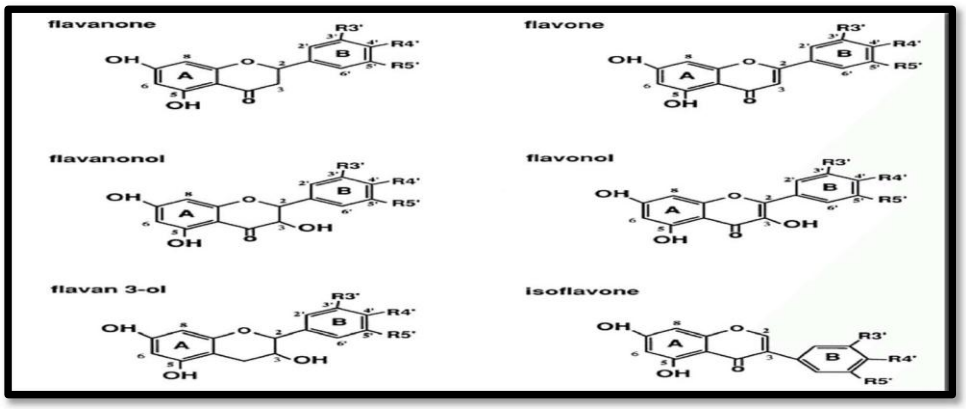

Figure 8 Structure of some flavonoid classes (Benzaoui and Houari, 2016)

10.3. Interests and biological effects of flavonoids

Flavonoids have the ability to modulate enzyme activity and modify the behavior of many cellular systems, suggesting that they may exert a multitude of biological activities, including significant anti-hepatotoxic, vasculoprotective, antiallerc, anti-inflammatory, anti-ulcer, and even anti-moral properties (Athamena, 2009; Bougandoura, 2011).

These compounds can prevent oxidative damage through different mechanisms of action: either by capturing hydroxyl, superoxide, alkoxyl and peroxide radicals; or by chelating metals (iron and copper) that are of major importance in initiating free radical reactions; or by inhibiting enzymes responsible for generating free radicals (Gori, 2014; Ngene *et al.,* 2015).

Flavonoids also protect plant tissues by absorbing ultraviolet rays and thus protecting essential materials (proteins and nucleic acids) from the toxic effects of these radiations, and also help to reduce the phenomenon of transpiration in dry areas (Mohammedi, 2013).

It should also be noted that flavonoids, by repelling certain insects by their irritating taste, can play a role in plant protection. Flavonoids exhibit other properties of interest or involved in photosensitization, morphogenesis, sex determination, photosynthesis, and regulation of plant growth hormones (Berdjouh *et al* .,2022).

11. Tannins

11.1. Definition

Tannins are a compound made up of polyhydroxyphenol groups, soluble in water, alcohols, acetone and slightly soluble in ether. It has a varied structure, with large molecular weights between 3000 and 500 Daltons (Abaydi *et al.,* 2020; Smaili, 2014). It has been discovered by modern analytical methods that the molecular weight of tannins reaches 20000 (Hawa, 2013). Where having in common the property of tanning the skin, i.e. making it resistant to mold and with low permeability, it is therefore called tannins (tannins). With an

unpleasant taste, with an astringent effect, these substances have the property of combining with proteins, which explains their ability to acquire a tan. Very common in the plant kingdom, it is found in various organs, but there is more accumulation especially in old or

Sick. They are located in vacuoles, sometimes combining with proteins and alkaloids (Roux and Catier, 2007; Smaili, 2014).

11.2. Classification des tannins

Three groups of tannins are generally distinguished in higher plants. They are differentiated by their structure as well as by their biological origin: hydrolyzable and non-hydrolyzable tannins (condensed) and complex tannins (Bruneton, 1999) as shown in Figure 6.

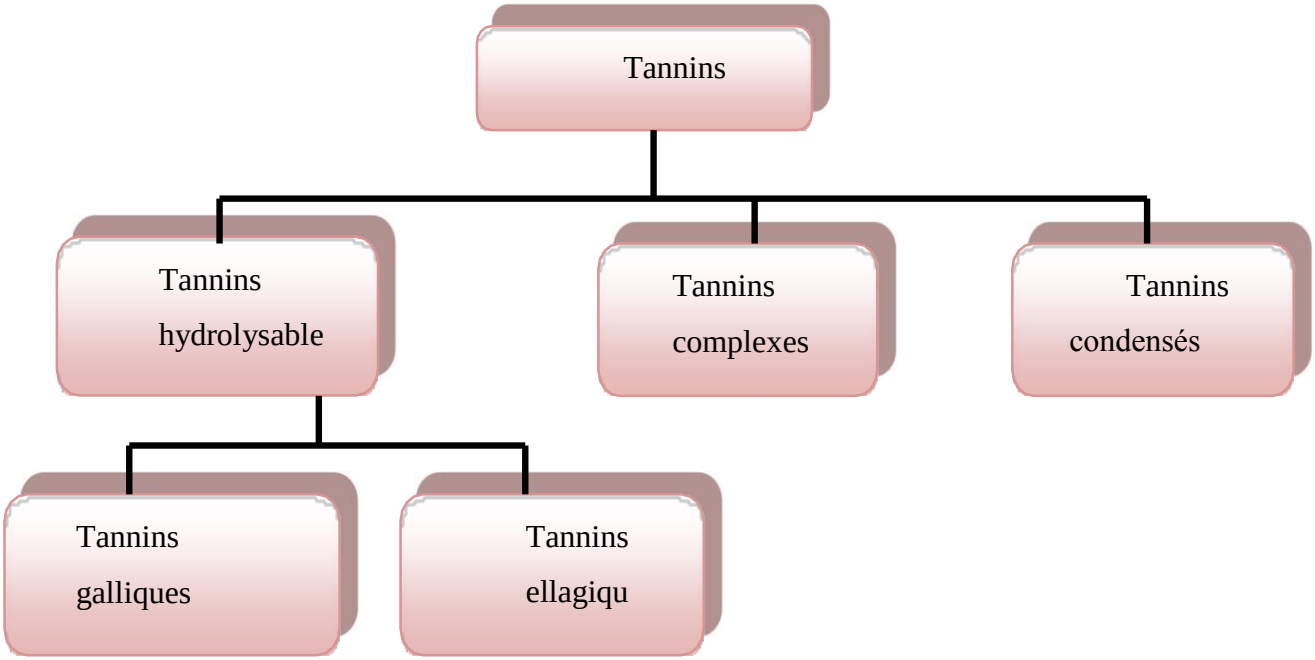

Figure 9 Classification of tannins according to their chemical structure (Wilfred and Ralph, 2006)

❖ **Tannins hydrolysables**

They are esters of a sugar (very often glucose but in some cases polysaccharides) and a variable number of phenol acid molecules (Figure 7). This group of tannins is characteristic of Dicotyledons (Hmid, 2014). As their name suggests, their capacity is characterized by acid and basic hydrolysis, and hydrolyzes under the action of enzymes and hot water (Djenidi, 2012).

Depending on the nature of the hydrolyzable tannins, a distinction is made between:

S Gallic tannins: which are the esters of oses (glucose) and gallic acids

S ellagic tannins: are split by enzymes into oses and ellagic acids (Figure 8) (Asres *et al.*, 2005)

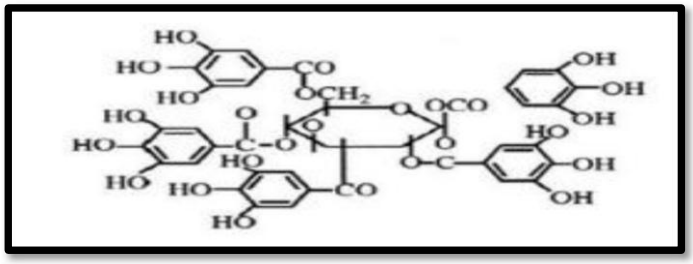

Figure 10 The structure of hydrolyzable tannins (Azzi, 2016).

