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بسم الله الرحمن الرحيم

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Dedication

To my dear parents

To my dear Brothers

To all my family

To my friends

To all those who supported me, directly or indirectly, in carrying out this work.

Zineb

Dedication

I dedicate this work to my mum, my best friend, my source of positive energy, the one who never stopped encouraging me, supporting me and backing me up. This work is an achievement for her as much as for me, and no matter what I say or do, I can't thank her enough.

To my dad, the man in my life, my hero, my example of wisdom, this work is for him, he who has always been there, who helped me so much and accompanied me throughout my studies, may God grant him good health and long life.

To my brothers, for their good humor and zest for life. To my family, whom I love dearly and who make me happy.

To my dear husband who has encouraged me and who is always there in the best of times and in the worst of times.

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Summary:

Quercus spp or oak, belonging to the *fagaceae* family, is an important tree in the Algerian forest, but its biological properties and interest in phytotherapy are little known. In order to contribute to the improvement of the Algerian flora, The objective of this study was to prepare alcoholic and aqueous extracts of both oak leaves and fruits using two different extraction methods. The phytoconstituents present in the oak extracts were then detected and spectrophotometrically measured, with a focus on secondary metabolites such as polyphenols and flavonoids. Finally, the biological activities of the extracts were evaluated.

Extraction by maceration and ultrasound was carried out using 2 solvents of different polarities (ethanol, distilled water). A quantitative analysis was carried out to determine the polyphenols contained in the extracts using the Folin Ciocalteu reagent, as well as the flavonoids present in the extracts using the aluminium chloride (AlCl3) method.

The results of the various assays show that the highest content of phenolic compounds was obtained in the leaf extracts. In terms of flavonoid content, the aqueous acorn extract had the highest flavonoid content. Phytochemical screening of *Quercus* acorn and leaf extracts revealed the presence of tannins, gall tannins, catechetic tannins, saponins, favonoids, polyphenols, terpenes and carbohydrates.

The results of the DPPH test demonstrated that *Quercus* extracts exhibited significant antioxidant activity. As the in vitro investigation indicated that the extracts, when subjected to the protein denaturation method and haemolysis test, exhibited good anti-inflammatory properties.

This study has demonstrated that the ethanolic and aqueous extracts of this plant have the potential to act as a potent antioxidant and anti-inflammatory agent, with significant biological and therapeutic implications.

الملخص:

تعد شجرة *Quercus spp* أو البلوط، التي تنتمي إلى فصيلة الفاجاسية، شجرة مهمة في الغابات الجزائرية، ولكن خصائصها البيولوجية وفائدتها في العلاج بالنباتات غير معروفة إلا قليلاً. من أجل المساهمة في تحسين النباتات الجزائرية، كان الهدف من هذه الدراسة هو إعداد مستخلصات كحولية ومائية لكل من أوراق وثمار البلوط باستخدام طريقتين مختلفتين للاستخلاص. تم بعد ذلك الكشف عن المكونات النباتية الموجودة في مستخلصات البلوط وقياسها بالقياس الطيفي، مع التركيز على المستقلبات الثانوية مثل البوليفينول والفلافونويد. وأخيراً، تم تقييم الأنشطة البيولوجية للمستخلصات.

تم إجراء الاستخلاص عن طريق النقع والموجات فوق الصوتية باستخدام مذيبين من أقطاب مختلفة (الإيثانول والماء المقطر). وأُجري تحليل كمي لتحديد البوليفينول الموجود في المستخلصات باستخدام كاشف فولن سيوكالتو، وكذلك الفلافونويدات الموجودة في المستخلصات باستخدام طريقة كلوريد الألومنيوم .(AlCl3)

أظهرت نتائج المقايسات المختلفة أنه تم الحصول على أعلى محتوى من المركبات الفينولية في المستخلصات الإيثانولية للأوراق. وفيما يتعلق بمحتوى الفلافونويد، كان مستخلص البلوط المائي يحتوي على أعلى محتوى من الفلافونويد. كشف الفحص الكيميائي النباتي لمستخلصات بلوط *Quercus* ومستخلصات الأوراق عن وجود العفص، والعفص المراري، والعفص الكاتيكي والعفص الكاتيكي، والصابونين، والفافونويد، والبوليفينول، والتربينات، والكربوهيدرات.

أظهرت نتائج اختبار DPPH أن مستخلصات *Quercus* تُبدي نشاطاً كبيراً مضاداً للأكسدة. كما أشارت الفحوصات المختبرية إلى أن المستخلصات، عند إخضاعها لطريقة تمسخ البروتين واختبار انحلال الدم، أبدت خصائص جيدة مضادة للالتهابات .

وقد أثبتت هذه الدراسة أن المستخلصات الإيثانولية والمائية لهذا النبات لديها القدرة على العمل كمضاد أكسدة قوي ومضاد للالتهابات، مع آثار بيولوجية وعلاجية كبيرة.

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Q: *Quercus*

(GBIF): The Global Biodiversity Information Facility

(PGE2): Prostaglandin E2

(NO): Nitric oxide

(COX-2): Cyclooxygenase-2

(iNOS): Inducible nitric oxide synthase

(O_2^-): Superoxide

(COX-1 and COX-2): Cyclooxygenases 1 and 2

(NSAIDs): Non-steroidal anti-inflammatory drugs

(UAE): Ultrasonic assisted extraction

QAE: Aqueous extract of *Quercus*

QEE: Ethanolic extract of *Quercus*

(DM): Demineralised water

EAG: Gallic acid equivalent

DW: Dry matter weight

LAF: Leave aqueous filtration

LAU: leave aqueous ultrasound

LEF: leave ethanolic filtration

LEU: leave ethanolic ultrasound

OAF: Oak aqueous filtration

OAU: Oak aqueous ultrasound

OEF: Oak ethanolic filtration

OEU: Oak ethanolic ultrasound

RBC: Red Blood Cells

Introduction

Introduction:

In our contemporary era, the prevalence of various diseases has led to an increased reliance on chemical medications, often accompanied by undesirable side effects. This trend extends beyond pharmaceuticals to encompass everyday products like foods containing additives, industrial antioxidants, and cosmetics. Consequently, there has been a resurgence of interest in natural remedies, particularly those derived from medicinal plants **(Petrovska, 2012)**.

Medicinal plants represent a valuable natural resource due to their minimal environmental impact, renewability, and therapeutic efficacy with fewer adverse effects compared to synthetic medications. Algeria, renowned for its rich biodiversity, has a long-standing tradition of utilizing plant-based treatments. A striking example of such natural matrices is the *Quercus* trees **(Tantray *et al*., 2018).**

Renowned for their diverse applications, Quercus species have been traditionally utilized for various purposes. Primarily, the wood and bark, along with the universally known fruit, acorn, have been valued for both human and animal consumption. Additionally, the leaves are commonly used to prepare infusions with medicinal or nutritional purposes, serving as remedies for ailments such as gastrointestinal disorders and as antiseptics. Due to their nutritional richness, acorns serve as a food source for both humans and animals, further highlighting the multifaceted benefits of *Quercus* species **(Popović *et al.*, 2013;Marrelli, 2021)**.

The pharmacological effects attributed to plants of the Quercus genus are wide-ranging and hold potential for therapeutic applications, including antioxidant, anti-inflammatory, and neuroprotective properties. These attributes are particularly noteworthy in the context of combating oxidative stress and mitigating inflammatory responses within the body. However, alongside these beneficial effects, there exists a nuanced concern regarding the impact of oxidative stress. This oxidative stress, in turn, can exacerbate inflammatory reactions, complicating the evaluation of the plants' medicinal potential. By understanding and addressing these intricacies, researchers can better assess the therapeutic value of Quercus plants, focusing on harnessing their anti-inflammatory and antioxidative properties while mitigating the effects of fungal contamination **(Burlacu et al., 2020).**

However, amidst this resurgence, we must confront the challenges posed by fungal infections and oxidative stress, which can incite inflammatory reactions and complicate disease management. These factors present a pressing need for effective and safer alternatives.

Therefore, the main objectives of this work include:

- Preparing hydro-alcoholic and aqueous extracts of both oak leaves and fruits with two types of extraction methods,
- Detecting phytoconstituents from oak extracts and spectrophotometrically measuring secondary metabolites such as polyphenols and flavonoids,
- Alongside evaluating various biological activities.

Part I:

Bibliographic synthesis

Chapter I: Bibliographic synthesis

I.1. Botanical description and systematic classification:

The oak tree belongs to the *Fagaceae* family and is one of the most distinctive trees in the Mediterranean basin, growing up to 20-25m tall. Its trunk is often twisted and branched, and its crown is rounded. The bark is brownish-black or black, with small, thin, square patches that are dry and often curved. The oak leaves are deciduous and alternate, except in the case of holm oak and cork oak, where they are evergreen for 2 or 3 years. The width of the leaves is generally greater towards the upper third of the blade. Male oak flowers are grouped together in discrete hanging strings in April, at the same time as the leaves appear, while the female flowers are isolated or grouped together in small upright spikes. The flowers bear 6 to 8 stamens. The plant has unisexual flowers, with male flowers in long-stalked, flexible catkins and female flowers in erect spikes. The fruit is an acorn, usually ovoid, half-protected by a long, grey, downy, scaly cup. The acorns ripen annually in September and October and fall off, leaving the cup. Cork from the cork oak is used to make high-quality corks. The cork oak tree can be harvested about 12 times over its lifetime. The wood of the cork oak is very dense and hard, making it difficult to work with and therefore rarely used.

There are over 300 varieties of oak trees, including the cork oak (*Quercus suber*) and the holm oak (*Quercus ilex*). The cork oak is the rarest of the oaks and is primarily used to make high-quality bottle corks. The botanical name for the oak tree is derived from the Latin word: *Quercus*, which is thought to come from the Celtic word '*kaerquez*', meaning 'beautiful tree'. The Greek name for oak is drus, which is a generic term for 'tree'. In addition to these names, oak is also referred to as aigilops or phegos, which comes from the Greek word '*phagein*', meaning 'to eat', in reference to the acorn. The French name of oak is Chêne, and in Arabic it is known as شجرة البلوط **(Charef, 2015)**.

- **Systematic classification of *Quercus*:**

Règne: Plantae

Subdomain: Tracheobionta

Phylum: Spermatophyta

Class: Magnoliopsida

Subclass: Hamamelididae

Order: Fagales

Family: Fagaceae

Genus: Quercus L.

Species: Quercus ilex L, Qurcus suber L, Quercus canariencis willd **(Djellit et Moualed., 2021).**

Figure 1: *Querqus ilex* **(Charef, 2015)**.

Figure 2: *Querqus suber* **(Charef, 2015)**.

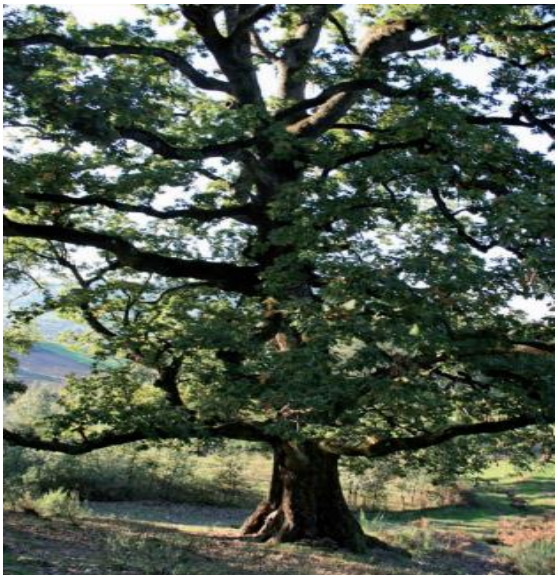


Figure 3: *Querqus canariensis* **(Messaoudène *et al*., 2008)**

I.2. Geographical repartition:

The *Quercus L.* genus, commonly known as Oak, belongs to the *Fagaceae* family, which includes important woody plants in the form of shrubs or trees. These plants are characterized by their simple alternate leaves, wind-pollinated flowers, acorn fruits, and their ability to survive for centuries. The genus comprises approximately 600 species worldwide. The *Quercus* genus is a significant group of woody angiosperms in the northern hemisphere due to its species diversity, ecological dominance, and economic value. Oaks dominate various habitats, such as temperate deciduous forests, temperate and subtropical evergreen forests, subtropical and tropical savannahs, and woodlands. They thrive in well-drained, loamy soils. The roots of an oak tree are extensive, reaching up to three times the height of the tree and as deep as 15-40 ft (4.6-12.2m), depending on site conditions. Oaks vary greatly in size, ranging from small shrubby species to trees with impressive dimensions. The tallest oak on record stands at 123 ft (37.5m) with a trunk circumference of 21.6 ft (606m) and a canopy spread of over 83.6 ft (25.5m). The Quercus genus first appears in the fossil record during the early Tertiary period in North America, approximately 50-55 million years ago. The genus also includes *Fagus* (beeches), *Castanea* (chestnuts), other '*castaneoid*' genera (*Chysolepis*, *Castanopsis* and *Lithocarpus*), and three monotypic tropical genera (*Trignobalanus*, *Formanodendron* and *Colombobalanus*). In the current writing, Oaks are dealt with either as a solitary genus with two sub genera (*Quercus* and *Cyclobalanopsis*), or as two particular genera. In the flora of China, *Quercus* and *Cyclobalanopsis* are two separate genera, with 35 species classified under *Quercus* and 69 species under *Cyclobalanopsis.* However, in the New World, the *Quercus* genus has been divided into three distinct groups: the white Oaks (section *Quercus,* also known as subgenus or section *Leucobalanus* or *Lepidobalanus*), the red or black Oaks (section *Lobatae;* also sometimes referred to as subgenus or section *Erythobalanus*), and the intermediate or golden Oaks (section *Protobalanus*). A fourth group, Cerris, is restricted to Eurasia and North Africa. North America has the largest number of oak species, with approximately 91 present in the United States. Mexico has 160-165 species, of which 109 are endemic. In India, the genus *Quercus* has 35 species, which are mainly confined to the Himalayan region between 1000-3500m in height and are the dominant climax tree species of the wet mild forests. Five species of evergreen oak, namely *Quercus glauca* (Phaliyant/harinj), *Q. leucotrichophora* (banj), *Q. lanuginosa* (rianj), *Q. floribunda* (moru), and *Q. semecarpifolia* (kharsu), grow naturally in the Western Himalaya. The genus *Quercus* has significant

conservation value in the Western Himalayas and provides numerous ecosystem services, such as conserving soil, water, and native flora and fauna **(Tantray *et al.*, 2018)**.

