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Présenté par :

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Thème

Optimisation d'extraction et activités biologiques de plantes médicinales.

Soutenu le: 25 /06/2024 **Devant le jury composé de :**

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SUBMITTED FOR THE DEGREE OF MASTER

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Domain: Nature and Life Sciences **Sector:** Biological Sciences

Option: Microbial Biotechnology

Presented by:

AISSAOUI Wissam & HAMAD Rima

Title

Optimization of extraction and biological activities of medicinal

Supported on: 25/06/2024 **Member of the Jury:**

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Dedication

With the help of GOD, the Almighty, this work is completed

To the one who changed from night to day to ensure the right conditions for me. Thank you for Your unwavering support, wisdom and love. Your dedication, perseverance and sacrifice, every success I have achieved is a reflection of your unconditional love and support. You are my compass throughout my journey. May this work be for you, May ALLAH the Almighty preserve you, grant you Health, happiness and protect you from all harm. *To my dear father*

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To the one who has the advice of a brother The support of the friend I am so lucky to have you as a brother. Thank you for your unconditional love, which has been a source of motivation for me and which inspired me to pursue my dreams. Thank you for everything you do for me. Thank you for your support and constant presence in my life. *To my only brother*

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To my little family who have always supported me

To all my dear friends

I dedicate this modest work

WISSAM

Dedication

I dedicate this work:

To the two people who mean the most to me, to those who have supported me throughout my life and my studies, to my dear parents. May God grant them good health and long life, my whole life would be insufficient to express my deep gratitude to you, may God bless you and protect you.

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To anyone who adds a grain of joy to my life.

Mima

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List of Abbreviation

ABTS	Aluminum salt of 2,2-azinobis-(3-ethylbenzothiazothiazoline-6sulfonic acid).
ADN	Deoxyribonucleic acid.
AG	Gallic acid.
$Alcl_3$	Aluminum trichloride.
AMPs	Aromatic and Medicinal Plants
ARN	Ribonucleic acid.
A. V	*Artemisia vulgaris.*
EAG	Gallic Acid Equivalent.
EQ	Quercetin Equivalent.
EXT	Extract.
FRAP	Ferric Reducing Antioxidant power.
FVT	Total flavonoids.
G	Gram.
GPS	Satellite positioning system.
H	Hour.
HCl	Hydrogen chloride.
KHZ	Kilohertz.
Mg	Milligram.
Ml	Milliliter.
Ms	Dry mass.
Nm	Nanometer.
OMS	World Health Organization.
PPT	Total polyphenols.
Q	Quercitaine.
UV	Ultraviolet.
µg	Microgram.
µL	Microliter.

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Introduction

Introduction

In the past and until now, aromatic and medicinal plants have always played a crucial role in medicine (FAO, 2003). They are one of the major important sources of secondary metabolites that have been widely used in most pharmaceutical and cosmetic companies (Pandey & al., 2017).

Algeria is one of the countries whose flora is extremely rich, varied and is represented by aromatic and medicinal plants, most of which exist in a spontaneous state (Amroune, 2018). Among the medicinal plants which constitute the vegetation, is the genus *Artemisia*. Several species of this genus are used in traditional medicine due to their richness in molecules endowed with therapeutic activities, among the best-known species is *Artemisia vulgaris*.

Artemisia vulgaris, or the common mugwort known in Arabic as "chih" of the *Asteraceae* family, is a large perennial plant that generally grows in dense clumps (Couplan & Styner,2018).

Various applications of this plant have been made possible due to its rich chemical composition, which includes flavonoids, lactones, phenolic acids, coumarins, as well as other groups of metabolites (USDA-ARS-NGRL, 2004).

This dissertation aims to optimize the extraction of polyphenols and flavonoids from the roots of *Artemisia vulgaris* as well as the evaluation of the antioxidant and anti-inflammatory activities of the plant. So that it is a reference concerning the best parameters to follow and therefore make it easier for researchers to make a choice for future studies.

Our manuscript is composed of two parts, the first part concerns the bibliographical study, which is subdivided into two (02) chapters.

The first chapter focuses on the general information about herbal medicine and the phytochemical composition of the medicinal plant; the second chapter gives a bibliographical overview of the plant *Artemisia vulgaris.*

The second part is devoted to the experimental part, it contains two Chapters: the third chapter sets out the equipment and methods used to extract the active biomolecules from our medicinal plant, as well as the search for their phenolic components, their antioxidant activities and anti-inflammatory potential.

The last chapter in this part examines in detail the results obtained during this study and makes a comparison with previous studies with discussion.

Finally, this study comes to a conclusion which highlights the consequences for future investigations in this area. This is followed by a list of references and finally the annexes.

Part I: Literature review

Chapter I
Phytotherapy

I-1- General information on herbal medicine

For centuries, medicinal plants have been used as remedies for human illnesses due to their components with therapeutic value (Nostro & al., 2000).

Phytotherapy is made up of two Greek words: “phyton”, meaning plant, and “therapein”, meaning to cure (Chaachouay, 2020). It is part of alternative medicine aimed at preventing and treating certain diseases using plants, parts of plants (such as roots, leaves, bark, fruits, etc.) or herbal preparations, containing one or more active ingredients (Bouacherine & al., 2017).

Phytotherapy is characterized by a diversity of methods, each with its own technique for preparing and applying plants (Chaachouay, 2020).

We can distinguish two main categories of herbal medicine. On the one hand, traditional phytotherapy, also known as herbalism, which involves the use of aromatic and medicinal plants, either fresh or dried. On the other hand, contemporary phytotherapy, which includes aromatherapy, gemmotherapy and homeopathy, uses phytomedicines developed from the active ingredients extracted from these plants (Strang, 2006).

I-2- Phytochemical composition of medicinal plants

The active ingredients of they plant are therapeutic compounds found in medicinal plants or in their preparations. They are contained in various parts of plants and are classified as primary or secondary compounds (Nsemi, 2010).

I-2-1-Primary metabolites

Primary metabolites are essential organic elements present in all plant cells, necessary for their survival. They perform various functions, including protection against hostile environmental factors. Although crucial, they are not always absolutely essential for the survival of the plant. These metabolites are generally grouped into four main categories: carbohydrates, lipids, amino acids (Alamgir, 2017).

- **Carbohydrates**, Carbohydrates play an important role in carbon resource allocation and plant growth.
- **Lipids,** are cellular macromolecules with structural, energy storage, and signaling roles in plant biological systems.

- **Amino acids,** Amino acids are essential metabolites in plants for protein synthesis and cellular function (Alamgir, 2017).

I-2-2- Secondary metabolites

Secondary metabolites, also called specialized metabolites, are distinguished by their more complex composition. They are often grouped into chemical families such as polyphenols, terpenoids and alkaloids (Fatiha, 2019).

This group of metabolites includes the molecules most commonly used in therapy. They are also of great importance for the plant because they protect it against the sun's rays, oxidation processes, and act as signals to interact with its environment (in order to defend itself against other species or to attract pollinating insects). Despite considerable research efforts on plant substances, very few secondary metabolites have been isolated and identified (Fatiha, 2019; Chabrier, 2010). Secondary metabolites can be classified according to:

- Chemical structure (having rings, containing a sugar)
- The composition (containing nitrogen or not)
- Solubility in various solvents or pathway (Biozot & Charpentier, 2006).

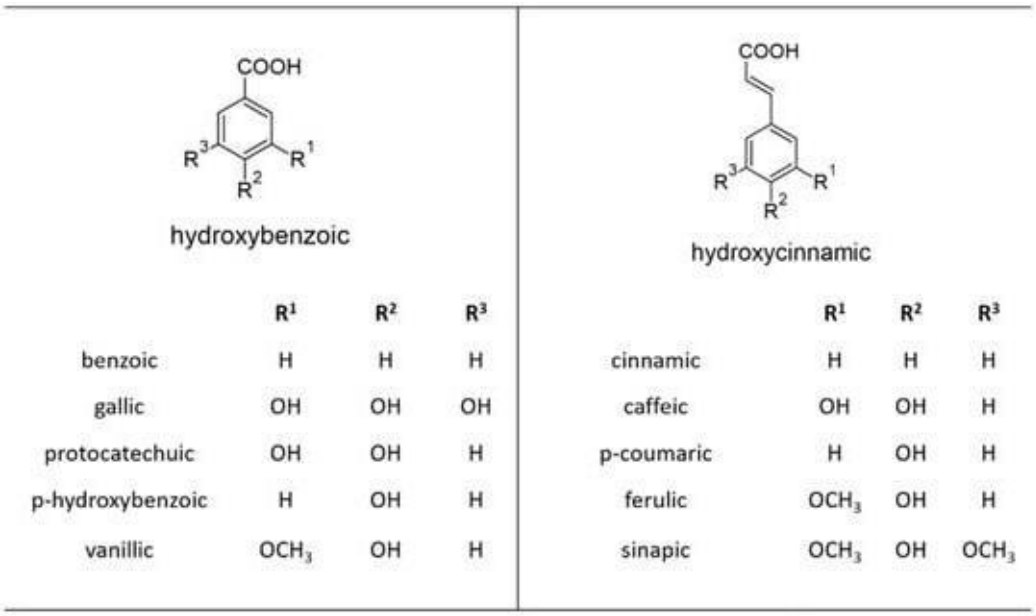
They can be classified into three types, depending on their origin: terpenoids, alkaloids, and phenolic compounds (N'Guessan & al., 2011).

Taking for example the latter, polyphenols, which constitute the largest andmost widespread group of metabolites in the plant kingdom and are an integral part of the human and animal diet (Martin & Andriantsitohaina , 2002).

I-2-2-1- Polyphenols

Polyphenols are natural compounds synthesized only by plants, characterized by their chemical properties linked to phenolic substances.

These molecules are simple in structure as phenolic acids (figure1), flavonoids and isoflavones or more complex such as tannins, anthocyanins and lignanes. They are renowned for their powerful antioxidant properties, top of form such as flavonoids, tannins, coumarins, etc. (Singla & al., 2019).



	R¹	R²	R³
benzoic	H	H	H
gallic	OH	OH	OH
protocatechuic	OH	OH	H
p-hydroxybenzoic	H	OH	H
vanillic	OCH_3	OH	H

	R¹	R²	R³
cinnamic	H	H	H
caffeic	OH	OH	H
p-coumaric	H	OH	H
ferulic	OCH_3	OH	H
sinapic	OCH_3	OH	OCH_3

Figure 1: Chemical structure of the main phenolic acids (Šamec & al., 2021).

- **Flavonoids**

Flavonoids are phytochemicals (figure 2) widely distributed in many plants, fruits and vegetables, as well as leaves, and have potential applications in medicinal chemistry due to their anti-inflammatory and antiviral effects, as well as their liver protection.

These medicinal components possess significant biological activities, the effectiveness of which has been demonstrated for various diseases. Flavonoids have been successfully isolated, and their numbers continue to grow steadily through ongoing research (Manzoor & al., 2020).

- **Coumarins**

Coumarins are aromatic secondary metabolites (figure 3), with a benzo-α-pyrone ring, belonging to the family of phenolic compounds (Adou & al., 2019). They have numerous beneficial biological activities, notably cytotoxic and antiviral (Yerer & al.,2020).

- **Tannins**

The name "tannin" is derived from the French "tanin" (tannin substance) and is used for a range of natural polyphenols produced by plants during their secondary

metabolism (figure 4). They bind to food macromolecules like proteins and carbohydrates, which can affect their digestion (Besharati & al., 2022).