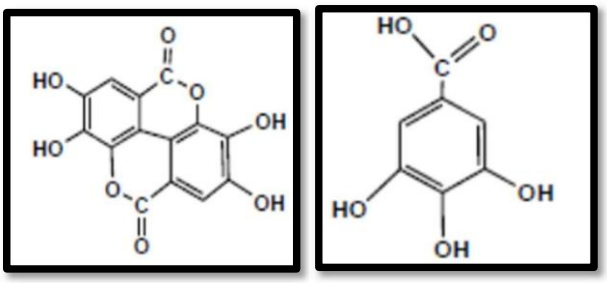

Figure 11 Chemical structure of some hydrolyzable tannins (Crestini and Lange, 2015).

Acide élagallique Acide gallique

- **Tannins condense**

Condensed tannins, proanthocyanidin, are polyphenols belonging to the flavonoid family and are widely distributed in the human diet. Are chemically defined as are oligomers or polymers of flavan-3-ols (Figure 9) that have the property of releasing anthocyanins in a hot acidic environment by breaking the inter-monomeric bond (Gori, 2014).

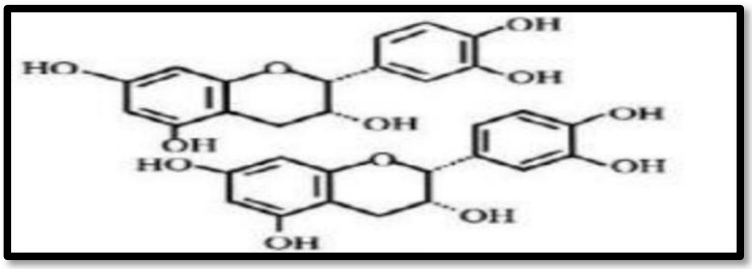

Figure 12 The structure of condensed tannins (Azzi, 2016).

- **Tannins complexes**

Both are hydrolyzed tannins and condensed tannins. In this case, the hydrolyzable tannin, which can be gallic or ellagic, is bound to one or more Flavon-3-ol units that are characteristic of condensed tannins (Bruneton, 2009).

essential oils EOs as products obtained either from natural raw materials by water or

steam distillation, or from Citrus peels by mechanical processes and which are separated from the aqueous phase by physical processes (LAWRENCE, 1995)

The biological activity of essential oils

Essential oils have been known and used for a long time, at that time their use was based on traditional practices and applications without precise scientific bases (HELLAL, 2011). Nowadays, their use is done on a scientific and rational basis since many research works have focused on the antimicrobial and antioxidant activities of the EOs of aromatic plants, which allows them to be used in different fields such as conservation (HELLAL, 2011).

Physiological role of essential oils

Many plants produce essential oils as secondary metabolites, their exact role in the plant's life process is still poorly understood. According to (BAKKALI, 2008), essential oils can have several "useful" effects for the plant:

- Repel or on the contrary attract insects to promote pollination.

- As an energy source.

- Facilitating certain chemical reactions. -Allowing to preserve the humidity of desert plants.

- Reduction of competition from other plant species by chemical inhibition of seed germination.

- By protection against infectious microbial flora.

Chemical composition

Chemically speaking, EOs are mixtures of extremely complex structures (HELLAL, 2011), which can contain more than 300 different compounds belonging to two groups characterized by specific biogenetic origins, terpenes and phenylpropane derivatives (BOUGUERRA, 2012). They may also contain various products resulting from degrading processes involving non-volatile constituents (Bruneton, 1999).

12. Terpenes

are the major group of compounds of the generally most volatile EOs with low molecular weights (NAIT, 2012). From a structural point of view, terpenes are natural hydrocarbons, with a cyclic or open chain structure, this structure is the result of the combination of several isoprene (C5H8)n units, thus forming a more or less long hydrocarbon backbone (HELLAL, 2011). On this basic skeleton, we find the presence of one or more similar or different

functional sites, the majority of functional sites are oxygenated sites with one or more oxygen atoms, for some nitrogenous or sulfur functional groups (Bruneton, 1999). They are also referred to as terpenoids, which are considered to be modified terpenes with methyl groups added or removed, or oxygen atoms added (NAIT, 2012).

12.1. Aromatic compounds

Phenylpropane (C6-C3) derivatives or so-called phenolic compounds are less responsive than terpenoids (HELLAL, 2011). They are generally characterized by the presence of a hydroxyl group attached to a phenyl ring (BRUNETON, 1999).

12.2. Compounds of various origins

There are a significant number of products resulting from the transformation of non-volatile molecules resulting either from the degradation of non-volatile terpenes that come from auto-oxidation, for example carotenes or fatty acids (HELLAL, 2011)

Different

methods are used to extract vegetable oils, this diversity is due to the variety of materials and the considerable sensitivity of their certain constituents. The choice of suitable method is made according to the nature of the plant material to be treated, the physico-chemical characteristics of the species to be extracted and the use of the extract, the main extraction methods are:Hydro-distillationSteam entrainment. Extraction by fats. Hydro-diffusion. Cold expression. Solvent extraction. Microwave extraction. Regardless of the type of extraction used, the steps in the extraction of essential oils of plant origin remain identical, it is necessary first to extract from the plant material the aromatic molecules constituting the essential oil, then to separate these molecules from the medium by distillation (LUCCHESI, 2005).

13. Extraction by hydrodistillation

This is the simplest method and therefore the oldest used. The plant material in a still filled with water placed on a heat source, the whole is then brought to a boil, the heterogeneous vapors are condensed in a refrigerant and the essential oil separates from the hydrosol by simple difference in density. As the essential oil is lighter than water, it floats above the hydrosol (PIOCHON, 2008) (Figure N° 02)

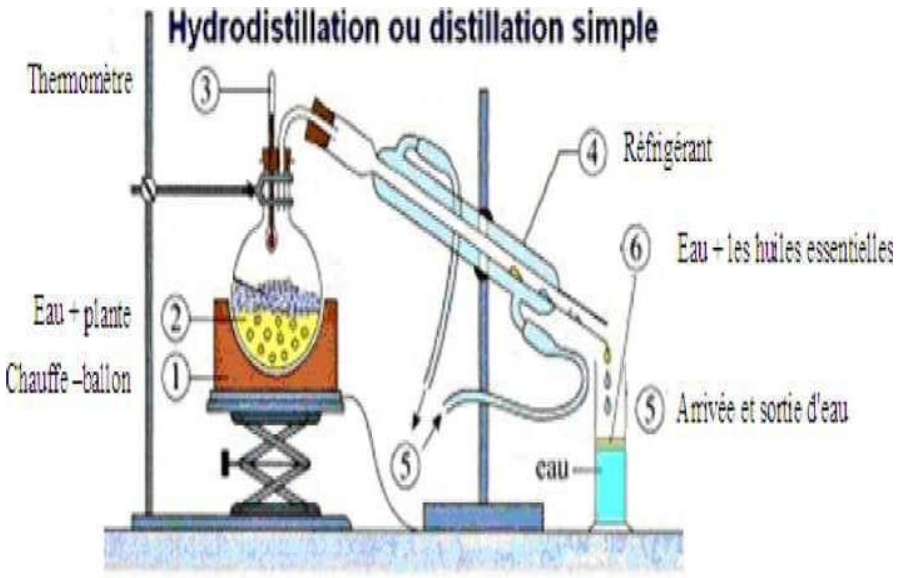

Figure 13 Extraction assembly by hydro-distillation. (PIOCHON, 2008)

Chapter II :

Biological Nanoparticles

I. Nanotechnology

Nanotechnology is the branch that involves synthesizing, engineering and utilizing materials ranging in size from 1 to 100 nm, known as nanomaterials. (Joudeh and Linke, 2022).

Nanoparticles possess unique physical and chemical properties due to their high surface area and nanometer size (Khan et al., 2019).Nanoparticles are generally called particles controlled or tricked at the atomic level. The intrinsic properties of metal nanoparticles such as zinc oxide NPs are mainly characterized by their dimension, composition, morphology, and crystallinity. Reducing the size to the nanoscale can transform their chemical, mechanical, technological, architectural, morphological, and optical properties (Sirelkhatim et al., 2015).