The cork oak (*Quercus suber*) is a species of oak native to the western Mediterranean region. It is commonly found in the Mediterranean area, along with the holm oak (*Quercus ilex L.*) and the kermes oak (*Q. coccifera L.*). These three species are collectively known as the most common evergreen arboreal oaks in the Mediterranean area. They occur in three out of ten biodiversity hotspots detected in the Mediterranean Basin. The species' modern distribution is discontinuous, extending from the Atlantic coasts of Morocco, the Iberian Peninsula and France to the southeastern regions of Italy, including the main west-Mediterranean islands as well as the coastal belts of Maghreb **(Khedidja *et al.*, 2023)**. Algeria has six species of oak trees: *Quercus canariensis Willd, Quercus afares Pomel, Quercus suber L, Quercus coccifera L, Q.faginea,* and *Quercus ilex L*. These species cover almost 40% of the Algerian forest and play an important role in the ecological, economic, and social aspects. The evolutionary history of most oak species is complex due to interspecific hybridization and introgressions. Interspecific hybridization is a frequent occurrence in oaks and can have significant evolutionary consequences on taxonomy. Due to the high variability and gene flow between these species, the current representation of the range of these taxa needs to be reviewed and explored **(Amel *et al.*, 2022)**.

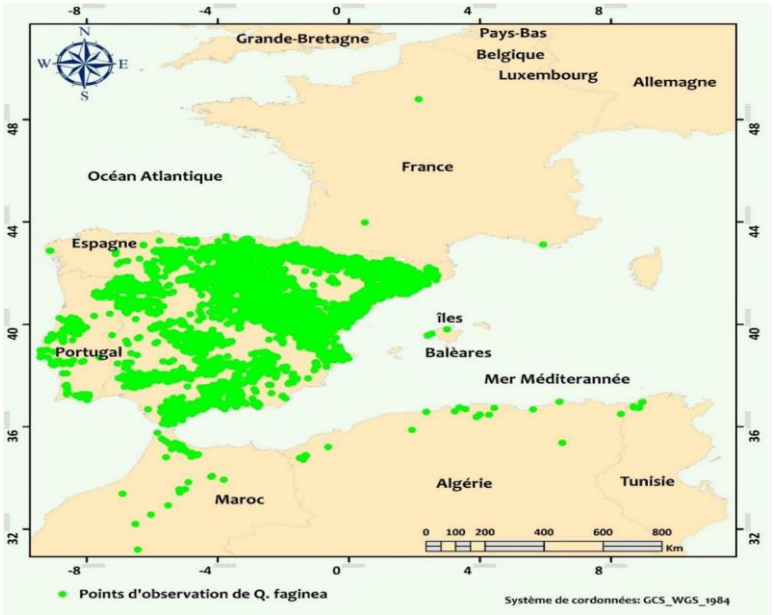


Figure 4: The following points have been identified as suitable for observation of *Quercus faginea*. The map was created using data from the Global Biodiversity Information Facility (GBIF) on the distribution of this species **(Aissi *et al.*, 2021)**.

I.3. Phytochemical composition:

Quercus species has been reported to contain several phytoconstituents with significant differences between species due to their high variability. However, certain classes of compounds are present in all *Quercus* species (see **Figure 4**). The main bioactive phytochemical compounds are phenolic compounds, which are generally found in the form of glycosides. The other compounds found in *Quercus* species include volatile organic compounds, vitamins (especially vitamin E), sterols, aliphatic alcohols, and fatty acids.

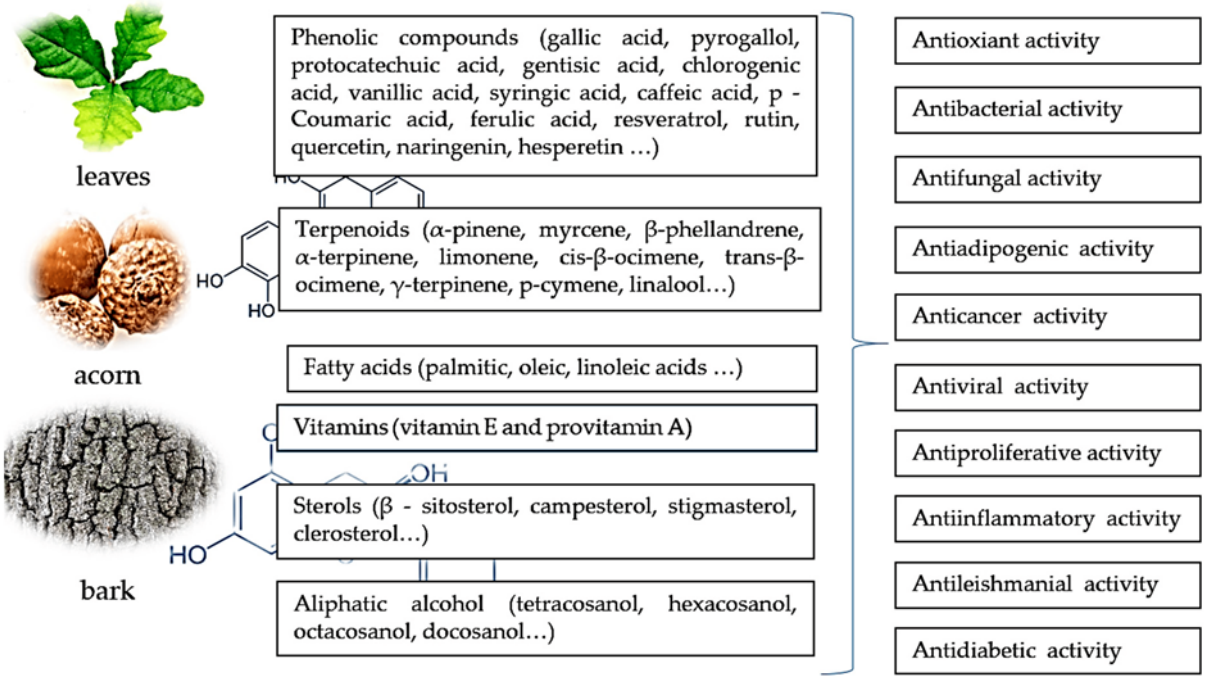


Figure 5: The main phytochemical compounds and biological activities of *Quercus sp* **(Burlacu *et al*., 2020).**

Phenolic compounds (**figure 5)** are one of the largest groups of secondary metabolites in plants due to their occurrence and pharmacological properties **(Tanase *et al*., 2019)**. They show a diverse range of structures, from simple molecules such as phenolic acids to polyphenols like stilbenes, flavonoids, and derived polymers **(Cheynier, 2012)**. Phenolic compounds protect plants against herbivores and have positive effects on mammals, including humans, due to their antioxidant, antimicrobial, anti-inflammatory, and anticarcinogenic properties **(Engström, 2016)**. While phenolic compounds have a wide range of biological activities, most of them are secondary to their antioxidant activity, which has several proposed mechanisms of action. The main idea is that phenolic compounds may donate an electron or transfer the whole hydrogen atom, from the O–H bond, to free radical molecules, thus transforming them into harmless

species. This process also transforms the phenolic into a radical, with an odd electron, but due to the aromatic structure, the odd electron can spread over the entire molecule, resulting in radical stabilization **(Leopoldini *et al*., 2011)**.

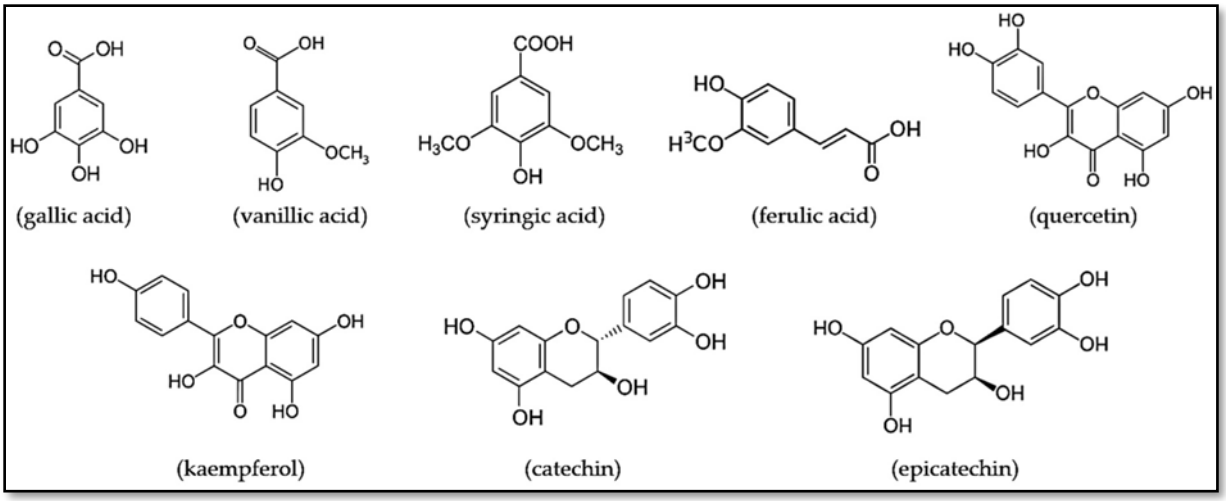


Figure 6: Chemical structures of the main phenolic compounds found in *Quercus* species **(Yarnes *et al*., 2006)**.

Phenolic compounds are present in all organs of *Quercus* species, including leaves, bark, and acorns. The production of individual phenolic compounds varies across *Quercus* species, with significant differences in both absolute and relative concentrations of polyphenols. For instance, ellagitannin production in white *Quercus* leaves can be characterised as products of castalagin and vescalagin. In contrast, black *Quercus* species contain these compounds in relatively lower amounts without derivatives. These differences in oak phenolic compounds account for ecological variations between species **(Yarnes *et al*., 2006)**.

Phenolic compounds identified in *Quercus* species are primarily flavonoid and non-flavonoid constituents that are involved in the phenylpropanoid metabolism via the shikimate pathway. The resulting hydroxycinnamic acids and esters participate in reductase, oxygenase, and transferase processes, resulting in a characteristic pattern of secondary metabolites for each plant species. For instance, *Q. ilex* extracts were found to contain flavan-3-ols, flavonols, and acylated kaempferol glucosides. This section will now present the primary bioactive compounds found in *Quercus* bark, leaves, and acorns. Phenolic compounds identified in *Quercus* leaves include phenolic acids (such as gallic acid, ellagic acid, protocatechuic acid, gentisic acid, chlorogenic acid, vanillic acid, syringic acid, caffeic acid, p-coumaric acid, and

ferulic acid), as well as flavonoids (like rutin, quercetin, epicatechin, naringenin, hesperetin, formononetin, naringin, and kaempferol), and tannins. These compounds are found primarily in the leaves of *Q. glauca, Q. incana, Q. ilex, Q. mongolica, Q. salicina, Q. petraea,* and *Q. robur*, with varying quantities of each compound **(Burlacu *et al*., 2020)**.

The *Quercus* species produce a well-known fruit, commonly referred to as an acorn. Historically, acorns have been used as animal feed. Due to their nutritional value and high content of phytochemical compounds with biological activity, acorns have also been incorporated into the human diet. There is a significant body of literature dedicated to the phenolic compounds found in acorns. Phenolic acids such as gallic acid, ellagic acid, and their derivatives, flavonoids including quercetin, catechin, and naringin, as well as tannins are the main compounds identified in *Quercus* acorn extracts. These compounds has been found in acorns of various *Quercus* species, including *Q. brantii*, *Q. floribunda*, *Q. glauca*, *Q. incana*, *Q. mongolica*, and *Q. robur*. It has been reported that *Quercus* acorns contain significant amounts of carbohydrates, amino acids, proteins, lipids, and various sterols. Terpenoid metabolites play a role in plant growth and development, but their primary function is to facilitate more specialized chemical interactions and protection in the abiotic and biotic environment. For example, terpenes such as -thujene, -pinene, camphene, sabinene, -pinene, myrcene, -phellandrene, -terpinene, limonene, cis--ocimene, trans--ocimene, -terpinene, p-cymene, linalool, and 3 The presence of -methyl-3-buten-1-ol and 3-methyl-3-buten-1-yl acetate was identified in the leaves of *Quercus ilex*. In addition, the following compounds were found in the sapwood of *Quercus faginea*: -amyrin, betulinic acid, lupeol, betulin oleanolic acid, hederangenin, corosolic acid, and arjunic acid. A study demonstrated that *Q. robur* emits a considerable quantity of isoprene, accompanied by relatively low levels of -pinene, camphene, myrcene, -pinene, and in some instances, 1,8-cineol and limonene. In a study it was demonstrated that *Q. canariensis* emitted predominantly isoprene, whereas *Q. suber* exclusively released monoterpenes, primarily -pinene, -pinene, sabinene, myrcene and limonene. Occasionally, -ocimene was identified in the emissions of both species, while traces of -thujene, camphene, -terpinene, and terpinolene were found only in *Q. suber* **(Burlacu *et al*., 2020).**

I.4. Traditional utilization:

Oak is a widely used tree in cabinet-making and has been historically valued for its medicinal properties. It is considered a 'noble' tree. The leaves, which remain on the branches throughout the winter, were boiled and used to treat frostbite. Additionally, the tannin in oak bark can help heal minor burns when boiled in water. White oak bark has been used as an astringent, tonic, and antiseptic. The bark was boiled and mixed with white vervain (*Verbena urticifolia*), milk, and water to create an antidote for poison ivy (*Rhus radicans*). The oak tree was considered sacred by the Druids in Europe and was used for its astringent properties found in its bark, leaves, and acorns. Medical practitioners use oak bark decoction to treat sore throats and angina. It is used to treat haemorrhoids, anal fissures, minor burns, and skin ailments. It is also used to treat diarrhoea, dysentery, and rectal bleeding, although less frequently. The powdered bark can be inhaled to treat nasal polyps or applied to eczema to dry the affected area. The gall is highly astringent and can be used as a substitute for the bark **(Charef, 2015).**

I.5.Biological activities of *Quercus* genus:

Quercus spp. can be valuable plants, especially for the treatment of gastrointestinal disorders, skin and urinary tract infections. The *Quercus* acorns are edible (animal feed or human diet), astringent and diuretic, used in diarrhea, indigestion and asthma **(Burlacu *et al*., 2020).** Several pharmacological activities have been reported to be exhibited by different extracts as well as single compounds, such as antioxidant, antibacterial, anti-inflammatory, and cytotoxicity activity **(Taib *et al*., 2020).**

