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Evaluation of the antioxidant activity of extracts from the leaves of *Olea europaea* from the wilaya of Bejaïa

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Dedication

I humbly dedicate this work to my precious parents, my guides and sources of inspiration along the way.

To my dearest mother,

No matter what I do or say, I'll never be able to thank you properly. Your love covers me, your kindness guides me and your presence by my side has always been my source of strength in overcoming obstacles.

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And here I am, concluding my dissertation with all its activity

I dedicate this modest work, which is the fruit of several years of study:

To the only person I miss today, and that is my father "Ahmed" Who wanted to be with me in these moments) رحمه هلا

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List of abbreviations

LIPOX : Lipoxygenase ;

HO˙ : Hydroxyl radical ;

DNA : Deoxyribonucleic acid ;

ROS : Reactive oxygen species ;

P-450 : Cytochrome P-450 ;

P53 : tumour protein P53 ;

LDL : low-density lipoprotein ;

NO : Nitric oxide ;

SOD : superoxide dismutase ;

CAT : catalase ;

GPx : glutathione peroxidase ;

BHA : butylated hydroxyanisole ;

BHT : butylated hydroxytoluene ;

ug EQ/mg : microgramme of quercetin equivalent per milligramme ;

µg/ml : microgram per millilitre ;

DPPH : 2,2-Diphenyl-1-picrylhydrazyl ;

ABTS : 2,2'-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid).

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Introduction

Introduction

According to the World Health Organisation, the vast majority of people in Africa use pharmacopoeia and traditional medicine. In Algeria, for example, various medicinal plants are used to remedy certain illnesses, such as diabetes and rheumatism, as well as gastrointestinal, inflammatory and cardiovascular diseases **(Addab et al., 2020).**

Despite the reputed use of medicinal plants, and even though some medicinal plants are a source of molecules with important biological activities, it is important to ensure that there are no toxic effects associated with their use. Nevertheless, scientific evaluation of the pharmacological activities of medicinal plants has enabled humans to be assured of the positive health effects of these plants **(Arab et al., 2013).** In short, medicinal plants are rich in secondary metabolites, which has made them one of the main sources of medicines **(Addab et al., 2020).**

An example of a medicinal plant is the olive tree or *Olea europaea*. This species has been cultivated for centuries in countries around the Mediterranean basin. It is best known for producing an oil that is used for medicinal, cosmetic and culinary purposes. It is also known for its leaves. Indeed, because of their medicinal properties, *Olea europaea* leaves have been the subject of several studies **(Djenane et al., 2012).**

The positive effects of *Olea europaea* leaves on human health are due to their richness in bioactive compounds, particularly phenolic compounds. The latter have significant biological activities, including antimicrobial, antioxidant and anticancer activities, giving *Olea europaea* leaves particular importance in the fields of food and health **(Addab et al., 2020).**

In order to evaluate, in vitro, the antioxidant activity of *Olea europaea* leaves from the region Sidi-Aïch (wilaya of Béjaïa), the first section of this modest work focuses on general information about the olive tree, free radicals, antioxidants and phenolic compounds. The second concerns the experimental aspect. It consists of : (i) using dried *Olea europaea* leaves to obtain different extracts ; (ii) determining the total phenol and flavonoid content of the extractsobtained ; and (iii) evaluating, in vitro, the antioxidant activity of these extracts.

Theoretical part

Chapter 1

Bibliographic synthesis

I. Generalities on the olive tree

1. Historic

In Asia, the majority of names given to the olive tree (*Olea europaea*) are of semitic origins. Candolle proposes that the cultivation of *Olea europaea* began in Asia. More precisely, in Palestine or further to the East. While at the time of Homer, the development of the olive tree was remarkable in Greece. Furthermore, even if it was little cultivated by the ancient Egyptians, its expansion in Italy dates from the sixth century before J. C.. Then, its culture spread to the Iberian Peninsula, southern Gaul and North Africa **(Chevalier, 1948).**

Although its culture has spread to new regions beyond the Mediterranean basin (such as Punjab, Syria, Anatolia, Iran, Mesopotamia and the South Caucasus), the olive tree, whose roots date back approximately 6 millennia, is the best example of a tree cultivated throughout the Mediterranean basin. In addition, in order to produce quality fruits and oil, several varieties of olives are cultivated, however their origin and dispersal constitute a subject of debate **(Besnard et al., 2018; Chevalier, 1948).**

2. Botanical classification

The table I indicates a botanical classification of *Olea europea*.

Table I : Botanical classification of *Olea europea* **(Ghedira, 2008).**

Kingdom	Plantae
Division	Magnoliophyta
Subdivision	Magnoliophytina
Class	Magnoliopsida
Subclass	Dialypétales
Order	Lamiales
Family	Oleaceae
Genus	*Olea*
Species	*Olea europea* L.

Table I : Botanical classification of *Olea europea* (continued) **(Ghedira, 2008).**

Subspecies	*O. europeasubsp. europaea var. sylvestris* *O. europeasubsp. europaea var. Europaea*

3. Botanical description

The olive tree belongs to the Oleaceae family that can live for several centuries. It usually measures six to eight meters in height, but sometimes it can reach fifteen meters. It is characterized by gnarled branches and grayish smooth bark. It has green evergreen leaves and a slow growth rate. The flowers adorning its branches and appearing from May to June are small and greenish-yellow in color (**Abdoun, 2013; Aillaud, 1985; Ghedira, 2008).** Its fruits (olives) are mainly oval in shape and protect a bony core. They have smooth skin and a fleshy envelope. They are green in color, but when fully ripe, their color turns black. They are foods that produce edible oil by grinding their fleshy envelope **(Gigon et al., 2010).**

The olive tree has the following plant parts:

- ✓ Trunk : pruning is able to transform a hurled trunk into a spreading trunk, characterized by a short size and zigzagging branches. Sometimes, this trunk can become massive, despite a shallow root system, and it tends to develop a significant woody layer underneath, serving as a crucial storage site for reserves **(Aillaud, 1985)**;
- ✓ Leaves : the leaves of the olive tree keep their green color throughout the year. They are simple, with an elongated shape and smooth edges. They are strong and have a thick texture. Their upper side is an intense green, while the lower side has a silver tint. Additionally, the leaves are covered by numerous hairs which help to limit water loss **(Aillaud, 1985)**;
- ✓ Flowers : the flowers of the olive tree are small and have a greenish-yellow color. They grow in clusters from the axils of leaves. They are regular, i.e., they have an axis of symmetry. More accurately, they have four sepals, four petals, two stamens and two carpels **(Aillaud, 1985)**;
- ✓ Fruits : when fully ripe, olives take on a black-tinted ovoid shape, containing a sturdy spindle-shaped grain (Figure 1) **(Ghedira, 2008).**

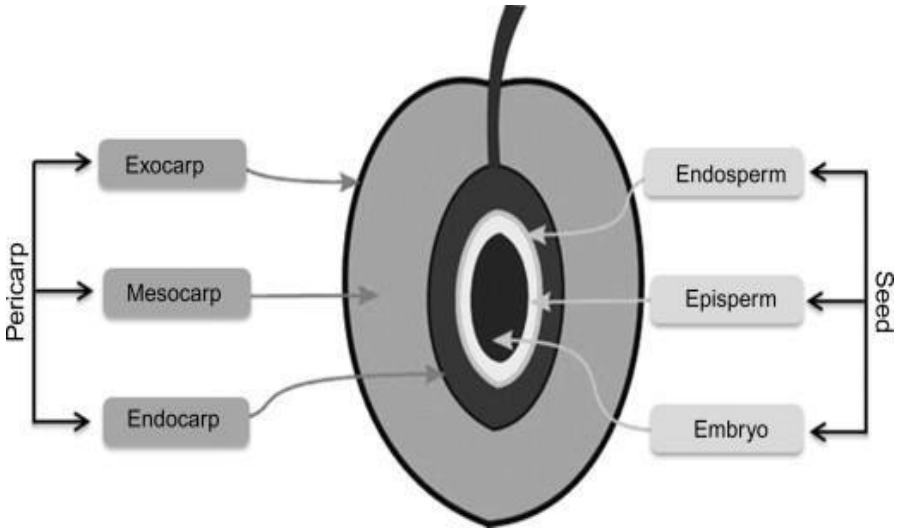


Figure 1 : Composition of the olive **(Calabriso et al., 2015).**

4. Leaves

The leaves of the olive tree have a very high value, because in addition to their antioxidant activity, they contribute to lower blood pressure, blood sugar and cholesterol. They are also considered antiseptic and antispasmodic. In addition, they are used in herbal medicine as diuretics because of their valuable compounds, such as triterpenes, flavonoids and secoiridoids, such as the oleuropeoside and the phenolic acids (Figure 2) **(Ghedira, 2008).**

Figure 2 : Leaves of *Olea europaea* **(Acila, 2018).**

4.1. Chemical composition

The leaves of the olive tree present a chemical composition, which varies depending on several factors, including the cultivated variety, climatic conditions, the age of the tree and wood content. They contain different levels of organic matters (from 76.4 to 92.7 g/100 g of dry matter), moderate levels of crude proteins (from 6.31 to 10.9 g/100 g of dry matter) and significant amount of amino acids (89.9 g/100 g of dry matter). They also present a variation in crude fat content (from 2.28 to 9.57 g/100 g of dry matter), as well as two main soluble carbohydrates, which are mannitol and glucose. The latter represent together from 27.1 to 30.8 %. In addition, they also contain lignocellulosic compounds, which may have a negative environmental impact. Finally, the nitrogen content fixed on the cell walls is high, but fluctuating (from 49.2 to 35.4 g/100 g of total nitrogen) **(Khelouf et al., 2023).**

The leaves of the olive tree are rich in bioactive compounds, such as phenolic compounds (tyrosol, hydroxytyrosol, ferulic acid and caffeic acid, flavonoids, such as apigenin, luteolin and luteolin-7-O-glucoside) and secoiridoids (e.g., ligstroside, oleuropein, and dimethyloleuropein) **(Khelouf et al., 2023).**

Qualitative and quantitative analysis of olive leaves extracts revealed six main polyphenolic compounds, headed by oleuropein (24.54 %), followed by hydroxytyrosol (1.46 %), luteolin-7-O-glucoside (1.38 %), verbascoside (1.11 %), apigenin-7-O-glucoside (1.37 %) and tyrosol (0.71 %). Furthermore, traces of luteolin, vanillic acid and caffeic acid were also detected **(Khelouf et al., 2023).**

4.2. Uses

The Table II presents some applications of olive tree leaves.

Table II : Fields of use of olive tree leaves.

Field	Application
Animal feed	The leaves of the olive tree can be included in the sheep's diet, which provides them additional nutrients **(Fegeros et al., 1995).** They can also be included in the goats' diet **(Ayeb et al., 2019).**

Table II : Fields of use of olive tree leaves (continued).

Agri-food industry	The leaves of the olive tree can be used to preserve turkey meat, allowing thus to extend its shelf life **(Djenane et al., 2012).**
Herbal medicine	The leaves of the olive tree can be used as an infusion for therapeutic purposes due to, among others, their anti-inflammatory properties **(Khan et al., 2007).**
Pharmaceutical industry	Phenolic compounds extracted from the leaves of the olive tree are used as additives in the pharmaceutical industry. These compounds have beneficial properties and are incorporated into different pharmaceutical products for their therapeutic effects **(Lkrik et al., 2015).**
Cosmetic industry	Bioactive compounds extracted from the leaves of the olive tree can be used as effective antioxidants to improve skin health. As a result, their use makes it possible to create a preliminary barrier against reactive oxygen and nitrogen species, which are involved in the skin aging process. Indeed, currently, these compounds are being studied to evaluate their potential for use in the cosmetic industry. This opens new perspectives for the development of cosmetic products beneficial for the skin **(Cádiz-Gurrea et al., 2021).**

In traditional medicine, different parts of *Olea europaea* are used for their healing properties. The ingestion of leaves, for example, is used to treat stomach and intestinal problems, and also serves as a method of maintaining oral hygiene. Furthermore, the dried leaves can be transformed into decoctions to take orally for alleviating the symptoms of diarrhea, respiratory illnesses and infections affecting the urinary tract **(Khan et al., 2007).** In addition, in order to combat high blood pressure levels and to stimulate urine production, some people choose to ingest an oral solution produced by infusing freshly picked leaves, whereas others choose to orally consume a decoction made from dried leaves to control

diabetes **(Khan et al., 2007)**. Lastly, the application of the leaves of the olive tree on skin wounds is a common method of disinfection **(Lakache et al., 2021).**

5. Geographic distribution

5.1. In the world

With its distinguishing features, *Olea europaea* can be considered a Mediterranean tree. Indeed, it is cultivated in various regions surrounding the Mediterranean Sea, from Madeira and the Canary Islands to the Arabian Peninsula and Mesopotamia, and currently there are approximately 5.3 million olive trees in the Mediterranean basin. In addition, in Africa, it moves south to Ethiopia. However, economic constraints have prevented its widespread adoption, although attempts have been made to cultivate it beyond its natural habitat, such as in the United States and South America (e.g., Argentina, Chile, Peru and Uruguay) **(Tissot, 1937).**