2Magnesium Oxide Nanoparticules

Magnesium is an essential mineral found abundantly in the human body, particularly in bones, muscles, and soft tissues. It plays a vital role in numerous biological processes, including enzyme activation, energy production, and regulation of muscle and nerve functions (Al Alawi, A. M. et al, 2018).

Magnesium oxide nanoparticles (MgO NPs) have several advantages over other metal nanoparticles, including low cost, non-toxicity, biocompatibility, stability under harsh processing conditions, relevant biomedical applications, and potent antimicrobial activity without photoactivation. In this regard, magnesium oxide (MgO) is currently classified as a GRAS (Grade A Safe) substance and suitable for human food. (Abdel-Aziz, M. M. et al, 2020).

The surface area, particle size, and crystallinity of MgO NPs can be tailored depending on the synthesis method used, including sol-gel, hydrothermal, or green synthesis approaches. These properties significantly influence their applications in various fields such as biomedicine, environmental remediation, and catalysis (Gatou, M. A. et al,2024). Due to the above properties, magnesium oxide is used as a semiconductor material, a catalyst in organic transformations, an adsorbent for organic and inorganic pollutants from wastewater, in electrochemical biosensors, as a photocatalyst, and as a heat-resistant material. It also exhibits antibacterial, anticancer, and antioxidant properties. (Abinaya, S. et al., 2021). Additionally, their non-toxic nature, low cost, and ease of production make them an attractive alternative for developing advanced nanomaterials for both therapeutic and industrial applications.

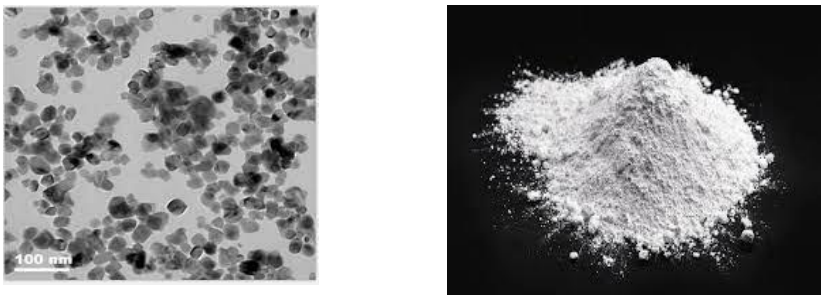

Figure 13. Magnesium Oxide Nanoparticles and powder (Azom, 2011; Azonano, 2013).

3.2 MgO NPs synthesis methods

3.1 .Physical synthesis methods

Magnesium oxide (MgO) prepared using physical methods, such as vapor deposition, plasma irradiation, and ultrasound. These techniques generally require high energy and robust equipment to produce MgO nanoparticles (Abinaya, S. et al., 2021).

3.3 Chemical synthesis methods

Recently, chemical methods for synthesizing magnesium oxide (MgO) nanostructures have received considerable attention. Mg(OH)2 nanostructures can be prepared in a variety of forms using several methods, such as electrodeposition, sol-gel techniques, precipitation, hydrothermal, solvothermal, bubbling, and microwave-assisted synthesis. Several reports have indicated this. (Pilarska, A *et al*., 2013).

3.3 Green synthesis methods

Biosynthesis, or green synthesis, is an eco-friendly and cost-effective method for producing nanoparticles using non-toxic materials from plants and microorganisms. It avoids harsh reaction conditions and toxic chemicals, aiming to reduce waste and support sustainability. While currently limited to laboratory-scale production, ongoing research suggests that large-scale manufacturing without heavy machinery could soon be achievable.(. Gatou, M. A, et al.2024. Abinaya, S. et al., 2021)

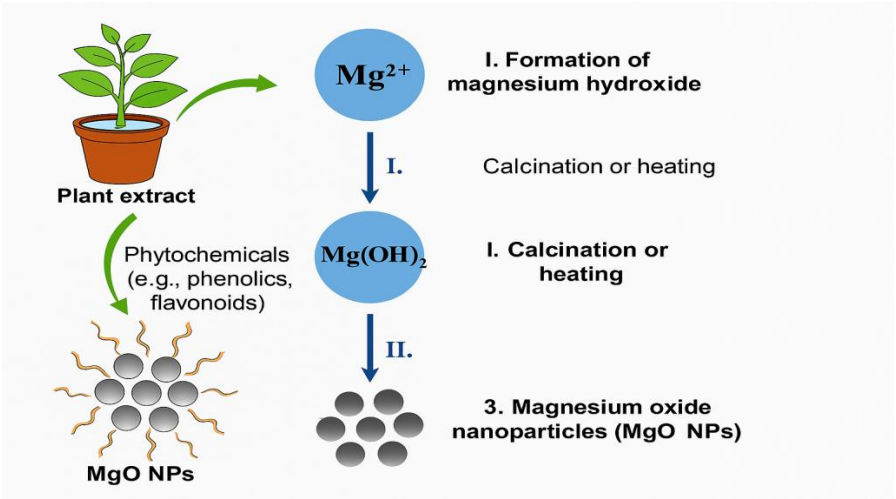

Figure 14. green synthesis mechanism of MgONPs using plant extract

3. Characterizations of MgO nanoparticles

The biosynthesized MgO NPs can be characterized using different analytical techniques.

4.1. UV-visible spectroscopy

The basic principle of UV spectroscopy is based on the absorption of samples at specific wavelengths of light, providing valuable insights into the material's response to this absorption. This method demonstrates the application of Beer's law and is characterized by its accuracy, simplicity, and broad applications, including drug discovery and structural elucidation of organic molecules. The instrument operates in the wavelength range of 200–800 nm. Finally, UV spectroscopy is an essential part of the gathering and evaluation of scientific data because of its many uses.(Mandru, A.et al,2023)

4.2.Fourier Transform Infrared Spectroscopy (FTIR)

FTIR identifies chemical bonds within compounds by observing vibrational transitions, producing spectra with distinct absorption bands characteristic of specific bond types.(Chen, Y.et al,2015)

4.3.X-ray diffraction

Ideal for powdered samples, XRD identifies crystal structures by analyzing diffraction patterns of X-rays scattered off the sample. Peaks in the pattern reveal details like crystal size, shape, and phase according to Bragg's law. (Bunaciu, A..et al,2015)

4.4. Scanning electron microscopy (SEM)

SEM uses high-energy electrons to scan the surface of a sample, producing high-resolution images of its surface morphology. It creates 3D images but necessitates coating the sample with a conductive material (Goldstein, J. I.et al , 1992).

4.5. Energy Dispersive Spectroscopy (EDS)

Spectroscopic methods in microscopy, like Energy Dispersive X-ray Spectroscopy (EDS), allow direct analysis of a sample's chemical composition without requiring prior information or simulations. (Moreira, M. H. M .et al ,2022).

5. Biological activities of MgO nanoparticles

5.1. Antimicrobial activity

MgO-NPs are recognized as effective and safe antibacterial agents (see Figure 4). Many debates have centered around their application in coatings or nanocomposite materials. Additionally, several studies have proven the antimicrobial properties of natural biological substances. The particle size of these nanoparticles also plays a significant role. (Gatou, M. A, et al.2024)

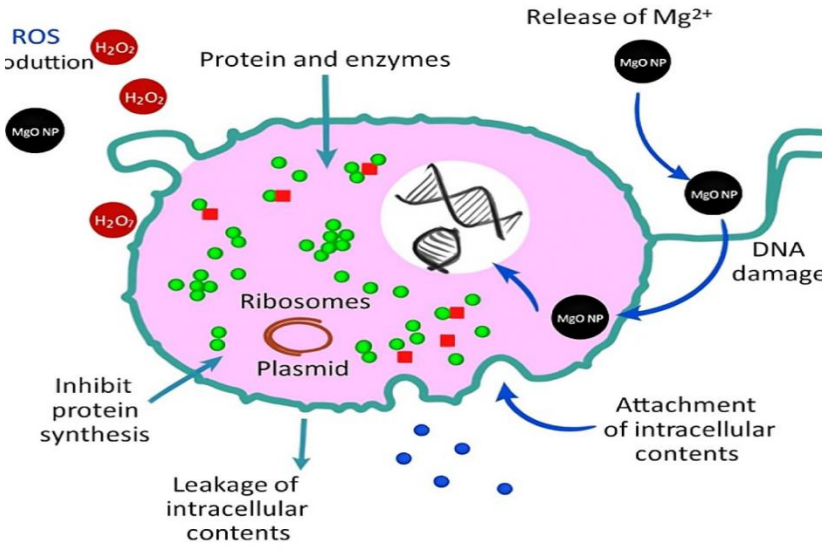

Figure 15. Schematic illustration shown Magnesium oxide nanoparticles' antibacterial mechanism

Magnesium oxide nanoparticles not only have antibacterial and antibiofilm effects but also possess antifungal properties. Research has shown that these nanoparticles impact fungal pathogens through multiple mechanisms, such as causing physical damage, interacting with fungal cells, destabilizing cell membranes, and inducing oxidative stress. Additionally, the antifungal effectiveness of biosynthesized magnesium oxide nanoparticles has been tested on two fungal strains, Aspergillus flavus and Fusarium solani. (Ramezani Farani, M. et al., 2023).