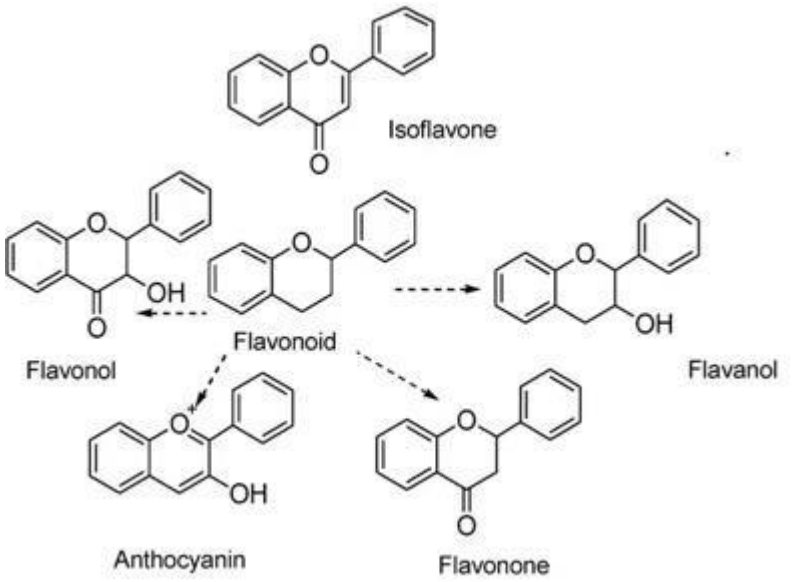


Figure 2: Chemical structure of flavonoids *(Ullah, 2020).*

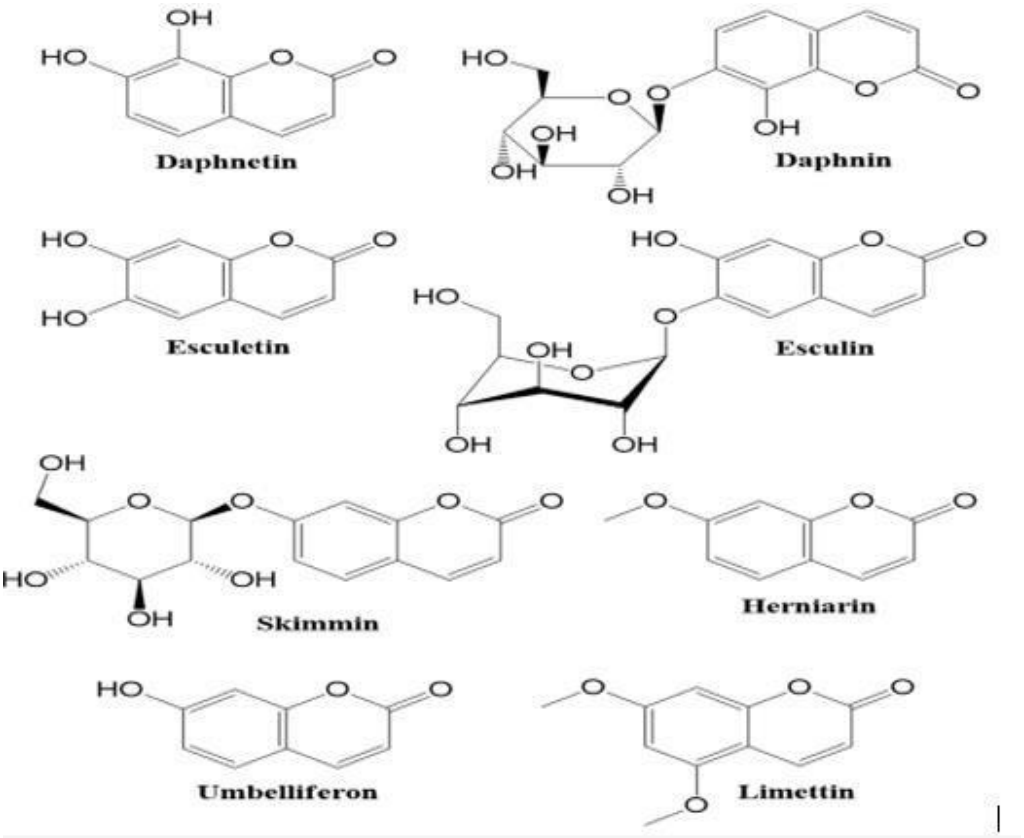


Figure 3: Chemical structure of some coumarins (Küpeli Akkol & al., 2020).

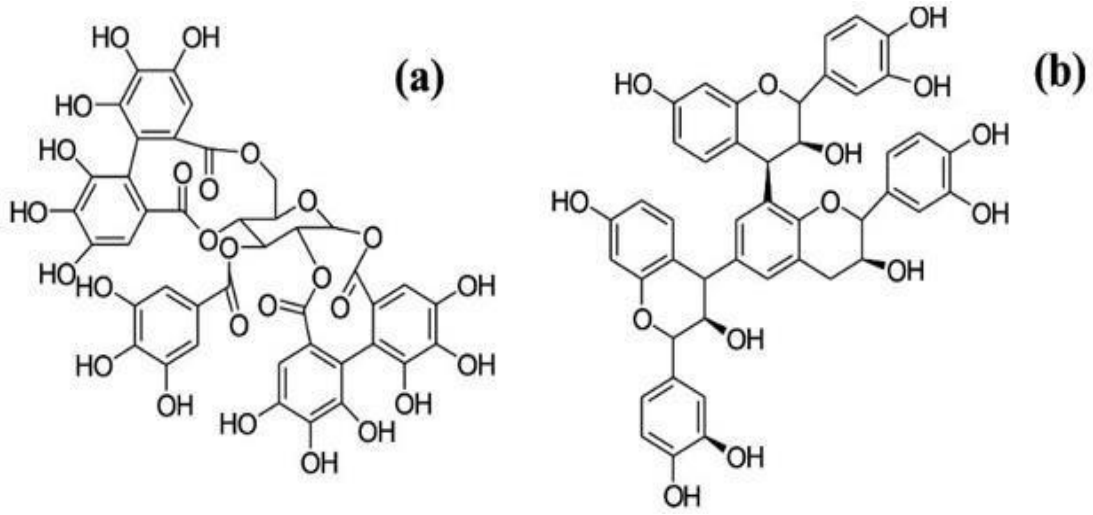


Figure 4: Chemical structure of (a) hydrolyzable (b) condensed tannins (Bayart, 2019).

I-3- Extraction methods of active ingredients

Extraction is method used in chemical engineering to selectively separate one or more compounds from mixture based on their chemical and/or physical characteristics. Extraction of high-value active ingredient is a very important step in the isolation as well as identification of natural bioactive molecules. Depending on the way and means used, there are different methods (Benabdallah, 2016).

I-3-1- Decoction methods

Decoction is a method of extracting substances from a plant or plant substance (the hard parts of plants: bark, roots, hard or leathery leaves, stems, fruit, berries, seeds or seeds) (Oubibete & Slimani, 2023). By boiling them in water for an extended period of time (Létard & al., 2015). This technique releases water-soluble active compounds, such as tannins, flavonoids and essential oils, to create a concentrated solution used for medicinal or culinary purposes.

I-3-2- Brassage Methods

It is usually made from the leaves and flowers of the plant, but it can also be the roots and bark. Simply pour boiling water over the plants and let them soak for ten to twenty minutes (Ouedraogo & al., 2021). It is possible to maintain it refrigerator for up to 48 hours. It is best to avoid sweetening herbal tea in general.

I-3-3- Maceration method

Macerations generally concern plants whose active substances risk disappearing or degrading under the effect of heat (by boiling). They can be defined as long-lasting (several days) cold infusions. This preparation is obtained by putting the plants in contact, cold, with any liquid. This liquid can be wine, alcohol, water or oil (Bekhechi & Abdelouahid, 2010). The contact time is sometimes very long; in fact, aromatic or bitter plants will have to macerate between two and twelve hours (Hidayat & Wulandari, 2021).

I-3-4- Ultrasound method

Ultrasound, which travels at a frequency of 20 to 100 kHz, is used to extract flavors and other molecules from plants. The use of ultrasonic extraction provides increased performance, higher quality extracts with a complete compound. Ultrasound likely has a mechanism of action that involves improving overall extraction rates and yield, enabling the use of fewer solvents, using less expensive raw product sources, improving the extraction of heat-sensitive compounds (Fabiano-Tixier & al., 2016).

I-3-5- Microwave-assisted method

Is a method that uses microwaves to extract desired compounds from plant materials. This technique involves exposing the plant sample to high frequency microwaves in a suitable solvent. Microwaves induce molecular agitation in the sample, thereby speeding up the process of extracting the desired compounds into the solvent. This method is often chosen for its speed, efficiency and ability to reduce the use of solvents compared to conventional extraction methods (Boukhatem & al.,2019).

I-4- Biological activities of medicinal plants

Medicinal plants are known for their various biological activities such as antibacterial, anti-inflammatory, antioxidant and anti-diabetic and others (Li & al., 2020).

I-4-1- Antibacterial activity

Some medicinal plants contain active molecules with antibacterial properties, meaning they can inhibit the growth of bacteria. These active molecules are often secondary metabolites. The antibacterial activity of plant extracts is mediated by many different mechanisms (Ginovyan & al., 2017). They act by disrupting the cell membrane, inhibiting DNA synthesis, and disrupting cellular metabolism (Nazzaro & al., 2013).

I-4-2- Antioxidant activity

Medicinal plants are often rich in antioxidants, compounds that protect cells against diseases linked to oxidative stress which occurs when the balance is disturbed, either by a lack of antioxidants, or by a significant overproduction of free radicals (Favier, 2003).

Method of evaluating *in vitro* antioxidant activity can be estimated by different methods, we distinguish: the DPPH free radical trapping method, the FRAP iron reduction method, and that of carotene decolorization, reduction of the radical-cation ABTS and the peroxyl radical capture method (ORAC) (Himed & al., 2016; Ouibrahim & al., 2015; Sarr & al., 2015; Techer, 2013; Bourkhiss & al., 2010).

I-4-3- Anti-inflammatory activity

Inflammation is a localized protective immune response of the body to injury and infection. It encompasses a set of enzymatic processes, mediator release, fluid efflux, cell migration, and tissue degradation and regeneration. This defensive process is triggered by various stimuli such as infections, irritants or cellular and tissue damage. The sensation of pain may also be associated with inflammation, resulting in increased protein degradation, increased blood vessel permeability, and membrane alterations, among other responses (Djellouli & et., 2022).

Various mechanisms of action have been proposed to explain the anti-inflammatory activity of medicinal plants, namely: Inhibition of inflammatory enzymes (Kim & al., 2004), inhibition of pro-inflammatory cytokines (Oguntibeju, 2018).

I-4-4-Antidiabetic activity

Diabetes mellitus, which is a common and severe metabolic disorder, is manifested by persistent elevation of blood sugar and total or partial insulin deficiency (Wang & Zhu, 2016).

The use of medicinal plants and phytochemicals in the management of diabetes could constitute a partial solution compared to pharmaceutical treatments which only temporarily lower blood sugar levels (Pan & al., 2017).

In fact, the antidiabetic effectiveness of plants acts through various mechanisms: stimulation of insulin production by the pancreas; reduction of glucose synthesis in the liver and increased assimilation of glucose by muscle and adipose tissues; hinders the absorption of glucose (by inhibiting digestive enzymes) (Shanak & al., 2019).

Chapter II

Bibliographic overview on the plant *Artemisia vulgaris.*

Chapter II Bibliographic overview on the plant *A.vulgaris*

II-1- Generalities

The use of medicinal plants for therapeutic reasons is an ancient practice which is regaining interest, even with the evolution of synthetic chemistry and the progress of modern medicine. Traditional medicine is used by more than 80% of the world's population to treat health problems, according to the World Health Organization (WHO). Their effectiveness in different therapeutic procedures has allowed them to maintain an important place. Today they play an essential role in the life of human beings. Ancestral knowledge is in fact passed down from generation to generation, which allows this knowledge to be preserved (Lazli & al.,2019). Bulletin of the Royal Society of Liège Sciences. This shift in interest is due to the fact that many diseases can be treated effectively and satisfactorily and at lower cost by plants. The active substances present in these plants are responsible for their therapeutic power. It is essential to carry out adequate biological tests and use appropriate chemical screening methods in order to evaluate the biological activity of these plants (Bohui & al.,2018).

II-2- *Artemisia* plant type

The Asteraceae family, also known as the "Aster family" or "Compositae", is the largest among the Spermatophytes and is among the most evolved. With more than 1,530 genera and more than 23,000 listed species, it presents great diversity. Among the most significant species (Filleul & al., 2019), we find:

- *Artemisia vulgaris.*
- *Artemisia dracunculus.*
- *Artemisia absinthium.*
- *Artemisia annua.*
- *Artemisia frigida Willd.*
- *Artemisia leucodes Schrenk.*
- *Artemisia scoparia Waldst. & Kit*
- *Artemisia sieversiana Ehrh. ex Willd.*
- *Artemisia Eclipta prostrata* (Liu & al., 2020).