I.6.Oxidative stress and antioxydants

I.6.1. Oxidative stress

Oxidative stress is an imbalance between the generation of reactive oxygen species ‘ROS’ (free radicals) and antioxidant defenses in the body, leading to various diseases and harming tissues and organs. Free radicals are oxygen-containing molecules with unpaired electrons that can cause oxidation reactions, which can either benefit or harm the body. The body produces both free radicals and antioxidants, with antioxidants neutralizing free radicals by donating an electron, thereby stabilizing them and making them less reactive. Maintaining a healthy lifestyle can help maintain this balance **(Betteridge, 2000; Moore, 2020).**

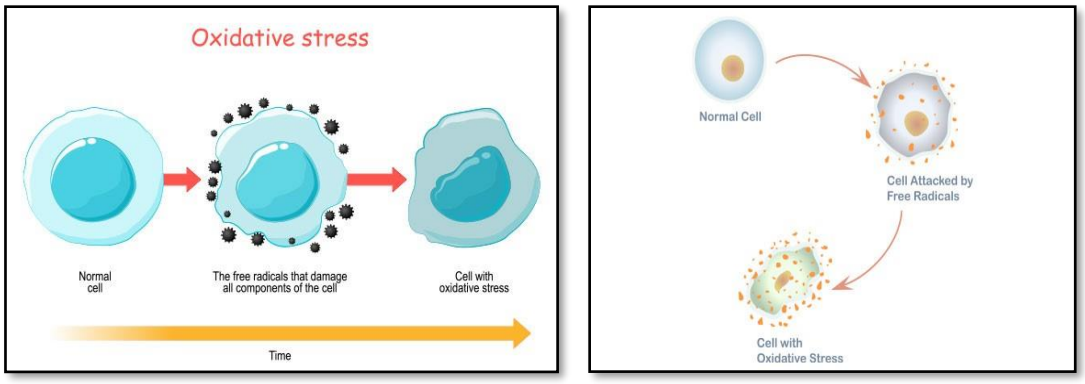


Figure 7: oxidative stress **(Moore, 2020).**

I.6.2. Free radicals

Free radicals are waste products generated during cellular metabolism. A free radical is any atom, group of atoms, or molecule that has one or more unpaired electrons on the outer most layer of its structure. Reactive oxygen species (ROS) are commonly defined as superoxide radicals ($O_2^{\bullet-}$), hydrogen peroxide (H_2O_2), hydroxyl radicals (•OH), and singlet oxygen (O_2). These ROS are produced as byproducts during metabolic processes in biological systems **(Aissani *et al*., 2022; Pizzino *et al*., 2017).**

All tissues, including their lipid, protein, carbohydrate, and DNA constituents, may be impacted. Over thirty distinct disease processes become more likely because of all these changes. Alzheimer's, Parkinson's, meningo-encephalitis, inflammatory and cardiovascular disorders, edema, accelerated aging of the skin, and cancer are a few of them **(Aissani *et al*., 2022).**

I.6.3. Antioxidant agents

Antioxidants are essential molecules that play a crucial role in protecting our bodies from free radicals, preventing premature aging, reducing the risk of cardiovascular disease, and even contributing to skin health by limiting the damaging effects of free radicals on cell membranes.

- **Enzymatic defenses**

These are highly effective defense systems because enzymes have the property of being able to carry out their work permanently. This line of defense includes

  - Superoxide dismutase (SOD): catalyzes the dismutation of superoxide anion.

$2O_2^{\bullet-} + 2H^+ \rightarrow H_2O_2 + O_2$

- Catalase (CAT): metabolizes H2O2 into water.

$2H_2O_2 \rightarrow 2H_2O + O_2$

- Glutathione peroxidase (GPx-1): reduces hydrogen and transforms organic hydroperoxides, particularly lipid hydroperoxides, from ROOH to ROH) **(Rajendran *et al.*, 2014).**

- **Non-Enzymatic defenses**

Vitamins E (α-tocopherol) and C (ascorbic acid), along with various plant-derived polyphenols such as flavonoids, xanthones, coumarins, carotenoids, phenolic acid derivatives, tannins, and anthocyanins, are among the essential nutrients not synthesized by the body and therefore must be obtained through the diet **(Chaudhary *et al*., 2023;Pizzino *et al*., 2017).**

I.7. Inflammatory response:

I.7.1 Definition:

Inflammation is a synchronised process induced by tissue damage or microbial infection. It is characterized by host tissue destruction, multiple organ failure, and sometimes even death. It is regarded as a crucial step in the wound healing cascade. Wound healing is an intricate network of overlapping biological processes that resolve tissue injuries. The healing process involves several phases, including haemostasis, inflammation, tissue proliferation, and maturation. These phases require the involvement of various cell types, some from the local area and others recruited upon injury. The ultimate objective of these processes is to eliminate invading microorganisms, remove damaged cells and tissue, and reestablish the skin barrier. Any fault in these steps may result in impaired healing **(Shukla *et al*., 2019)**. Macrophages and neutrophils are implicated in the inflammatory response, secreting a range of mediators such as eicosanoids, oxidants, cytokines, and lytic enzymes. These mediators are responsible for initiating, progressing, and sustaining acute or chronic inflammation. Amongst these mediators, prostaglandin E2 (PGE2) and nitric oxide (NO) are the most significant, produced in macrophages by cyclooxygenase-2 (COX-2) and inducible nitric oxide synthase (iNOS), respectively. PGE2 is involved in inducing the production of various chemoattractants and pro-inflammatory cytokines, while NO is responsible for vasodilation, an increase in vascular permeability, and edema formation at the site of inflammation. Additionally, (NO) along with superoxide (O_2^-) and the products of their interaction, initiates a wide range of toxic oxidative reactions that cause tissue injury. Similarly, neutrophils also produce oxidants and release granular constituents, including lytic enzymes that play an important role in causing inflammatory injury. Inhibiting the release of these mediators is a potential strategy for

controlling inflammation and is implicated in the mechanism of action of several anti-inflammatory drugs, including representative ones such as dexamethasone **(Kaur *et al*., 2004)**.

I.7.2 Anti-inflammatory agents:

I.7.2.1 Non-steroidal anti-inflammatory drugs (NSAIDs):

They are commonly used to manage inflammation. They possess anti-inflammatory, antipyretic, analgesic, and thrombotic properties due to the inhibition of cyclooxygenases 1 and 2 (COX-1 and COX-2). NSAIDs are frequently prescribed for pain relief, soft tissue injuries, osteoarthritis, gout, and inflammatory disorders, with an estimated usage of over 30 million per day.

The mode of action of NSAIDs is to decrease prostaglandin production by inhibiting cyclooxygenase and lipooxygenase transformations of arachidonic acid. They also inhibit oxygen radicals and suppress neutrophil migration. However, it could be argued that the short-term use of these drugs has been found to accelerate the healing of combat-related extremity wounds by altering the levels of inflammatory cytokines. Additionally, aspirin has been found to have a positive effect on the healing of chronic wounds by inhibiting inflammatory pathways through the upregulation of anti-inflammatory molecules responsible for repair **(Ghlichloo and Gerriets, 2024)**.

I.7.2.2 Cyclooxygenase (COX) inhibitors:

Are used to stall the expression of COX enzymes responsible for the synthesis of prostaglandin that induces inflammation. Cyclooxygenase has two isoforms, COX-1 and COX-2, which impact the course of inflammation through induction by growth factors, cytokines, and hormones. COX-1 is present ubiquitously in most cells, while COX-2 is an inducible form located on fibroblasts, macrophages, and other immune cells, and is predominantly upregulated during inflammation. Several inhibitors have been developed for COX-2 to control the cytotoxic effects of inflammation. COX-1 inhibitors have been found to have adverse effects on the gastrointestinal mucosa, whereas COX-2 inhibitors are considered safe. These inhibitors are commonly used after post-operative procedures to temporarily relieve inflammation and promote wound closure **(Shukla *et al*., 2019)**.

I.7.3 Anti-Inflammatory activity of natural products:

Extracting plant materials is the first major step towards testing their biological activities. Using whole extracts has advantages and disadvantages compared to isolating pure active compounds. One advantage is the potential for synergism between active components, which may be lost when each component is isolated. Several medicinal tests have discovered synergism, including those for anti-inflammatory activity. However, the mixture of different compounds may also lead to inhibitory effects, where one component reduces the biological activity of the other. According to some studies, pure compounds like amentoflavone, pseudohypericin, and hyperforin, which are isolated from extracts of Hypericum perforatum, have higher anti-inflammatory activity than the extracts themselves. Medicinal purposes also employ essential oils, plant juices, and plant powders, in addition to plant extracts. The selection of solvents for extracting plant materials is a crucial factor in determining the potential activity of the extract. This is because the polarity of the solvent determines which compounds will be extracted and which will not. For instance, water, which is highly polar, is unlikely to extract the active anti-inflammatory compound monoterpene 1,8-cineole (*Achillea millefolium*), but will easily extract protocatechuic acid (*Boswellia dalzielii*). Therefore, in the case of newly studied plants, various extracts are often prepared using solvents with a wide polarity range. The following is a summary of selected research articles that have reported on the anti-inflammatory activity of plant extracts **(Azab *et al*., 2016)**.

The anti-inflammatory effects of *Quercus* species have been widely studied. The anti-inflammatory activities of the leaves infusion of *Q. sideroxyla*, *Q. durifolia*, and *Q. eduardii* have been evaluated in HT-29 cells. The results demonstrated that *Q. sideroxyla* modulated the expression of NF-Kb, leading to decreased levels of the inflammatory markers COX-2 and IL-8. *In vitro* studies have shown that triterpenes isolated from acorns of *Quercus* serrata var. brevipetiolata inhibit nitric oxide (NO) production and other pro-inflammatory cytokines. Additionally, lupeol isolated from white oak leaves (*Quercus resinosa*, *Q. grisea*, *Q.laeta*, and *Q. obtusata*) was evaluated for its ability to inhibit COX-1 and COX-2 enzymes using the *in vitro* colorimetric COX (ovine) inhibitor assay. The study found that lupeol from *Q. obtusata* had a differential effect in inhibiting COX-2 without affecting COX-1. Furthermore, the bark of *Quercus* gilva Blume yielded (−)-epicatechin, procyanidin B3, and procyanidin B4, which demonstrated anti-inflammatory and antioxidative properties. These three compounds exhibited dose-dependent inhibitory activities on the gene expression of COX-2 and IL-1β. Studies

indicate that flavonols, such as quercetin glucuronide and kaempferol 3 O-glycoside, are glucuronidated by the kombucha consortium. These metabolites has been found to be effective antioxidant and anti-inflammatory agents in human macrophages **(Taib *et al*., 2020).**

Part II

Experimental study

Chapter II: MATERIALS AND METHODES

II. Materials and methods

The work was carried out in the biochemistry teaching laboratory (Faculty of SNV & STU) at Université 8 Mai 1945 in Guelma, Algeria. The aim was to evaluate the antioxidant, anti-fungal and anti-inflammatory potential of different extracts of *Quercus* leaves and acorns.

II.1. Vegetal material

- **Drying and crushing**

The material used in our study consists of *Quercus* leaves and acorns. The plant was collected in the Guelma region MAOUNA in December 2023.

II.2. Sample preparation and phenolic Compound Extraction

Due to the intricate nature of the majority of samples, the method employed for their preparation exerted a discernible influence on the outcomes of the entire extraction process. Several standard sample preparation methods, such as drying, homogenisation, filtration, and grinding, were commonly employed prior to the extraction process.

Regarding the oak acorn and leaves samples, their preparation before the extraction of phenolic compounds consisted of cleaning, drying, and grinding. The cleaning of the acorn and leaves samples was conducted using a variety of methods, including washing and tap water. The acorn and leaves were air-dried at room temperature, in the shade for approximately 15 days. The grinding of the plant material into fine particles was conducted using either an electric grinder or a vibrating-type ultrafine grinder (electric coffee grinder CB-MAC-7120) **(Banc *et al.*, 2023)**.

Figure 8: leaves and acorns of *Quercus* plant.

II.2.1 Extraction method

The solvent extraction method was a common approach for the preparation of crude extracts. Phenolic compounds were extracted through the utilization of solvents with varying degrees of polarity, including methanol, ethanol, and water. Among the conventional extraction techniques employed were maceration extraction, decoction technique, digestion technique, and ultrasound technique **(Banc *et al.*, 2023).**

II.2.2. Preparation of samples:

II.2.2.1 ultrasound-assisted extraction

Ultrasound-assisted extraction involves using sound waves at frequencies ranging from 20 kHz to 2 MHz. These waves affect elastic mediums like liquid solvents and plant tissues, creating cavitation bubbles that collapse and release energy, generating localized high-pressure

and temperature zones. This phenomenon, known as cavitation effect, facilitates the extraction of phytochemicals from plant materials. as illustrated in **Figure 9**.

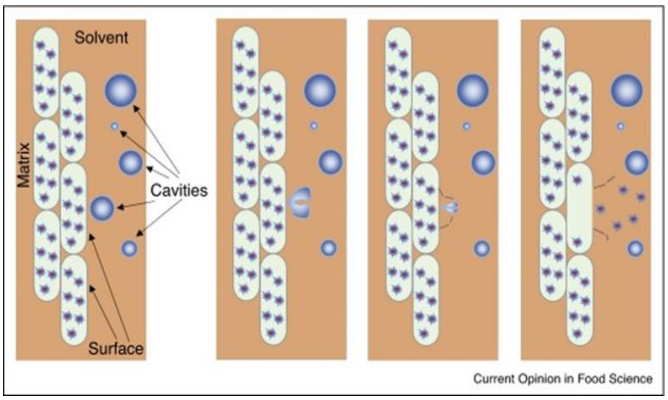


Figure 9: Schematic representation of the mechanism of releasing active components from cells using ultrasound waves **(Panja, 2018).**

Ultrasonic assisted extraction (UAE) was performed at 40 kHz ultrasonic power. Briefly, 57g of leaves and 33g acorn was placed in a volumetric flask containing 100 mL of water (100%) or ethanol/water (70:30 v/v) solvent. The extraction mixture have been ultrasonicated for 15 min at a constant, which was monitored throughout the extraction times, to ensure proper and reproducible recovery of bioactive compounds from the extracted matrix **(Tanase *et al*., 2022)**. The extraction mixture has been subjected to further centrifugation, after which the supernatants were recovered. The ethanolic and aqueous extracts were concentrated under reduced pressure (using a rotary evaporator R-215 (Buchi)) in order to evaporate the ethanol and water. Finally, both aqueous and concentrated ethanolic extracts were freeze-dried using a freeze dryer, two dried extracts has been obtained: QAE-UAE and QEE-UAE. The aqueous extract of *Quercus* was obtained through ultrasound-assisted extraction, designated as QAE-UAE. Similarly, the ethanolic extract of *Quercus* was obtained through ultrasound-assisted extraction, designated as QEE-UAE **(Tanase *et al*., 2023).**

II.2.2.2 Preparation of aqueous extracts

The aqueous extraction was conducted using the decoction method, which is a classical method. Demineralised water (DM) was added to the coarse ground powder in a stainless-steel extractor and boiled for two hours. The resulting filtrate was then filtered through a 149 µm nylon cloth with a mesh size of 60. This process was repeated twice to ensure a complete extraction. The obtained filtrate was concentrated in a vacuum tray drier at 45°C under reduced pressure **(Saini *et al*., 2023)**.