5.3. Anti-inflammatory Activity

Bioinspired magnesium oxide nanoparticles can enhance their anti-inflammatory potential by activating pro-inflammatory cytokines and interleukins that initiate the inflammatory process. Ultimately, bioinspired magnesium oxide nanoparticles show increasing potential as a topical therapeutic agent for the treatment of numerous diseases, thanks to their exceptional biocompatibility and antioxidative and anti-inflammatory properties. However, further research is needed to investigate their pharmacokinetics as an alternative to natural drugs available on the market. (Al-Harbi, L. M ., 2024)

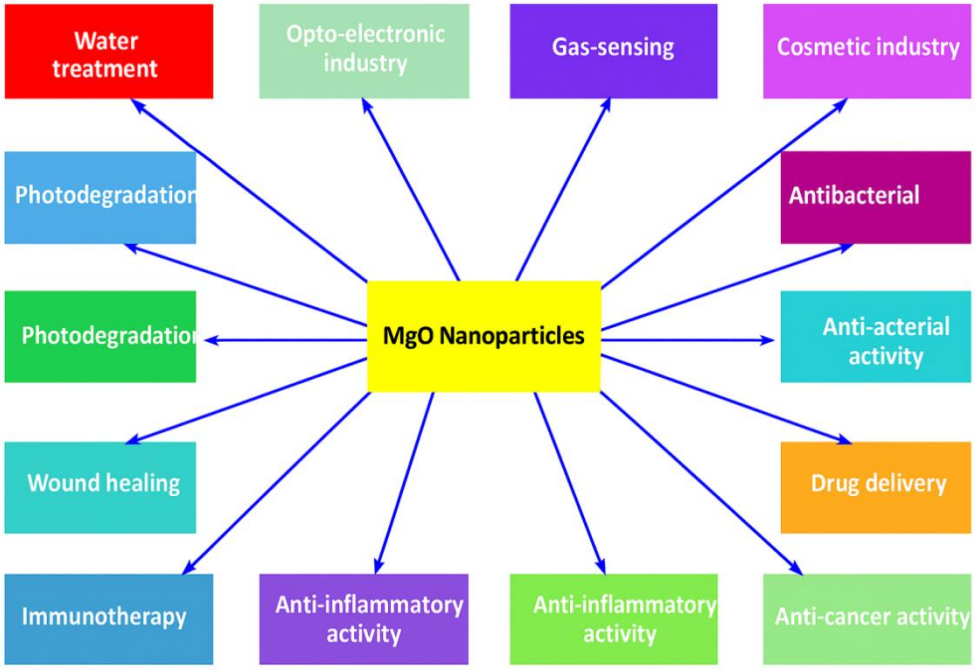

Figure 16. MgO Nanoparticles activities and applications (Raha & Ahmaruzzaman, 2022)

Part Two

Experimental

Chapter I Material and Method

In this chapter, we discuss the principle adopted, the materials used, and the methods used to study the aqueous extract of the wild medicinal plant *Artemisia Herba Alba*.
The aim was to identify and evaluate its antidiabetic, anti-inflammatory, and in vitro activities, as well as its antioxidant activities.

Our research was carried out in the teaching laboratory of the Faculty of Natural and Life Sciences at Echahid Hamma Lakhdar University in El Oued and the El Medjed Medical Analysis Laboratory.

I. Study material

Study materials include laboratory equipment, equipment and chemicals in addition to biological materials.

I.1. Biological materials

It is represented by the plant species chosen and the animal model adopted.

A. Plant material

The plant species used in this experimental study is to Artemisia Herba Alba the family Asteraceae, and its taxonomy, phytochemistry, activity and all data relating to it have been previously reported. The plant sample was chosen for this study based on its common use in traditional medicine for the treatment (Hammiche et al., 2006) and its antioxidant (Khettaf et al., 2016) and anti-inflammatory effect, and its importance has been recognized by (Ozenda, 1958; Quézel and Santa,1963) and are often found in arid or desert regions.

The aerial part (the stems and leaves) was chosen to carry out the experiments in this study because it is at this level that the majority of the active substances are found, i.e. it is the place where the main compounds of primary and secondary metabolism are synthesized and temporarily stored.

Collection, Drying, and Grinding of Artemisia herba-alba

The plant Artemisia herba-alba was collected in March 2022 from the Wilaya of Djelfa. After collection, the plant samples were placed in suitable cloth bags and immediately transported to the laboratory for drying.

The drying process was carried out at room temperature, away from direct sunlight, in order to preserve the active constituents of the plant. Once drying was complete, the stems and leaves were ground into a fine powder using an electric grinder, to be used later in various chemical and biological analyses.

impurities, then spread out in thin layers. It is then dried at room temperature, in a shaded and well-ventilated area.

The aerial part of the plant is washed with water to remove .After the drying process is completed, it is partially ground using an electric grinder to obtain a plant powder. The powder is then stored in tightly sealed containers and placed in a dry, dark place, away from heat sources, until he beginning of the experiments.

Figure 14 Plante *Artemisia herba-alba* (Mekhadmi, 2024)

Geographic distribution of the harvest area *Artemisia herba-alba*

Djelfa Province, from which the aerial parts of *Artemisia herba-alba* were collected, is located in northern Algeria within the High Plateaus region. It is characterized by a semi-arid climate, with significant temperature variation between summer and winter, and low precipitation levels. The altitude is approximately 1,200 meters above sea level.

It is bordered to the north by Médéa Province, to the south by Laghouat Province, to the east by M'Sila Province, and to the west by Tiaret Province (see Figure 18).

Figure 15 Geographical map of Algeria showing the harvesting area of the plant studied El Djelfa

B. Animal material

Our study was carried out on 353 male white rats of the *Wistar* Albino strain aged between 9 and 11 weeks and weighing between 150 g and 180 g. These animals were brought from the Pasteur Institute in Algiers, and raised in an animal facility of the Faculty of Natural and Life Sciences of the University of El-oued.

- **Breeding**

The fingerling potatoes are raised in plastic cages after being cleaned. four rats are distributed in each cage with labels that distinguish the batches according to the study, and in order to track each rat during the treatment period, they are numbered with permanent marker. These cages contain sawdust which is changed three times a week until the end of the experiment.

The rats are kept in the adaptation phase for two weeks before the start of the experiment under controlled light and temperature conditions (12 hours of lighting/24 °C temperature) where they have free access to water and standard food. All rats are processed and handled according to the standards set out in the Manuals for the Care and Use of Experimental Animals (CCAC, 1984). To monitor the weight evolution of the rats, measurements were carried out régulièrement deux fois par semaine à l'aide an electronic scale.