In our research, we explored the specie *Artemisia vulgaris.*

Chapter II Bibliographic overview on the plant *A.vulgaris*

II-3-*Artemisia vulgaris* plant through time

The genus *Artemisia* takes its name from the Greek goddess of the hunt Artemis. The etymology of *Artemisia* also reveals another possible origin of the name: Queen *Artemisia*, wife of the Greco-Persian king Mausolus, was famous as a medical and botanical specialist, and she oversaw the construction of the mausoleum, which later became famous. The epithet *vulgaris*, for its part, means usual, common or vulgar (Abiri & al., 2018).

This herbaceous species is also known as mugwort, tobacco, common wormwood, wild wormwood, criminal herb, also known as "the mother of herbs", occupies an important place in the history of medicine. It is one of the most emblematic species of its genus, presenting a global distribution in various natural habitats such as (Europe, Asia, North and South America), as well as Africa. For many centuries, this herb has been used primarily to treat gynecological conditions and gastrointestinal diseases.

Recent studies have demonstrated that *Artemisia vulgaris* has antioxidant, hypolipidemic, hepatoprotective, antispasmodic, analgesic, estrogenic, cytotoxic, antibacterial, antifungal, hypotensive and broncholytic properties (Anwar & al., 2019). The diversity of applications of this plant arises from its varied chemical composition, including essential oils, flavonoids, sesquiterpene lactones, phenolic acids, coumarins and other metabolites. The presence of essential oil in *A. vulgaris* makes it an important culinary spice in the food industry. Currently, this medicinal plant provides a wide range of therapeutic properties, including antimalarial, anti-inflammatory, antihypertensive, antioxidant, antitumor, immunomodulatory, hepatoprotective, antispasmodic and antiseptic effects (Malik & al., 2019).

The growing use of *Artemisia vulgaris* now extends to cosmetic production in Europe, Asia and North America. A recent review on *A. vulgaris* highlights its importance, both from a South American-Asian and European perspective. This review offers traditional information on the therapeutic and dietary use of *A. vulgaris* in Europe, while exploring new potential applications of this plant in herbal medicine, notably as a hepatoprotective, broncholytic, anthelmintic and cytotoxic agent. Additionally, it is examining its use in the cosmetics industry as a raw material in Europe, East Asia (especially Korea) and North America (Ekiert & al., 2020).

Chapter II Bibliographic overview on the plant *A.vulgaris*

II-4- Phylogenetic classification

Artemisia vulgaris, a member of the *Asteraceae* family, is one of more than 500 species found in temperate and cold regions around the world. When fresh, this plant gives off a distinctive pungent odor and has a bitter, spicy and pungent taste (Zubair & al., 2020). It is classified as illustrated in the table below.

Table 1: Phylogenetic classification of *Artemisia vulgaris* (Zubair & al., 2020).

Kingdom	Plantae
Subkingdom	Plants
Division	Spermatophyta
Subdivision	Magnoliophyta
Class	Magnoliopsida
Subclass	Asteridae
Order	Asterales
Family	Asteraceae
Subfamily	Asteroideae
Genus	*Artemisia*
Specie	*Artemisia vulgaris*

II-5- Nomenclature

Artemisia vulgaris, commenly known as mugwort, has various names and synonyms depending on the region and context. Here are some details regarding its nomenclature:

- **Scientific name**: *Artemisia vulgaris*
- **French Name**: *Artemisia vulgaris*
- **English Name**: Mugwort
- **Italian name**: Amaranth
- **Spanish name**: altamis, altamiza, altamisa
- **Arabic name**: الشيح

- **Vernacular name**: mugwort, *artemisia*, belt of Saint-Jean, crown of Saint-Jean, herb of a hundred tastes, herb of Saint-Jean, herb of fire, royal herb, discount, tobacco of Saint Peter (Siwan & al., 2022).

II-6- Botanical description

Artemisia vulgaris is an herbaceous plant that can reach up to 2.5 m in height and 75 cm in width (figure 5). Its leaves, 5 to 10 cm long, are densely arranged in an alternating manner, mainly on the upper parts of the stem (Ekiert & al., 2020).

The plant has a thick main root as well as numerous small fibrous lateral roots. The roots have a light brown tint and can measure up to 1 cm in diameter.

The stems of the plant are slightly wavy, straight or branched, showing a brown coloring at the base, which becomes woody over time. They display a more pronounced green tint towards the top and can take on a purple tint at the upper end (Abiri & al., 2018).

This plant is distinguished by its intense aroma which is easily released when you crush its leaves. It offers a spicy taste and a slightly aromatic odor, accompanied by a bitter flavor (Serve, 2006).

Figure 5: Botanical description of *A. vulgaris* (Fleischhauer & al. 2019).

Chapter II Bibliographic overview on the plant *A.vulgaris*

II-7- Ecological niche

Artemisia vulgaris, commonly known as mugwort, is a tall, aromatic and perennial herbaceous plant, characterized by its bitterness. It grows abundantly in the wild in mountainous regions of the temperate, humid and cold zones of the globe. This plant belongs to the Asteraceae family (Zubair & al., 2020).

- **Habitat**: *Artemisia vulgaris* is a plant adapted to several habitats, including roadsides, wastelands and abandoned lots.
- **Climate**: Plant adaptable to various climates, prefers temperate and subtropical.
- **Soil**: It can often colonize disturbed areas and quickly establish itself in new habitats, prefers nutrient-rich, slightly acidic to neutral, well-drained soils.
- **Symbiotic relationships**: *Artemisia vulgaris*: symbiotic relationships with fungi for better absorption of nutrients from the soil (Abd-El Gawad & al.,2023).

Part II: Experimental procedures

Chapter III
Material and methods

Our experimental work focused on the phytochemical analysis and biological activities of a medicinal plant widely used which was *Artemisia vulgaris*. Our investigations were carried out within pedagogical laboratory of the Faculty of Natural and Life Sciences and Earth Science at the University of Akli Mohand Oulhadj Bouira.

III-1-Biological material

III-1-1- Plant matter

Samples of the root part of *Artemisia vulgaris* were collected from Lakhdaria Wilaya of Bouira (figure 6).

The different data and information are mentioned in the table below (Table 2).

Table 2: Summary of plant data studied.

Plant	Location	GPS Coordinates	Harvest Period	Plant Part
A.vulgaris	Lakhdaria, Wilaya of Bouira	36° 37′ 00″ N, 3° 35′ 00″ E	March 2023	Root

Figure 6: Photograph of the plant material used AL-Jubouri & al. (2013).

III-1-2- Non-biological material

All the equipment, glassware and devices used are shown in the annexes I.

III-2- Methods

III-2-1- Preparation of the powder

The root plant of *Artemisia vulgaris* (Chih), which has been cleaned to eliminate all traces of dust then crushed using an electric grinder to obtain fine and homogeneous powders (Figure 7).

The glass containers were used to store the powder obtained in a dry place and protected from humidity and light until use.

Figure 7: Photograph of the plant *Artemisia vulgaris*.

III-2-2- Extraction of biomolecules (phenolic compounds)

Artemisia vulgaris is rich in active components, namely: phenolic components. To extract these components that are trapped inside the plant cell, the use of solvents is recommended. There are many extraction methods referenced and used today. In our study we chose using two methods, the maceration method (classical method) and the sonication method (modern method), using ethanol as solvent.

The extraction is developed following the protocol presented by Hasni (2021) and Hamia & al. (2014) with some modifications (figure 8).

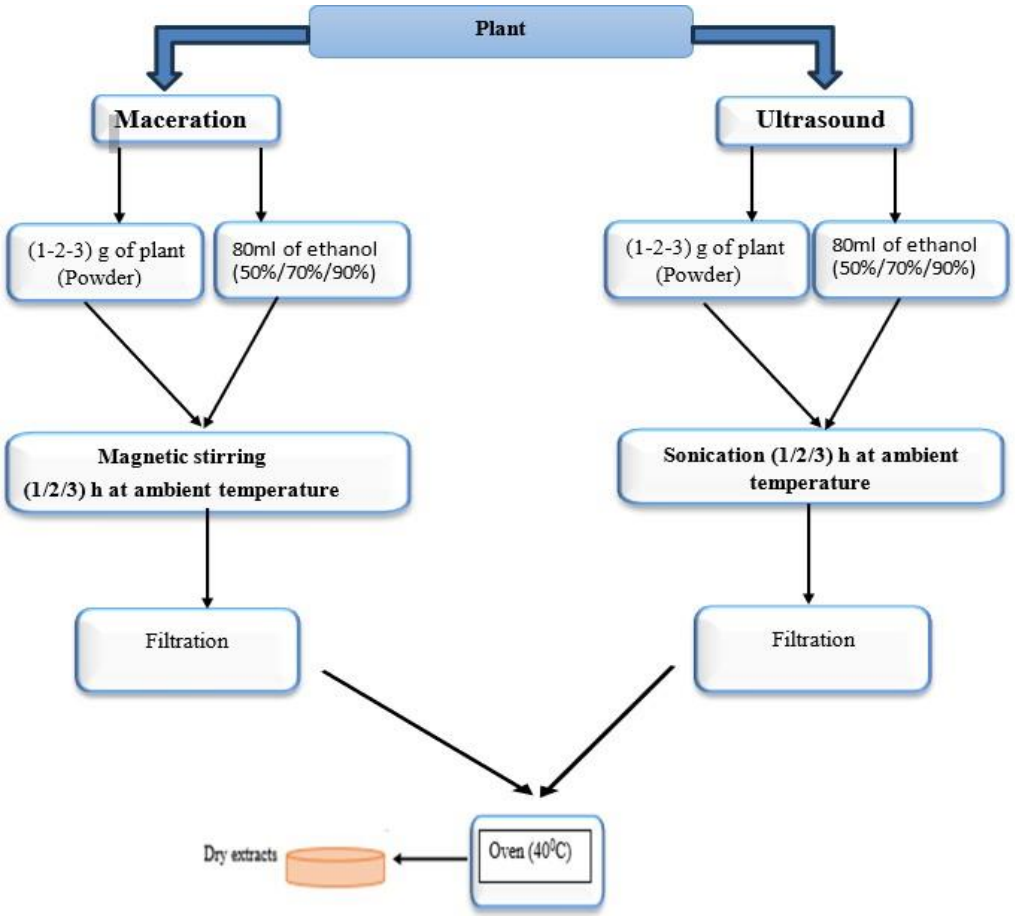


Figure 8: Diagram illustrating extraction by maceration and ultrasound (Hasni, 2021; *Hamia & al., 2014*).

Then, the dry extracts were recovered by reconstituting them in adequate solvent and stored in sterile glass bottles at 4°C, protected from light, until that they are used.

III-2-3- Extraction yield

The yield of the dry extract was calculated the by this formula to obtain the percentage:

$$\text{Extraction yield (\%)} = (Pe / Ps) \times 100.$$

Pe: Weight of the crude extract in grams (g).

Ps: weight of dry plant matter in grams (g).

III-2-4- Dosage of different components

The measurement of phenolic compounds such as total polyphenols (PPT) and total flavonoids (FVT) was carried out using a UV-visible spectrophotometer model SP-3000 nano.

III-2-4-1- Determination of total polyphenol content

- **Principle**

The method of Singleton & Rossi (1965), allows polyphenols to be measured using the Folin-Ciocalteu reagent. This colorimetric mixture with a wavelength of 725nm is obtained by the reduction by the action of the polyphenols of phosphotungstic acid and phosphomolybdic acid.

In this way, the PPTs are evaluated by comparing the absorbance of the solution studied to that obtained by a standard, namely gallic acid.

- **Operating mode**

200 µL of each extract was introducet into a test tube, 1 mL of Folin-Ciocalteu reagent (diluted 1/10 in distilled water) was added, 800 µL of aqueous sodium carbonate solution (7.5%) were added. The final solution was mixed and kept in the dark for 5 min in a water bath. The absorbance is measured at 725 nm against a blank using a spectrophotometer. are added

A calibration curve is carried out in parallel under the same operating conditions using gallic acid (0.02 mg/ml). The results are then presented in mg of gallic acid equivalent per g dry weight of the extract (mg EAG/g Ech). All measurements are repeated 3 times.

III-2-4-2- Determination of flavonoids content

- **Principle**

Aluminum chloride ($AlCl_3$, 3.61%) was utilized in the method. This process involves oxidizing carbons 4 and 5 of flavonoids, leading to the formation of a yellow complex that absorbs light at a wavelength of 430 nm (Lagnika, 2005; Arvouet-Grand & al., 1994).