II.2.2.3 Preparation of hydro-alcoholic extracts

Involved the powder being taken in a round-bottom flask fitted with a condenser and refluxed for five hours using water and alcohol (70:30) as a solvent ratio. The filtrate was then filtered through (60 mesh) 149-micron nylon cloth. This process was repeated twice after three hours, using a fresh solvent, until the drug appeared exhausted. The filtrate was concentrated by a vacuum tray drier at 45°C and under reduced pressure. After drying, the extracts (aqueous and hydroalcoholic) were stored in airtight containers for subsequent use **(Saini *et al*., 2023)**.5

The extraction rate is calculated using the following formula:

Extraction rate (%) = [W1 – W0 /S] ×100

W1: weight of dry material after evaporation.

W0: initial weight of flask

S: initial sample weight (powder in grams)

II.3 Phytochemical Screening

Test for Phenols:

The different plant extracts were mixed with 2 mL of 2% FeCl3 solution and observed for the formation of a blue, green or black color **(Shrestha *et al*., 2024)**.

Flavonoids test:

Two hundred milligrams of each extract was dissolved in sodium hydroxide, after which hydrochloric acid was added. The transformation of the solution from yellow to colourless confirmed the presence of flavonoids **(Adil *et al*., 2024**).

Test for alkaloids:

A quantity of approximately 15 mg of each extract (acorn and leaf) was separately stirred with 1% hydrochloric acid (6 mL) on a water bath for 5 minutes and filtered. These filtrates were divided into three equal parts.

Mayer's test: One portion of the filtrate was treated with Mayer's reagent (potassium mercuric iodide solution) (1 mL). The formation of a cream-colored precipitate indicates the presence of alkaloids **(Iqbal *et al*., 2015)**.

Test for terpenoids:

The Salkowski test was employed to identify the presence of terpenoids. A crude extract (approximately 100 mg) was separately shaken with chloroform (2 mL) and concentrated H_2SO_4 (2 mL) was added to the test tube alongside the extract. The formation of a reddish brown coloration at the interface indicated the presence of terpenoids **(Iqbal *et al*., 2015).**

Test for tannins:

The presence of tannins is detected by adding 1 ml of water and 1 to 2 drops of 1% $FeCl_3$ solution to 1 ml of each extract. The appearance of a dark green or blue-green color indicates the presence of tannins. The appearance of a dark green color indicates the presence of catechic tannins. The appearance of a blue-green color indicates the presence of gallic tannins **(EL-Haoud *et al.*, 2018)**.

Test for Saponins:

Approximately 200 mg of extract was obtained from the plant sample and placed into test tubes. Distilled water (10 mL) was added each extract and boiled. The froth persisted for more than three minutes, indicating the presence of saponin **(Adil *et al*., 2024)**.

Carbohydrate test:

Fehling's test

Distilled water was added to each sample to extract the solution, which was then filtered. A small quantity of Fehling's solution A and B was added to the filtrate, which was then boiled for a period of time. The precipitation of a brick-red color indicated the reduction in the presence of sugars **(Adil *et al*., 2024).**

II.4 Determination of Total Phenolic content

Total polyphenols are determined using the Folin-Ciocalteu method, which is the most widely used method for this purpose **(Azab *et al*., 2016)**.

- **Principle:**

The method is based on the oxidation of phenolic compounds by the Folin-Ciocalteu reagent in a basic medium. The reagent is a complex of phosphotungstic acid and yellow phosphomolybdic acid, and its colorimetric properties change when it is complexed with certain

molecules. The reagent reacts with the -OH function of phenols, resulting in the formation of a new blue molybdenum-tungsten complex that absorbs at 760 nm. The coloration's intensity is directly proportional to the amount of polyphenols found in the plant extracts **(SAIDI, 2019)**.

- **Protocol:**

To determine the total phenolic content, 125µL of each extract at defined initial concentrations were mixed with 500µL of distilled water and 125µL of Folin-Ciocalteu reagent diluted 10-fold. After vigorous shaking, the mixture was left to stand for 6 minutes. Next, 1250 µl of 7% sodium carbonate (Na_2CO_3) solution was added, and the final volume was adjusted to 3ml with distilled water. The tubes were kept in the dark at room temperature for 90 minutes, and the absorbance was measured at 760 nm. The extracts' total phenolic content was determined using the gallic acid standard curve, which was conducted under the same analytical conditions. The total polyphenol content of the extracts is expressed as milligrams (mg) of gallic acid equivalent per gram (g) of dry matter weight (mg EAG/g DW) **(SAIDI, 2019).**

II.5 Determination of Total Flavonoid content

The objective was to quantify flavonoids in extracts of Quercus and seeds using the aluminium trichloride method **(Djamilatou *et al*., 2021)**.

- **Principle**

The fundamental principle of this colorimetric method is that aluminium chloride forms stable acid complexes with the C4 ketone group and the C3 or C5 hydroxyl group of flavones and flavonols. It also forms labile acid complexes with the ortho-dihydroxyl groups in the A or B rings of flavonoids **(SAIDI, 2019)**.

- **Protocol**

25 µL of extract and 75 µL of ethanol were added, followed by the addition of 5 µL of an $AlCl_3$ solution (10% in methanol) and 140 µL of distilled water. The mixture was then stirred and left at room temperature for 30 minutes. The absorbance was measured against a blank prepared without $AlCl_3$. The total flavonoid content was calculated in terms of quercetin equivalents (mg EQ/g DW) by reference to the calibration curve plotted with 10, 20, 40, 60, 80

and 100 mg/L quercetin The method was employed to ascertain the total flavonoid content of the sample extracts. was combined with an equal volume of a 2% solution of AlCl3; 6H2O. after which the absorbance was measured at 430 nm **(Djamilatou *et al*., 2021)**.

II.6 Evaluation of antioxidant activity

The 1.1-diphenyl-2-picrylhydrazyl (DPPH) assay is commonly used to determine antioxidant activity and estimate the radical scavenging capacity of plant extracts. DPPH is a stable free radical that acts as a hydrogen radical scavenger. The assay measures the reduction of the DPPH radical by an antioxidant, which can be observed by measuring the decrease in absorbance at 517 nm **(Carmona-Jiménez et al., 2014; Baliyan et al., 2022).** Thus, Substances can convert DPPH (which is purple) into the non-radical form 1,1-diphenyl-2-picrylhydrazine (which is yellow).

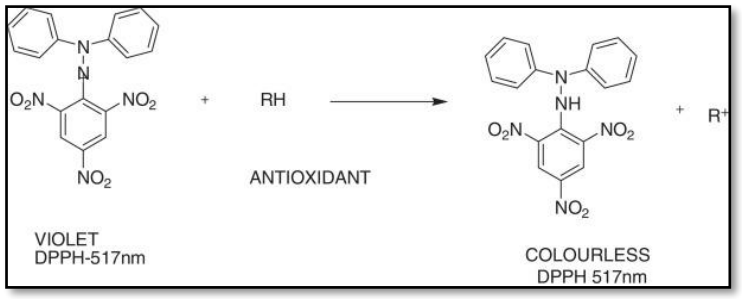


Figure 10: DPPH radical scavenging activity **(Nithya and Madhavi, 2017).**

- **Operating mode**

The scavenging ability of the extract towards the DPPH radical was determined using the method of **(Blois, 1958)** and **(Baliyan *et al*., 2022)** with few modifications.

- **Preparation of DPPH**

3.94 mg of DPPH (2,2-diphenyl -1-picrylhydazine) is dissolved in 100 ml methanol (CH3-OH) to obtain a stock solution of DPPH

- **Preparation of extracts and ascorbic acid standard range**

- **Dissolution:** Each 2 mg extract is thoroughly dissolved in 2 mL of solvent.
- **Preparation of concentrations:** From the dissolved extract solution, different concentrations are prepared by serial dilution, resulting in concentrations of 400 µg/ml, 200 µg/ml, 100 µg/ml, 50 µg/ml, 25 µg/ml, and 12.5 µg/ml.

- **Reaction setup:** Subsequently, 25 µL of each prepared extract solution is added to 975 µL of DPPH solution.
- **Incubation:** The reaction mixtures are allowed to incubate for 30 minutes at room temperature in darkness to facilitate the reaction between the extracts and DPPH.
- **Absorbance measurement:** Following the incubation period, the absorbance of each reaction mixture is recorded at 517 nm against a blank using a UV-visible spectrophotometer.

Note

- The mixture of methanol, DPPH and standard (BHA, BHT, a-tocopherol, ascorbic acid and quercetin) will serve as positive control for comparison.
- Methanol alone will serve as blank
- DPPH solution and methanol serves as negative control.

- **Percentage of DPPH radical inhibition**

The percentage of inhibition of radical scavenging activity was calculated using Equation 1. The concentration of each sample (represented as legends on the graph) and was obtained from the plotted graph of the percentage of inhibition versus the concentrations of samples.

Percentage of Inhibition (%) = (A control – A extract) / A control × 100 (Equation 1)

Where: A control is the absorbance of the positive control reaction and, An extract is the absorbance of the test samples (extract/standard).

II.7. Evaluation of antifungal activity

In this study, the antifungal properties of different plant extracts have been tested on the growth of the phytopathogenic fungus *Fusarium oxysporum f. sp lycopersici.* The fungus was identified by the team under the leadership of the head of the mycology laboratory at the "Biotechnology Research Center" in Constantine, Algeria.

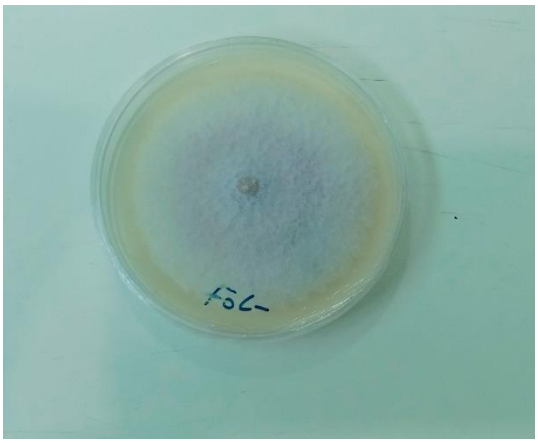


Figure 11: *Fusarium oxysporum f. sp lycopersici.*

The evaluation of the antifungal activity of the extracts was carried out using the agar disk diffusion method, in which disks were soaked with 25 µl of each extract.

II.7.1. Preparation of the culture medium

Prepar In the quest to provide a suitable medium for microbial culture, the preparation of potato medium stands as a pivotal procedure. The process begins by meticulously peeling and washing potatoes before cutting them into moderate-sized cubes.

These cubes are then subjected to boiling at 40°C using an isotemp, followed by filtration of the resultant potato water.

To enrich the medium, 20g of glucose is added to the filtered solution, which is then adjusted to the desired volume with distilled water. Subsequently, the mixture undergoes heating and agitation, with 20g of agar gradually introduced until transparency is achieved.

Crucially, the final volume of the medium is meticulously calibrated to 1 liter, ensuring consistency and reproducibility. Following preparation, sterilization of the medium is executed via autoclaving for a duration of 2 hours, safeguarding against contamination. Finally, the sterilized medium is carefully poured into labeled petri dishes, sealed, and readied for microbial inoculation.

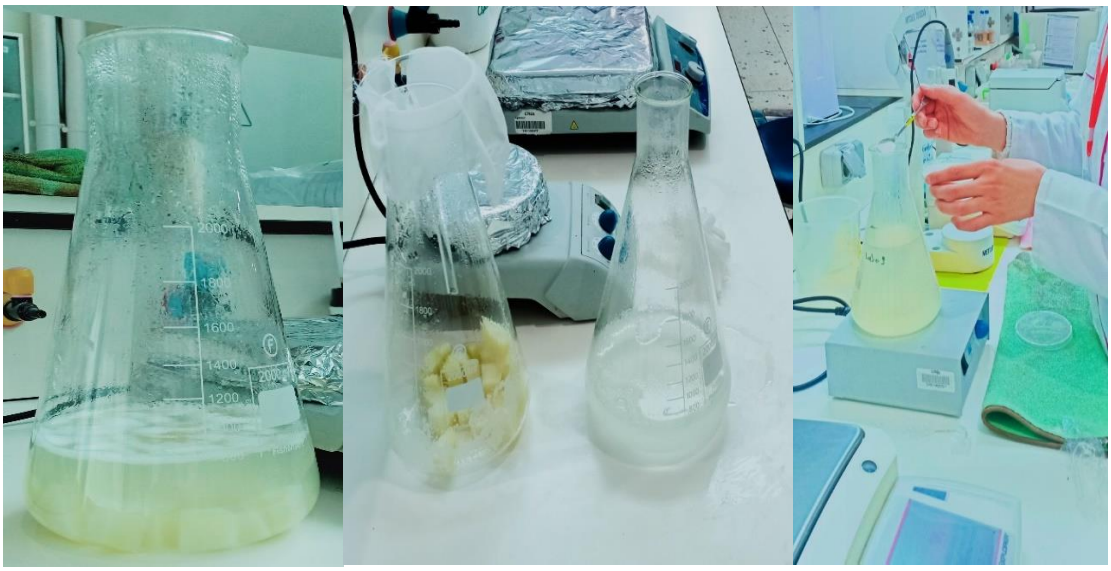


Figure 12: Preparation of the Culture Medium steps.

II.7.2. Preparation of extracts

To prepare the desired concentrations of the extract, initially dissolve the specified amount of extract in distilled water.

Following this, in an Eppendorf tube, weigh 20mg of the extract, then add 200 µL of the chosen solvent, typically distilled water. Subsequently, from the resulting mother solution, proceed to prepare a series of concentrations, including 100, 50, 25, and 12.5 mg/mL, thus establishing a range of concentrations for subsequent experimentation or analysis

- **Mycelial disc applications (Inoculation):**

Following the solidification of the culture medium, proceed to aseptically place a young colony of *F. oxysporum* mycelial discs, each with a diameter of 0.5 mm, at the center of a petri dish. Next, for each concentration of the extract, replicate the procedure three times by using a micropipette to add 25 microliters each time. Once completed, ensure the proper closure of the petri dishes before incubating them at 28°C for a duration of 48 hours.