5.2. In Algeria

With more than 25 million trees spread over approximately 389000 hectares, the national olive orchard encompasses a vast area. The majority of these traditional groves are located on rugged terrain, mainly in the central region. Actually, they represent around 85% of the entire orchard and are specific to the production of olive oil **(Lamani et al., 2016).**

Olives cultivation is mainly concentrated in six main regions, three of which are located in the central region and three in the eastern region. These regions, namely Bejaia, Tizi-ouzou, Bouira, Bourdjbourreridj, Setif, and Jijel, represent more than half of the country's total olive-growing area. The remaining olive groves, mainly dedicated to the production of table olives, are mainly found in three other regions, namely Tlemcen, Mascara, and Relizane **(Lamani et al., 2016).**

Even if Bejaia claims first place in terms of olive oil production, the Algerian olive industry is distinguished by its varied landscape such that, the mountainous regions of Kabylie serving as a focal point for olive tree cultivation. Indeed, it is in these areas that the production of olive oil thrives, giving rise to a product that is both natural and beneficial for health **(Lamani et al., 2016).**

II. Generalities on free radicals and antioxidants

1. Free radicals

A free radical represents a chemical entity, which can be a molecule, a fragment of a molecule or even a simple atom that contains one or more single electrons (unpaired) on its outer orbital. This particularity gives it high reactivity and therefore a very short half-life. In fact, a free radical tends systematically to complete its outer orbital by capturing an electron, thus seeking to achieve increased stability. This process results in its reduction *via* the oxidation of another compound (Figure 3) **(Goudable and Favier, 1997).**

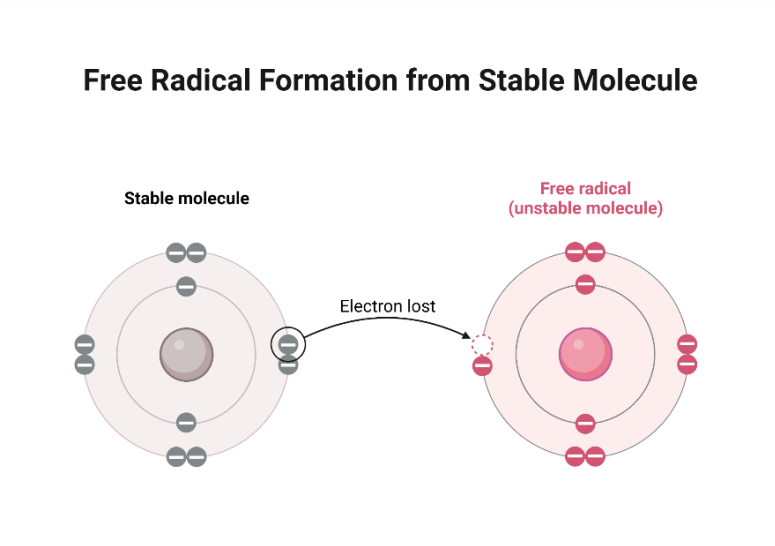


Figure 3 : The difference between a stable molecule and a free radical **(Anon, 2024).**

1.1. Examples

The Table III shows some examples of free radicals.

Table III : Some examples of free radicals **(Tessier and Marconnet, 1995).**

O_2	Molecular oxygen	Biradical, stable, low oxidizing power.
$O_2^{\bullet -}$	Superoxide anion	Less reactive, toxic, oxidizes catecholamines, can form $HO^{\bullet}$.
$^1O_2$	Singlet oxygen	Non radical, highly reactive, can initiate LIPOX.
H_2O_2	Hydrogen peroxide	Non radical, stable, less toxic, diffusible, antiseptic, can form $HO^{\bullet}$.
$HO^{\bullet}$	Hydroxyl radical	Highly reactive, less diffusible, main initiator of lipoperoxydation, alters proteins and DNA.

1.2. Origins (sources)

Free radicals, among others, reactive oxygen species (ROS), can be generated, either from fundamental metabolic processes present in the human body, or from external sources, such as smoking, exposure to X-rays, ozone, air pollutants, and industrial chemicals. The production of free radicals occurs constantly within cells, resulting from enzymatic and non-enzymatic reactions. Among the enzymatic reactions generating free radicals, we find those involved in the respiratory chain, phagocytosis, prostaglandin synthesis, and the cytochrome P-450 system. On the other hand, free radicals can also arise from non-enzymatic reactions between oxygen and organic compounds, as well as from reactions initiated by ionizing processes (Figure 4) **(Lobo et al., 2010).**

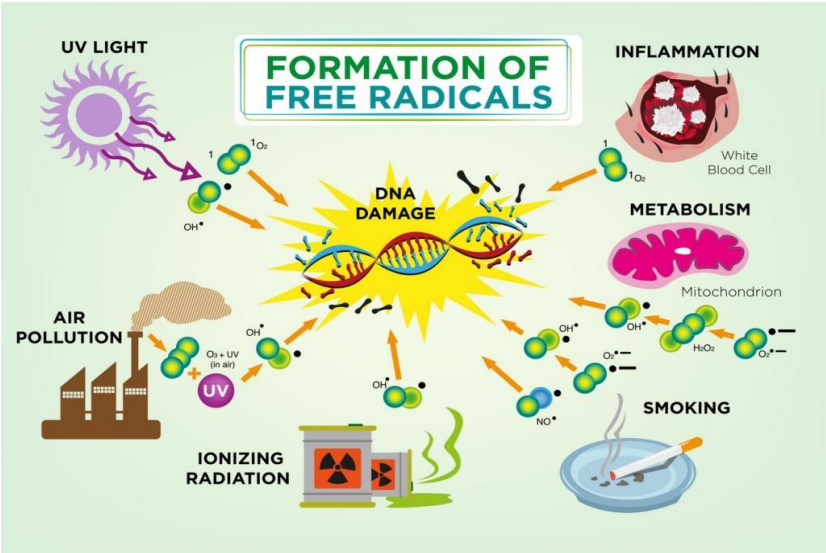


Figure 4 : Some factors causing the formation of free radicals **(Piron, 2024).**

1.3. Oxidative stress

In its ground state, air oxygen (O_2) shows low reactivity towards most biological molecules, but some oxidized forms are significantly more reactive and therefore more harmful **(Rolland, 2004).** The production of free radicals is a normal process of aerobic metabolism in humans. However, an imbalance between the production of these radicals and antioxidant defense mechanisms can lead to oxidative stress **(Goudable and Favier, 1997).**

The underlying sources of oxidative stress result frequently from nutritional factors (such as deficiencies in vitamins and trace elements, or the excess of pro-oxidants, such as iron and fatty acids), although they can also result from accidental events (such as inflammation and the exposure to pro-oxidant xenobiotics), or genetic events. Frequently,

these different factors interact to form the pathogenic mechanism **(Favier, 2003).**

Briefly, oxidative stress is generally described as an imbalance between the systems that promote oxidation and the abilities of the organism, cells or cellular compartments to counteract these processes **(Barouki, 2006).** This imbalance can result from various factors, including activation of ROS production systems. Indeed, under normal conditions, the antioxidant response can compensate the production of ROS, but in certain pathological situations (such as cancer), the production of ROS is exacerbated and prolonged, exceeding thus the antioxidant defense capacity. This prolonged disruption of redox homeostasis may also result from other factors, such as exposure to environmental pro-oxidants or radiation, an intoxication with heavy metal, dietary antioxidant deficiency, or genetic abnormalities (Figure 5) **(Migdal and Serres, 2011)**.

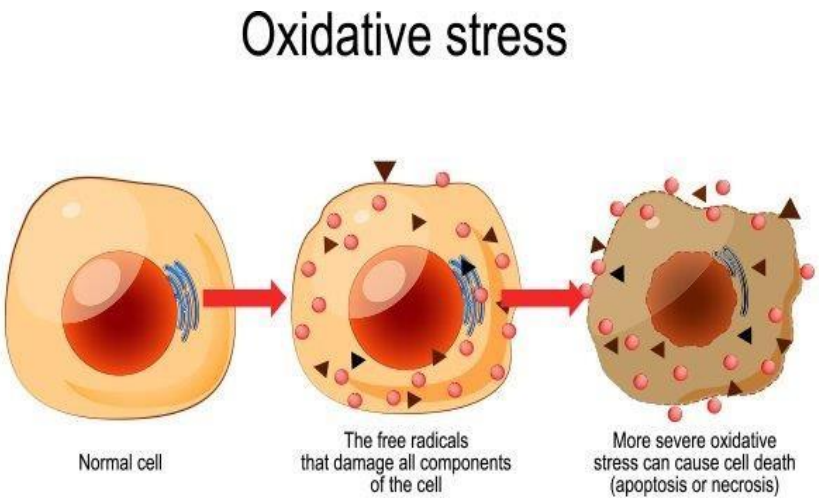


Figure 5 : The impact of oxydative stress on cells **(Nuzzo, 2020).**

1.4. Pathologies related to oxidative stress

Oxidative stress can cause damage to cells and tissues. Lipids and DNA are particularly vulnerable to the action of free radicals **(Goudable et Favier, 1997).** Indeed, oxidative stress plays a crucial role in many diseases, either as a trigger factor or as a complicating factor. The variety of health impacts of this stress is not surprising, as it can target different tissues and cell types. In other words, a varied range of free radicals interacts with different types of cells, inducing thus some pathologies specific to each individual. In addition, most diseases induced by oxidative stress generally manifest with advancing age,

because aging weakens antioxidant defense mechanisms and increases the production of mitochondrial radicals **(Favier, 2003).**

By inducing alterations in certain biological molecules and by regulating the expression of some genes, oxidative stress is considered the main cause of the appearance of certain pathologies, such as cancer, cataract, amyotrophic lateral sclerosis, acute respiratory distress, pulmonary edema and premature aging. For example, some free radicals activate carcinogenic precursors and damage DNA, thereby promoting cell proliferation and the inhibition of the expression of tumor suppressor genes, such as P53 **(Favier, 2003).** Furthermore, oxidative stress is a major contributing factor in the development of diabetes, Alzheimer's disease, rheumatisms, and cardiovascular diseases. For example, in the case of atherosclerotic plaque formation, LDL oxidation plays an important role in converting monocytes into foam cells. Oxidative stress is also involved in insulin resistance, the activation of endothelial cells which release pro-oxidant mediators (such as prostacyclin, cytokines, fibrinolysis factors, superoxide, and NO), as well as in the proliferation of smooth muscle fibers. In addition, some free radicals can induce an increase in homocysteine concentration, constituting thus a cardiovascular risk factor **(Favier, 2003).**

2. Antioxidants

Antioxidants are a category of biologically active substances whose main role is to protect the cells and tissues of a living organism from some damages caused by free radicals. Their action consists of neutralizing free radicals, leading thus to their deactivation **(Mucha et al., 2021).**

2.1. Types

It can be distinguished primary antioxidants and supplementary antioxidants. A primary antioxidant, also called a true antioxidant (AH), reacts with a free radical ($R^{\bullet}$) to form another free radical ($A^{\bullet}$) more stable ($AH + R^{\bullet} \rightarrow A^{\bullet} + RH$). This stability is generally due to the subsequent conversion of the radical $A^{\bullet}$ into a non-radical compound:

$A^{\bullet} + A^{-} \rightarrow AA$ or $A^{\bullet} + R^{*} \rightarrow AR$ **(Rolland, 2004).**

Supplementary antioxidants, also called prophylactics, act by inhibiting the production of free radicals. For example, glutathione peroxidase can break down hydroperoxides into

alcohols, whereas ascorbic acid is an example of an oxygen sequestrant. It acts by capturing free molecular oxygen before it can react to form free radicals **(Rolland, 2004).**

Depending on the mode of action of antioxidants, it can be distinguished enzymatic antioxidants (which act by breaking down free radicals with the help of cofactors such as copper and zinc) and non-enzymatic antioxidants (which interrupt chain reactions leading to the formation of free radicals), while based on their solubility, antioxidants can be divided into water-soluble antioxidants (which are present in cellular fluids, such as vitamin C) and liposoluble antioxidants (which can cross lipid membranes, such as vitamin E) **(Aziz et al., 2019).**

Depending on the size of the antioxidants, it can be differentiated between small-sized antioxidants (which include vitamin C, vitamin E, carotenoids and glutathione) and large-sized antioxidants (such as, the enzymes SOD, CAT et GPx), whereas based on their origin, it can be distinguished natural antioxidants (which include minerals: selenium, copper, iron, zinc, manganese; vitamins: C, E, B; and phytochemicals: flavonoids, catechins, carotenoids) and synthetic antioxidants (such as butylated hydroxylanisole (BHA) and butylated hydroxytoluene (BHT) **(Aziz et al., 2019).**