➢ **Operating mode**

The level of flavonoids is evaluated by colorimetry using the aluminium trichloride $AlCl_3$ method cited by (Djeridane & al., 2006), by introducing 1 ml of each solution. Then, 1 ml of aluminum trichloride ($AlCl_3$) prepared in methanol is added. In another tube we have a volume of 1ml of ethanol and 1ml of aluminum trichloride ($AlCl_3$).

A calibration curve is created using standard rutin solutions prepared at different concentrations. The absorbance of the mixture obtained can be directly measured using a UV-visible spectrophotometer at 430 nm.

The results were then expressed in mg of rutin equivalent per gram of dry weight of the extract (mg ERU/g E). Each measurement is carried out 3 times to guarantee the accuracy of the data.

III-2-5- Determination of the antioxidant activity of plant extracts

III-2-5-1- The DPPH test (2,2-diphenyl-1-picrylhydrazil radical)

➢ **Principle**

The reduction of an alcoholic solution of the stable radical species DPPH in the presence of a hydrogen-donating antioxidant (HA) makes it possible to form a non-radical form, DPPHH (diphenyl picryl-hydrazine).

When DPPH was reduced to DPPH-H, it turns yellow (figure 9), with color intensity inversely proportional to the ability of antioxidants in the medium to donate protons. Due to the rapidity of color loss, hydrogen donor is seen as a powerful antioxidant (Mansouri & al., 2005).

➢ **Operating mode**

The determination of antioxidant power is based on the reaction between the oxidant and the antioxidant. The UV-Visible spectrophotometer model SP-3000 nano. Was calibrated using a blank consisting of pure methanol at a wavelength of 517 nm.

The anti-free radical activity of DPPH in phenolic extracts is determined according to the method described by Brand-Williams & al. (1995).

1950 µL of the DPPH solution was added to 50 µL of each extract prepared at different concentrations. After homogenization, the mixture was incubated at room

temperature in the dark, protected from light. After 30 minutes of incubation, the absorbance of the extracts tested was read at 515 nm against that of a blank which contains only 1950 µL of the methanolic solution of DPPH and 50 µL of ethanol. The anti-radical activity of the extracts tested corresponding to the inhibition percentage (PI) of the DPPH radical is calculated according to the following equation:

$$\%\ d'inhibition = [abs.\ contrôle - abs\ échantillon/abs\ controle] \times 100$$

Where:

Abs control: the absorbance of the DPPH solution alone (control).

Abs sample: the absorbance of DPPH in the presence of the extract.

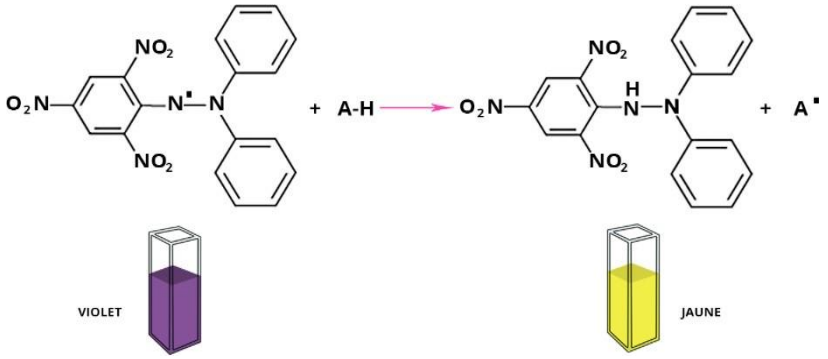


Figure 9: Chemical structure of the DPPH˙ radical and its reduced form (Brand-Williams & al., 1995).

III-2-6- Evaluation of the anti-inflammatory activity of plant extracts

In vitro anti-inflammatory activity of extract of *A. vulgaris* was evaluated using bovine serum protein denaturation method described by (Williams & al., 2008).

- **Operating mode**

After preparing the 0.05% BSA solution (5g BSA in 100 ml distilled water) and adjusting its pH to 6.8, we proceeded directly to the evaluation of the anti-inflammatory activity of our extracts.

For a volume of 0.45ml of BSA, 0.05ml of extract was added then incubated in a water bath at 37°C for 15 minutes. At the end, the incubation was extended for 3 minutes but at a temperature of 57°C. After, the reaction was stopped by adding 2.5 mL of buffer at pH 7.4.

A blank containing the buffer solution instead of the extract, then we proceeded to measure the absorbance at 660nm.

The standard was used contains: 1litre of 1xPBS (Phosphate-Buffered Saline).

- Begin with 800ml of distilled water in duran bottle
- Measure out and add 8g of NACL
- Add 1,44g of Na_2HPO_{4-}
- Add o,24g of KH_2PO_4
- Bring the pH 7,4 or 7,2

The percentage of anti-inflammatory activity is calculated according to the following equation:

$$\%\ d'inhibition = [abs\ c - abs\ t/abs\ c] \times 100$$

Abs c: Absorption of control.

Abs t: Absorbance of extract or standard.

Chapter VI
Results and discussions

IV-1- Extraction rate

In this study the yields (Table 3) were calculated based on the dry plant material of three masses (1g, 2g, 3g) examining the root part of *Artemisia vulgaris* under the action of ethanol solvent by three different concentrations (90% 70% 50%) and time variation, extracted by two methods ultrasound and maceration. The yield rates were measured as a percentage (%) of the amount of extract relative to the amount of dry matter of the plant.

Table 3: Extraction yield values of *Artemisia vulgaris* of ethanol by two methods ultrasound and maceration.

Type / Extract	Time (h)	Powder quantity (g)	Ultrasound (90%)	Maceration (90%)	Ultrasound (70%)	Maceration (70%)	Ultrasound (50%)	Maceration (50%)
1	2	2	6.60	13.48				
3	4	2	9.39	12.47				
11	3	3	8.35	12.33				
15	3	1	15.06	12.94				
2	4	3			12.30	11.56		
4	2	3			10.16	14.02		
5	4	1			19.32	18.42		
8	3	2			10.60	17.86		
12	2	1			29.74	37.44		
6	3	3					11.88	12.89
7	4	2					12.26	9.55
13	2	2					9.14	17.72
14	3	1					15.83	10.26

The study reveals that maceration outperforms ultrasound in terms of extraction yield for the plant examined. This highlights the superiority of maceration in extracting the desired compounds. These results sorted despite the fact that both extraction methods were carried out under comparable conditions, with respect to the amount of plant material and the type of solvent used.

Comparing the yields, the best results were obtained with samples (1-3 and 11) by maceration (13.48% and 12.47% and 12.33% respectively) with (2g ,2g, 3g) powder and (2h ,4h ,3h) time. On the other hand, for sample (15) by sonication with 15.06%) (1g , 3h).

The highest yield was obtained by the maceration method with Ethanol (70%) with (37.44%) for the sample (12) with (1g and 2h). On the other hand, at the concentration (50%), the two samples (6 and 13) extracted by maceration are higher (12.89% and 17.72%) (3g ,3h) (2g ,2h) respectively. By contribution to the samples (7 and 14) of percentage (12.26% and

15.83%), respectively which are higher with the sonication method with (1g, 3h) for extract 14 and (2g, 4h) for extract7.

Regarding the choice of extraction solvent, the best results were obtained with concentration of 50% and 90% ethanol for extract 14 and 15 with same method of extraction ultrasound also same condition (1g ,3h).

Ethanol and acetone were found to be the best solvents for flavonoid extraction. Ethanol and water are preferred for their non-polluting nature, lower cost and non-toxicity, compared to other solvents such as methanol. This is supported by the work of (Mahmoudi & al., 2013), who concluded that ethanol is the most suitable solvent for extraction.

The results obtained show that the use of ultrasound has a positive effect on the extraction of natural substances in terms of speed (Chabir & al.,2009). However,the amplitude of sonication can have an inverse effect on yield (Gutte & al.,2015). The Ultrasound proves useful for extraction because it increases the diffusion mechanisms internal by creating cavities inside the root pores (Rodriguez & al., 2015). This improvement in yield can be attributed to two physical phenomena: diffusion at through the cell wall and rinsing with the solvent (ethanol/water) followed by disruption of the cell (Chabir & al.,2009).

IV-2- Phenolic compound content of extracts

IV-2-1- Content of flavonoids

The flavonoid content is also estimated using the aluminum trichloride method. Rutin is considered as a positive control, which made it possible to carry out the calibration curveand the calculation of the flavonoid content in our extract. The results are expressed in mg rutin equivalent per g of extract (Annexe II).

The evaluation of the flavonoid content of extracts from maceration, sonication of *A. vulgaris* plants revealed variables results presented in (figure 10), it is assured that the content of total flavonoids varies considerably depending on the part of the species used, the extraction method applied as well as the time and quantity of the powder.

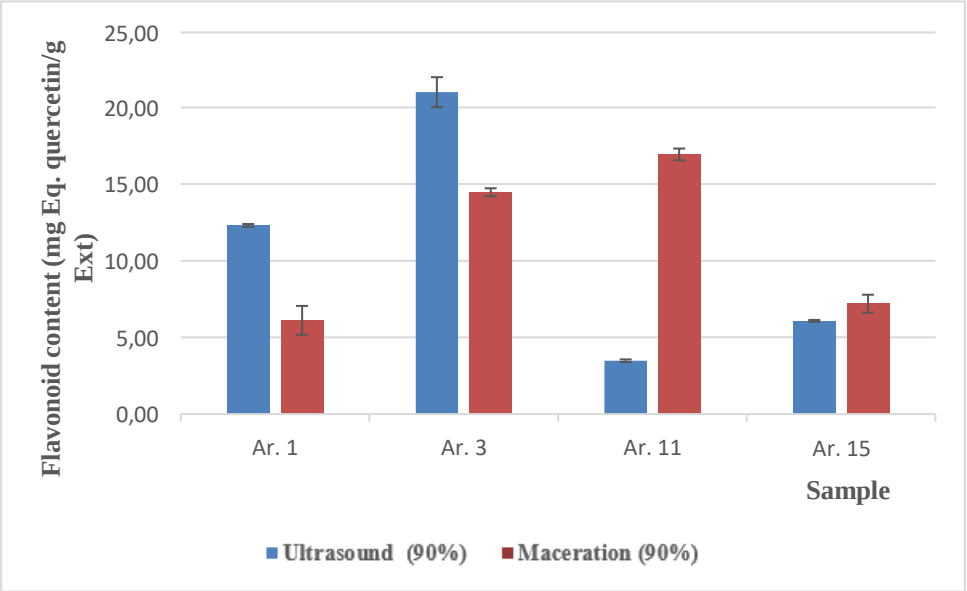


Figure 10: Flavonoid content of extracts from sonication and macerations (90%).

Ar.1: (2h, 2g)**; Ar.3:** (4h, 2g)**; Ar.11:** (3h, 3g)**; A.15:** (3h, 1g)**.**

The extracts (1 and 3) obtained by ultrasound have the highest concentration of flavonoids (12.31 mg EQ/g EXT and 21.00 mg EQ/g EXT, respectively), ultrasound being more effective for shorter times (2h, 4h) and moderate quantities. Conversely, the macerations method gave a higher flavonoid concentration than ultrasound for the extracts (11and15) presented (16.93mg EQ/g MS and 7.19mg EQ/g DM), respectively. Maceration can be more effective for intermediate durations (3 hours) with a larger quantity of powder.

From the results presented in (figure 11), it is assured that the content of total flavonoids varies considerably depending on the part of the species used, the extraction method applied as well as the time and quantity of the powder.