- **Measurement of mycelial growth**

The mycelial growth of the phytopathogenic agent is measured in millimeters. The results are expressed as a percentage of radial growth inhibition compared to the mean average diameter of fungal colonies grown in a control medium (positive).

Thus, the inhibitory activity is expressed as a percentage and calculated according to the formula

Percentage of Radical Growth Inhibition= (*Dc*−*Dt* /*Dc*) ×100

Where: *Dc* is the mean diameter of fungal colonies in the control medium and,

Dt is the mean diameter of fungal colonies in the treatment medium.

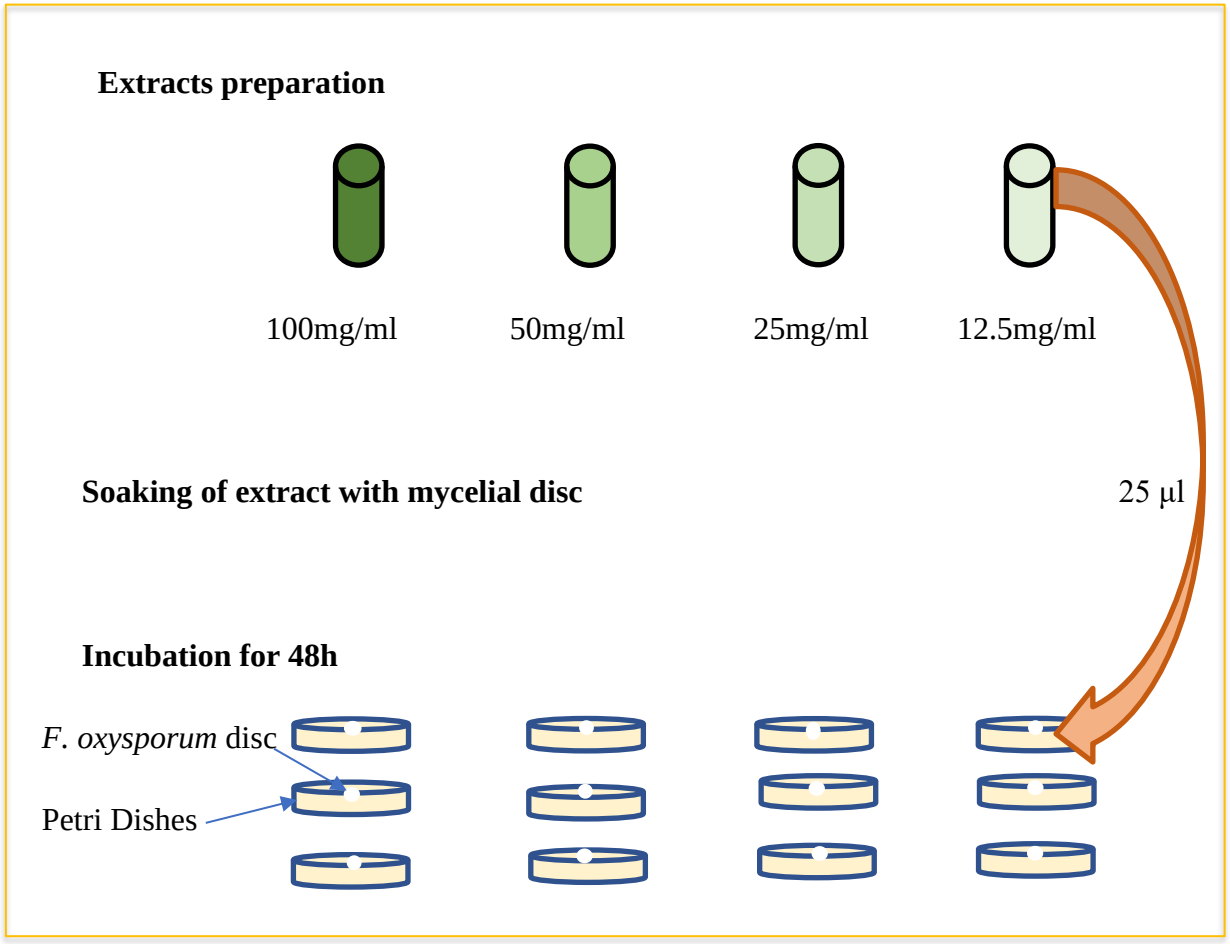


Figure 13: Antifungal activity report

***In-vitro* anti-inflammatory activity evaluation**

The bovine serum albumin 'BSA' denaturation inhibition test caused by heat (72°) has been applied with slight modifications **(Derbal and Fedali, 2015).**

- **Phosphate Buffer preparation (Annexe N° 01)**

The test solution (0.5 ml)

Consists of 0.45 ml of the Bovine Serum Albumin (BSS) 5% and 0.05 ml of each extract with a concentration of 250 µg/ml.

The control test solution (0.5 ml)

Consists of 0.45 ml of 5% BSA and 0.05 ml of distilled water.

The product control solution (0.5 ml)

Consists of 0.45 mL distilled water and 0.05 ml of each extract with a concentration of 250 µg/mL.

The standard test (0.5 ml)

Consists of 0.45 ml of 5% BSA and 0.05 ml of Aspirine standard solution with a concentration of 250 µg/mL.

All the above solutions were adjusted to pH 6.3 with a solution of HCl (1N), the samples were incubated at 37°C for 20 min, then the temperature was raised to keep the samples at 57° for 3 min. After cooling the tubes, 2.5ml of phosphate buffer saline solution (pH: 6.3) was added to the above solutions, and the absorbance was read by the spectrophotometer at 416 nm, and the percentage inhibition of protein denaturation was calculated as follows:

Percentage of inhibition = [100-(OD of test solution-OD of product control/ OD of test control)] × 100

II.8. Evaluation of antihemolytic activity

II.8.1. Preparation of the erythrocyte suspension

Fresh blood samples (approximately 8 ml) were collected in heparinized tubes, at the laboratory level, where the blood sample was taken, from volunteers. The various human blood samples collected are centrifuged at 3000 rpm, for 10 min, in order to eliminate the plasma and polymorphonuclear cells. Then, the RBC pellet is washed three times, with an equitable volume of iso-saline solution (0.9% NaCl). Washing involves centrifuging the pellet suspension for 10 minutes at 1400g. After this step, the volume was measured and reconstituted in the form of a 10% (v/v) suspension (RBC), with an iso-saline and used immediately.

II.8.2. Extract cytotoxicity test

Before undertaking the test of the antihemolytic activity of the plant extracts, a toxicity test is necessary, in order to target the concentrations to be used. The principle of this test is to bring red blood cells into contact with the extracts at different concentrations, in an isotonic solution and to monitor the level of Hb released by the hemolyzed cells, with the aim of evaluating the cytotoxicity of these extracts towards RBC.

- **Procedure**

The protocol followed is that of **Bulmus and colleagues (2003), (Djolu RD *et al*.,2023),** where a volume of 1.6 ml of the extract or/and aspirine (250-500-1000 µg/ml), reference molecule was mixed with a volume of 0.4 ml of RBC suspension (10%).

Two controls were carried out under the same conditions, replacing the extract with physiological water (negative control) or with distilled water (positive control) corresponding to 100% hemolysis. The reaction mixture was incubated at 37°C for 30 min, then centrifuged at 3000 rpm for 10 min and the absorbance of the released Hb was measured at 560 nm.

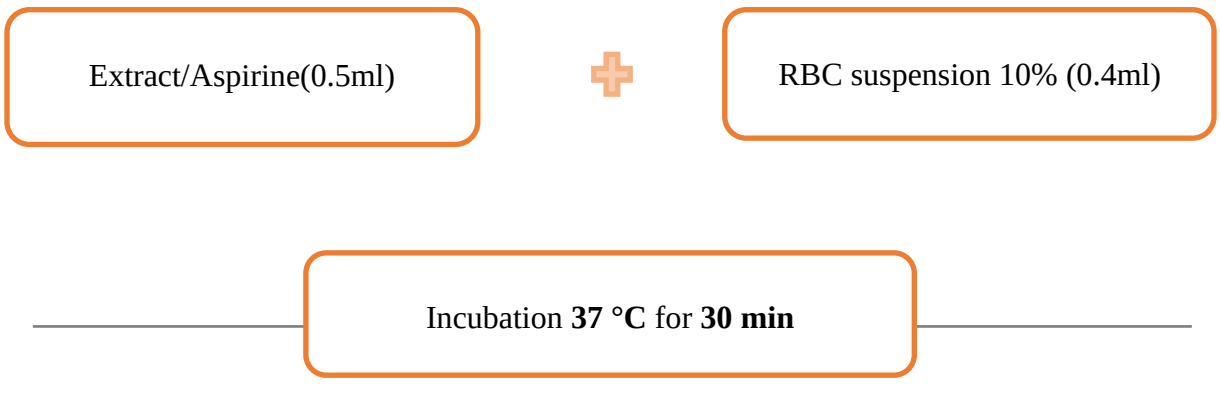
- **Expression of results**

The percentage of hemolysis was calculated from the formula

% hemolysis=(At/Ac) X 100

Where: Ac=Absorbance of the control; At Absorbance of the test.

The percentage of hemolysis is evaluated for each extract, by measuring the absorbance of the Hb released from the RBC by hemolysis, in comparison with the negative control (C-, solution of RBC in physiological water, having a rate of very low hemolysis, 15%) and positive control (C+, solution of RBC in distilled water to cause total hemolysis, 100% hemolysis).



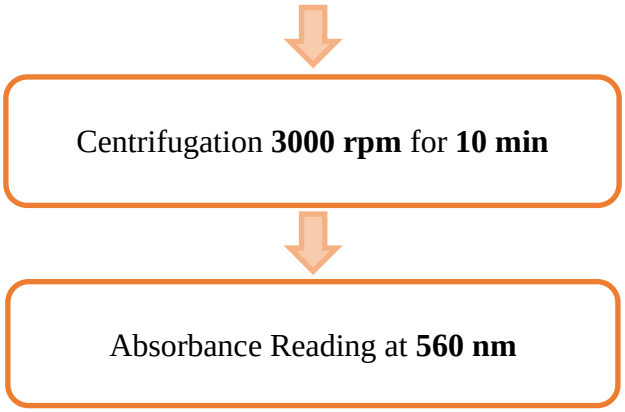


Figure 14: Summary diagram of the red blood cell membrane destabilization protocol

II.8.3. Antihemolytic activity

This test was carried out according to the method described by **(Sadique *et al,* 1989, Oyedapo *et al,* 2010), (Ganesh Gadamsetty *et al* ,2013).**

- **Procedure**

The reaction medium containing 0.5 ml of the extract, aspirin or gallic acid at different concentrations (125-250-500 µg/ml), mixed with 1.5ml of phosphate buffer (0.9% NaCl, pH=7.4) and 2ml of a hypo-saline solution (0.36% NaCl), is incubated at 37°C for 20 min. Then 0.5 ml of the RBC suspension (10%) is added to each concentration and a second incubation is carried out at 56°C for 1 hour.

Finally, the tubes are cooled under running water and followed by centrifugation at 2500rpm/for 5min. The absorbance of the supernatant is measured at 560 nm.

In parallel, a control is carried out by replacing the extract with 0.5 ml of phosphate buffer.

- **Expression of results**

The percentage of membrane stability was estimated from the following expression

% Membrane Stability = (Ac-At/Ac) X 100

where: Ac = Absorbance of control. At = Absorbance of the test

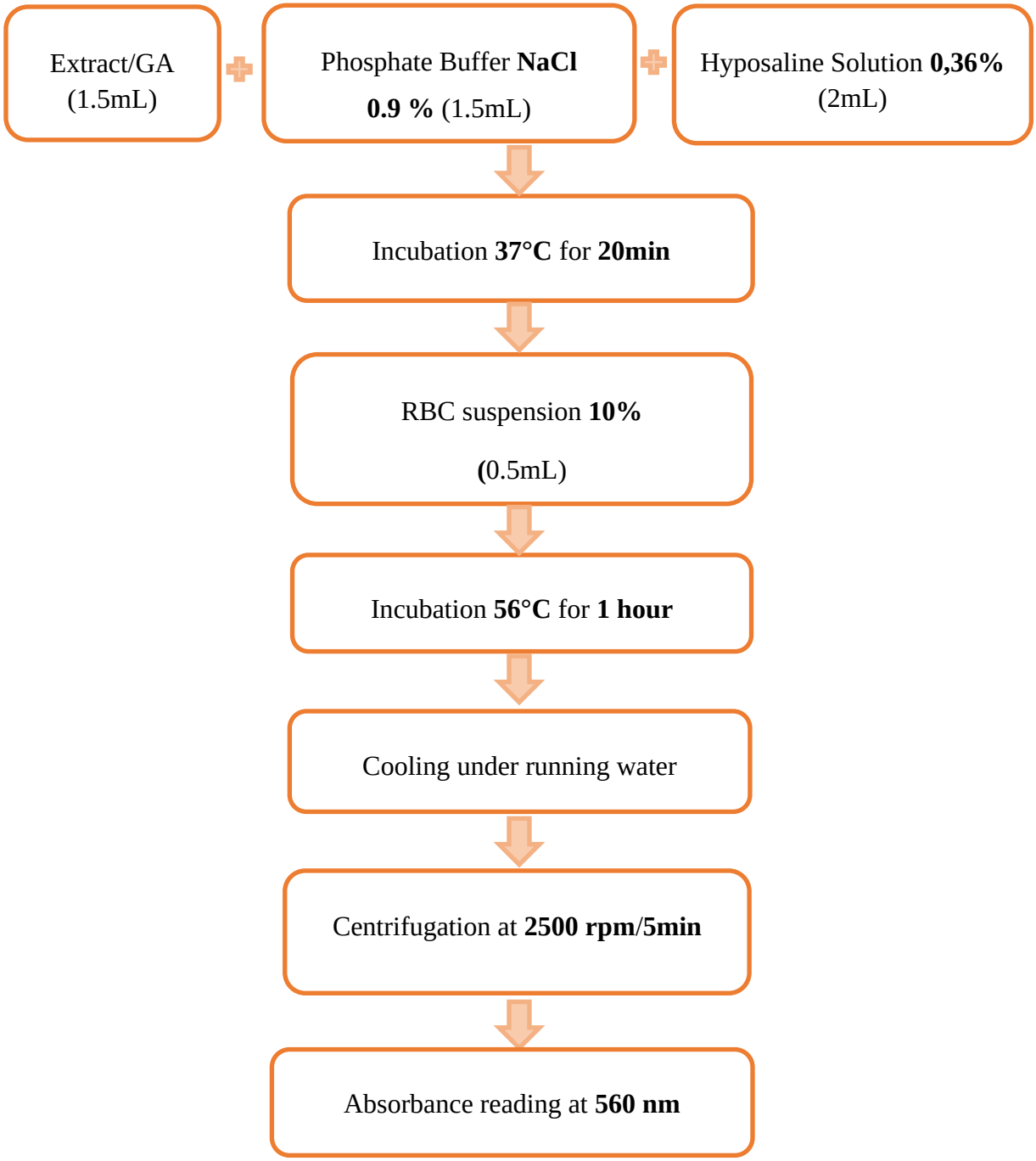


Figure 15: Summary diagram of the red blood cell membrane destabilization protocol

- **Statistical analysis**

The curves and histograms are plotted using Microsoft Excel 2019.

tests are expressed as mean ± standard deviation. Each experiment was performed in

triplicate.