2.2. Mode of action

Some antioxidants, such as vitamins E, C, Q, and carotenoids found in foods, act by capturing free radicals and by neutralizing their single electron, thereby transforming them into stable molecules or ions. Once a vitamin has trapped a radical, it becomes itself a radical, and can either be eliminated or regenerated by another system **(Favier, 2003).** Another antioxidant strategy relies on enzymatic processes designed to remove superoxide anion and hydrogen peroxide. For example, superoxide dismutase is able to neutralize superoxide anions by catalyzing a dismutation reaction in which two superoxide anions combine to form oxygen and hydrogen peroxide molecules. Superoxide dismutases exist in different forms, but their overall structure is remarkably conserved through evolution, forming a hydrophobic site at the center of the protein that hosts the superoxide anion. However, a metal located at the heart of the enzyme makes it possible to distinguish between manganese superoxide dismutase (which protects the mitochondria) and copper-zinc superoxide dismutase (which protects the cytoplasm and the cell membrane) **(Favier, 2003).** It should also be noted that the role of superoxide dismutases and peroxidases is complementary, because an effective protection against free radicals requires the coordinated action of these enzymes **(Favier, 2003).** Indeed,

there is a synergistic action between some antioxidants **(Aziz et al., 2019).**

Briefly, antioxidants act primarily via two modes. The first involves an interruption of the chain reaction inducing the formation of free radicals, where the primary antioxidant donates an electron to the free radical. The second mechanism involves removing the initiators of ROS and reactive nitrogen species, where the secondary antioxidant neutralizes the chain reaction catalyst. To summarize, antioxidants can exert their action on biological systems by various means, notably by donating electrons, by binding with metal ions, by acting as co-antioxidants or by regulating gene expression **(Lobo et al., 2010).**

III. Generalities on phenolic compounds

Polyphenols, also known as phenolic compounds, are found as secondary metabolites in various parts of higher plants, including roots, stems, leaves, flowers, pollen, fruits, seeds, and wood. Their distinctive characteristic is the presence of an aromatic ring containing free hydroxyl groups (Figure 6) or linked to a carbohydrate **(Boizot and Charpentier, 2006).**

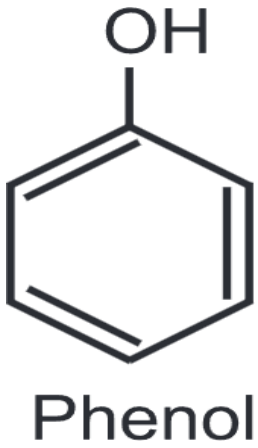


Figure 6 : Chemical structure of a phenol group **(Madani, 2017).**

1. Types

Phenolic compounds, which belong to a broad group of organic compounds, can be classified based on their carbon skeleton or the pathway by which they are synthesized to form the benzene or the aromatic ring **(Elie, 2022).** The Table IV shows the basic skeleton and the types of phenolic compounds.

Table IV : Basic skeleton and the types of phenolic compounds **(Elie, 2022; Caballero et al., 2015).**

The carbon skeleton	Aromatic rings, composed of 6 carbon atoms, are designated by C_6. The structures attached to the aromatic ring are represented by C_p, where p indicates the number of carbons in these structures. If these structures are also linked to another C_6 aromatic ring, then the notation is extended to $(C_6\text{-}C_p\text{-}C_6)_n$, where n represents the number of repeats of this structure.
Simple phenolic compounds	There is a lack of simple phenolic compounds in their natural form, including phenol, catechol, and hydroxyquinone or hydroquinone. These compounds, classified among simple phenols (C_6), are not common.
Phenolic acids	Phenolic acids can be either derivatives of hydroxylated benzoic acid (C_6H_5COOH), designated by $C_6\text{-}C_1$ and forming the category of hydroxybenzoic acids, or derivatives of cinnamic acid ($C_6H_5\text{-}CH=CHCOOH$), designated by $C_6\text{-}C_3$ and comprising the group of hydroxycinnamic acids. These compounds are widespread throughout the plant kingdom. Examples: hydroxybenzoic acids, including para-hydroxybenzoic acid, salicylic acid, protocatechuic acid, gallic acid and vanillic acid, are a group of phenolic acids. Another group of phenolic acids are the hydroxycinnamic acids, such as caffeic acid, chlorogenic acid, and paracoumaric acid. These hydroxycinnamic acids have the ability to produce coumarins, which are phenols derived from hydroxylated lactones (coumarin itself is considered a lactone).
Polyphenols	Polyphenols can be divided into several categories, including naphthoquinones, stilbenoids, flavonoids, lignans, lignins, and tannins. ✓ Naphthoquinones, derived from naphthoquinone ($C_{10}H_6O_2$), are quinones derived from naphthalene. Juglone or juglandin are examples. ✓ Stilbenoids are hydroxylated derivatives of stilbene ($C_{14}H_{12}$), which is an aromatic hydrocarbon. Transresveratrol is an example.

	✓ The reducing group of flavonoids that are phenol glycosides is linked either to the oxygen (O) resulting from one of the hydroxyl functions (-OH), or to the aglycone by a carbon (C). Kaempferol, isoflavonoids such as daidzein, and anthocyanins or anthocyanosides are examples of flavonoids. ✓ Lignans $(C_6\text{-}C_3)_2$ and lignins $(C_6\text{-}C_3)_n$, $n > 2$. ✓ Condensed tannins are polyphenols capable of precipitating proteins, polysaccharides and alkaloids. Procyanidin is an example. ✓ In addition to condensed tannins, tannins also include the group of hydrolyzable tannins, which includes ellagitannins and gallotannins.

2. Biosynthesis

According to Elie (2022), phenolic compounds can be synthesized either by the pathway that uses shikimic acid or by the pathway that uses acetic acid. However, some phenolic compounds are synthesized by a mixed pathway.

2.1. Shikimate pathway (or of shikimic acid)

In plants, phenolic compounds biosynthesis by the shikimate pathway (or of shikimic acid) is a complex process that has evolved over time. It involves a series of reactions that produce the precursors of three aromatic amino acids: phenylalanine, tyrosine, and tryptophan. Subsequently, these amino acids undergo transformations, notably by deamination, to give rise to phenolic compounds such as cinnamic acid and coumaric acid, which are then converted into other phenolic compounds by hydroxylation reactions catalyzed by enzymes **(Elie, 2022).**

2.2. Acetic acid pathway (or polyacetate pathway)

This pathway occurs when compounds containing an aromatic ring previously synthesized by the shikimate pathway are involved. It allows the synthesis of compounds from the polyketide class, which include some phenols. Polyketides are a very diverse group of secondary metabolites, present in microorganisms, fungi, plants (such as plant pigments)

and some marine animals. Their complex biosynthesis, similar to that of fatty acids, involves specific enzymes. Due to their structural diversity and numerous applications, polyketides are attracting great interest in the fields of biology, medicine (as antibiotics), agronomy and industry **(Elie, 2022).**

2.3. Mixed pathway

If a polyphenol is produced by a polyacetate pathway that is followed by a shikimate pathway, then the pathway is called mixed pathway, but if the aromatic core was formed by another pathway than shikimate, then it is not a mixed pathway **(Elie, 2022)**.

3. Roles

Anthocyanins, flavonoids, and tannins are among the most common phenolic compounds in plants. These compounds play essential roles in many physiological processes, such as cell growth, rhizogenesis, seed germination, and fruit ripening **(Boizot and Charpentier, 2006).** Indeed, phenolic compounds play important roles in plant life and in humans' use of plants. They can intervene:

- in some processes, such as lignification and growth regulation;
- in the interactions between plants and their biological and physical environment. For example, in molecular interactions with some symbiotic or parasitic microorganisms. These interactions occur either in nature or after collecting some plants;
- in the quality criteria which influence man's choices for his consumption of plant organs and the products derived from them by transformation;
- in the variations of certain characteristics of plants during transformation processes. For example, during the preparation of some fruit juices, because enzymatic browning may appear, leading thus to a change in the quality of the finished product;
- in strengthening antioxidant defense, contributing thus to the protection of human health **(Macheix et al., 2005).**

4. Biological activities

The polyphenols found in our food play a crucial role in our health. They help protect us from oxidative stress and degenerative diseases **(Han et al., 2007).** In fact, flavonoids are associated with many brain health benefits. They can halve the risk of dementia, preserve

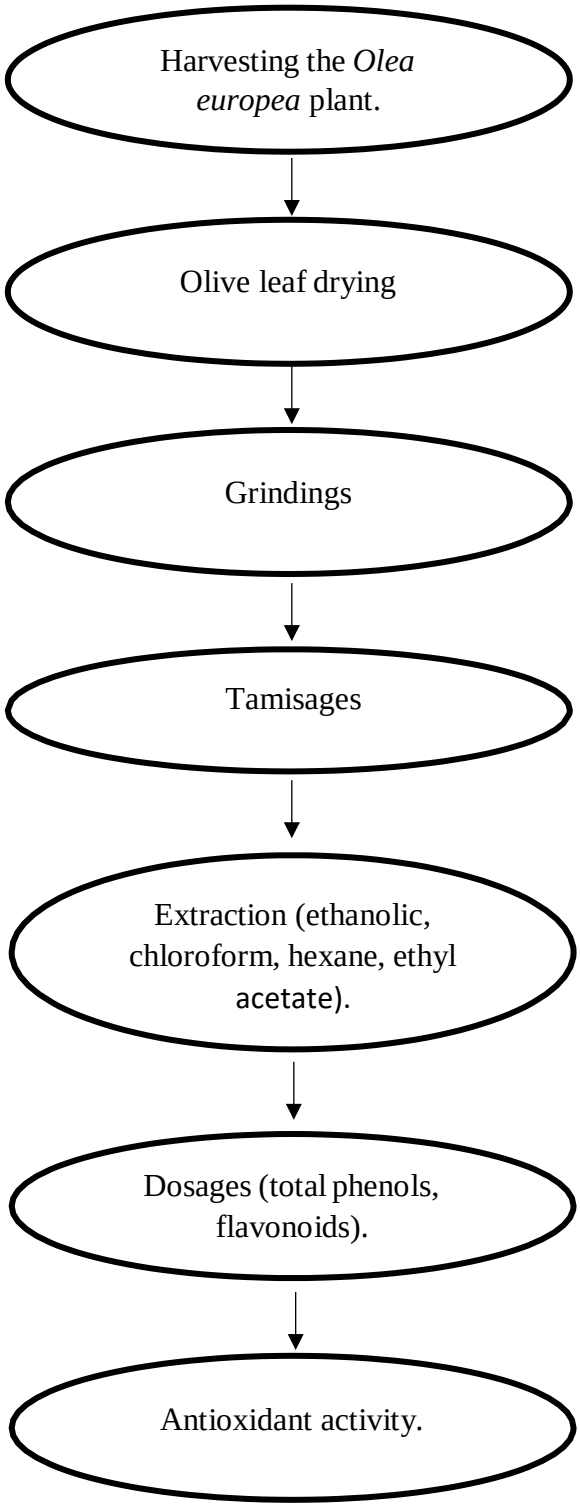
cognitive functions with age, delay the onset of Alzheimer's disease, and reduce the risk of developing Parkinson's disease **(Vauzour et al., 2010).** Furthermore, as antioxidant agents and by strengthening antioxidant defenses, polyphenols may have good effects on the vascular system **(Iqbal et al., 2023; Vauzour et al., 2010).** In addition, polyphenols have demonstrated anticancer properties thanks to their multiple mechanisms of action. They can eliminate carcinogens, modulate cancer cell signaling, influence the cell cycle, promote apoptosis and regulate enzyme activities **(Vauzour et al., 2010).** Moreover, polyphenols also have anti-inflammatory properties **(Han et al., 2007).**

Experimental part

Chapter 2
Materials and methods

Objective

The work was carried out in the biochemistry laboratory of the Faculty of Natural and Life Sciences and Earth Sciences. The general objective of this work is to determine the total polyphenol and flavonoid content of crushed dried leaves of *Olea europea* L. Then, to measure the antioxidant power of the phenolic compounds contained in the extract of the studied plant.



I. Preparation of plant materials

1. Origin of the plant

Olive leaves of the subspecies *Olea europaea var. europaea*, were harvested in February 2024 in the Sidi-Aïch region of the province of Béjaia.

2. Olive leaves drying

After harvesting, the leaves are rinsed twice with water to remove any dust, then placed on absorbent paper to absorb the water. They are then dried in the open air, away from light and humidity.

Figure 7 : Preparing olive leaves for drying.

3. Grinding

The harvested leaves are processed in an electric grinder to reduce them to fine particles.

Figure 8 : Crushing olive leaves.

4. Sieving

After crushing the olive leaves, a sieving stage is carried out using a 200mm × 50mm sieve to separate the finest particles from the largest. The powder is then stored in a glass bottle, protected from light.

Figure 9 : Olive leaves powder after grinding and sieving.

II. Preparation of plant extracts

1. Extraction of phenolic compound

This method uses maceration, a very simple extraction technique, following the method used by Atmani et al. (2009), with some modification. It involves contacting plant material with a solvent, possibly stirred, to extract specific chemical species.