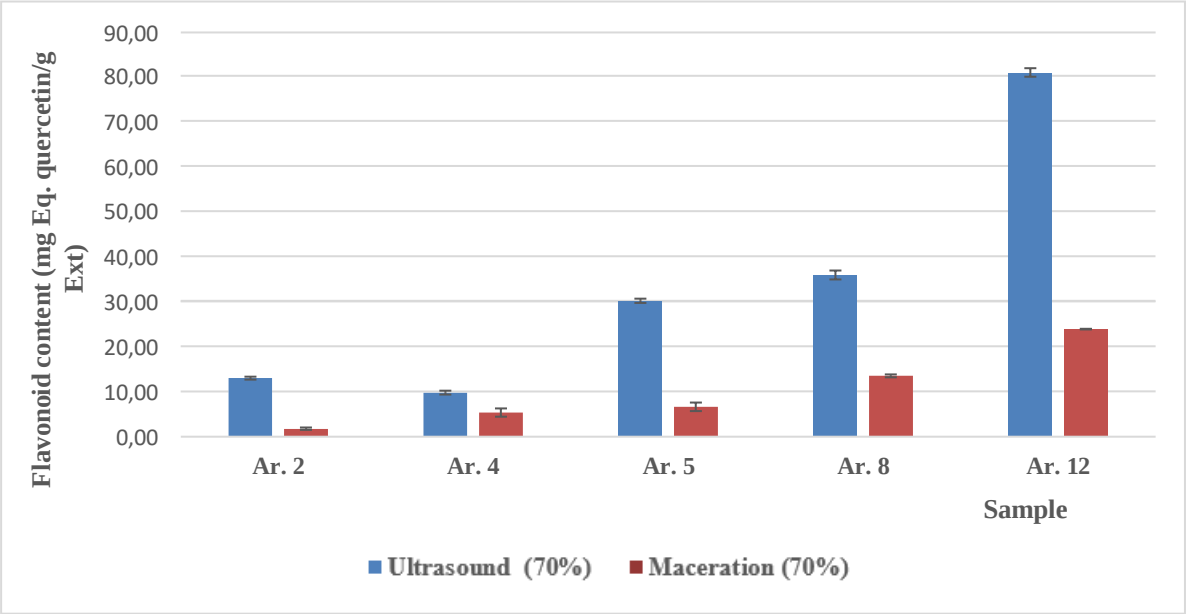


Figure 10: Flavonoid content of extracts from sonication and macerations (70%).

Ar.2: (4h, 3g)**; Ar.4:** (2h, 3g)**; Ar.5:** (4h, 1g)**; Ar.8:** (3h, 2g)**; Ar.12:** (2h, 1g)**.**

According to the results presented in (figure 11), shows the flavonoid content of all the extracts (2,4,5,8 and 12) obtained by ultrasound present the highest concentrations of flavonoids (12.98 mg EQ/g EXT and 29.76 mg EQ/g EXT and 30.13 mg EQ/g EXT and 35.86 mg EQ/g EXT and 80.77 mg EQ/g EXT), respectively, for short (2h) and intermediate (3h) times, whatever the quantity of powder used. In contrast, the maceration method resulted in a lower concentration of flavonoids. Conversely, the macerations method gave a lower flavonoid concentration.

From the results presented in (figure 12), it is assured that the content of total flavonoids varies considerably depending on the part of the species used, the extraction method applied as well as the time and quantity of the powder.

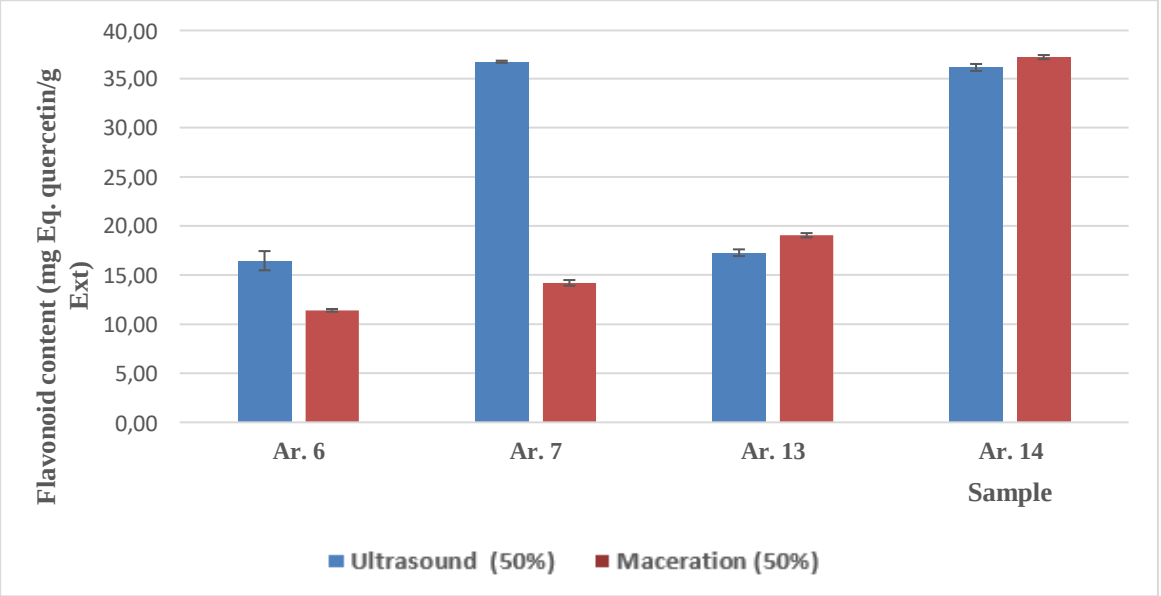


Figure 11: Flavonoid content of extracts from sonication and macerations (50%).

Ar.6: (3h, 3g)**; Ar.7:** (4h, 2g)**; Ar.13:** (2h, 2g)**; Ar.14:** (3h, 1g)**.**

The figure shows that the extracts obtained by sonication (extracts 6 and 7) present the highest concentrations of flavonoids (16.45 mg EQ/g EXT and 36.73 mg EQ/g EXT), respectively, for longer durations (3h and 4h), with variations depending on the quantity of powder. On the other hand, the extracts (13 and 14) displayed the highest values with maceration (19.06 mg EQ/g EXT, 37.20 mg EQ/g EXT), respectively.

The results of the analysis of the AV extracts (figure 10,11,12), show that the Ar.12 extract, obtained by ultrasound with 70% solvent, an extraction time of 2 hours and 1 gram of powder, represents the highest amount of flavonoids among all samples tested. This result highlights the remarkable efficiency of ultrasonic extraction under these specific conditions.

Artemisia vulgaris contain a higher concentration of flavonoids. After two hours of sonication, these flavonoids undergo significant degradation, resulting in a marked decrease in the yield of extracted compounds. These findings suggest that there is an optimal sonication duration to maximize flavonoid extraction, whereas longer periods can lead to their degradation. (Draban & al., 2021),

IV-2-2- Determination of total polyphenol components

The total polyphenol content is also estimated using the colorimetric technique by UV-visible spectrophotometry. To achieve effective extraction, two elements are crucial: the extraction method and the choice of solvent.

To determine the quantitative analyzes of total phenols, the linear regression equation of the calibration curve. Gallic acid considered as a positive control. The results are expressed as the absorbance of a gallic acid solution as a function of its concentration in (µg/ml, Annexe II).

The evaluation of the content of phenolic compounds in extracts from maceration, sonication of *Artemisia vulgaris* plants revealed different contents.

The results of the analysis of A. vulgaris extracts by the concentration of ethanol (90%) (figure 13).

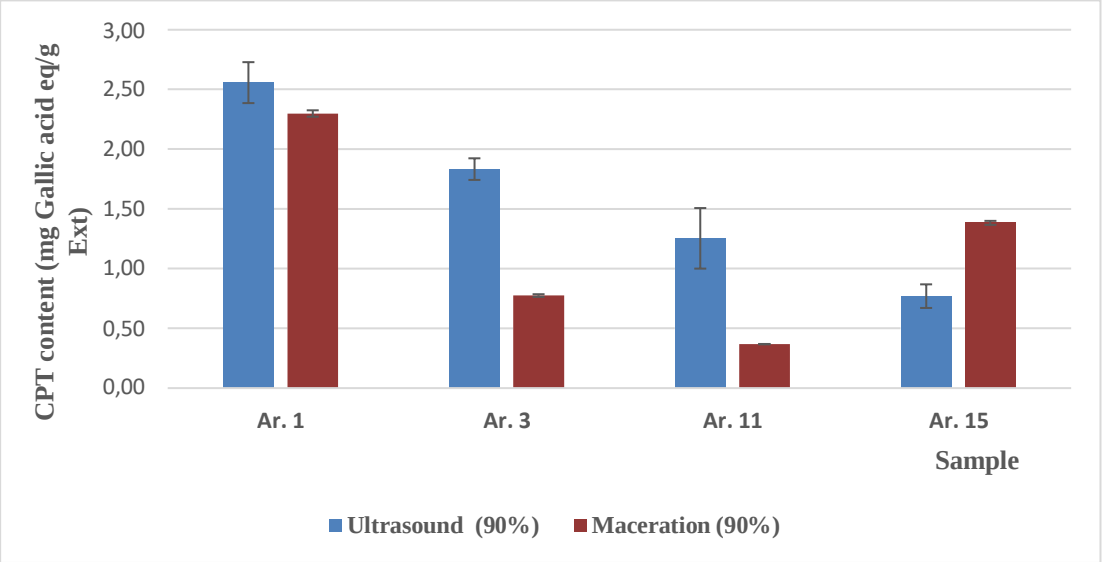


Figure 12: Polyphenol content of sonication extracts and macerations (90%).

Ar.1: (2h, 2g)**; Ar.3:** (4h, 2g)**; Ar.11:** (3h, 3g)**; Ar.15:** (3h, 1g)**.**

The results of the analysis of A. vulgaris extracts by the concentration of ethanol (90%) (figure 13), show that the samples (1,3 and 11), with larger quantities of powder (2 g, 3 g) and variable durations (2 h, 3 h, 4 h), have a higher content of phenolic compounds than that of sample (15) with (0.77mg Eq.gallic acid/g Ext) obtained by the ultrasonic extraction method.

The results of the analysis of A. vulgaris extracts by the concentration of ethanol (70%) (figure 14).

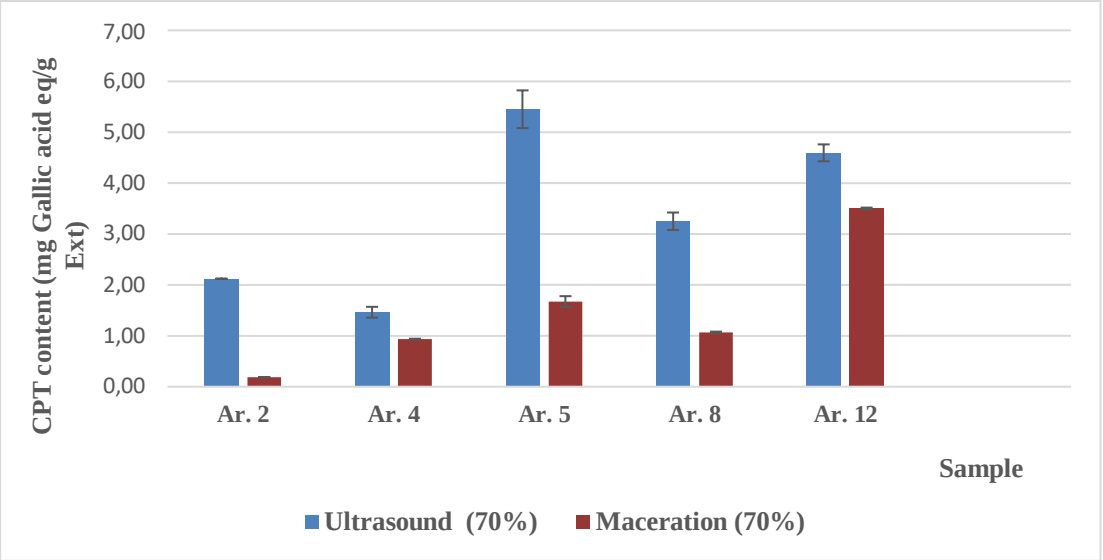


Figure 13: Polyphenol content of sonication extracts and macerations (70%).

Ar.2: (4h, 3g)**; Ar.4:** (2h, 3g)**; Ar.5:** (4h, 1g)**; Ar.8:** (3h, 2g)**; Ar.12:** (2h, 1g)**.**

In view of the results obtained in (Figure 14), by the concentration of (70%) all the samples have the high content of total polyphenols by the ultrasonic extraction technique, knowing that the highest concentration is noted (5 .45 mg Eq.gallic acid/g Ext) for sample (5) with an extraction time of 4 hours and 1 gram of powder.

The results of the analysis of A. vulgaris extracts by the concentration of ethanol (50%) (figure 15).