- **Diet**

The diet used in our experiment is marketed as El ALF cattle, as a mixture of different products ground, this feed is composed of barley, corn and bran (Southon *et al.,* 1984)

Figure 16 photograf *a* (Mekhadmi, 2021)

II. Laboratory Equipment

II.1. Apparatus and equipment

The equipment used is grouped in the following table (Table 04):

Table 4 Equipment used in experiments

Device	Laboratory equipment
Spectrophotométre UV-Vis+ Cuves	Graduated test tube
Bain-marie	Eprouvette graduée
Horizontal Centrifuge	Glass Test Tubes + Stand
Drying oven	Beaker + Crystallizer
Magnetic stirrer + magnetic bar	Micropipettes + Pipette graduée
Analytical balance	Pissette
Electric Scale	Spatule + Règle
Light Microscope	Glucometer + strips

Electric Mill	**Glass Funnel + Filter Paper**
Microtome	**Glass bottle**
Automatic Tissue Sample Preparation System	

II.1.1

. Reagents and Chemicals

Several reagents and chemicals were used in this work, among these products (Table 04):

Table 5 Chemicals used in the experiment

Réactif de Drajendorf	**DPPH**
Réactif de Mayer	**Acide ascorbique**
HCL	**Tampon phosphate de sodium (pH 6.6)**
Magnésium (Mg)	**Ferricyanure de potassium (K3[Fe(CN)6]) (1%)**
Chlorure d'hydrogène FeCl3 (à 0.1%)	**Acide trichloracétique (TCA) (10%)**
Méthanol	**Chlorure d'hydrogène FeCl3 (0.1%)**
Chloroforme	**Tampon phosphate de sodium (pH 6.4)**
Anhydride acétique	**Acide acétylsalicylique (Aspirine)**
Acide sulfurique H2SO4	**Albumine d'œuf**
Folin-Ciocalteu	**Alloxane**
Bicarbonate de sodium Na2CO3 (7.5%)	**Sérum de glycosyle**
Acide gallique	**Carragénine**
Quercetine	**Sérum de salée (eau physiologique)**
Chlorure d'aluminium AlCl3 (2%)	**Formol**
Eau distillé	**Tubes Héparinés**

Ethanol	**Eosine aqueux**
Xylène	**Hémataxyline**

III. Study Method

III.1. Preparation of the aqueous extract by maceration

Traditionally, water extraction is already the most widely used method in traditional medicine. This method offers simplicity and comfort, with some modifications depending on the grass and the method.

This type of extraction was carried out using the method (Majhenic *et al.*, 2007; Bougandoura and Bendimerad, 2012), with some modifications to the plant of ***Artemisia herba-alba*.** This method was done by pouring 50grams of the powder from the aerial part of the ***Artemisia herba-alba*** plant into 500 ml of distilled water and Then the extract was macerated for 3 hours and then we filtered it using filter paper to obtain our extract .After filtration, it is dried in an oven at a temperature not exceeding 50°C. An extract is then obtained in the form of a thin film, solid to be scraped off with a flat spatula and then stored in the refrigerator at 4 °C in closed glass bottles covered with aluminum foil

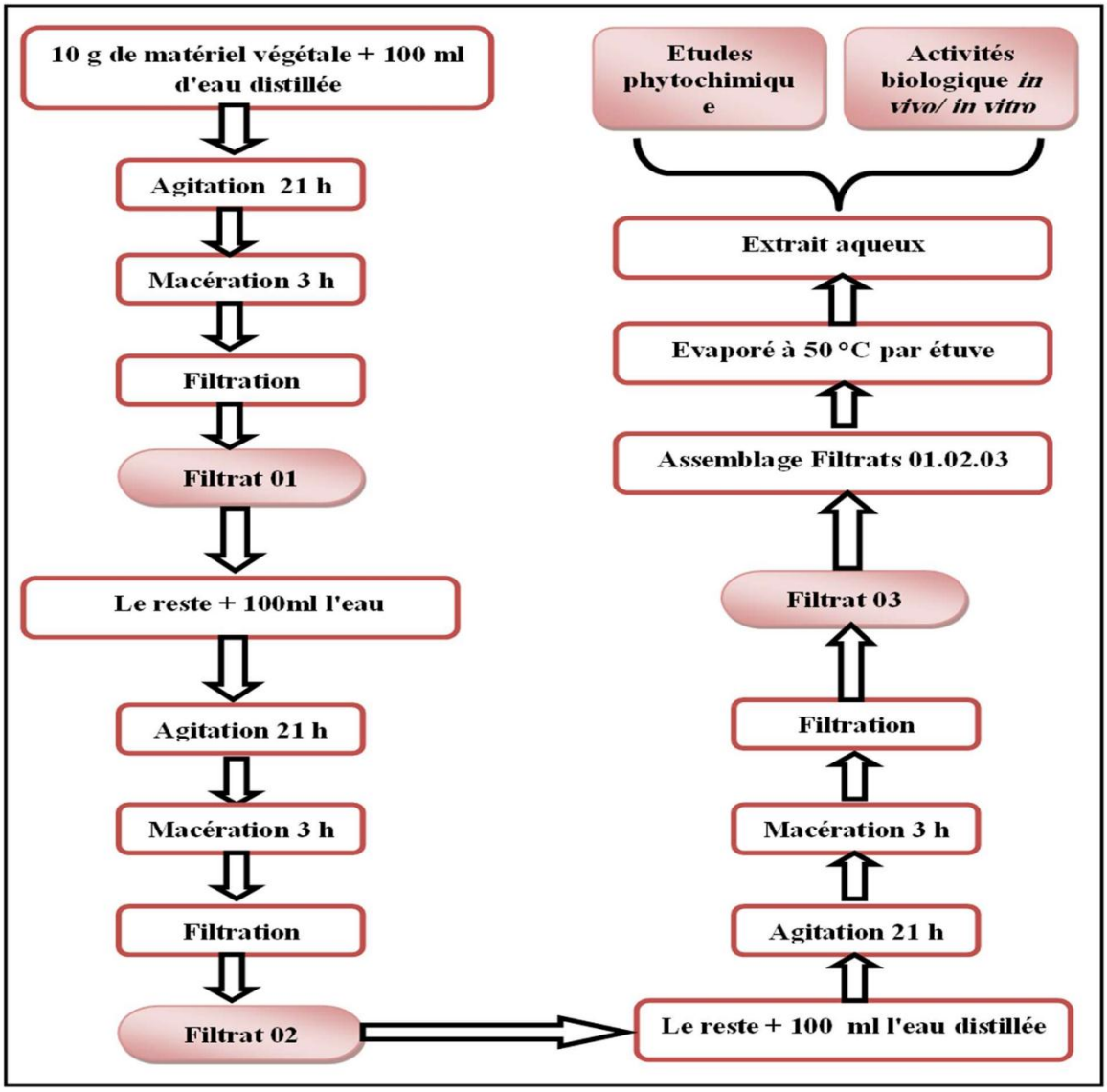

Figure 20 Protocol for the preparation of the aqueous extract of *Artemisia Herba Alba*

until used (Figure 16)

A. Calculation of *Artemisia Herba Alba* dry matter yields

The yield of the plant in extract is the ratio between the weight of the extract and the weight of the plant to be treated (Truong et al., 2019). The return, which is expressed as a percentage, has been calculated by the following formula:

$$R\ \% = (PEB/PMV) \times 100$$

Where **:**

R: is the yield in %.

PEB: Weight of Crude Extract (g)

PMV: Weight of plant material (g)

In this chapter, we deal with the principle adopted, the materials used and the methods of study of the aqueous extract of the spontaneous medicinal plant *Artemisia Herba Alba*. In order to identify and evaluate their antidiabetic activities, their anti-inflammatory activities *in vivo* and *in vitro* and their antioxidant activities *in vitro*.

Our research work was carried out in the pedagogical laboratory which is part of the Faculty of Natural and Life Sciences of the Echahid Hamma Lakhdar University of El Oued, and the El Medjed Medical Analysis Laboratory.

Preparation of the Nanoparticle Extract:

Magnesium oxide nanoparticles (MgO-NPs) were synthesized using a green synthesis method based on the aqueous extract of *Artemisia herba-alba*, following the protocol of Sangeetha et al. (2019) with some modifications.

A quantity of 2.5 g of the aqueous extract was weighed and mixed with 100 mL of distilled water in a beaker. Separately, 0.7 g of magnesium acetate was weighed and dissolved in 10 mL of distilled water. The mixture was then placed on a magnetic stirrer at a temperature of 70 °C. The magnesium acetate solution (0.1 M) was slowly added dropwise to the extract solution until a color change was observed, indicating nanoparticle formation.