1.1. Preparation of the crude ethanolic extract

- ✓ The sample powder, weighing 50 grams, was mixed with 500 milliliters of ethanol (96%) in a beaker and stirred for 24 hours using a magnetic bar on a stirring plate, all at room temperature ;
- ✓ The extracts obtained were placed in a test tube for separation for 24 hours ;
- ✓ After separation, the ethanolic extract is recovered and placed in glass petri dishes (the dishes are weighed empty before filling and after drying) and placed in the oven at 37°C for drying until a constant weight is reached ;

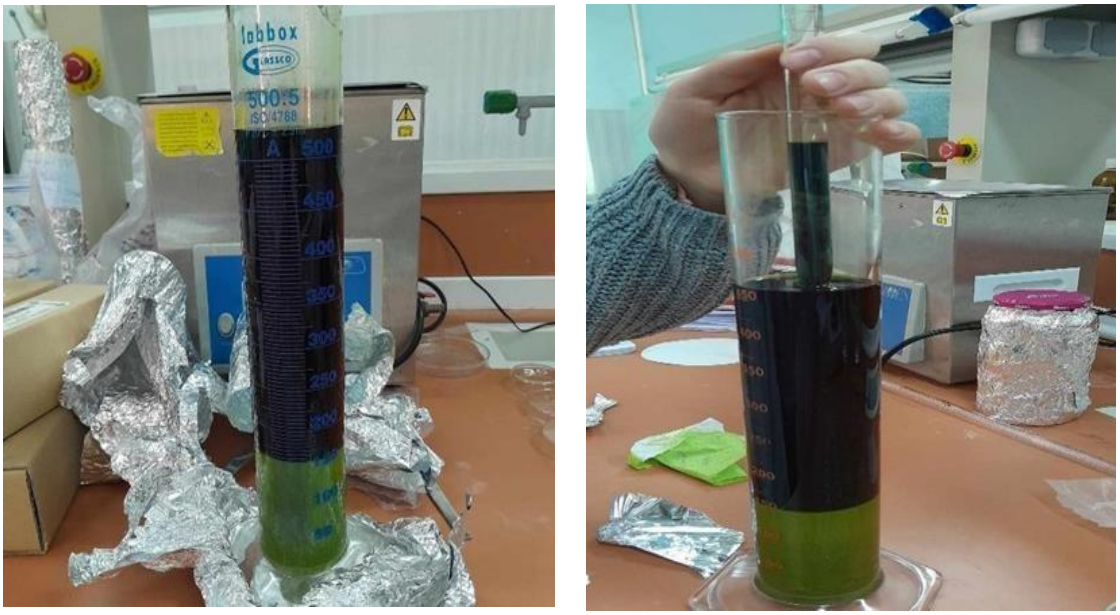


Figure 10 : Separation and recovery of the extract.

- ✓ After drying, the powder obtained by scraping the petri dishes is placed in a glass bottle and stored in the refrigerator at 4°C, protected from light.

Figure 11 : The crude ethanolic extract after drying and scraping.

1.2. Preparation of chloroform, ethyl acetate and hexane extracts

- ✓ Chloroform, ethyl acetate and hexane extracts are prepared from crude ethanolic extract ;
- ✓ Quantity of ethanolic extract (5g) mix with 15ml of chloroform and 5ml of distilled water (1:3:1, w/v/v) and stir in a beaker for 15 minutes using a glass spatula, all in the laboratory ;

Figure 12 : Preparation of chloroform extract.

- ✓ After stirring, the extract remains inside the hood and is sealed with parafilm until separation ;
- ✓ After separation, two phases are obtained : one organic and one aqueous. These phases are then separated and placed in Petri dishes in the fume hood to dry ;
- ✓ Once dried, the powder obtained by scraping the Petri dishes is placed in a glass vial and stored in the refrigerator at 4°C, protected from light.

NB : the same steps apply to the preparation of ethyl acetate and hexane extracts.

1.3. Calculation of yield

The extraction yield (*R*) of each extract can be calculated using the following formula :

(Mahmoudi et al., 2013).

$$R\ (\%) = 100\ (X / Y).$$

- R : is the yield in % ;
- X : weight after solvent evaporation - empty crucible weight ;
- Y : weight of starting powder or extract.

III. Phenolic compounds assay

1. Determination of total phenols

1.1. Principle

The principle of total phenol measurement is based on the reducing power of phosphomolybdic acid ($H_3PMo_{12}O_{40}$) and phosphotungstic acid ($H_3PW_{12}O_{40}$) (yellow Folin-Ciocalteu reagent) through polymeric ionic complexes of phenolic compounds. This results in the formation of a blue complex, which is accompanied by the oxidation of phenolic compounds and stabilized by the addition of sodium carbonate ($NaCO_3$) **(Dif et al., 2015).**

Total phenols were determined by comparing the observed absorbance with the absorbance obtained from gallic acid standards of known concentrations **(Dif et al., 2015).**

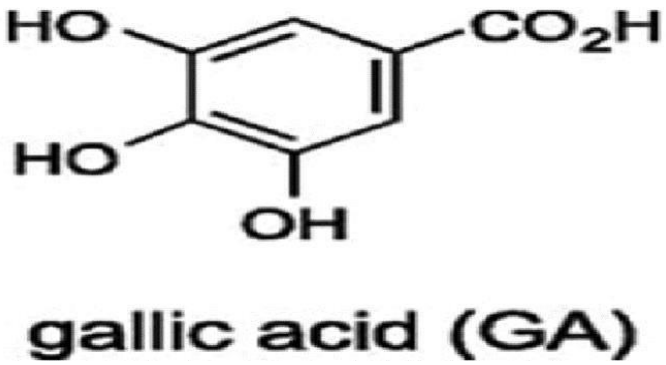


Figure 13 : Chemical structure of gallic acid **(Sourani et al., 2016).**

1.2. Experimental protocol

Total phenol content was quantified using spectrophotometry at 765 nm, following the protocol detailed by Hismath et al. (2011), with some modification.

a. Preparation of solutions

- ✓ Folin ciocalteu reagent solution : 3ml Folin reagent diluted in 27ml distilled water ;
- ✓ Sodium carbonate solution (Na2Co3) : 7.5g sodium carbonate mixed with 100ml distilled water.

b. Preparation of extract solution

1mg extract (ethanolic, chloroform, hexane or ethyl acetate) mixed with 1ml distilled water.

Figure 14 : Prepared ethanolic extract solution.

NB :

- A dilution of 0.5mg extract in 1ml methanol for hexane and chloroform extracts in aqueous phase.

c. Preparation of test solutions

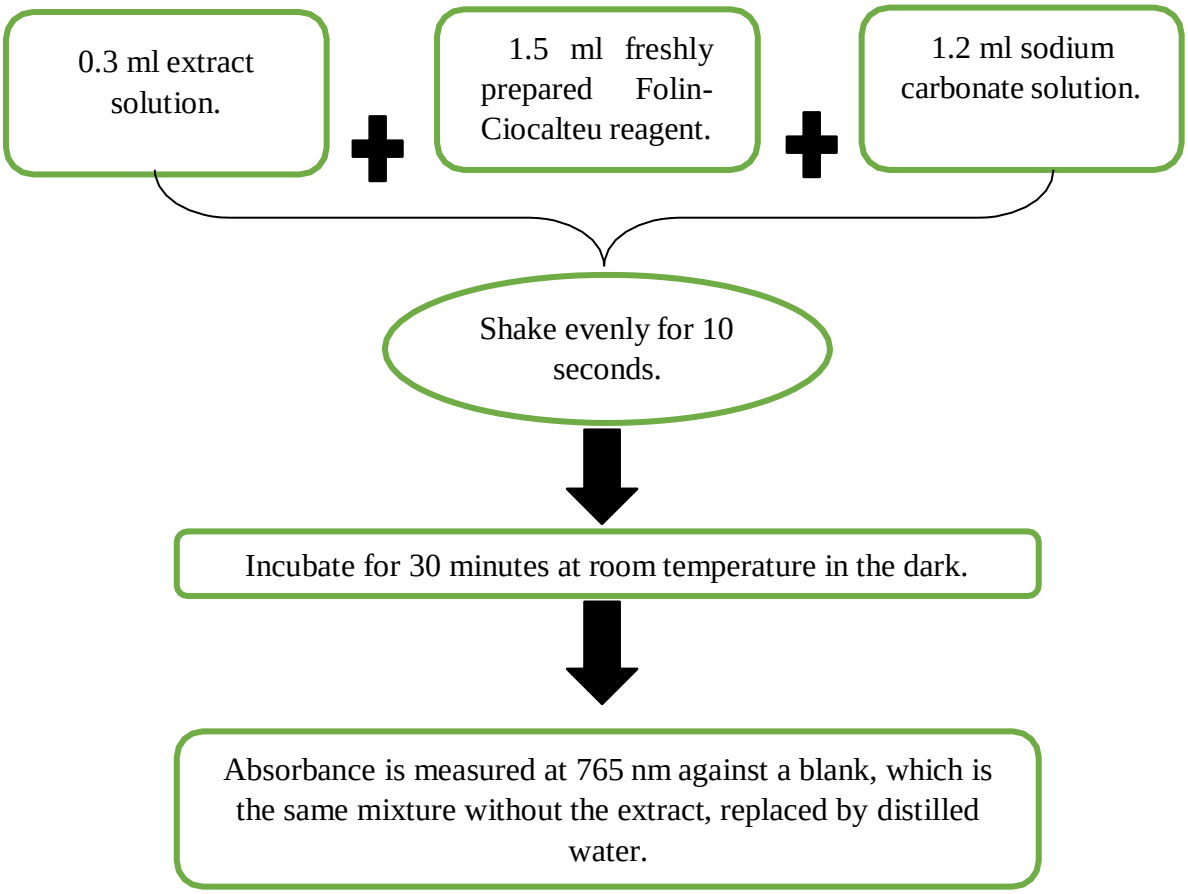


Figure 15 : Diagram showing the process used to measure total phenols **(Hismath et al., 2011).**

NB :

- The same steps are followed for chloroform, ethyl acetate and hexane extracts, and for both the organic and aqueous phases of each extract.

2. Determination of flavonoids

2.1. Principle

Flavonoids were quantified using a method based on the formation of an extremely stable complex between aluminum chloride and the oxygen atoms present on carbons 4 and 5 of flavonoids **(Ali-Rachedi, .2018).**

2.2. Experimental protocol

Flavonoid content was quantified using spectrophotometry at 430 nm, following the protocol detailed by Bouba et al. (2010), with some modification.

a. Preparation of aluminum chloride solution AlCl3

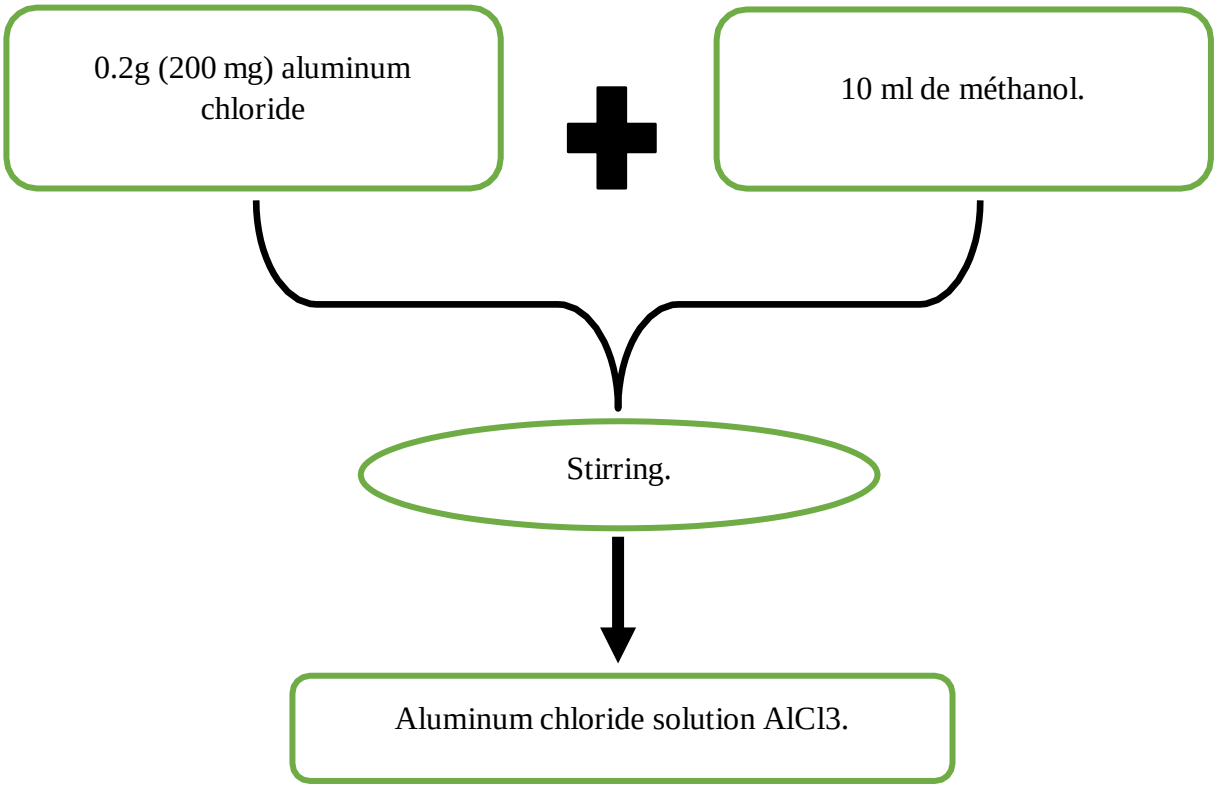


Figure 16 : Preparation of aluminium chloride solution (ALCL3) **(Bouba et al., 2010).**

b. Preparation of extract solution

Combine 10 ml methanol with 2.5 mg ethanolic extract in a flask, and shake until a homogeneous solution is obtained.

c. Preparing the test solution

.