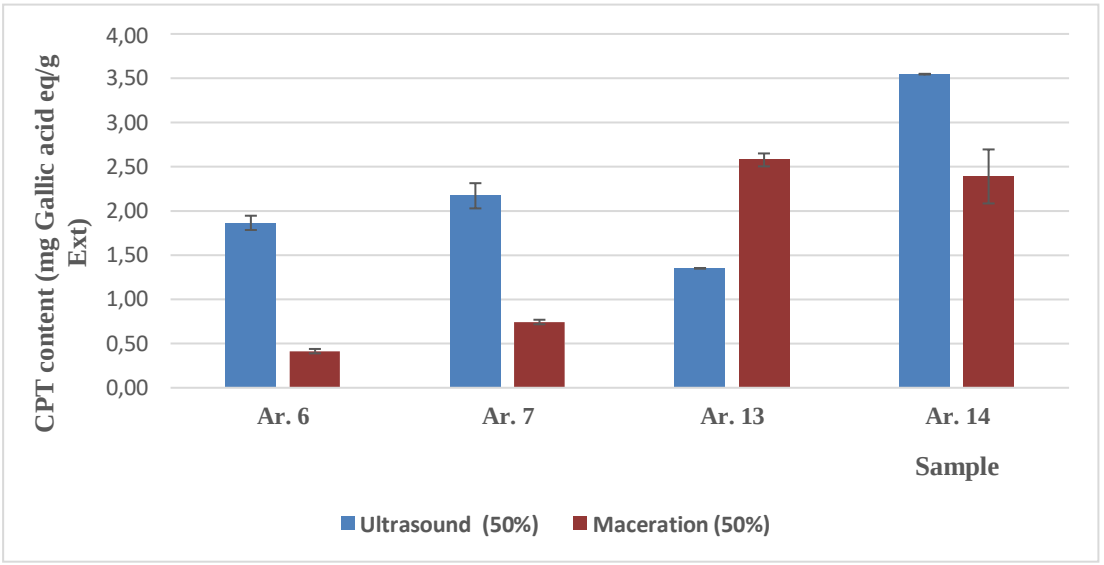


Figure 14: Polyphenol content of sonication extracts and macerations (50%).

Ar.6: (3h, 3g)**; Ar.7:** (4h, 2g)**; Ar.13:** (2h, 2g)**; Ar.14:** (3h, 1g)**.**

The results, presented in the (figure15), show that the three samples (6,7 and 14), with powder quantities of (3 g, 2g,1g), as well as a time variation (2 h, 3 h, 4 h), were revealed significant results when using ultrasound (1.87 mg Eq. gallic acid/g of extract and 2.17 mg Eq. gallic acid/g of extract and 3.54 mg Eq. gallic acid/g of extract), respectively. In comparison, sample (13) showed a higher value when processed by maceration technique compared to ultrasound (2.58 mg Eq. gallic acid/g extract). The values also demonstrated a progressive increase in total polyphenol yield in parallel with increasing sonication duration.

The results of the analysis of the AV extracts (figure 13,14,15), show the Ar.5 extract, obtained by ultrasound with 70% solvent, an extraction time of 4 hours and 1 gram of powder, represents the highest quantity of polyphenols among all samples tested.

Increasing the duration of sonication could have an effect of increased cell wall disintegration and subsequent release of phenolic compounds.

The results obtained show that the use of ultrasound has a positive effect on the extraction of natural substances in terms of speed and quantity extracted. These results are in agreement with those found by Chabir & al. (2009). Ultrasound is useful for extraction becauseit increases the internal diffusion mechanisms by creating cavities inside the root pores. This improvement in yield can be attributed to two physical phenomena: diffusion through the cell wall and rinsing by the solvent (ethanol/water) followed by cell rupture (Chabir & al., 2009).

The number of phenolic compounds in plant extracts varies from one plant to another, this is probably due to geographical location, harvest season, climatic conditions and environment, maturity of the plant and duration of conservation (Gheffour & al., 2015).

IV-3- Optimization of antioxidant activity

In our research, we conducted various experiments to evaluate the antioxidant properties of isolated *A. vulgaris* root substance. The DPPH test is widely used to more accurately assess antioxidant activity. It makes it possible to measure the anti-radical power, the capacity of an antioxidant to neutralize the chemical radical DPPH by providing it with an electron or a proton, which transforms it into a non-radical molecule. This approach involves monitoring the color change of the DPPH radical, which evolves from purple to yellow. The reduction capacity of this radical is determined by the decrease in absorbance at 517 nm (Krache & al., 2018). The results are presented in (figures 16,17 and 18).

The results of the analysis of A. vulgaris extracts by the concentration of ethanol (90%) (figure 16).

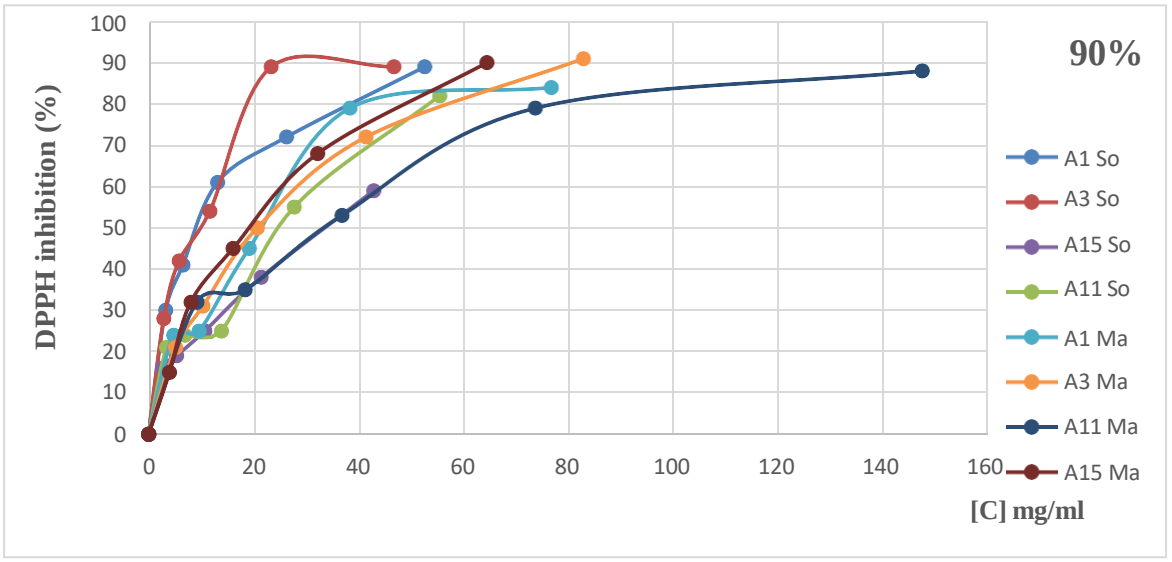


Figure 15: DPPH anti-radical activity of the extracts *A. vulgaris* (90%).

So: Test with sonication**; Ma:** Test with maceration**.**

A1-So & Ma: (2h, 2g)**; A3-So & Ma:** (4h, 2g)**; A11-So & Ma:** (3h, 3g)**; A15-So Ma:** (3h, 1g)**.**

Extracts (3, 11, and 15) showed the highest inhibition percentages, respectively, for long (4 hours) and intermediate (3 hours) maceration times, regardless of the amount of powder used. Even small quantities (as in the case of A15 extract with 1 gram) have shown greater effectiveness. On the other hand, the sonication method gave lower inhibition percentages.

The results of the analysis of A. vulgaris extracts by the concentration of ethanol (70%) (figure 17).

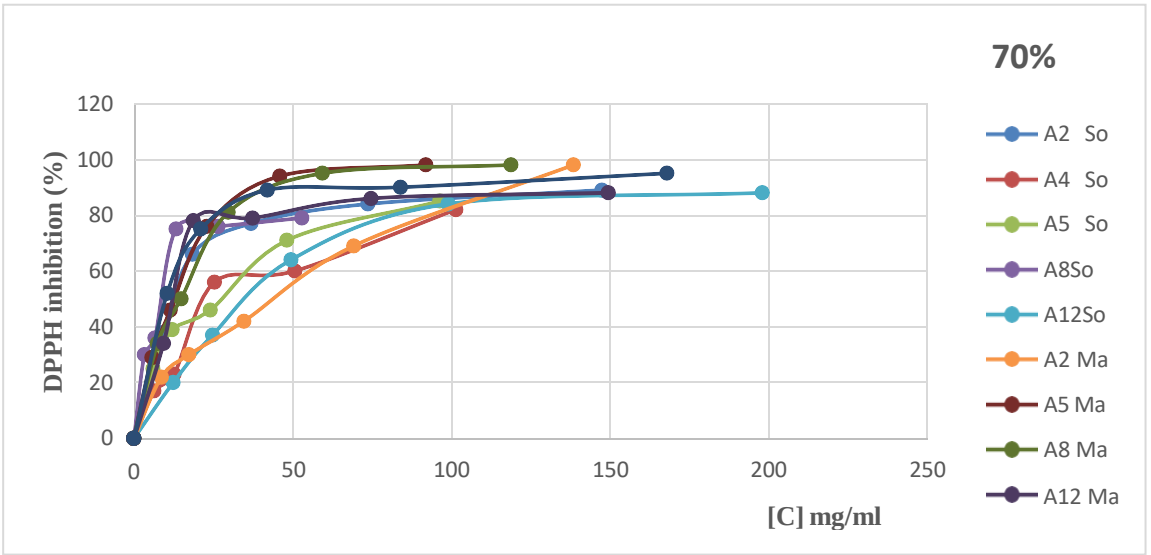


Figure 16: DPPH anti-radical activity of the extracts *A. vulgaris* (70%).

So: Test with sonication**; Ma:** Test with maceration**.**

A2-So & Ma: (4h, 3g)**; A4-So & Ma:** (2h, 3g)**; A5-So & Ma:** (4h, 1g)**; A8-So & Ma:** (3h, 2g)**;**
A12-So Ma: (2h, 1g)**.**

The extracts (2,4,5,8 and 12) obtained by maceration present the greatest percentages of DPPH˙ inhibition (98%,95%,98%,98%,95%), respectively, whatever the time extraction and the quantity of powder used. On the other hand, the sonication method resulted in a lower inhibition percentage. On the other hand, the sonication method gave lower inhibition percentages.

The results of the analysis of A. vulgaris extracts by the concentration of ethanol (50%) (figure 18).

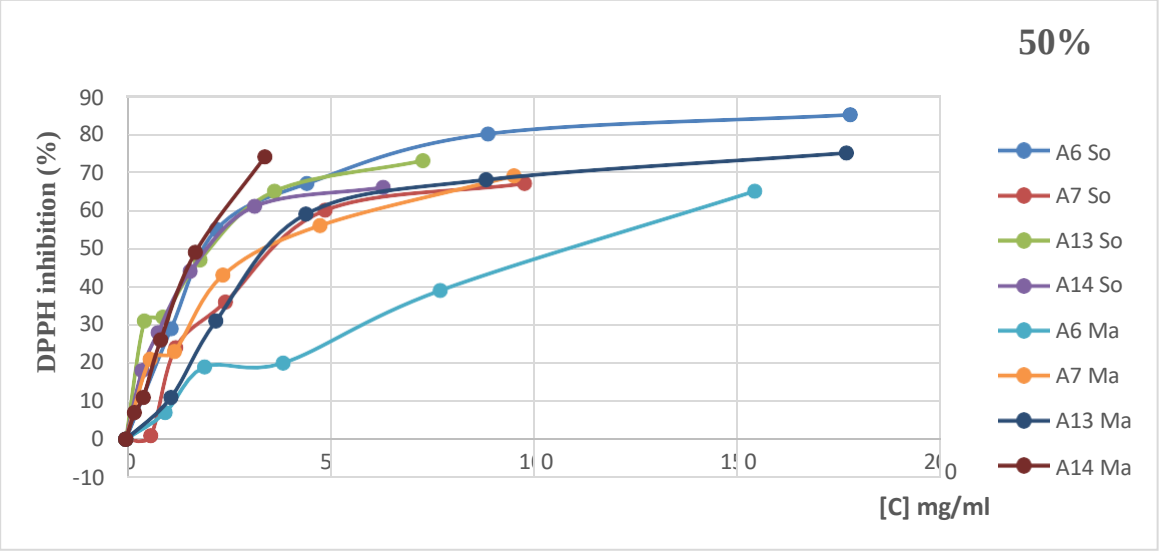


Figure 17: DPPH anti-radical activity of the extracts *A. vulgaris* (50%).

So: Test with sonication**; Ma:** Test with maceration**.**

A6-So & Ma: (3h, 3g)**; A7-So & Ma:** (4h, 2g)**; A13-So & Ma:** (2h, 2g)**; A14-So Ma:** (3h, 1g)**.**

For root extracts of *A. vulgaris* solubilized in 50% ethanol (figure 18), the percentages varied from 1% to 66% for sonication and from 7% to 75% for maceration.