The resulting mixture was centrifuged at 3000 rpm for 10 minutes to collect the precipitate. The obtained precipitate was then dried in an oven at 60 °C for 30 minutes and ground thoroughly into a fine powder. The final product was placed in a furnace and calcined for 5 hours.

The yield was calculated using the following formula:

R% = (n1 / E2) × 100

Where:

- **R%**: Productive yield of the nanoparticle extract (%)
- **n1**: Mass of the dry nanoparticle extract obtained after evaporation
- **E2**: Mass of the dry extract used in the synthesis process.

IV. Study material

IV.1. Phytochemical test

The qualitative phytochemical test carried out on the aqueous extract of *Artemisia Herba Alba*, makes it possible to detect the different families of secondary metabolites present in it through a set of chemical reactions, precipitation by specific chemical reagents carried out on the extract (Himour *et al.,* 2016; Batah *et al.,* 2016). The main secondary metabolites are: polyphenols (flavonoids, tannins),

IV.2. Quantitative analysis of phenolic compounds

IV.2.1. Determination of total polyphenols

IV.2.1.1. Principle:

The principle of this method is based on the reduction of phosphotungstic acid (H3PW12O40) or Folin-ciocalteu reagent in an alkaline solution. During oxidation, it is reduced to a mixture of blue oxide: tungsten blue oxide (W8O23), the color obtained whose maximum absorption at about 760-765 nm is proportional to the amount of polyphenols it contains extracted that has been analyzed (Kessimi, 2006).

IV.2.1.2. Procedure

Polyphenols were quantified using Folin's reagent. Ciocalteau by taking 200µl of extract and mixing in 1ml of ciocalteau-Folin reagent, wait 5 minutes then add 800µl of sodium carbonate Na2CO3 (7.5%) and place in the dark for 2 hours, then measure the absorbance of the prepared solution at a wavelength of 765 nm using a spectrophotometer (Normala and Mardhiah, 2010) (Figure 17).

The calibration curve was performed by gallic acid at different concentrations (0.187 - 3mg/ml), under the same conditions and the same dosing steps. The results are thus expressed in mg gallic acid equivalent per mg dry matter (mg EAG/mg) (Wong *et al.,* 2006).

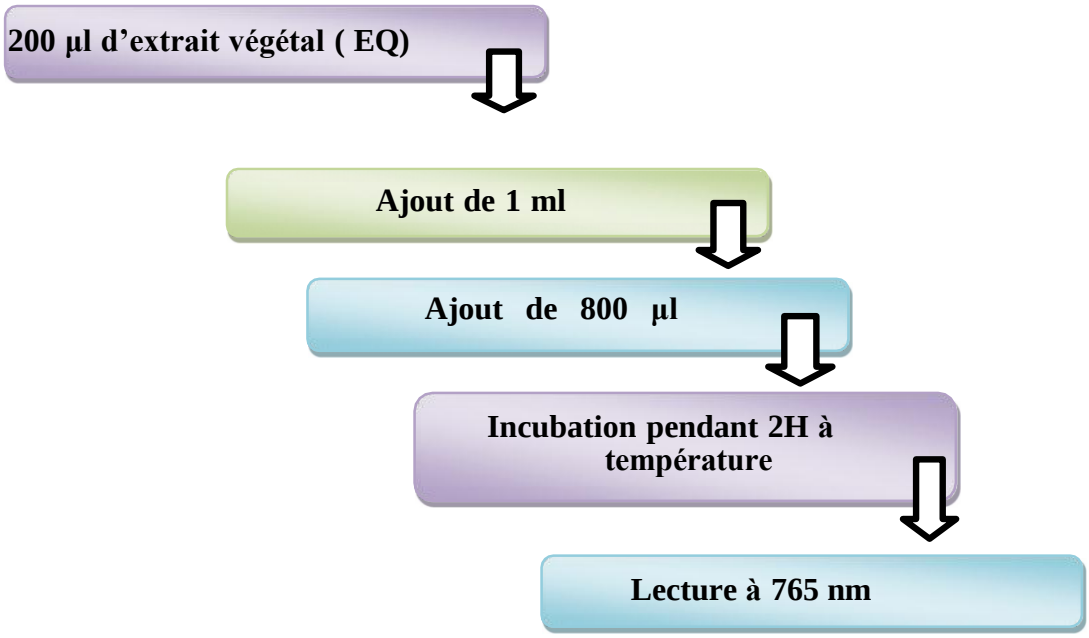

Figure 17 Different steps in the determination of total polyphenols (TPPs) of aqueous extract

IV.3. Flavonoid Determination

IV.3.1. Principle

Flavonoids contain a free hydroxyl group in positron 5 which is capable of giving a yellowish compound by chelating ions, in the presence of aluminum chloride Al+3. The yellow colouration produced is proportional to the amount of flavonoids present in the extract (Basli *et al.,* 2012).

IV.3.2. Procedure

Determination of total flavonoids was performed: 1 ml of distilled water is added to 1 mg of sample to be analyzed is added to 1 ml of AlCl3 solution (02% dissolved with methanol). Read absorbances using a UV-visible spectrophotometer at 420nm, after starting incubation for 1 hour at room temperature (Ghedadba *et al.,* 2015; Lahmer and Messai, 2017) (Figure 18).

The concentration of flavonoids contained in plant extracts was calculated by referring to the quercetin-based calibration curve as standard (0.187-3 mg/ml), The results were expressed in mg quercetin equivalents per mg extract (mg EQ/mg) (Kosalec et al., 2004; Mbaebie *et al.,* 2012).

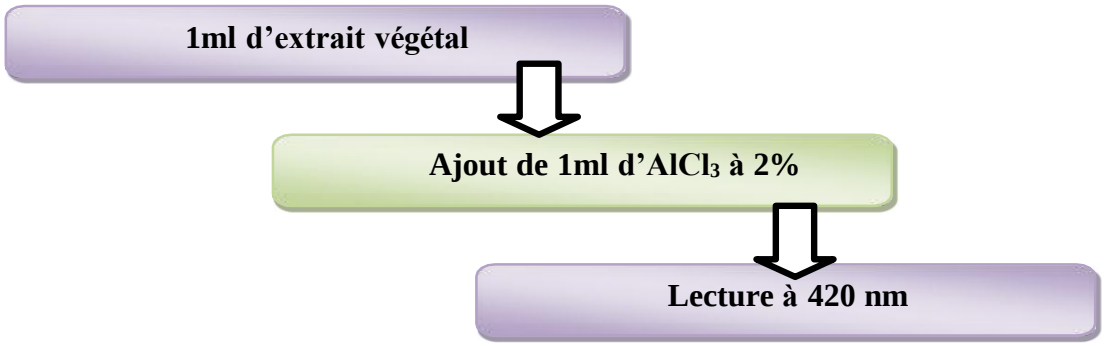

Figure 18 Different steps in the determination of flavonoids (VF) of aqueous extract

Determination of total tannins (TTC)

The tannins assay was done by the vanillin-HCl[4] method, this method is based on the production of a measurable color complex at 500 nm, and the Catechin (Fig.22) is used as a standard at wavelength λ = 500 nm.

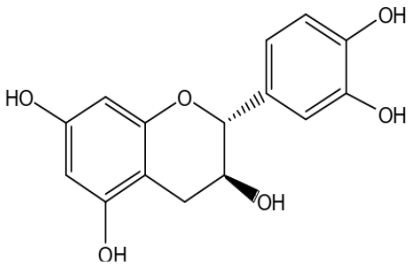

Fig.23 Structure of Catechin

The protocol:

3 ml of 4 % of a vanillin solution prepared in ethanol and 1.5 ml of concentrated HCl are added to 0.5 ml of extract. After incubation for 15 min, the absorbance is measured at 500 nm by a UV-Visible spectrophotometer. Calibration is performed with catechin (0.01-0.1 g/l) and the results are expressed in milligram equivalents of catechin per gram of dry vegetable matter (mg CE/g DM) ().

IV.4. Toxicity Study of Aqueous Extract of *Artemisia Herba Alba*

IV.4.1. Toxicity test animals

The acute toxicity study is a qualitative6 and quantitative analysis of the irreversible impairment of vital functions after a single administration of a substance within a few days **(**Ruckebusch, 1981).