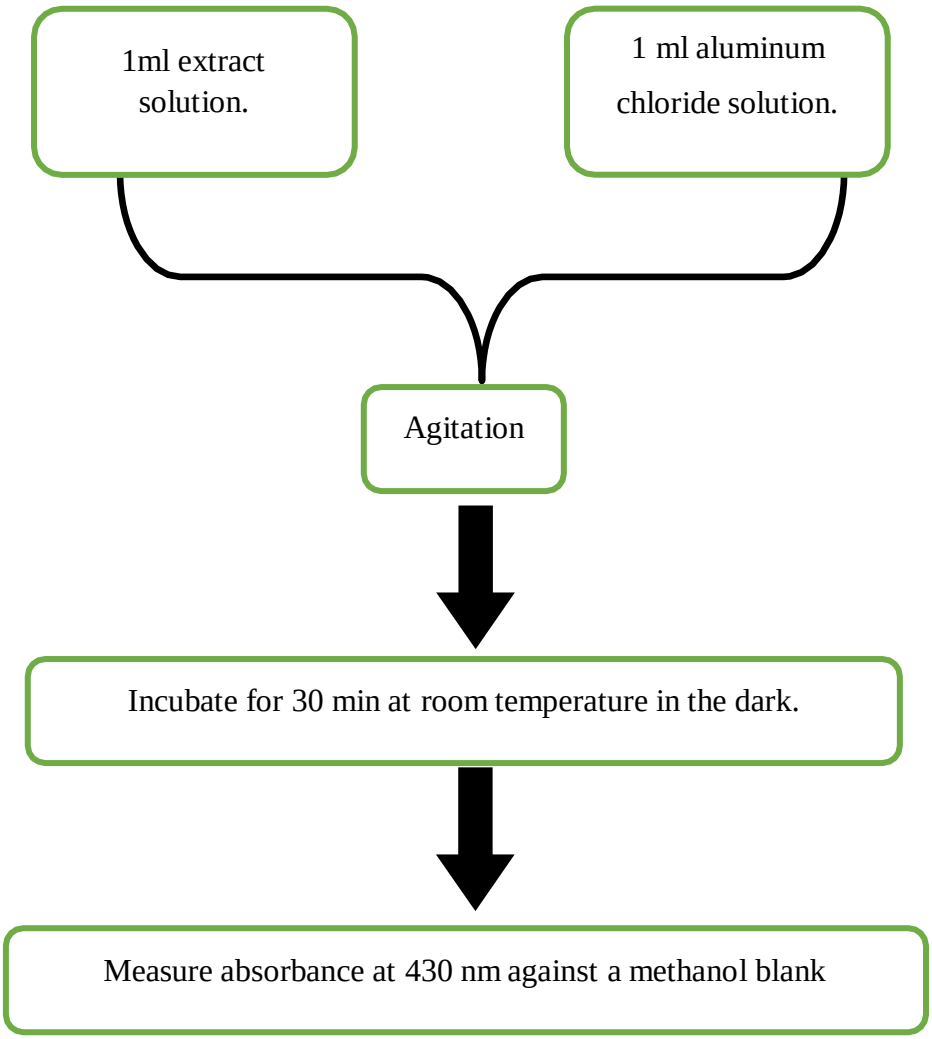


Figure 17 : Diagram illustrating the steps involved in flavonoid determination **(Bouba et al., 2010).**

NB :

- Perform the same steps for each type of extract: organic and aqueous chloroform, organic and aqueous hexane, and organic and aqueous ethyl acetate.
- After incubation, observe a yellow color in the samples, while the blanks show no color change.

Figure 18 : Test solution of aqueous phase chloroform extract after incubation.

IV. Antioxidant activity

1. DPPH radical scavenging

1.1. Principle

The DPPH (2,2-Diphenyl- picrylhydrazine) antioxidant measurement method is based on a substance's ability to reduce the DPPH• radical. This reduction causes the color of the solution to change from violet to yellow in the presence of an anti-free radical compound. The reaction is assessed by measuring the absorbance of the solution spectrophotometrically at a wavelength of 517 nm. This change in color from violet to yellow is proportional to the antioxidant power **(Habibou et al., 2019).**

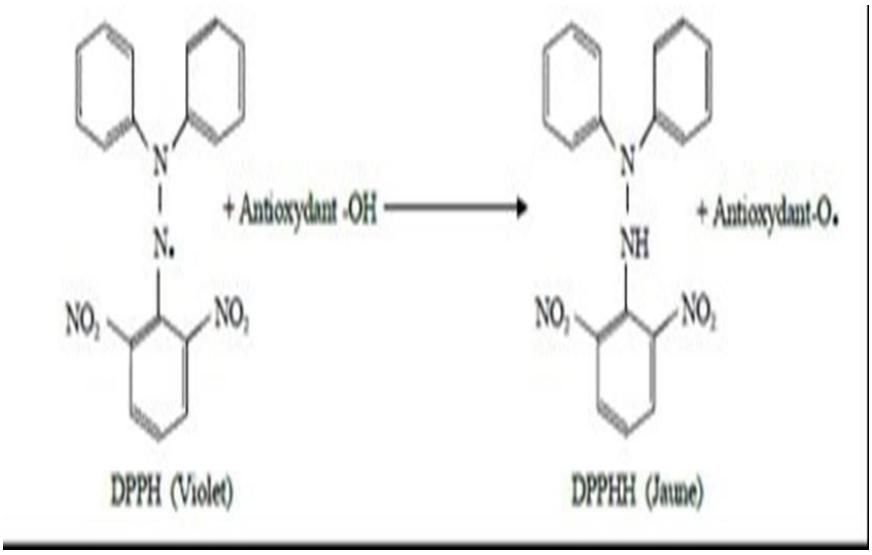


Figure 19 : DPPH• radical reduction reaction **(Habibou et al., 2019).**

1.2. Experimental protocol

The DPPH$^{\bullet}$ radical trapping test was carried out using the method described by Hemalatha et al. (2010), with a few modifications.

a. Preparation of the DPPH solution

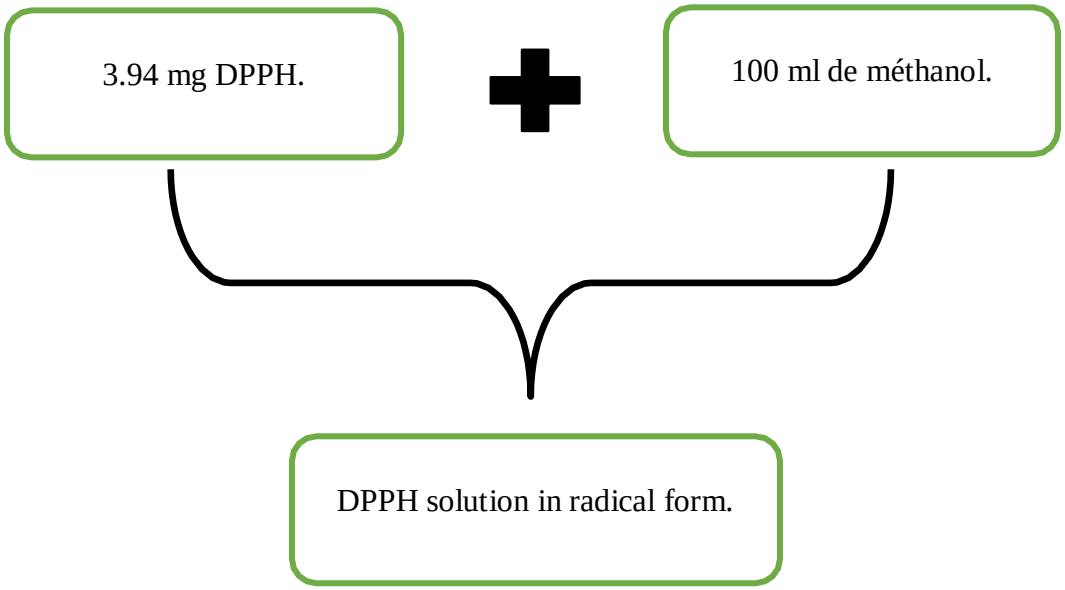


Figure 20 : Preparation of a DPPH solution **(Hemalatha et al., 2010).**

b. Preparation of general blank (control)

In three clean tubes, add 1 ml DPPH solution to 3 ml methanol. Shake each tube for 10 seconds, then incubate for 30 minutes. After this, measure absorbance at a wavelength of 517 nm. (Keep these absorbances for the whole experiment).

Figure 21 : General DPPH blank.

c. Preparation of extract solution

Combine 2 mg ethanolic extract with 20 ml methanol in a glass vial.

d. Preparation of extract blanks

For each extract, prepare an extract blank. For example, for ethanolic extract, use three tubes for the blanks. In each tube, add 3 ml ethanolic extract with 1 ml methanol.

e. Preparation of test solutions

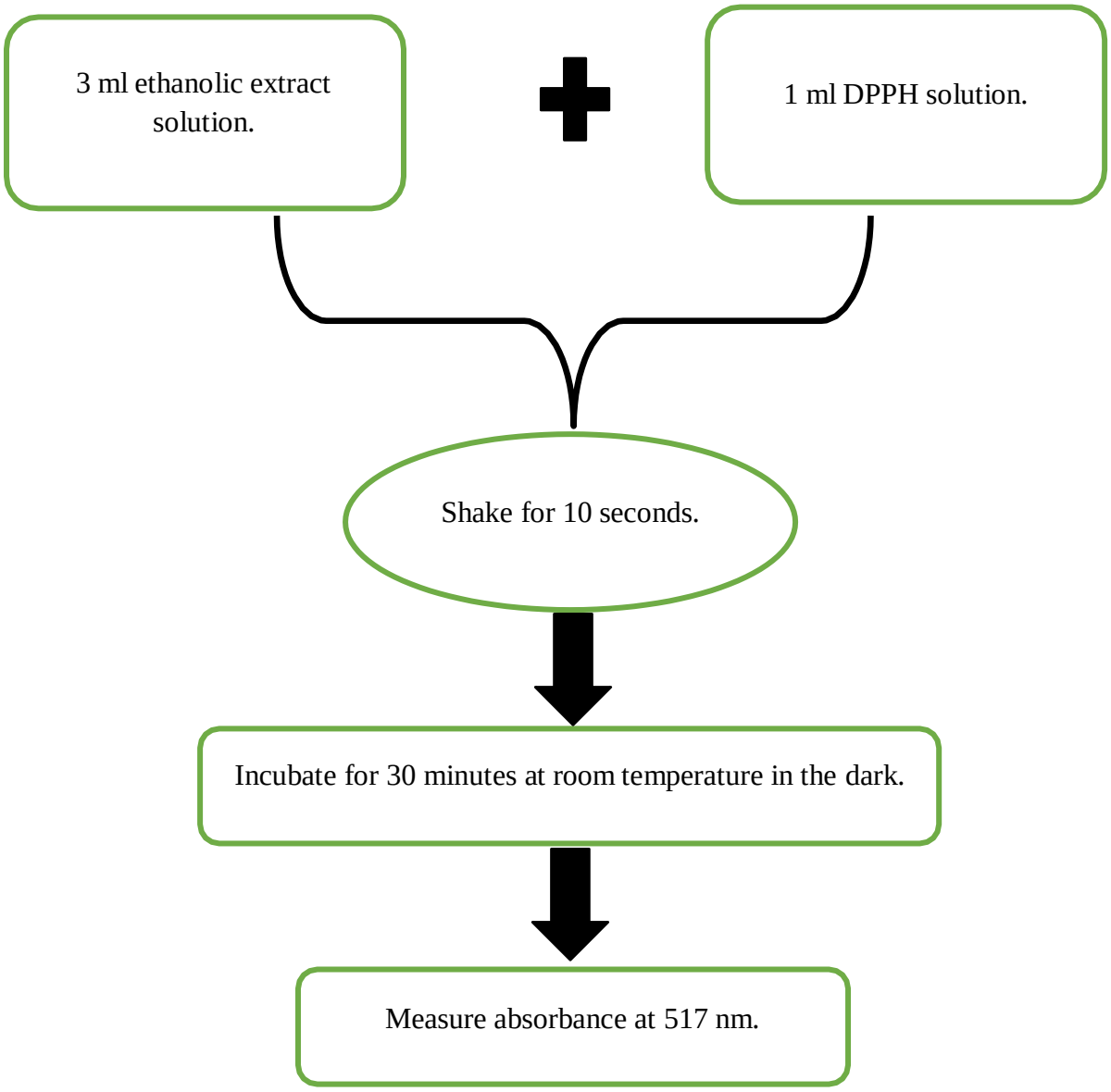


Figure 22 : Schematic diagram describing the steps involved in the DPPH$^{\bullet}$ radical capture assay **(Hemalatha et al., 2010).**

NB :

- If the DPPH solution does not show a violet color but takes on a yellow hue, this indicates a reduction reaction.