Chapter III: Results and discussion

III. Results and discussion

III.1. Extraction yield

The extraction yield is an important measure in evaluating the efficiency of extracting desired compounds from plant materials. It is calculated as the mass of the dry extract obtained divided by the mass of the plant powder used, expressed as a percentage.

Table 1: *Querqus spp* extracts yield calculation

Extracts	**Mass of the dry extract (g)**	**Mass of the plant powder (g)**	**Yield**
LAF	71.05	75	0.95%
LEF	50.17	75	0.67%
LEU	46.28	75	0.62%
OEF	45.28	63	0.72%
OEU	41.02	63	0.65%

L: leaves**, O:** oaknut(acorn), **U:** ultrasound extraction, **E:** ethanolic extract, **A:** aqueous extract, **F:** filtration.

We can see from **table1.** that the extraction method used for LAF is relatively effective in extracting compounds from the plant material, yielding almost 1 gram of extract per 100 grams of plant powder. However, the lowest yield was found in LEU with 0.62%, indicating the least efficient extraction process.

These variations in yield can be attributed to differences in the extraction methods, the solubility of compounds in the solvents used, or the inherent properties of the plant materials themselves. For further optimization, one could explore different solvents, extraction times, or temperatures to potentially increase the yields.

Our results do not align with the findings of **(Spigno *et al*.,2007)**, as we achieved 100% water LAF effectiveness in extraction. The solvent used in their study for extracting bioactive compounds from Q. infectoria consisted of 70% ethanol and 30% water. It has been reported

that alcoholic solvents are commonly recommended for extracting phenolics from natural sources because they yield higher amounts of total extract compared to other solvent types.

III.2. Phytochemical screening

The table below shows the presence and relative abundance of various phytochemical groups in different plant extracts.

Table 2: phytochemical screening of *Quercus* extracts.

Extracts / **Phytochemical Groups**	LEU	LAU	OEU	OAU	ELF	EOF	LAF	OAF
Phenols	+	+	+	+	+	+	+	+
Flavonoids	+	+	+	+	+	+	+	+
Alkaloids	+	+	+	+	+	+	+	−
Terpenes	+	+	+	−	+	+	+	−
Saponins	+	+	+	+	+	+	+	+
Tannins	+ **Catechic**	+ **Catechic**	+ **gallic**	+ **Gallic**	+ **catechic**	+ **Gallic**	−	−
Carbohydrates	+	+	+	+	+	+	+	−
Lipid	−	−	−	−	−	−	−	−

(-): Negative**, (+):** Present**, L:** leaves**, O:** acorn, **U:** ultrasound extraction, **E:** ethanolic extract, **A:** aqueous extract, **F:** filtration.

Across all tests, lipids were absent in all the extracts. Polyphenols showed a low presence in acorn extracts and a high presence in leaf extracts. Flavonoids were more abundant in leaf extracts compared to acorn extracts, except for the aqueous seed extract. Saponins and carbohydrates were found in high amounts in all aqueous and ethanolic extracts. Catechic tannins were present in the leaf extracts, while gallic tannins were found in the acorn extracts

but were absent in the aqueous extracts. Additionally, alkaloids and terpenes were present in all extracts, except for the aqueous acorn extracts.

Our findings were in accordance with those of a previous study, which revealed the presence of diverse groups of compounds, including saponins, alkaloids, tannins, glycosides, triterpenes, phenols and flavonoids, in various extracts of *Quercus* **(Yusof & Abdullah, 2020).**

The results obtained for acorns are consistent with those of **Al Hawani et al. (2020)**, who identified the presence of saponins, alkaloids, phenols, tannins, and carbohydrates in ethanolic extracts. However, **Al Hawani et al. (2020)** reported the absence of flavonoids, whereas our findings showed a significant presence of flavonoids in the ethanolic extracts **(Al Hawani *et al*., 2020).**

Regarding the leaves, our results differ from those of **Djellit and Moualed (2021)**, who found that almost all ethanolic and aqueous extracts lacked flavonoids. Our findings suggest that the phytochemical composition of *Quercus* plants varies widely due to the presence of numerous species distributed globally. These species exhibit different characteristics depending on their environment and species **(Djellit & Moualed, 2021)**.

A phytochemical analysis of the plant extracts revealed the presence of constituents known for their biological and physiological activities. These include phenols, tannins, flavonoids, saponins, carbohydrates, terpenoids, and alkaloids. Phenolic compounds are one of the largest and most widespread groups of plant metabolites. They possess various biological properties such as anti-apoptosis, anti-aging, anti-carcinogen, anti-inflammation, anti-atherosclerosis, cardiovascular protection, and improvement of endothelial function. Numerous studies have highlighted the antioxidant properties of medicinal plants rich in phenolic compounds, including flavonoids, phenolic acids, and tocopherols. Tannins bind to proline-rich proteins, interfering with protein synthesis. Flavonoids, hydroxylated phenolic substances synthesized by plants in response to microbial infection, have demonstrated antimicrobial activity against a wide range of microorganisms. This activity is likely due to their ability to complex with extracellular and soluble proteins and bacterial cell walls. Flavonoids also exhibit strong antioxidant and anticancer properties **(Yadav & Agarwala, 2011)**.

The plant extracts also contain saponins, known for their inhibitory effect on inflammation. Saponins can precipitate and coagulate red blood cells, form foams in aqueous solutions, exhibit hemolytic activity, bind cholesterol, and have a bitter taste. Alkaloids, long

associated with medicinal uses, are known for their cytotoxicity. They possess analgesic, antispasmodic, and antibacterial properties. Additionally, glycosides are recognized for their ability to lower blood pressure **(Yadav & Agarwala, 2011)**.

III.3. Determination of total phenolic compounds

Polyphenols are among the most significant groups of secondary plant metabolites, with extensive research highlighting their biological activities, particularly their antioxidant and free radical scavenging properties.

The total polyphenolic content of the plant extracts was determined using a colorimetric method with the Folin-Ciocalteu reagent. Polyphenol concentration was estimated from calibration curves based on gallic acid and expressed as micrograms of gallic acid equivalents per milligram of dry extract (mg GAE/g extract).

A calibration curve was established using a linear regression formula ($y = 0.1219x + 0.5024$) with a coefficient of determination ($R^2 = 0.9624$). The results, presented as a histogram in **Figure 16**, provide a visual representation of the polyphenol content in the various plant extracts.

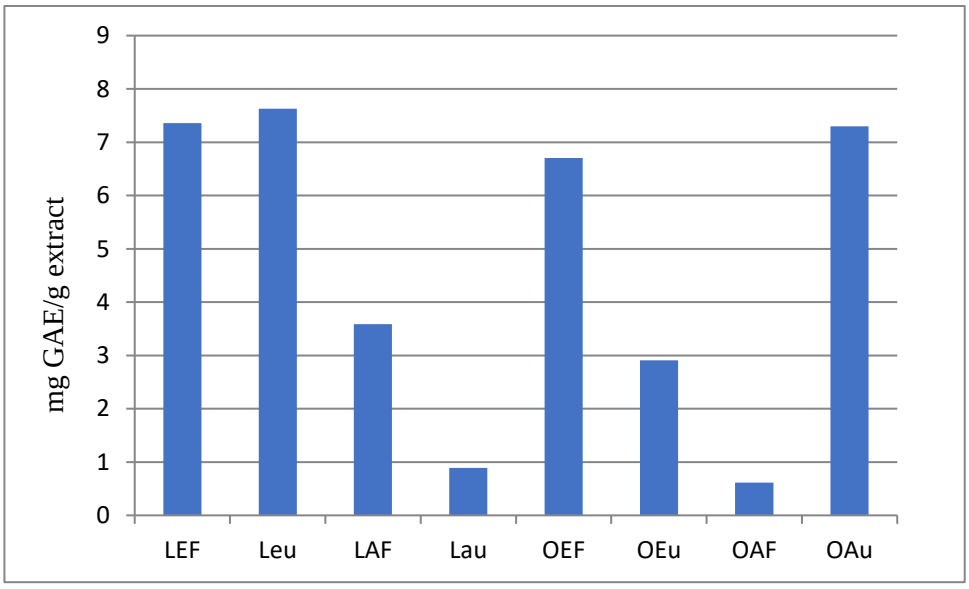


Figure 16: histogram showing the polyphenol content

Table 3: Total polyphenol content of various extracts from *Quercus spp.*

Plant part	Extracts	Total polyphenols (mµg EAG/g DM)
	LEF	7.360±0.217
Leafs	LEU	7.631±1.052
	LAF	6.701±0.277
	LaU	7.300±0.208
	OEF	3.589±0.833
	OEU	0.888±1.186
Acorn	OAF	2.908±0.312
	OAU	0.614±0.263

The **table 3** presents the total polyphenol content in various extracts from different parts of *Quercus* species, expressed in milligrams of gallic acid equivalents per gram of dry matter (mg GAE/g DM).

A significant difference was noted in the phenolic compound levels between the leaves and acorns. All extracts examined in this study (**table 2**) showed the presence of phenolic compounds. The findings outlined in Table 2 highlight the ethanolic extract of leaves obtained via ultrasonication (LEU) as the richest in polyphenols (7.631±1.052 mg GA/g extract), closely followed by the ethanolic extract (LEF) and the aqueous ultrasonic extract (LAU). The ranking of extracts based on their polyphenol content was as follows: LAu (7.360±0.217 mg GA/g extract), LAF (6.701±0.277 mg GA/g extract), and LAU (7.300±0.208 mg GA/g extract). This contrasts sharply with the lowest phenolic content observed in the acorns, ranging from 3.589 ± 0.833 mg GA/g extract to 0.614±0.263 mg GA/g extract.

The dosage of polyphenols depends on several factors, including temperature, storage conditions, and the extraction method and solvent used. It's important to recognize that solvents play a critical role in the solubility of phenolic compounds **(Tsao, 2010).**

The results obtained in this study align with those reported by **Djellit *et al*. (2021)**, who found values ranging from 8.50 to 2.63 ± 0.08 mg GA/g extract. These findings are consistent with the outcomes of our study.

Another study by **Vinha *et al*. (2016)** showed that the total polyphenol content in acorns of various *Quercus* species ranged from 18 to 32 µg GA/mg extract, considerably lower than our research findings. These disparities could be due to differences in the distribution of phenolic compounds among different plant parts or variations in climatic conditions **(Males *et al*., 2010)**.

III.4. Determination of flavonoid compounds

The quantification of flavonoids was determined using the aluminium trichloride method, with a calibration curve of quercetin. A linear trend curve was established with the formula y= 0.1419 and a coefficient of determination R^2= 0.9989 **(SAIDI, 2019)**.

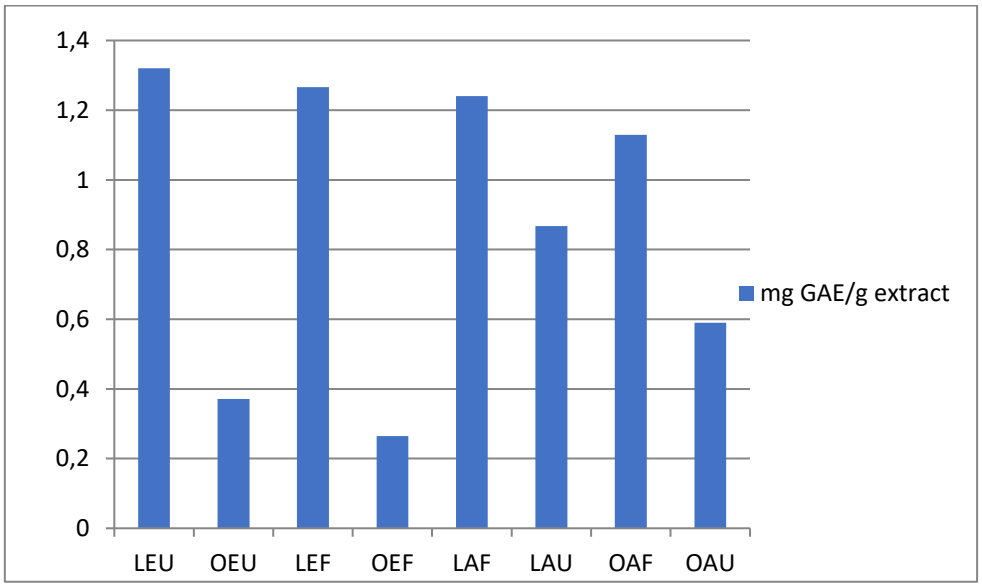


Figure 17: histogram showing the flavonoid content.

Table 4: Total flavonoid content of various extracts from *Quercus spp.*

Extract	Flavonoids (mg QE/g DM)
LEU	1.320±0.213
LEF	1.266±0.132
LAF	1.240±0.08
LAU	0.867±0.0928
OEU	0.371±0.004
OEF	0.265±0.066
OAF	1,129±0.158
OAU	0.590±0.122

The impact of solvents on total polyphenols differs from their effect on flavonoids. In both leaf and acorn fractions, the quantities of flavonoids are lower compared to total polyphenols. Among the various solvents, ethanolic fractions are notably richer in flavonoids for both plant parts. Nonetheless, all fractions exhibit varying levels of flavonoids, with leaves demonstrating the highest concentration **(SAIDI, 2019)**.

The total flavonoid content of *Quercus* extracts was measured, with ethanolic extracts exhibiting the highest flavonoid content across different solvents. Specifically, in descending order, ethanolic leaf extracts (LEU) displayed the highest flavonoid content, averaging 1.320±0.213 mg EAG/g, followed by ethanolic leaf extracts (LEF) with an average of 1.266±0.132 mg EAG/g. Aqueous extracts showed lower flavonoid amounts, with 1.240±0.08 mg EAG/g for leaf extracts (LAF) and 8.67 ± 0.0928 mg EAG/g for leaf extracts (LAU). In contrast, the flavonoid content of acorn extracts was generally lower than that of leaf extracts, with ethanolic extracts of acorn measuring 0.265±0.066 mg EAG/g for (OEF), except for one sample which exhibited the highest total flavonoid content among all the acorn extracts at 1,129±0.158 mg EAG/g for (OAF).