The extracts (7,13 and 14) obtained by maceration showed the highest inhibition percentages (69%, 75%, 74%), respectively, for powder quantities of 1 g and 2 g, whatever the time. extraction used. On the other hand, the sonication method resulted in a lower inhibition percentage.

Comparing our results to previous studies we observed significant similarities, our recent result also revealed that *A. vulgaris* maceration extract is much more effective than that obtained by sonication, which confirms our findings (Luliana & al., 2019). The results indicate

that the antioxidant activity is determined by the extraction method used, which is in agreement with previous research. The study carried out by Andriyani revealed that the ethanol extract obtained by maceration presents a high level of antioxidant activity distinct from that of the aqueous extract obtained by decoction (Andriyani & al., 2015).

A correlation was observed between antioxidant activity and levels of phenolic compounds in the roots, confirming that total polyphenols are the main contributors to this activity in Artemisia vulgaris extracts. the relative stability of root antioxidant activity could result from a higher concentration of stable phenolic compounds or better protection against degradation could be explained by the degradation of fragile phenolic compounds. (Trifan & al., 2022).

IV-4- Anti-inflammatory activity

The objective of this study was to demonstrate the *in vitro* anti-inflammatory activity of ethanolic extracts from the roots of *Artemisia vulgaris* by a method based on the calculation of the percentage of inhibition of protein denaturation.

IV-4- 1-Inhibition of denaturation of BSA (Bovine Serum Albumin)

In this study, we were interested in the ability of our plant to protect BSA against denaturation following increased temperature.

The curve shows Effect of *A. vulgaris* extracts on bovine serum albumin denaturation.

The graph in (Figure 19) depicts how the percentages of protein denaturation inhibition by A. vulgaris extracts vary with ethanol concentration (90%).

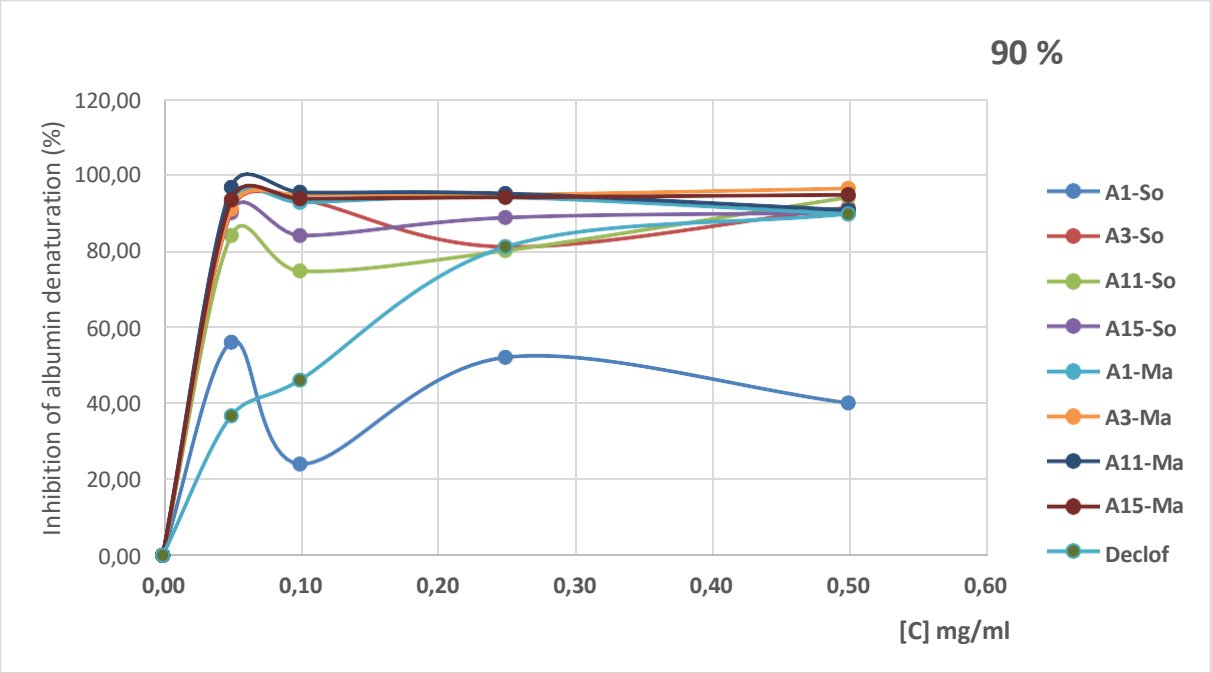


Figure 18: Effect of *A. vulgaris* extracts on bovine serum albumin denaturation.

So: Test with sonication**; Ma:** Test with maceration**; Declof:** Diclofenac (reference drug)

A1-So & Ma: (2h, 2g)**; A3-So & Ma:** (4h, 2g)**; A11-So & Ma:** (3h, 3g)**; A15-So Ma:** (3h, 1g)**.**

According to the curve of the percentages of inhibition of protein denaturation (figure 19), we observe that the ethanolic extracts of *Artemisia vulgaris* present a protective effect against the denaturation of BSA by heat.

- The curves for A1, A3, A11 and A15 maceration show a general trend of inhibition of albumin denaturation, reaching a maximum inhibition percentage of 96.67% at a concentration of 0.05 mg/ml then, we observed a reduction with increasing concentration.

- The curves for A1, A3, A11 and A15 sonication also show inhibition, but with greater variability in their effectiveness. For example, A11 appears to achieve inhibition close to that of diclofenac 80.00% at average concentrations 0.25 mg/ml, while other tests like A1 show reduced effectiveness between 24% and 56%.

- The diclofenac curve shows a stable and high effectiveness in inhibiting albumin denaturation, thus marking its anti-inflammatory effect by a percentage of 89.67% at the concentration of 0.5 mg/ml, and also at a low concentration of 0.05 mg /ml, the protective effect is (36.67%), sserving as a point of comparison for other tests.

The graph in (Figure 20) depicts how the percentages of protein denaturation inhibition

by A. vulgaris extracts vary with ethanol concentration (70%).

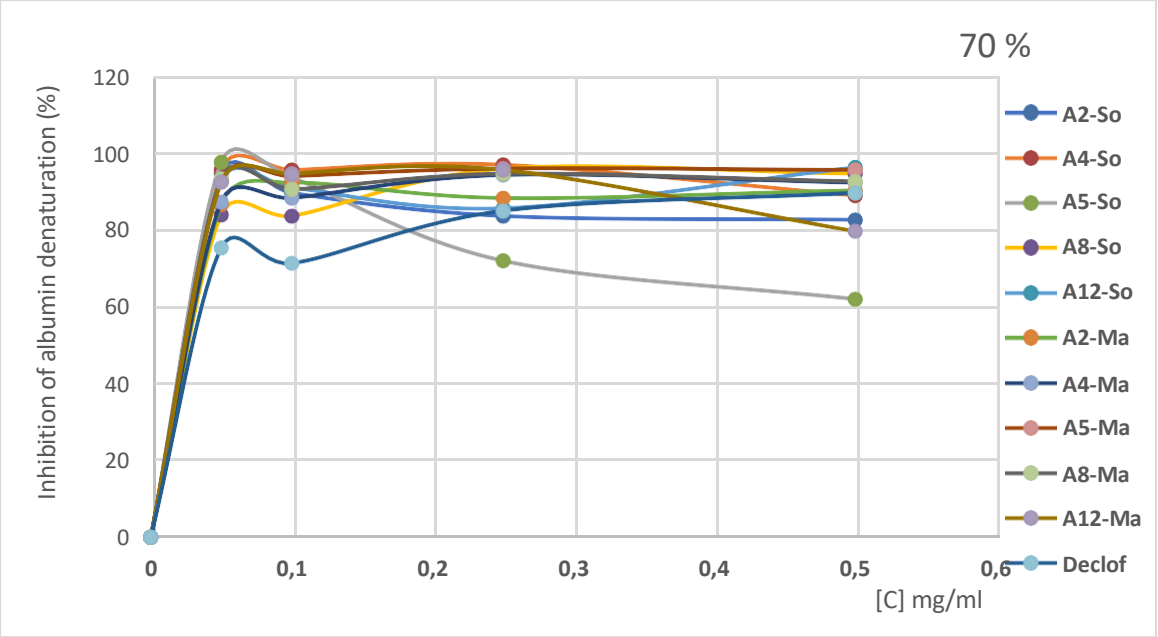


Figure 19: Effect of *A. vulgaris* extracts on bovine serum albumin denaturation.

So: Test with sonication**; Ma:** Test with maceration**; Declof:** Diclofenac (reference drug)

A2-So & Ma: (4h, 3g)**; A4-So & Ma:** (2h, 3g)**; A5-So & Ma:** (4h, 1g)**; A8-So & Ma:** (3h, 2g)**; A12-So Ma:** (2h, 1g)**.**

- The curves (figure 20) for A2, A4, A5, A8 and A12 sonication show relatively high BSA denaturation inhibition, up to 97.67% at lower concentrations 0.05mg/ml, although some variations are visible.
- The curves for A2, A4, A5, A8 and A12 maceration show greater variability in inhibition. For example, A5 shows a relatively constant effectiveness between (93% and 96%), while A2 and A12 have lower inhibition values compared to the other tests with (87.67%) at a concentration of 0.05mg/ml and (97.67%) at a concentration of 0.5 mg/ml.
- Diclofenac once again serves as a reference with a stable and high inhibition curve.

The graph in (Figure 21) depicts how the percentages of protein denaturation inhibition by A. vulgaris extracts vary with ethanol concentration (50%).

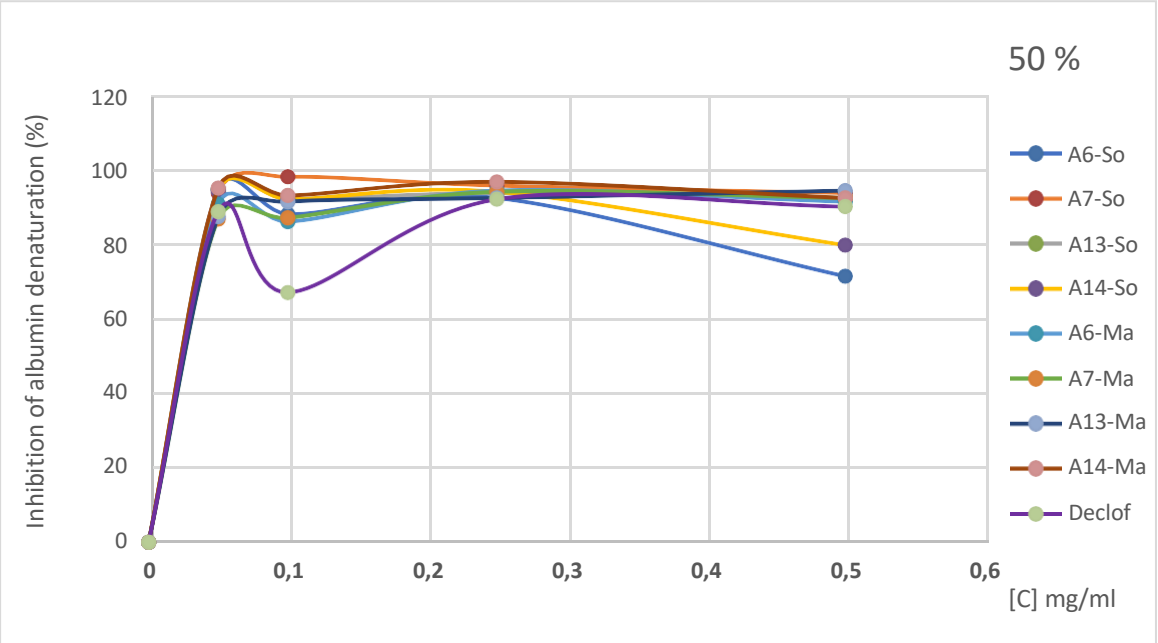


Figure 20: Effect of *A. vulgaris* extracts on bovine serum albumin denaturation.