1.3. Expression of results

Antioxidant activity is measured by the percentage inhibition (%I) of absorbance at 517 nm :

$$\%I = [(Ac - At) / Ac] \times 100$$

Ac : Absorbance of the general blank (DPPH solution + methanol) ;

At : Absorbance of test solution (DPPH solution + extract solution) ;

2. Trapping the $ABTS^{\bullet+}$ radical

2.1. Principle

The principle of $ABTS^{\bullet+}$ radical scavenging is based on the ability of a substance to neutralize the $ABTS^{\bullet+}$ radical. This radical is generated by the removal of an electron from a nitrogen atom in the ABTS molecule. When an antioxidant is introduced into the solution, it reacts with the $ABTS^{\bullet+}$ radical by supplying it with hydrogen, thus forming ABTS+, which results in discoloration of the solution **(Marc et al., 2004).**

2.2. Experimental protocol

The $ABTS^{\bullet+}$ radical trapping test was carried out following the method described by Re et al. (1999), with a few modifications.

a. Preparation of the ABTS stock solution

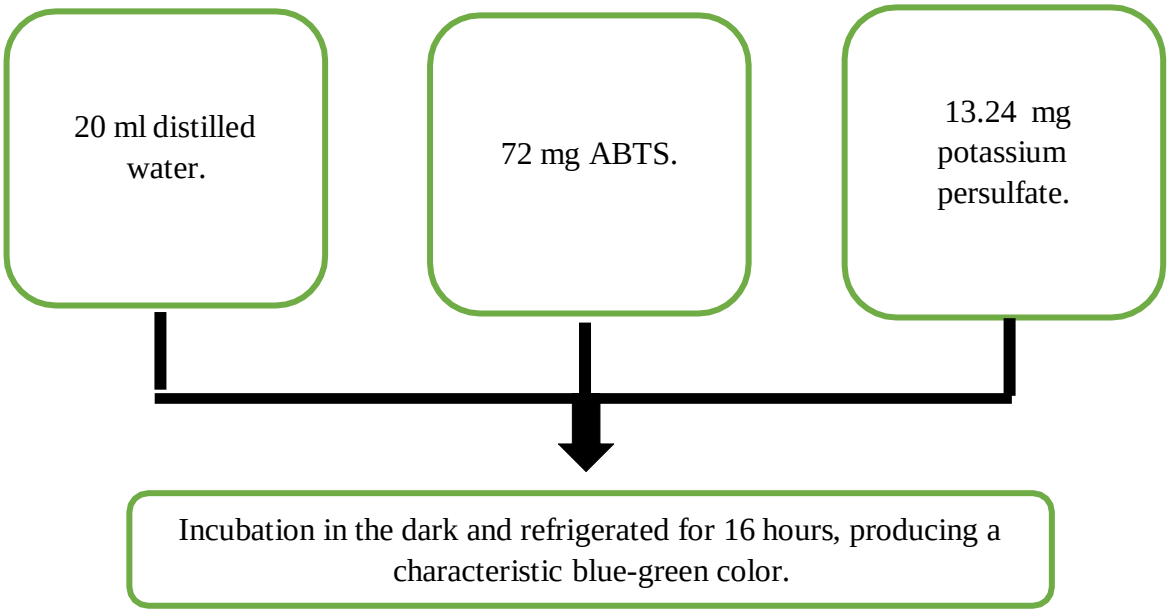


Figure 23 : Preparation of ABTS solution **(Re et al., 1999).**

NB :

- The ABTS solution obtained was diluted with distilled water to an absorbance of 0.720 ± 0.680 at a wavelength of 734 nm before use.

b. Preparation of general blank (control)

A volume of 0.6 ml of methanol was mixed with 1.9 ml of diluted ABTS solution (3 tubes = 3 measurements). Incubation for 7 minutes at room temperature in the dark. Finally, absorbance was measured at 765 nm against a methanol blank.

Figure 24 : General ABTS blank.

c. Preparation of extract solutions

1 mg ethanolic extract (from chloroform, hexane or ethyl acetate) was added to 10 ml methanol and stirred vigorously until the solution was homogeneous.

d. Preparation of test solutions

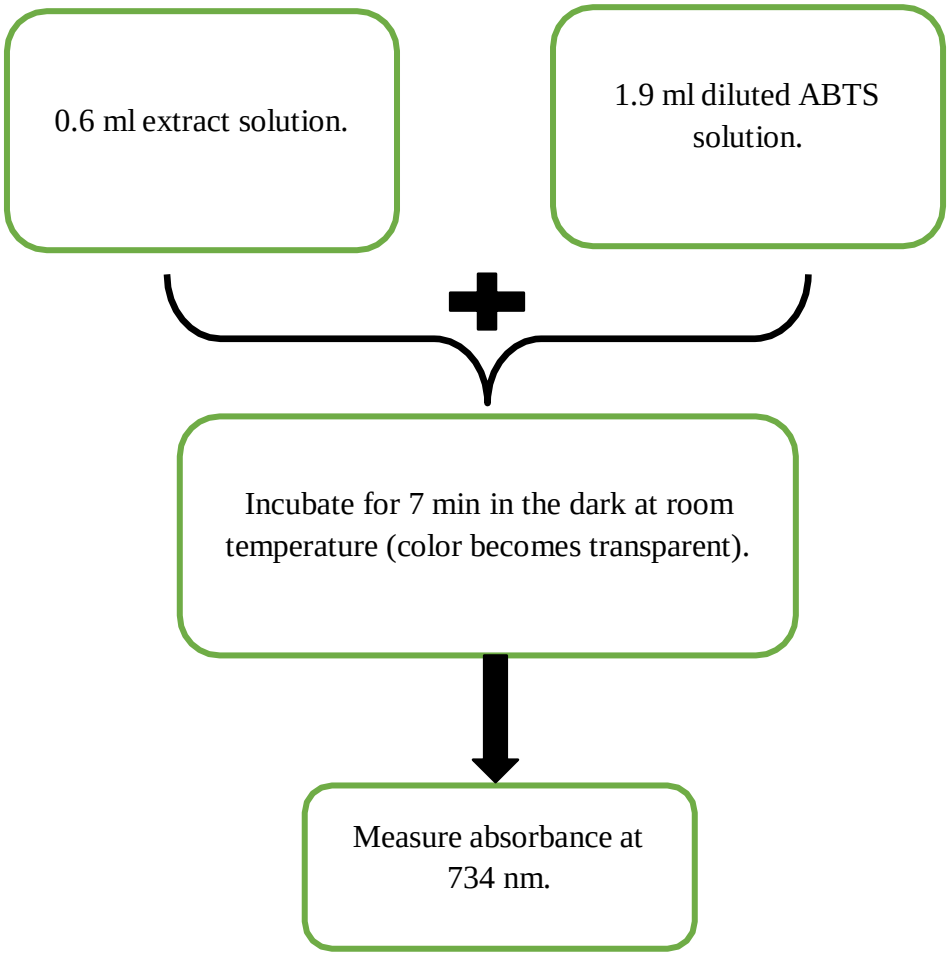


Figure 25 : Schematic diagram of the process for measuring $ABTS^{\bullet+}$ radical scavenging capacity **(Re et al., 1999).**

2.3. Expression of results

Antioxidant activity is then assessed by measuring the percentage inhibition (%I) of absorbance at 734 nm where the $ABTS^{\bullet+}$ radical shows a characteristic absorption band. The %I of the absorbance corresponds to : **(Sarr et al., 2015).**

$$(\%I) = (A0-A1/A0)100$$

A0 : absorbance of general blank ;

A1 : absorbance of solution tested.

V. Statistical analysis :

For each sample studied, three measurements were taken. Results are presented as mean ± standard deviation. Statistical comparisons were made using STATISTICA 5.5 software, by analysis of variance (ANOVA/MANOVA). Differences were considered significant at $p < 0.05$.

Chapter 3
Results and discussion

I. Extraction efficiency

The extraction of phenolic compounds plays a crucial role in the valorisation of these active substances, and its efficiency depends on both the method used and the choice of solvent, which is essential for preserving their biological properties **(Mahmoudi et al., 2013).**

The results of the extraction rate calculation for each extract are shown in Table V.

Table V : Extraction percentages for each *Olea europea* extract.

The extracts	Extraction yields
Crude ethanolic extract (C E E)	42,44%
aqueous chloroform extract (A C E)	12,4 %
organic chloroform extract (O C E)	12 %
aqueous hexane extract (A H E)	12,8 %
organic hexane extract (O H E)	13,6 %
aqueous ethyl acetate extract (A E A E)	7%
organic ethyl acetate extract (O E A E)	12,4%

The extraction yields of the different solvents used in the study, shown in table V The results show that the yields vary depending on the solvent used **(Mahmoudi et al., 2013).**

The results show that ethanolic extract was found to be the most efficient extraction solvent, with a yield of 42.44%. On the other hand, the yields of extracts obtained with hexane (in the organic and aqueous phases), chloroform (in the organic and aqueous phases), as well as ethyl acetate in the aqueous phase, were comparable, all around 13.6% to 12%. However, the lowest yield was observed for ethyl acetate in the organic phase, with an extraction yield of only 7%.

From our results compared with those of the study by Addab et al (2020), we found that the extraction efficiency of the ethanolic extract presented in the previous table was higher than that reported in their study (25.28% from the Biskra region, 27.91% <Skikda>, 30.60% <Mila> and 35.89% <Batna>).

The results show that ethanolic extract proved to be the most efficient extraction solvent, closely followed by hexane (in organic and aqueous phase), chloroform (in organic and aqueous

phase) and ethyl acetate in aqueous phase, while ethyl acetate in organic phase is given the significantly lower extraction yield. This observation can be explained by the fact that some compounds are more soluble in polar solvents such as ethanol, while others can be better extracted by apolar solvents such as chloroform. Specific interactions between solvents and olive leaf compounds and solvent selectivity can also influence extraction yields. In summary, variations in extraction yields between solvents can be attributed to solvent polarity, as polar molecules will be extracted by polar solvents, whereas non-polar molecules will be extracted by non-polar solvents **(Das and Chandra, 2012).**

In short, the very high level of ethanol extract can be explained by the richness of olive leaves in polar compounds.

II. Determination of phenolic compounds

1. Determination of total phenols

Appendix 1 shows the standard curve for gallic acid obtained by spectrophotometric reading. The formula for the linear regression obtained is Y= 0.009x+0.002 and R2= 0.997.

The total phenol content was determined using a gallic acid calibration curve. The total phenol content recorded in this study expressed in mg gallic acid equivalent/g extract (mg GAE/g extract) is :

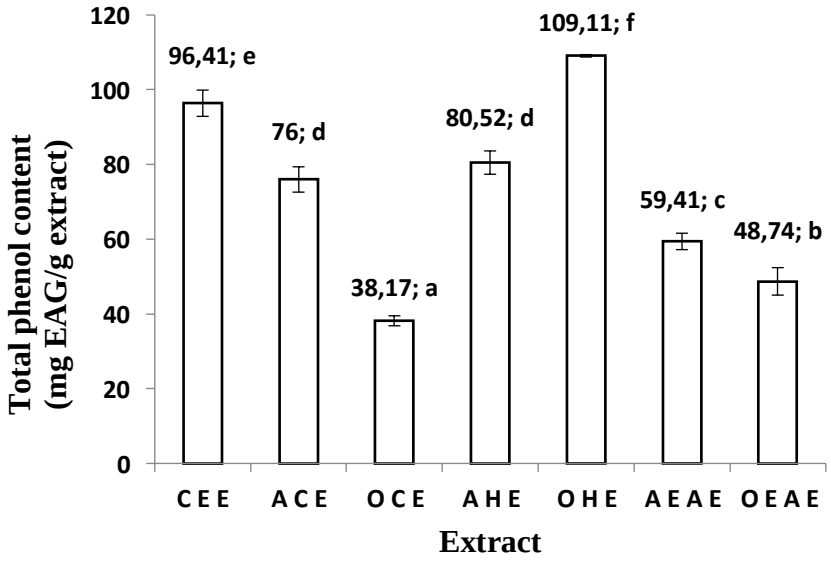


Figure 26 : Graphical representation of total phenol content as a function of olive leaves extracts.

With : C E E : Crude ethanolic extract ; A C E : Aqueous chloroform extract ; O C E : Organic chloroform extract ; A H E : Aqueous hexane extract ; O H E : Organic hexane extract ; A E A E : Aqueous ethyl acetate extract and O E A E : Organic ethyl acetate extract. Finally, using

STATISTICA 5.5 software (ANOVA/MANOVA), statistical comparisons were carried out, such that letters a, b, c, d, e and f indicate significant differences in total phenol content between extracts.

The hexane extract in the organic phase (O H E) stood out with a content of (109.11 ±0,31mg EAG/g extract), the highest of all, explaining that the extract is rich in compounds of reducedpolarity. It is closely followed by the ethanolic extract with (96.41±3,51mg EAG/g extract), the hexane aqueous phase with (80.52±3,12mg EAG/g extract) and the chloroform aqueous extract with (76±3,39mg EAG/g extract). This observation can be attributed to the high polarity of the total phenols, due to their high presence of hydroxyl groups, favouring their solubility in polar solvents.

The other solvents appear to have lower levels of total phenols, with significant variations between them. For example, ethyl acetate, in both its aqueous and organic phases, showed intermediate concentrations of total phenols, with values of (59.41±2,19 mg EAG/g extract) and (48.74±3,67mg EAG/g) extract respectively. In contrast, the organic chloroform extract recorded the lowest content, with only (38.17±1, 34 mg GAE/g extract). This observation could be explained by the presence of numerous phytochemical compounds in this extract, resulting in steric hindrance that could hinder the revelation of total phenols by the Folin Ciocalteu reagent **(Garcia-Alonso et al., 2004).**

A total phenol content of 115.35 ± 2.24 mg EAG/g extract was found by Lakache et al (2021), which is close to that of the organic hexane extract of our plant (109.11 ±0.31 mg EAG/g extract).