Leaf extracts generally showed higher flavonoid content compared to acorn extracts, with (LEU) exhibiting the highest flavonoid concentrations among all extracts. Conversely, acorn extracts demonstrated lower flavonoid content overall, showing a notably low flavonoid concentration. These results indicate variations in flavonoid content among different extracts from *Quercus* species, suggesting potential differences in their chemical compositions and biological activities.

These findings corroborate those of **Meziti *et al*. (2019)**, indicating that the flavonoid content of *Quercus* extracts is notably lower than the polyphenol content (3.11±0.04 µg EQ/mg extract).

Flavonoids constitute one of the most abundant groups of natural compounds and are arguably the most significant class of natural phenolic compounds. They boast a wide range of chemical and biological activities, including radical scavenging properties **(Djeridane *et al*., 2010)**.

III.8. DPPH antioxidant activity

The DPPH method was used to assess the antioxidant activity of both *Quercus spp*. leaves and fruits. The color transition from violet to yellow indicated the varying concentrations of the samples. Subsequently, absorption measurements were obtained using a spectrophotometer at 517nm. With the derived equation, the percentage of inhibition for each sample have been calculated.

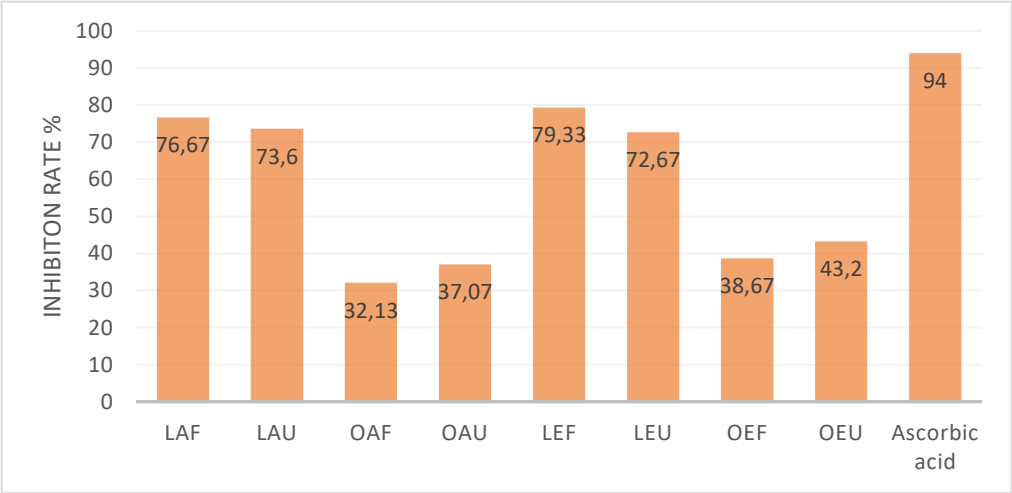


Figure 18: inhibition percentage of *Querqus spp* leaves and acorns.

According to the results depicted in Figure 15, the LEF extract exhibits the highest antioxidant activity with an inhibition rate of 79.33%. This could be attributed to its rich phenolic compound content, as demonstrated in studies by **(Phung *et al*.,2016)**, which found a strong correlation between phenol content and antioxidant capacity. Additionally, research by **(Aleebrahim-Dehkordy *et al*.,2019)** indicates that the alcoholic extract of *Quercus brantii* leaves possesses potent antioxidant properties.

Leaves demonstrate greater antioxidant activity compared to acorn fruit. Leaf extracts obtained through filtration (LEF) exhibit slightly higher antioxidant activity than those obtained through ultrasound (LAU, LEU), both in hydro-ethanolic and ethanolic solutions.

Ultrasonically extracted fruit samples show slightly higher antioxidant activity compared to those obtained through maceration, regardless of the solvent used. Conversely, ethanol solutions of acorn fruit (OEF: 38.67%, OEU: 43.2%) exhibit relatively higher antioxidant activity compared to aqueous ethanol solutions (OAF: 32.13%, OAU: 37.07%).

(Tahmouzi, 2014) reported that *Q. ilex* acorn extract provides protection against lipid oxidative degradation and protein carbonylation, likely due to the robust antioxidant activity of acorn polyphenols.

Antifungal activity

Our focus in this study is specifically on evaluating the inhibition of fungal growth caused by *Fusarium oxysporum f. sp lycopersici*, a notorious fungal pathogen known to affect tomato plants. To measure the antifungal activity, we have monitored the colony diameter of the fungi.

Table 5: the effects of *Quercus spp.* leaf extracts on the growth of *Fusarium oxysporum f. sp lycopersici.*

Extract	Concentration	Colony diameter (mm)	Inhibition rate (%)
LAF	100mg/ml	1,59	20,5
	50mg/ml	1,83	8.5
	25mg/ml	1,84	8
	12.5mg/ml	1,93	3.5
LAU	100mg/ml	1,53	23,5
	50mg/ml	1,83	8,5
	25mg/ml	2	0
	12.5mg/ml	2	0

LEF	100mg/ml	1,77	11,5
	50mg/ml	1,83	8,5
	25mg/ml	1.87	6,5
	12.5mg/ml	1,87	6,5
LEU	100mg/ml	1,79	10,5
	50mg/ml	2	0
	25mg/ml	2	0
	12.5mg/ml	2	0
Control (+)		2	
Control (-)		2.3	

Table 6: Quercus acorn the effects of *Quercus spp.* acorn extracts on the growth of *Fusarium oxysporum f. sp lycopersici.*

Extract	Concentration	Colony diameter (mm)	Inhibition rate (%)
SAF	100mg/ml	1.63	18,5
	50mg/ml	1.77	11,5
	25mg/ml	1.77	11,5
	12.5mg/ml	1.75	12,5
SAU	100mg/ml	1.67	16,5
	50mg/ml	1.8	10
	25mg/ml	1.8	10
	12.5mg/ml	1.7	15
SEF	100mg/ml	1.79	10,5
	50mg/ml	1,96	2
	25mg/ml	1.96	2
	12.5mg/ml	2	0
SEU	100mg/ml	1,83	8,5
	50mg/ml	1,83	8,5
	25mg/ml	2	0

	12.5mg/ml	2	0
Control (+)		2	
Control (-)		2.3	

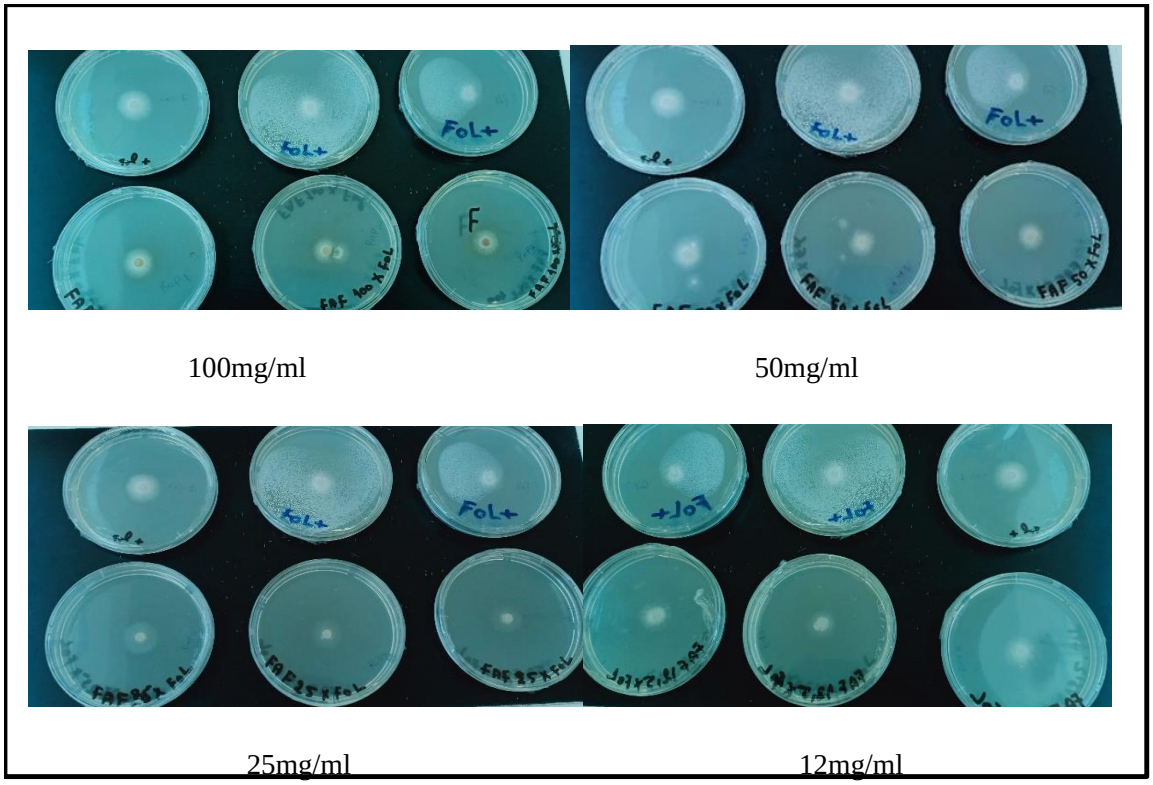


Figure 19: Antifungal activity of leaf aqueous extract of *Quercus spp.*

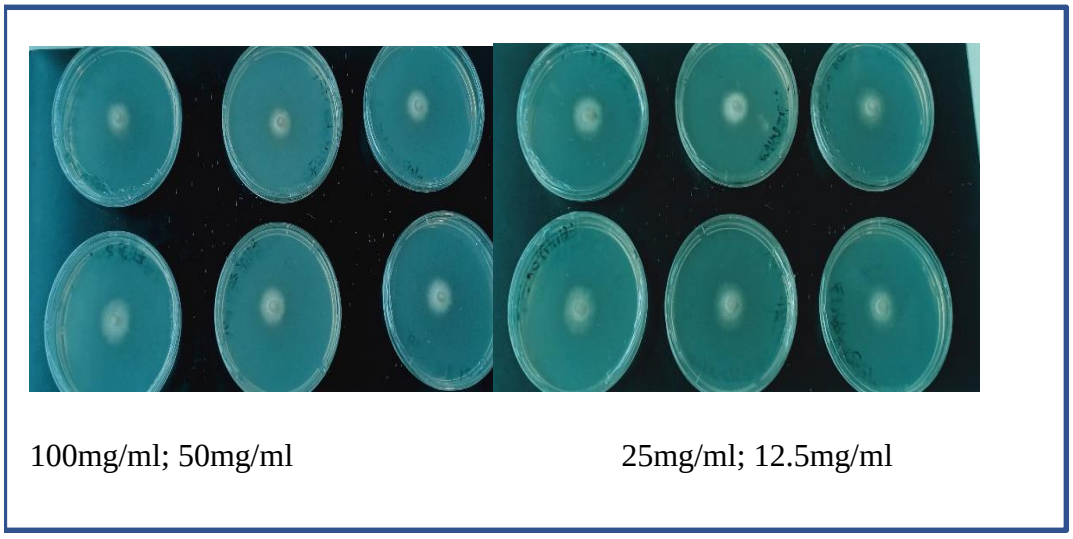
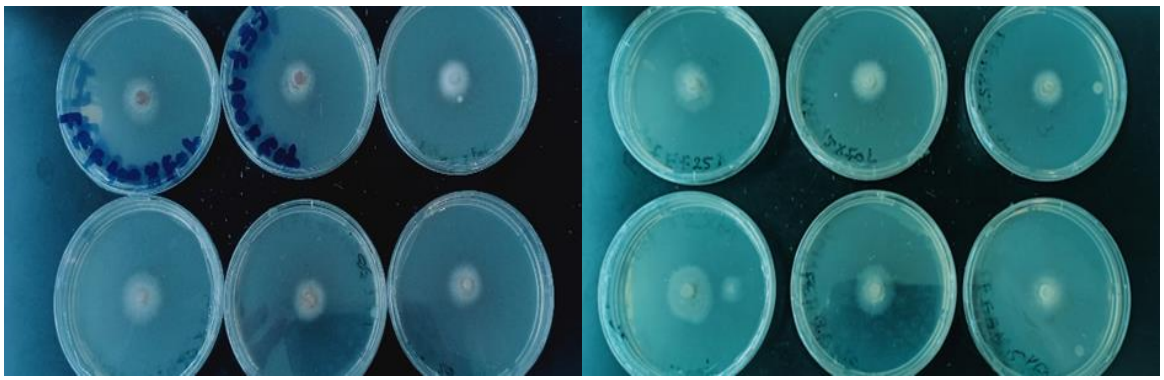
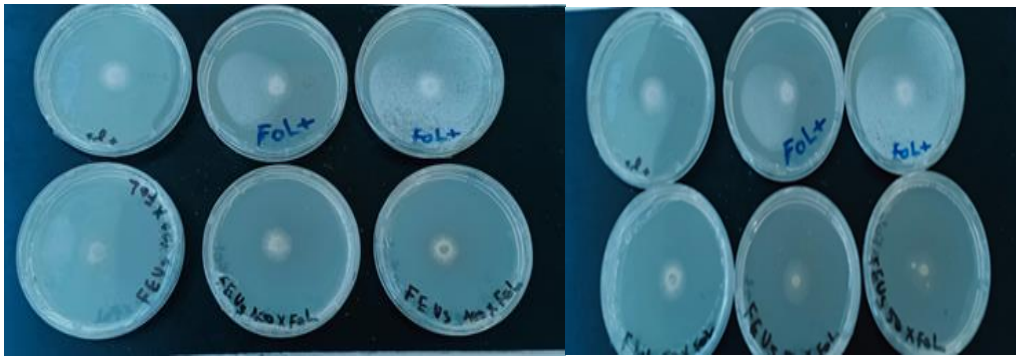


Figure 20: Antifungal activity of ultrasonicated leaf aqueous extract of *Quercus spp.*



100mg/ml; 50mg/ml 25mg/ml; 12.5mg/ml

Figure 21: Antifungal activity of leaf ethanolic extract of *Quercus spp.*



100mg/ 50mg/ml

Figure 22: Antifungal activity of ultrasonicated leaf ethanolic extract of *Quercus spp.*