The acute toxicity of *Artemisia Herba Alba* was evaluated in 12 *Wistar* rats (badly)

randomly divided into 4 batches of 4 rats each, after fasting for 16 hours except water, each group received a single daily dose of the aqueous extract of the test plant (500 or 1000 mg/kg bw) orally using a gavage syringe (Figure 19), kept in the same conditions. Signs of toxicity and the weight of the rats were recorded during the duration of the experiment (7 days) according to the method (Pissang *et al.*, 2022), with some modifications.

Animal groups:

- ✓ **Groupe 01:** Rattes receiving distilled water (control batches).
- ✓ **Group 02:** Rattes receiving aqueous extract of *Artemisia Herba Alba* 500 mg/kg bw
- ✓ **Group 03:** Rattes receiving aqueous extract of *Artemisia Herba Alba* 1000 mg/kg bw
- ✓ **Groupe 02:** Rats receiving Artemisia Herba Alba *oil* 500 mg/kg bw
- ✓ **Groupe 03**: Rats receiving Artemisia Herba Alba *oil* 1000 mg/kg p.c.
- ✓ **Groupe 02**: Rats receiving *Artemisia Herba Alba* nanoparticle 500 mg/kg bw
- ✓ **Group 03:** Rattes receiving *Artemisia Herba Alba* nanoparticle 1000 mg/kg bw.

❖ **Observation**

The rats were carefully and regularly monitored during the first 24 hours, with particular attention given to the first 4 hours after the administration of the solution. Monitoring then continued daily throughout the duration of the experiment (7 days). Visual observations included changes in motility, skin, hair, eyes, somatomotor activity, and general behavior. The weight of each animal was measured immediately before administration, and then regularly thereafter to detect any changes in weight or signs of toxicity.

The administration of the extract has been tested and then once a day. Weight changes are calculated and recorded (El Kabbaoui, 2019). This test was carried out in accordance with the World Health Organization Director for the Evaluation of the Safety and Efficacy of Herbal Me dicines (W.H.O., 2000).

Figure 19 Administration de extrait aqueux par voie orale à l'aide d'une seringue de gavage (Originale, 2023)

IV.5. Study of the biological effect of aqueous extract from *Artemisia Herba Alba*

IV.5.1. Evaluation of antioxidant activity *in vitro*

The ability of a plant extract or compound to eliminate free radicals is estimated in several ways, the most important of which are: free radical inhibition test "DPPH" or iron reduction test "FRAP".

A. Free radical scavenging test (DPPH)

1. Principle of the test

Free radical inactivation test method DPPH depends on the ability of plant extracts (antioxidants) to donate a hydrogen atom from phenolic hydroxyl groups and modify the DPPH free radical, and this results in the removal of the purple color of DPPH root* and its transformation into yellow DPPH-H (Figure 20) following its conversion into a stable compound (2,2-diphenyl-1-picrylhydrazyne) (Colak et al., 2017). In order to colorically monitor this interaction, a spectrophotometer is used at a wavelength of 517 nm**.**

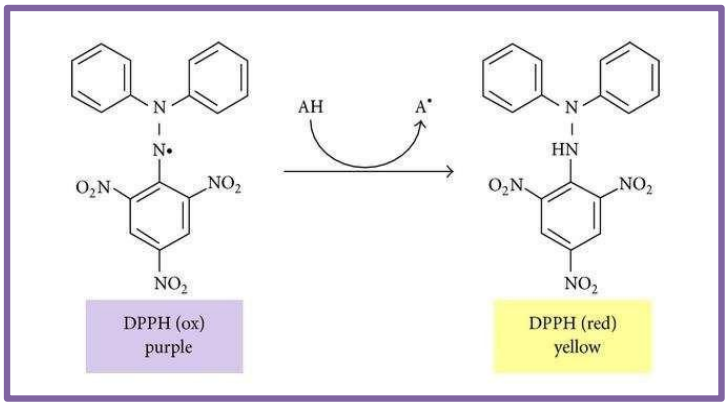

Figure 20 DPPH (2.2 Diphenyl 1 picryl hydrazyl) test reaction (Yacooba *et al.*, 2018)

2. Procedure

❖ **Preparation of DPPH Reagent**

✓ 4 mg of DPPH* is dissolved completely in methanol in a 100 mL vial

✓ This solution is put in the fridge (it must be protected from air and light)

❖ **Preparation of extracts**

Prepare the original solution by taking 12 mg of the extract and we dissolve it in 2 ml of methanol so that the concentration of the original solution becomes 6 mg/ml, and based on this concentration, we prepare the rest of the dilutions. Take 200 µL of the extract dissolved in methanol and add 800 µL of DPPH solution (3 iterations), homogenize the solution, incubate the tubes in the dark for 30 minutes, and then measure the absorbance of the solutions prepared at a wavelength of 517 nm by a spectrophotometer (Dziri *et al.*, 2012; Azouaou *et al.*, 2020).

For the positive comparison of the percentage of DPPH free radical inhibition, we use ascorbic acid, the absorbance of which has been measured under the same conditions as the samples and for each concentration.

3. Percentage of inhibition

Then, the curve expressing results as free radical scavenging activity or free radical inhibition activity in percentages (I%) as a function of the concentrations (µg/ml) of the different solutions is plotted using the following formula (Tailor *et al.,* 2014; Trabsa, 2015; Aouni *et al.,* 2017).

Avec :

$$I\% = [(AC-AE)/AC] \times 100$$

I % : Pourcentage d'inhibition

AC : Absorbance du contrôle (DPPH)

AT : Absorbance de l'extrait (Trabsa, 2015)

4. Calculation of the 50% inhibitory concentration (IC50)

Graphically, the IC50s are calculated by linear regressions of the plotted graphs; percentages of inhibition as a function of different concentrations of the fraction tested. CI50 or 50% inhibitory concentration (also known as EC50 for Efficient concentration 50), is the concentration of the test sample needed to reduce 50% of the DPPH radical (Mekhadmi., 2021).

B. Ferric Reducing-Antioxidant Power (FRAP) Iron Reduction Test

1. Principle

The activity of the reducing power is determined according to the method (Oyaizu, 1986; Benzie and Strain, 1996)

The reducing activity of an extract is evaluated by the redox reaction between the extract to be tested and the transition metal ions; in particular iron, Fe3+ participates in the formation of the hydroxyl radical by the Fenton reaction. The FRAP method is based on reducing the ferric iron (Fe3+) present in the K3Fe(CN6) complex to ferrous iron (Fe2+) (Khadhri *et al.,* 2013) by the ability of the antioxidants (HA) present in the plant extract to donate an electron (Li *et al*., 2009) (Figure 21), in an environment acidified by trichloroacetic acid (TCA) to maintain the solubility of iron (Luqman *et al*., 2012; Sushma *et al*., 2013).

Indeed, the FeCl3/K3Fe(CN)6 system makes it possible to determine the concentrations of antioxidants, which participate in the redox reaction (Amarowicz *et al.,* 2004). The reduced shape of this complex results in a change from yellow to blue (Chung *et al*., 2006), which allows it to be quantified by UV-Vis spectrophotometry, and an increase in absorbance corresponds to an increase in the reducing power of the extracts tested (Hubert, 2006).