2. Determination of flavonoids

Quercetin was used as a positive reference to establish the calibration curve and calculate the flavonoid content of our extract. The results are expressed as mg quercetin equivalent/g extract (mg QE/g extract) (figure 27).

Flavonoid content was determined using a quercetin calibration curve (Appendix 2). The flavonoid content recorded in this study is :

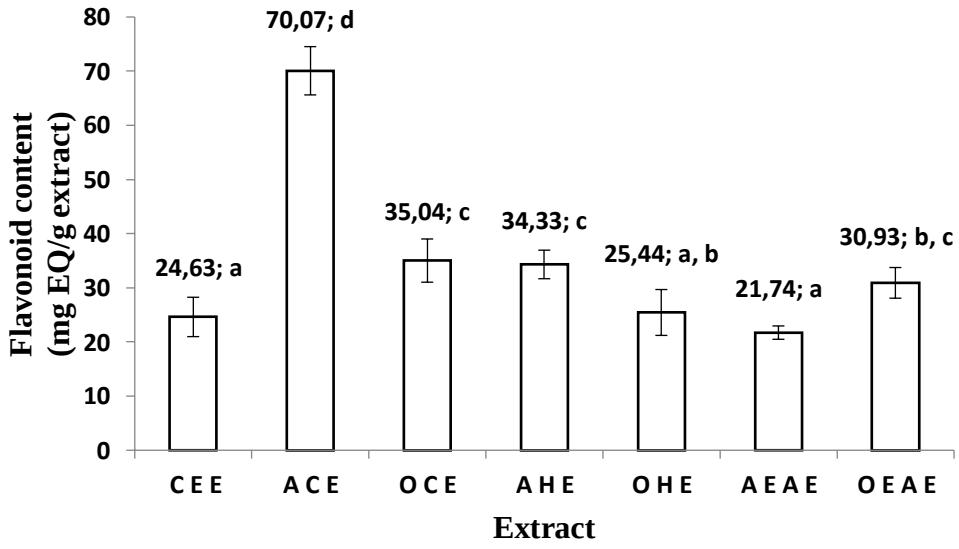


Figure 27 : Graphical representation of flavonoid content as a function of olive leaves extracts.

With : C E E : Crude ethanolic extract ; A C E : Aqueous chloroform extract ; O C E : Organic chloroform extract ; A H E : Aqueous hexane extract ; O H E : Organic hexane extract ; A E A E : Aqueous ethyl acetate extract and O E A E : Organic ethyl acetate extract. Finally, using STATISTICA 5.5 software (ANOVA/MANOVA), statistical comparisons were carried out, such that letters a, b, c and d indicate significant differences in flavonoid content between extracts.

The different solvents used to extract flavonoids have varying capacities to extract these compounds. Chloroform appears to be the most effective solvent for extracting flavonoids, with higher flavonoid contents per gram of extract, both in the aqueous phase (higher 70.07±4,46 Mg EQ/g extract) and in the organic phase (35.04±3,99 Mg EQ/g extract).

Flavonoid levels in the other extracts appear to be lower, with significant differences between them. For example, hexane in the aqueous phase and ethyl acetate in the organic phase appear to have significant intermediate flavonoid contents with values of (34.33±2,63 Mg EQ/g extract) and (30.93±2,83 Mg EQ/g extract) respectively, while the organic hexane extract, ethanolicextract and ethyl acetate in aqueous phase show even lower contents with values of (25.44±4,24 Mg EQ/g extract), (24.63±3,64 Mg EQ/g extract) and (21.74±1,23Mg EQ/g extract) respectively.

The results obtained show significant concentrations of flavonoids, with the aqueous chloroform extract showing the highest level at (70.07±4,46 mg EQ/g extract). Given that flavonoidsare small molecules rich in hydroxyl groups **(Ollila et al., 2002),** their

highly polar nature makes them soluble in polar solvents. Organic chloroform extract followed closely with (35.04±3,99 mg EQ/g extract), aqueous hexane extract (34.33±2,63 Mg EQ/g extract) and organic ethyl acetate extract (30.93±2,83 Mg EQ/g extract).

It appears that the amount of flavonoids in the organic hexane extract, ethanolic extractand aqueous ethyl acetate extract is slightly lower than that of the other extracts with values of (25.44±4,24Mg EQ/g extract), (24.63±3,64Mg EQ/g extract) and (21.74±1,23Mg EQ/g extract) respectively. This may be due to the fact that these extracts contain a significant amount of other phytochemicals that can interfere with the formation of the flavonoid-aluminium chloride complex. This complex is used to reveal the presence of flavonoids in plant extracts. The presence of these other phytochemicals can limit the ability of aluminium chloride to react with flavonoids, resulting in an underestimation of their quantity in these specific extracts.

A flavonoid content of 10.63±0.05 mg EQ/ g extract was found by Lfitat et al (2009), which is lower than that of our plant's ethyl acetate organic extract (30.93±2.83 mg EQ/ g extract).

III. Antioxidant activity

The antioxidant activity of olive leaf extracts was assessed using two separate methods. The first method involved measuring the capacity of the extracts to neutralise the ABTS (2,2'-azinobis (3-ethylbenzothiazoline-6-sulphonic)) radical, while the second involved the trapping of the DPPH (2,2-diphenyl-1- picrylhydrazyl) radical.

1. Trapping of the $ABTS^{\bullet+}$ radical

In this test, colourless ABTS undergoes initial oxidation with potassium persulphate (K2S2O8), forming the cationic radical $ABTS^{\bullet+}$, which gives the solution a blue-green colour. When an antioxidant compound is introduced, it reacts with the $ABTS^{\bullet+}$ radical, reducing it to the ABTS state, resulting in a discolouration of the solution **(Sarr et al., 2015).**

The results of the anti-free radical activity against the $ABTS^{\bullet+}$ radical are expressed as percentage inhibition for the standard (Trolox) as well as for *Olea europaea* extracts. These data are represented as a histogram in Figure 28 :

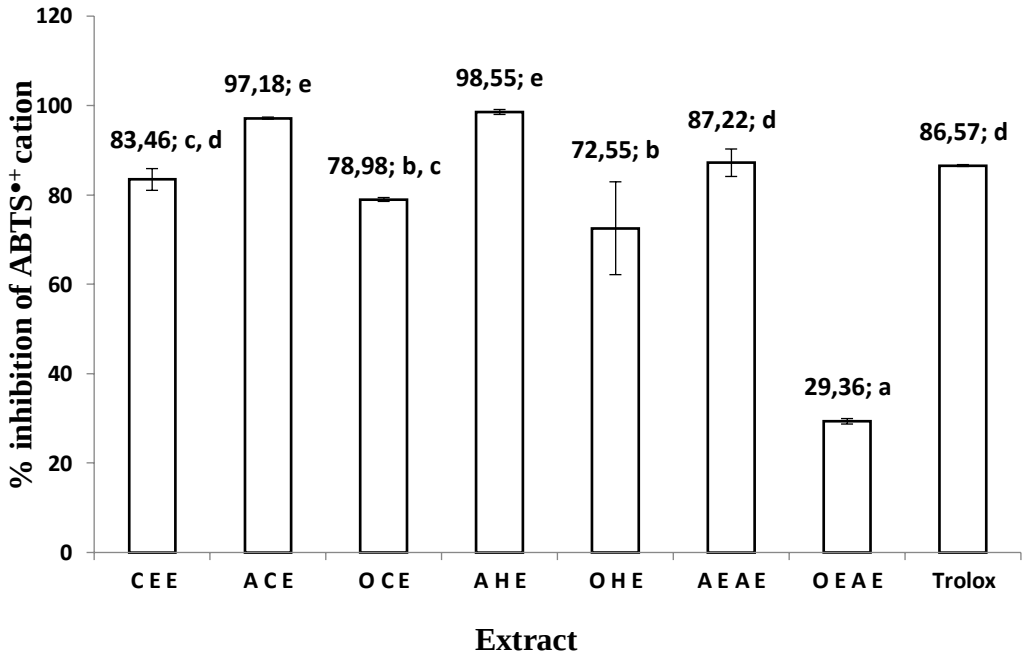


Figure 28 : ABTS$^{\bullet+}$ cation scavenging activity of dried olive leaves extracts.

With : C E E : Crude ethanolic extract ; A C E : Aqueous chloroform extract ; O C E : Organic chloroform extract ; A H E : Aqueous hexane extract ; O H E : Organic hexane extract ; A E A E : Aqueous ethyl acetate extract ; O E A E : Organic ethyl acetate extract and trolox : 6-hydroxy-2,5,7,8-tetramethylchro-man-2-carboxylic acid **(Lfitat et al., 2020).** Finally, using STATISTICA 5.5 software (ANOVA/MANOVA), statistical comparisons were performed, such that a, b, c, d and e indicate significant differences regarding the ABTS$^{\bullet+}$ cation scavenging activities of the extracts.

According to the statistical analysis, there was no significant difference between the highest percentages of ABTS$^{\bullet+}$ radical inhibition of aqueous hexane (98.55±0,54%) and chloroform (97.18±0,22%) extracts. However, the organic extracts of hexane (72.55±10,39%), chloroform (78.98±0,43%) and aqueous (87.22±3,07%) and ethanolic (83.46±2,44%) ethyl acetate are significantly lower than the other extracts, which have intermediate contents. On the other hand, ethyl acetate in the organic phase had the lowest content (29.36±0,61%).

From this histogram, it can be seen that our plant showed strong scavenging activity ofthe ABTS$^{\bullet+}$ radical compared with the standard used (Trolox) which gave a percentage inhibition of : (86,57±0,22)%.

The aqueous hexane extract and the aqueous chloroform extract showed the highest inhibitory activity of the ABTS$^{\bullet+}$ radical, with a percentage of (98.55±0,54%) and (97.18±0,22%) respectively. This is explained by its presence of phenolic compounds

(total phenols, flavonoids) which are molecules containing hydroxyl groups that provide hydrogen and electrons, allowing them to take a good $ABTS^{\bullet+}$ cation scavenging activity. Followed by ethanolic (83.46±2,44%), organic chloroform (78.98±0,43%), aqueous ethyl acetate (87.22±3,07%) and organic hexane (72.55±10,39%) extracts, due to the complexity of its compositions, and consequently the steric hindrances it generates, this may inhibit the trapping of the $ABTS^{\bullet+}$ radical. While the inhibition rate of the $ABTS^{\bullet+}$ cation is lower for the ethyl acetate extract in the organic phase (29.36±0,61%) than for the other extracts, this could be attributed to the presence of phenolic compounds with low polarity.

The choice of extraction solvent is crucial in the extraction process, particularly when working with antioxidant compounds. The polarity and type of solvent can influence the solubility of the target compounds **(Jayaprakasha et al., 2008).**

The results obtained for our olive leaves for the scavenging activity of the ABTS-+ radical are better than those obtained by Khelouf et al (2023). We can therefore see that our samples are more effective and this is probably linked to the difference in chemical composition or to seasonal factors.

2. DPPH radical scavenging

The DPPH test is based on the idea that antioxidants act as hydrogen donors. The $DPPH^{\bullet}$ radical, initially violet in colour and with an absorption at 517 nm, reacts with the antioxidant by accepting hydrogen to form DPPH. The effectiveness of the antioxidant is reflected by the decrease in the $DPPH^{\bullet}$ radical and the change in colour of the solution from purple to yellow **(Sarr et al., 2015).**

The results of the ability of *Olea europaea* extracts as well as the standard (BHA) to scavenge the $DPPH^{\bullet}$ radical are shown in Figure 29 :

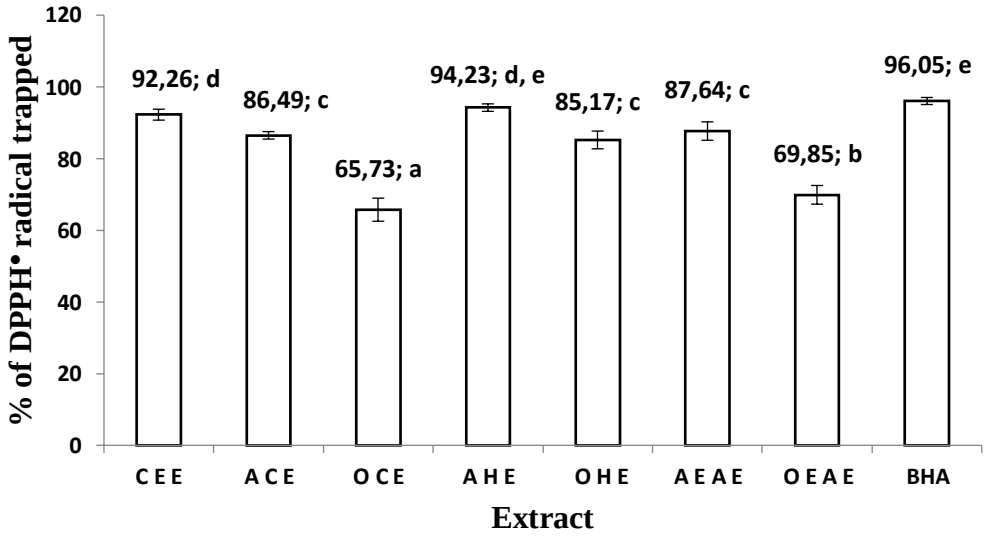


Figure 29 : DPPH• radical scavenging activity of dried olive leaves extracts.