So: Test with sonication**; Ma:** Test with maceration**; Declof:** Diclofenac (reference drug)

A6-So & Ma: (3h, 3g)**; A7-So & Ma:** (4h, 2g)**; A13-So & Ma:** (2h, 2g)**; A14-So Ma:** (3h, 1g)**.**

- The curves (figure 21) for A6, A7, A13 and A14 show an inhibition of BSA denaturation reaching values close to 100%, (98%) at lower concentrations 0.1 mg/ml.
- The curves for A6, A7, A13 and A14 maceration show greater variability in inhibition. Some curves, like A6 and A7, show reduced efficiency compared to others.
- The diclofenac curve for 50% ethanol shows an effectiveness in inhibiting the denaturation of BSA, with a percentage of 92% at the concentration of 0.25 mg/ml, and at a low concentration of 0.05 mg/ml, the protective effect is (88.67%).

Ultimately, sonication remains an effective method, but its effectiveness decreases as the concentration of ethanol decreases. The best results are obtained with 90% ethanol. Maceration shows great variability and reduced efficiency with 50% ethanol. On the other hand, the concentrations of ethanol (70% and 90%) are higher and produce better results.

To maximize inhibition of albumin denaturation, the use of sonication with 90% ethanol is recommended

The process of protein denaturation is a phenomenon where proteins lose their secondary and tertiary structures through the application of external stress or an agent such as a strong acid or base, high concentration of inorganic salt, an organic solvent or heat. Most

proteins lose their biological function when they undergo denaturation (Marliyah & Ananthi, 2015).

This denaturation can also occur through an autoimmune inflammatory process in which autoantigen production is increased. This increase can be one of the main triggers of inflammatory reactions regardless of the origin of the inflammation (Sridevi & al., 2015).

In our work, *Artemisia vulgaris* extract was able to inhibit heat-induced denaturation of BSA at concentrations 0.05; 0.1; 0.25 and 0.5 mg/ml with a maximum inhibition percentage of 98% which was observed at the lowest concentration 0.1 mg/ml. Diclofenac being a known anti-inflammatory, its results of inhibition of albumin denaturation serve as a reference. Assays that approach or exceed it in terms of efficiency are potentially promising for similar applications.

According to the results obtained by Pandey & al. (2021), the leaf extract of *A vulgaris* from the temperate zone had a maximum inhibition percentage of 54.05% at 400 mg/kg, and that obtained by Miloudi & Yala (2022), shows that the hydro-methanolic extract of *mastic pistachio* fruits had a maximum inhibition percentage of 36.37% at 0.25 mg/ml. Rashid & Shafi (2018), found that the methanolic extract of pomegranate flower showed a maximum inhibition of 71.24% at a concentration of 0.5 mg/ml. Also, according to the results obtained by Marliyah & Ananthi (2015), the ethanolic extract of Zeamays had an inhibition percentage of 54.51% at 0.1 mg/ml. This large difference observed could be due to the nature of the Bovine Serum Albumin (BSA) used, the solvent used, or even the part of the plant studied.

These results indicate that phenolic compounds, which are major constituents of the plant, have an inhibitory effect on various mechanisms leading to the triggering of the inflammatory reaction, such as the denaturation of BSA. Therefore, they are the origin of the healing effects observed in plants.

Conclusion

Conclusion

The use of phytochemicals, including polyphenols and flavonoids, to prevent and treat disease represents an innovative approach in the field of natural health. Medicinal plants produce a diversity of active compounds with varied biological properties, including anti-inflammatory and antioxidant effects.

To analyze the biological activities of the plant extracts, in particular their anti-inflammatory and antioxidant properties, total polyphenol and flavonoid were determined using two extraction methods: maceration and sonication, both with ethanolic solvents at different concentration. The results of this study highlighted the exceptional performance of the *A. vulgaris* plant in the two biological activities examined. This suggests that extracts from this plant have strong therapeutic potential. The extraction methods used, namely maceration and sonication, probably promoted an efficient release of the active compounds of the plant, thus explaining the promising results obtained. In addition, the polyphenol and flavonoid assays made it possible to quantify these compounds in the extracts, thus providing valuable information on their concentration and their contribution to the observed biological activities.

These discoveries open up new perspectives in the development of drugs and natural products aimed at treating or preventing various conditions, by exploiting the benefits of the plants studied and their active compounds.

It would be timely and stimulating to reinforce this research with other chemical and pharmacological studies, with the aim of isolating, characterizing and purifying the active ingredients responsible, in particular potential activities such as antioxidant, anti-inflammatory, analgesic and antibacterial extract. Additionally, it would be highly beneficial to explore other activities, such as antimutagenic and anticancer effects, and extend the study to other parts of the plant.

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Annexes

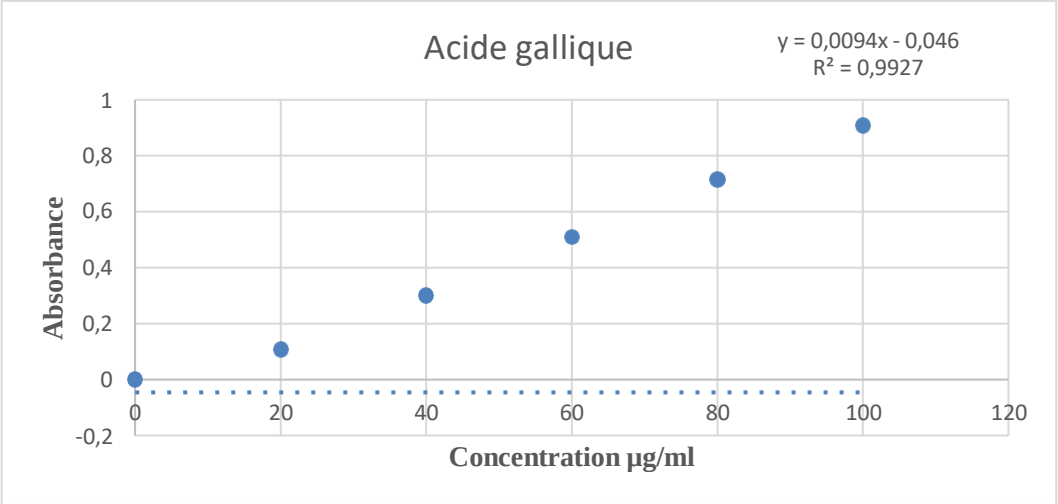
Annexes

Annexes I: Non-biological materials

Equipment	Glassware and others	Reagents and solutions
Precision scale	Spatula	**Ethanol and Methanol**
Stirring plate	Funnel	**Sodium carbonate (Na2CO3)**
Incubation oven	Burette	**Aluminum trichloride (AlCl3)**
Ultrasonic bath	Bottles	**Monobasic potassium phosphate (KH2PO4)**
pH meter	Magnetic bar	**Rutin, Gallic acid**
Fridge	Cotton	**Folin-Ciocalteu (0.1N)**
Micropipette	whatman paper	**Potassium chloride (KCl)**
Water bath	Glass petri dishes	**2,2-diphenyl-1-picrylhydrazyl (DPPH)**
Vortex	Beakers	**Hydrochloric acid (HCl)**
	Tanks	**Sodium hydrogen phosphate (Na2HPO4)**
	Vials	**Sodium chloride (NaCL)**
	Test tubes	
	Tube rack	
	Bits	

Annexes

Annexes II : Calibration curves



.**Figure**: Gallic acid calibration curve

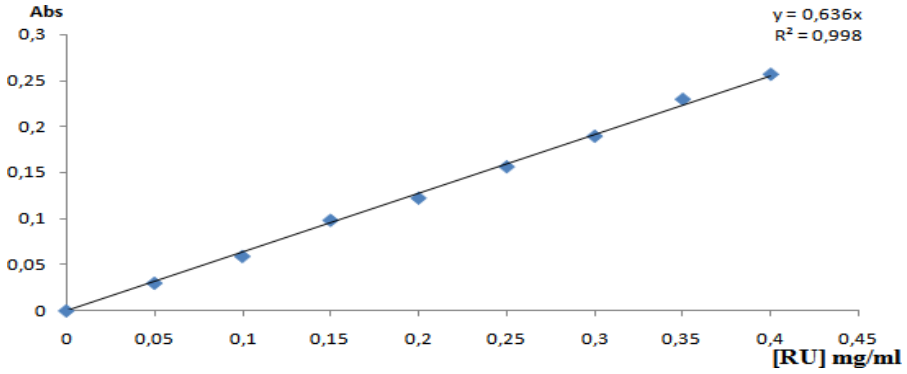


Figure : Flavonoid calibration curve.

Résumé

L'Armoise commune (*Artemisia vulgaris*), l'une des plantes connues pour son utilisation en médecine traditionnelle et qui occupe une grande importance à l'échelle mondiale et en Algérie, a été utilisée comme une pharmacopée naturelle pour l'homme grâce à ces propriétés curatives. Dans notre étude, une analyse des extraits éthanoliques des racines d'*A. vulgaris*, obtenus par deux méthodes distinctes : la macération et l'ultrason a été effectuée afin de déterminer sa teneur en substances bioactives en dosant les composés phénoliques (polyphénols totaux, flavonoïdes) d'une part, et évaluer ses activités biologiques : antioxydante (effet scavenger du radical DPPH) et activité anti-inflammatoire (test d'inhibition de la dénaturation de la BSA). D'après les résultats obtenus, les extraits obtenus avec ultrason sont les plus riches en polyphénols, quelle que soit le pourcentage d'éthanol, la portion d'échantillon utilisée, et le temps d'extraction. Comme ils enregistrent également la teneur la plus élevée en flavonoïdes. Concernant les activités biologiques, les extraits polyphénoliques de cette plante sont dotés d'un pouvoir antioxydant élevé allant jusqu'à 98%, et un potentiel antiinflammatoire important qui varie d'un test à l'autre selon les critères mentionnés.

Mots clés : *A. vulgaris*, activité antioxydante, activité anti-inflammatoire, composés phénoliques**.**

Abstract

Common Mugwort (*Artemisia vulgaris*), one of the plants known for its use in traditional medicine and which occupies great importance globally and in Algeria, has been used as a natural pharmacopoeia for humans thanks to these healing properties. In our study, an analysis of ethanolic extracts from the roots of A. *vulgaris*, obtained by two distinct methods: maceration and ultrasound was carried out in order to determine its content of bioactive substances by measuring phenolic compounds (total polyphenols, flavonoids) on the one hand, and evaluate its biological activities: antioxidant (effect scavenger of the DPPH radical) and anti-inflammatory activity (BSA denaturation inhibition test). According to the results obtained, the extracts obtained with ultrasound are the richest in polyphenols, whatever the percentage of ethanol, the portion of sample used, and the extraction time. As they also record the highest flavonoid content. Regarding biological activities, the polyphenolic extracts of this plant have a high antioxidant power of up to 98%, and a significant anti-inflammatory potential which varies from one test to another according to the criteria mentioned.

Key words: *A. vulgaris*, antioxidant activity, anti-inflammatory activity, phenolic compound.

الملخص:

نبات الشيح الشائع (*Artemisia vulgaris*)، أحد النباتات المعروفة باستعمالها في الطب التقليدي والذي يحتل أهمية كبيرة عالميا وفي الجزائر، تم استخدامه كدواء طبيعي للإنسان بفضل هذه الخصائص العلاجية. في دراستنا، تم إجراء تحليل للمستخلصات الإيثانولية من جذور *A. vulgaris*، التي تم الحصول عليها بطريقتين متميزتين: النقع والموجات فوق الصوتية لتحديد محتواها من المواد النشطة بيولوجيا عن طريق قياس المركبات الفينولية (إجمالي البوليفينول، الفلافونويدات) على من ناحية، وتقييم أنشطتها البيولوجية: مضادات الأكسدة (كاسح تأثير جذري DPPH) والنشاط المضاد للالتهابات (اختبار تثبيط تمسخ BSA). ووفقا للنتائج التي تم الحصول عليها، فإن المستخلصات التي تم الحصول عليها بالموجات فوق الصوتية هي الأكثر غنى بالبوليفينول، مهما كانت نسبة الإيثانول، ونسبة العينة المستخدمة، ووقت الاستخلاص. كما أنها تسجل أيضًا أعلى محتوى من الفلافونويد. وفيما يتعلق بالأنشطة البيولوجية، فإن المستخلصات البوليفينولية لهذا النبات تتمتع بقوة عالية مضادة للأكسدة تصل إلى 98%، وقدرة كبيرة مضادة للالتهابات والتي تختلف من اختبار إلى آخر وفقًا للمعايير المذكورة.

الكلمات المفتاحية: الشيح الشائع، نشاط مضاد للأكسدة، نشاط مضاد للالتهابات، مركب فينولي.