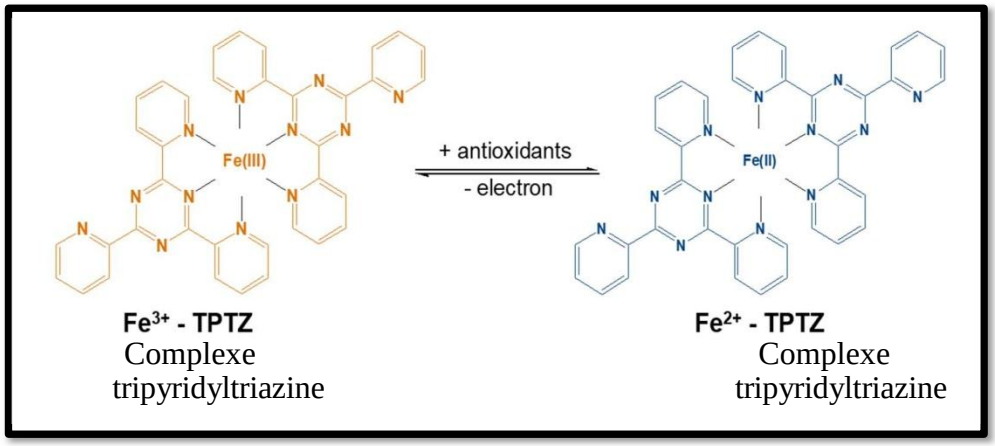

Figure 21 Reduction of a ferric tripyridyltriazine complex [(Fe(III)-TPTZ)2] to a ferrous tripyridyltriazine complex [(Fe(II)-TPTZ)2] by an antioxidant (HA) (Anh-Dao *et al.*, 2022)

2. Procedure

The reducing power of *Artemisia Herba Alba* aqueous extracts is determined by the method (Oyaizu, 1986) with some modifications.

The FRAP reagent is prepared by the following mixtures: in glass test tubes containing 0.25 ml of the aqueous extract of the plant under study at different concentrations between (0.0625 and 1 mg/ml), 0.625 ml of a sodium phosphate buffer solution (0.2M, pH = 6.6) were added, followed by 0.625 ml of a solution of potassium ferrycianide [K3Fe (CN)6] 1% in distilled water, all the test tubes are incubated in a water bath at 50 °C for 20 min. Then, we add 2.5ml of 10% trichloroacetic acid (TCA) is added in order to stop the reaction. Then, the tubes are centrifuged at 3000 rpm for 10 min.

Take 0.625 ml of supernatant and transfer to another tube to which 0.625 ml of distilled water and 0.125 ml of a solution of ferric chloride FeCl3 0.1% freshly prepared in distilled water have been added.

The absorbance reading of the reaction medium is at 700 nm compared to a similarly prepared blank, replacing the extract with distilled water to allow the apparatus to be calibrated (UV-VIS spectrophotometer). The positive control is represented by a test of ascorbic acid is used as the standard of an antioxidantin this experiment under the same operating conditions. The test is repeated 3 times.

Anti-inflammatory activity in vitro: **denaturation of egg albumin**

Protein denaturation is a well-recognized cause of inflammatory conditions, the ability of a compound or an extract to reduce or inhibit heat-induced protein (egg albumin) denaturation is an indication of possible anti-inflammatory activity.

The protocol:

Egg albumin was prepared gently drawing out the liquid white of fresh (lay-today) hen's eggs. A 5 ml reaction mixture was made by adding 0.2 ml of the fresh albumin, 2.8 ml of phosphate-buffered saline, and 2 ml of varying concentration (0.3125, 0.625, 1.25, 2.5, 5, and 10 mg/ml) extracts of *P. atlantica* Desf., The mixture was incubated at 37 °C for 15 min and then heated at 70 °C for 5 min. It was cooled under running water vortexed and absorbance was measured at 660 nm. Diclofenac sodium has been used as a reference medicine. The percentage inhibition of denaturation, which is an index of anti-inflammatory activity, was calculated using the following equation[17]:

Inhibition of protein denaturation % = [(A0 - A1) / A0] x 100

Where, A0: the absorbance in the absence of extract. A1: the absorbance in the presence of extract.

b. denaturation of bovine serum albumin BSA

The anti-inflammatory activity of *P. atlantica* Desf was performed using bovine serum albumin denaturation (BSA) with some modifications[18-21].

The protocol:

0.05 ml of the different concentrations of the extracts and drug reference Diclofenac sodium (0.3125, 0.625, 1.25, 2.5, 5 and 10 mg/ml) and 0.45 ml of bovine serum albumin (1 % p/v) mixed. The mixture was incubated at 37 °C for 20 min and then heated at 57 °C for 3 min. After cooling, 2.5 ml phosphate-buffered saline (PBS, pH 6.4) was added. The absorbance was measured at 416 nm.

The percentage of inhibition of protein denaturation was calculated as follows:

Inhibition % = 100 - [100- [(A1-A0) / A1) x 100]]

Where, A0: the absorbance in the absence of extract. A1: the absorbance in the presence of extract

- **Antibacterial**

- **Microorganisms used**

Four microbial strains (03 bacteria and 01 yeast) from the batches of the ATCC (American Type Culture Collection) were used: Escherichia coli (ATCC 25922), Pseudomonas aeruginosa (ATCC 25853), Staphylococcus aureus (ATCC 6538) and Candida albicans (ATCC 10231).

- **Solid-state technique (disk diffusion method)**

The technique used to determine the antimicrobial potency of plant extracts has a great influence on the results.

At present, the in vitro antimicrobial activity of a substance can be demonstrated by a large number of conventional techniques, both in solid and liquid media. In this study, we have chosen the solid medium technique [58]. After two successive transplants on broth (Muller Hinton broth for bacteria and Dextrose Sabouraud for yeast) and isolation on specific agar medium (Muller Hinton agar and Sabouraud Dextrose agar), well-isolated colonies were transferred to tubes containing sterile distilled water in order to obtain microbial suspensions with a turbidity (expressed by the measurement of the Optical Density at 600 nm) close to that of McFarland of 0.5. An OD of 0.08-0.1 corresponds to 108 CFU/ml [59]. Subsequently, the entire surface of the agar (Mueller Hinton agar for bacteria and Sabouraud Dextrose for yeast) was spread by this microbial suspension. The plant extracts are dissolved in DMSO at a rate of 100 mg/ml. Sterile discs of 6 mm in diameter (Whatman paper n°3) impregnated with 10µl of extract from each plant per disc, were sterile deposited on the surface of the agar. The cans are kept at 4°C for 1 to 2 hours, then incubated for 24 hours at 37°C for bacteria and 48 hours at 37°C for yeast [59]. Antimicrobial activity was determined using a ruler measuring the diameter of the zone of inhibition. All the experiments were carried out in triplicate.

CONCLUSION

Conclusion Générale

This work aims to study innovations in the field of nanoparticles, with a focus on exploring the biological activities of plant extracts from Artemisia, through the analysis of its chemical composition and testing its biological effectiveness in various areas. In the first phase of the study, a quantitative estimation of biologically active compounds was conducted. These compounds included phenols, tannins, and flavonoids, which are known for their antioxidant, anti-inflammatory, and antimicrobial effects.

This was followed by an evaluation of the biological activity of the aqueous extract of Artemisia through several standardized tests. The antioxidant activity was assessed using two different methods: the DPPH free radical scavenging assay and the Ferric Reducing Antioxidant Power (FRAP) assay. The results showed a high capacity of the extract to neutralize free radicals, confirming its richness in bioactive antioxidant compounds. The study also included the evaluation of the antidiabetic activity through the inhibition of the alpha-amylase enzyme, as well as the anti-inflammatory and antibacterial activities against multiple microbial strains. The extract demonstrated clear and promising efficacy in inhibiting the growth of certain microorganisms.

To confirm the laboratory findings, biological experiments were conducted on laboratory animals (male Wistar rats). Inflammation was locally induced in the rats' paws using carrageenan, followed by treatment with the aqueous extract of Artemisia. A significant reduction in inflammation was recorded compared to the untreated control group, and no signs of toxicity were observed during the experimental period, indicating the relative safety of using the extract at the tested concentrations.

Based on the obtained experimental results, it can be concluded that Artemisia represents a promising natural source of compounds with multiple biological activities. It has proven effective as an antioxidant, anti-inflammatory, and antibacterial agent, in addition to being safe for use in an animal model. Accordingly, this study recommends further research into this plant, through future studies focusing on the mechanisms of action of its active compounds at the molecular and cellular levels, expanding the scope of experiments to include various pathological conditions, and exploring advanced methods for preparing and delivering these extracts, such as the use of nanotechnology, with the aim of enhancing their biological efficacy and application in therapeutic and pharmaceutical formulations.

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ANNEXES

Photo 1: Préparation de l'extrait aqueux (Originale, 2024)

Photo 2: Bain-marie de type MEMMERT

Photo 3: Spectrophotométrie à transmission