With : C E E : Crude ethanolic extract ; A C E : Aqueous chloroform extract ; O C E : Organic chloroform extract ; A H E : Aqueous hexane extract ; O H E : Organic hexane extract ; A E A E : Aqueous ethyl acetate extract ; O E A E : Organic ethyl acetate extract and BHA : Butylated hydroxyanisole. Finally, using STATISTICA 5.5 software (ANOVA/MANOVA), statistical comparisons were performed, such that letters a, b, c, d and e indicate significant differences regarding the DPPH• radical scavenging activities of the extracts.

The aqueous hexanolic extract and the ethanolic extract show the highest %I and there is no significant difference between them ($p<0.05$), with values of (94.23±1.03%), (92.26±1.51%) respectively, are comparable to that of the (96.05±0,99 %) BHA standard. This suggests a strong capacity of the compounds present in these extracts to neutralise DPPH• radical free radicals, indicating significant antioxidant activity. The ethyl acetate aqueous phase, chloroform aqueous phase and hexane organic phase extracts also showed high %I, with values of (87.64±2,57 %), (86.49±1,03%) and (85.17±2,47%) respectively, also indicating significant antioxidant activity. Finally, the organic phase chloroform and organic phase ethyl acetate extracts showed relatively lower %I, with values of (65.73±3,22%) and (69.85±2,62%) respectively.This suggests that the compounds extracted from these organic phases have less antioxidant activity than the other extracts.

The results show that aqueous phase hexane extract and ethanolic extract, with values of (94.23±1,03%) and (92.26±1,51%) respectively, have the highest DPPH• radical scavenging activity percentages of all the samples tested, suggesting that these extracts are rich in phenolic compounds, such as total phenols and flavonoids, which are known

for their ability to donate hydrogen atoms. Thus, the higher the number of -OH groups in a molecule, the higher its capacity to donate hydrogen atoms, resulting in higher DPPH• radical scavenging activity **(Jayaprakasha et al., 2008).** Finally, the extracts with lower values than the others (ethyl acetate aqueous phase (87.64±2,57 %), Hexane organic phase (85.17±2,47%), chloroform aqueous phase (86.49±1,03%), chloroform organic phase (65.73±3,22%) and ethyl acetate organic phase (69.85±2,62%)) could be due to steric hindrance, which could hinder the reduction of the DPPH• radical.

Conclusion and outlook

Conclusion and outlook

Olive leaves and the olive tree (*Olea europaea* L.) are known for their abundance of phenolic compounds, substances with beneficial biological properties. Their age-old use in traditional medicine is supported by their ability to combat oxidative stress and a range of ailments. These plants are increasingly appreciated for their antioxidant, anti-inflammatory and antimicrobial effects, making them important players in many sectors, including natural medicine and cosmetics.

The main objective of this study was to evaluate the content of phenolic compounds extracted from olive leaves from the region Sidi-Aïch (wilaya of Bejaïa), using the maceration method. The study also aimed to assess the antioxidant activities of the extracts obtained.

The total phenol and flavonoid contents were assessed using the Folin Ciocalteu and aluminium chloride (AlCl3) methods respectively. These analyses highlighted the richness of this plant in total phenols, estimated at 109.11 mg of gallic acid equivalent per gram of extract, as well as the significant presence of flavonoids, estimated at 70.07 mg of quercetin equivalent per gram of extract.

Analysis of the anti-free radical activity of phenolic compounds extracted from olive leaves has demonstrated a strong capacity to neutralise radicals, reaching levels of up to 94.23% for DPPH and 98.55% for ABTS, at different concentrations.

Research revealing high concentrations of total phenols and flavonoids in olive leaves opens up vast prospects in many fields. In medical terms, these compounds offer promising therapeutic potential against oxidative stress and various diseases. In cosmetics, they can be used to develop natural skincare products. In short, these prospects open up new opportunities for research and applications in health and beauty.

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The appendices

Appendix 1 : products and equipment used during practice.

Products

The table showing the products used during practice and the raw product formulas.

Table I : Products used.

Products	**Formulas**
ABTS	$C_{18}H_{18}N_4O_6S_4$
DPPH	$C_{18}H_{12}N_5O_6$
Gallic acid	$C_7H_6O_5$
Quercetin	$C_{15}H_{10}O_7$
Ethyl acetate	C4H8O2
Aluminum chloride	$AlCl_3$
Methanol	CH_3OH
Distilled water	H_2O
Sodium carbonate	Na_2CO_3
Folin Ciocalteu	$H_3PW_{12}O_{40}$
Ethanol	C_2H_6O
Chloroform	$CHCl_3$
Hexane	C_6H_{14}
Potassium persulfate	$K_2S_2O_8$

Equipment

- Beaker ;
- Glass spatula ;
- Magnetic rod ;
- Test tube ;
- Pipette and bulb ;
- Stirring plate ;

- Petri dish ;
- Test tubes ;
- Tube holder ;
- Micropipette ;
- Spatula ;
- Analytical balance ;
- Spectrophotometer ;
- Spectrophotometer cuvettes ;
- Tips ;
- Flask ;
- Oven.

Appendix 2 : Calibration curves used to calculate flavonoid and total phenol content :

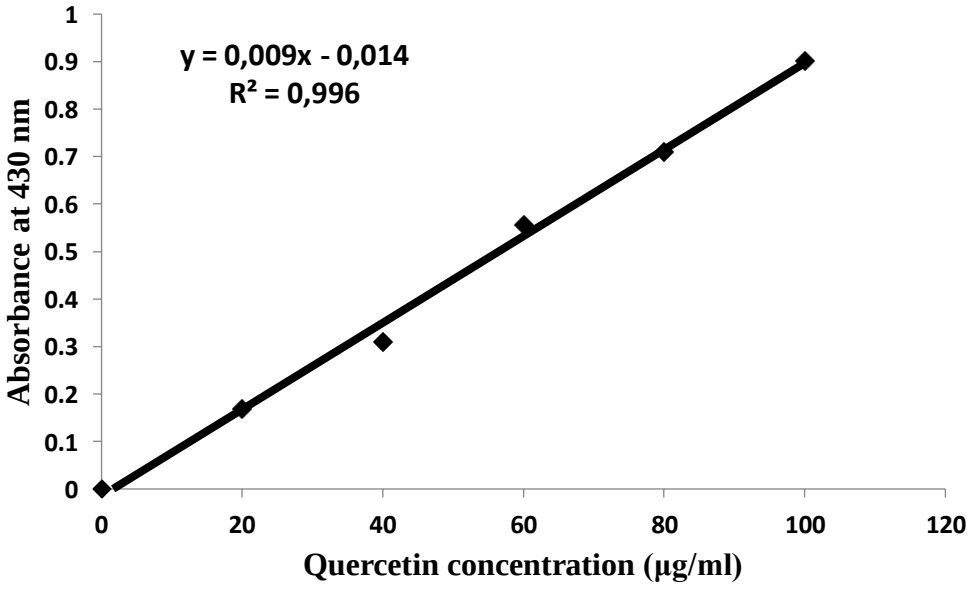

Figure 1 : Calibration curve (or straight line) used to calculate flavonoid content.

Where : y = 0.009 x - 0.014 : The equation of the calibration curve ; y : Absorbance at 430 nm ; x : Concentration in mg EQ/g extract and R : Correlation coefficient.

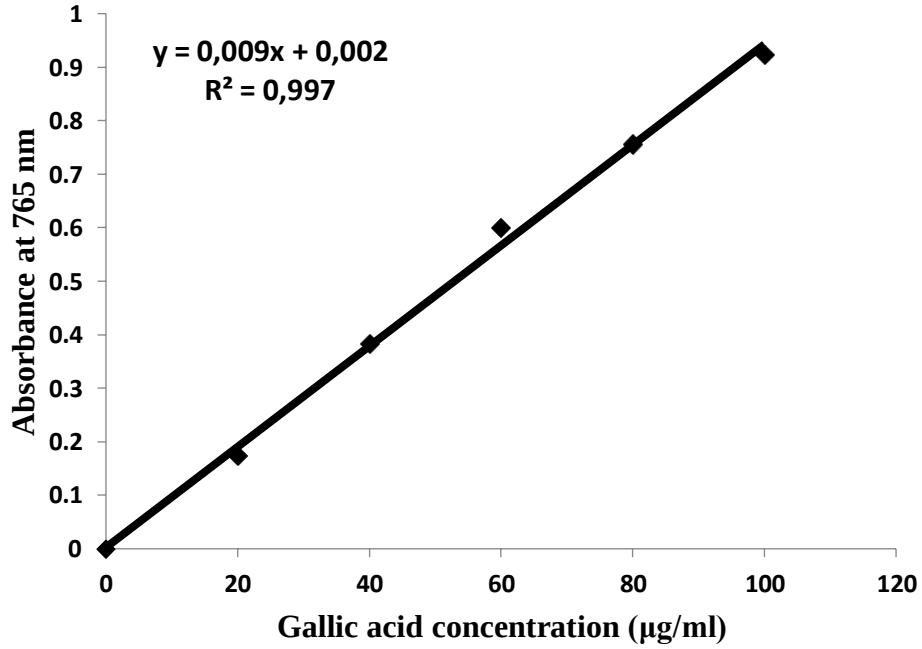

Figure 2 : Calibration curve (or straight line) used to calculate the total phenol content.

Where : y = 0.009 x + 0.002 : The equation of the calibration curve ; y : Absorbance at 765 nm ; x : Concentration in mg EAG/g extract and R : Correlation coefficient.

Abstract

The objective of this study was to assess the phenolic compound content and antioxidant activity of olive leaves from the region Sidi-Aïch of wilaya Bejaïa, To this end, the study was divided into several distinct parts: In the first part, the extraction yield of olive leaves by maceration was evaluated, showing that the crude ethanolic extract had the highest extraction rate (42.44%). The second part involved analysis of phenolic compounds, in particular total phenols and flavonoids. Results showed that the organic hexane extract had the highest total phenol content (109.11±0.31 mg EAG/g extract), while the aqueous chloroform extract showed the highest flavonoid content (70.07±4.46 mg EQ/g extract). Finally, the third part of the study focused on antioxidant activities, assessed using the DPPH radical and ABTS radical tests. In both tests, the aqueous hexane extract showed the highest activity, with percentages of 94.23±1.03% and 98.55±0.54% respectively.

Key words : olive leaves, phenolic compounds, antioxidant.

Résumé

L'objectif de cette étude était d'évaluer la teneur en composés phénoliques ainsi que les activités antioxydantes des feuilles d'olivier de la région Sidi-Aïch wilaya de Bejaïa. Pour cela, l'étude a été divisée en plusieurs parties distinctes : Dans la première partie, le rendement d'extraction des feuilles d'olivier par macération a été évalué, montrant que l'extrait éthanolique brut présentait le taux d'extraction le plus élevé (42,44%). La deuxième partie consistait en l'analyse du dosage des composés phénoliques, notamment des phénols totaux et des flavonoïdes. Les résultats ont indiqué que l'extrait organique d'hexane présentait la teneur la plus élevée en phénols totaux (109,11±0,31 mg EAG/g d'extrait), tandis que l'extrait aqueux de chloroforme montrait la plus haute teneur en flavonoïdes (70,07±4,46 mg EQ/g d'extrait). En fin, la troisième partie de l'étude s'est concentrée sur les activités antioxydantes, évaluées à l'aide des tests du radical DPPH et du radical ABTS. Dans les deux tests, l'extrait aqueux d'hexane a présenté l'activité la plus élevée, avec des pourcentages respectifs de 94,23±1,03% et 98,55±0,54%.

Mots clés : feuilles d'olivier, composés phénoliques, anti-oxydant.

ملخص

كان الهدف من هذه الدراسة هو تقييم محتوى المركبات الفينولية وكذلك الأنشطة المضادة للأكسدة لأوراق الزيتون من ولاية سيدي عيش بمنطقة بجاية. ولهذا تم تقسيم الدراسة إلى عدة أجزاء متميزة: في الجزء الأول تم تقييم إنتاجية استخلاص أوراق الزيتون بالنقع، وتبين أن المستخلص الإيثانولي الخام حقق أعلى نسبة استخلاص (42.44%). الجزء الثاني يتكون من تحليل جرعات المركبات الفينولية، وخاصة الفينولات الكلية والفلافونويدات. أشارت النتائج إلى أن مستخلص الهكسان العضوي أظهر أعلى محتوى من الفينولات الكلية (109.11±0.31 ملغ مكافئ حمض الغال /غ مستخلص)، بينما أظهر مستخلص الكلوروفورم المائي أعلى محتوى فلافونويد (70.07±4.46 ملغ مكافئ كيرسيتين/غ مستخلص). أخيرا، ركز الجزء الثالث من الدراسة على أنشطة مضادات الأكسدة، والتي تم تقييمها باستخدام فحوصات جذرية دي بي بي ش و أ بي تي اس. في كلا الاختبارين، أظهر المستخلص المائي للهكسان أعلى نشاط، بنسب مئوية على التوالي 94.23±1.03% و98.55±0.5

الكلمات المفتاحية: أوراق الزيتون، المركبات الفينولية، مضادات الأكسدة.