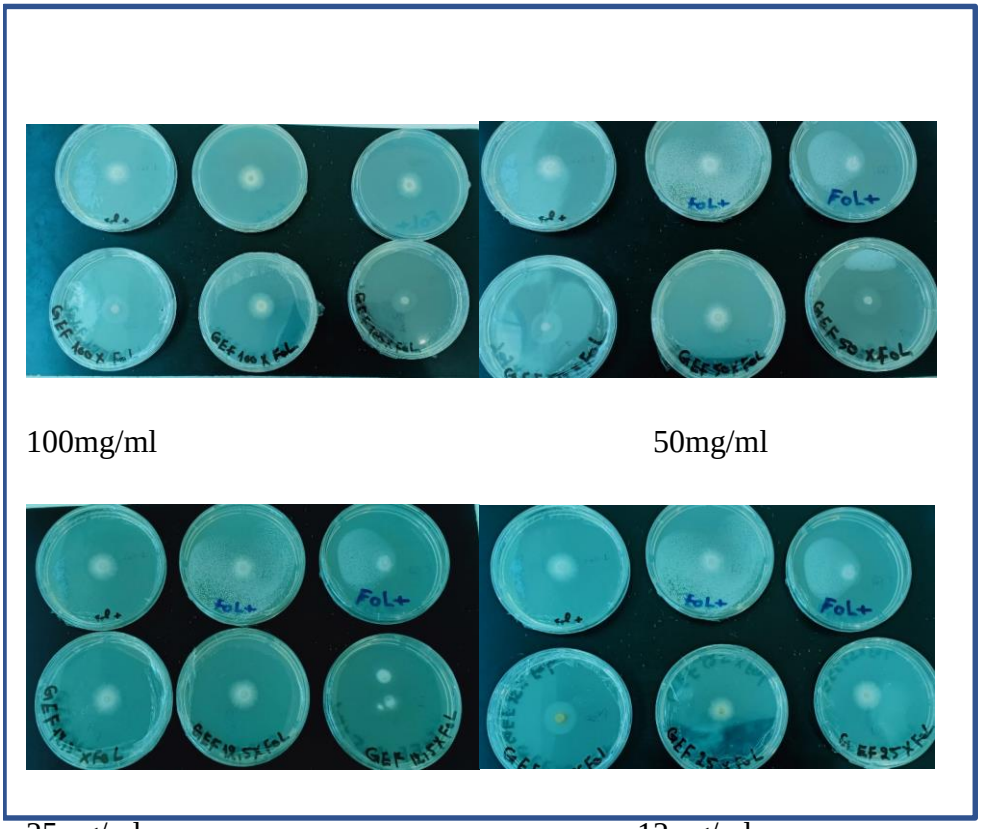


Figure 23: Antifungal activity of macerated acorn ethanolic extract of *Quercus spp.*

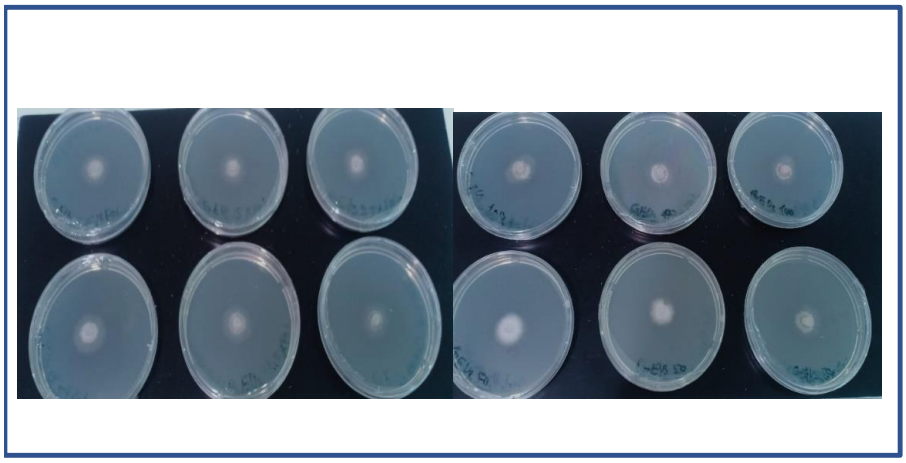
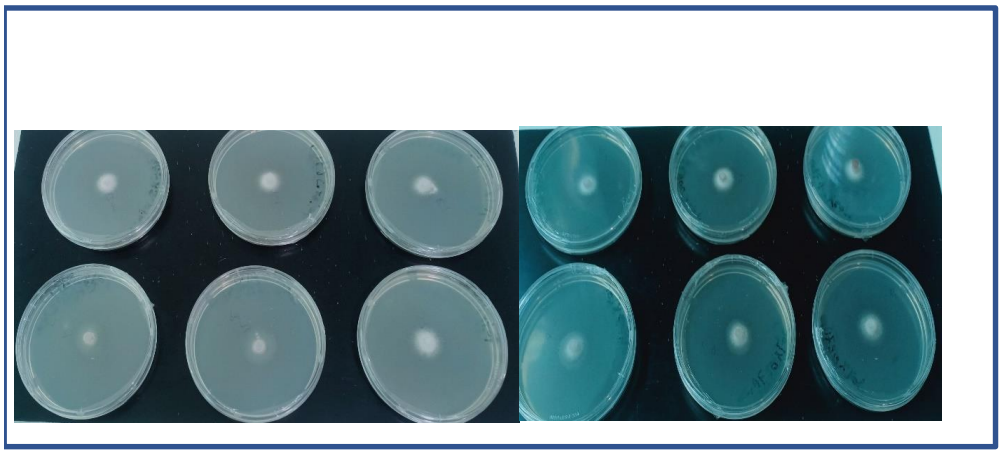
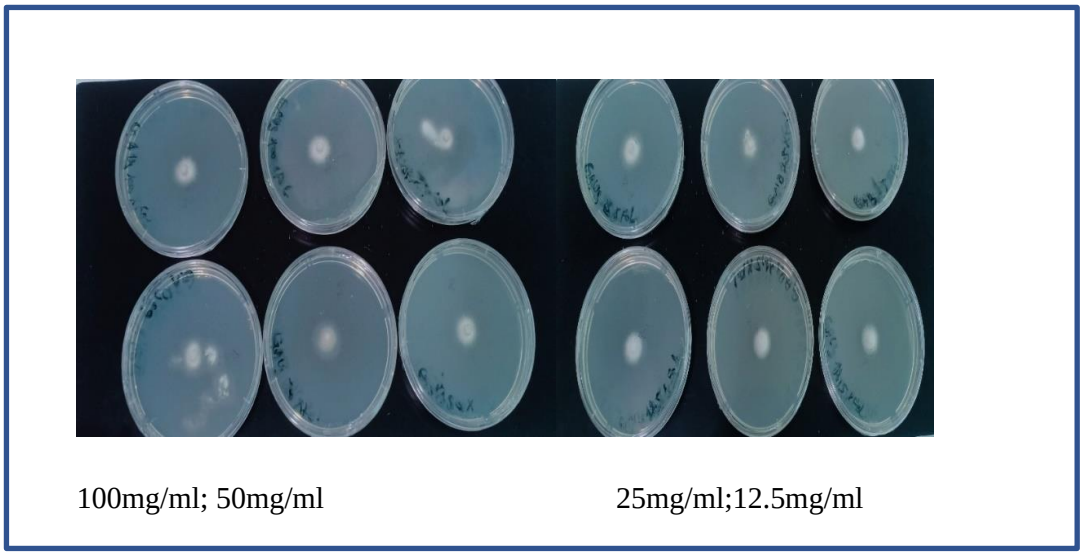


Figure 24: Antifungal activity of ultrasonicated acorn ethanolic extract of *Quercus spp.*



100mg/ml; 50mg/ml 25mg/ml; 12.5mg/ml

Figure 25: Antifungal activity of macerated acorn aqueous extract of *Quercus spp.*



100mg/ml; 50mg/ml 25mg/ml;12.5mg/ml

Figure 26: Antifungal activity of ultrasonicated acorn aqueous extract of *Quercus spp.*

- **In the presence of the aqueous extract**

Figure 26 shows that the aqueous extracts of *Quercus spp.* leaves and acorns exhibit a moderate antifungal effect, resulting in the following average colony diameters at concentrations of 12.5 mg/ml, 25 mg/ml, 50 mg/ml, and 100 mg/ml, respectively:

LAF: 1.93 mm, 1.84 mm, 1.83 mm, 1.59 mm.

LAU: 2 mm, 2 mm, 1.83 mm, 1.53 mm.

OAF: 1.75 mm, 1.77 mm, 1.77 mm, 1.63 mm.

OAU: 1.7 mm, 1.8 mm, 1.8 mm, 1.67 mm.

These results are compared to the control, which shows an average diameter of 2 mm.

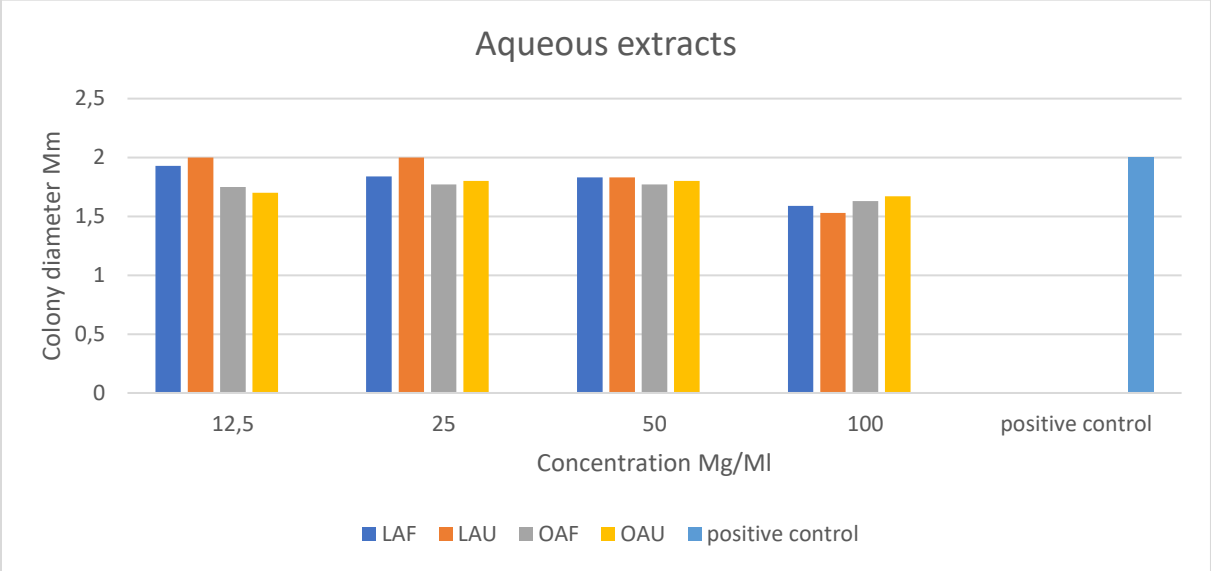


Figure 27: Effect of the aqueous extract of *Querqus spp* leaves and acorns on the growth of *F. oxysporum f. sp lycopersici*.

- **In the presence of the ethanolic extract**

Regarding the effect of the ethanol extract of *Quercus spp.,* the results presented in Figure 27 indicate that this extract also has a slight fungicidal effect. The average colony diameters for various concentrations are as follows:

LEF: 1.87 mm, 1.87 mm, 1.83 mm, 1.77 mm

LEU: 2 mm, 2 mm, 2 mm, 1.79 mm

OEF: 2 mm, 1.96 mm, 1.96 mm, 1.79 mm

OEU: 2 mm, 2 mm, 1.83 mm, 1.83 mm

These results suggest that the ethanol extract of *Quercus spp.* has a limited fungicidal effect compared to the control.

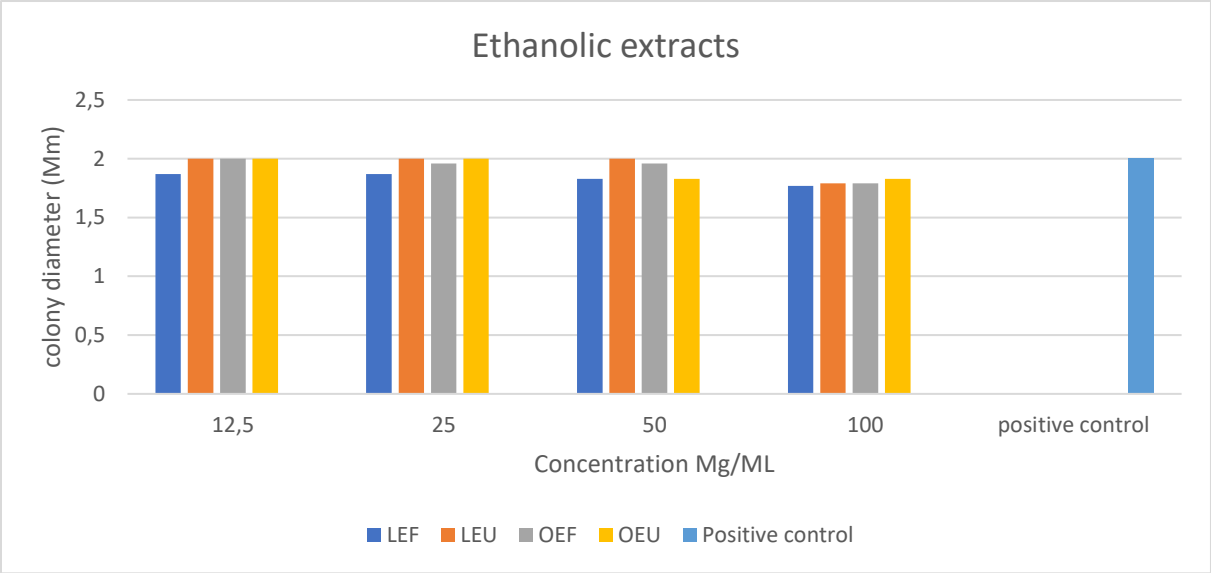


Figure 28: Effect of the ethanolic extract of *Querqus spp* leaves and acorns on the growth of *F. oxysporum f. sp lycopersici*.

The results of the inhibition percentage of *Quercus spp.* extracts (both aqueous and ethanolic) on the growth of *F. oxysporum f. sp lycopersici*.are presented in Figure 28. The findings indicate that aqueous extracts are more effective, with the ultrasonic method for leaves achieving an inhibition rate of 23.50% and the maceration method for acorns achieving 18.5% at a concentration of 100 mg/ml.

Aqueous extracts contain more potent antifungal substances compared to all ethanolic extracts tested in this study. For lower concentrations (12.5 mg/ml, 25 mg/ml, 50 mg/ml), both maceration and ultrasonic aqueous extracts of acorns maintain a consistent inhibitory activity. Conversely, the ethanolic extracts exhibit very low inhibition rates, ranging from 0% to 11.5% for leaves and 0% to 10.5% for acorns.

Overall, the results suggest that aqueous extracts are more effective than ethanolic extracts. This difference in activity may be attributed to the varying chemical compositions of the extracts.

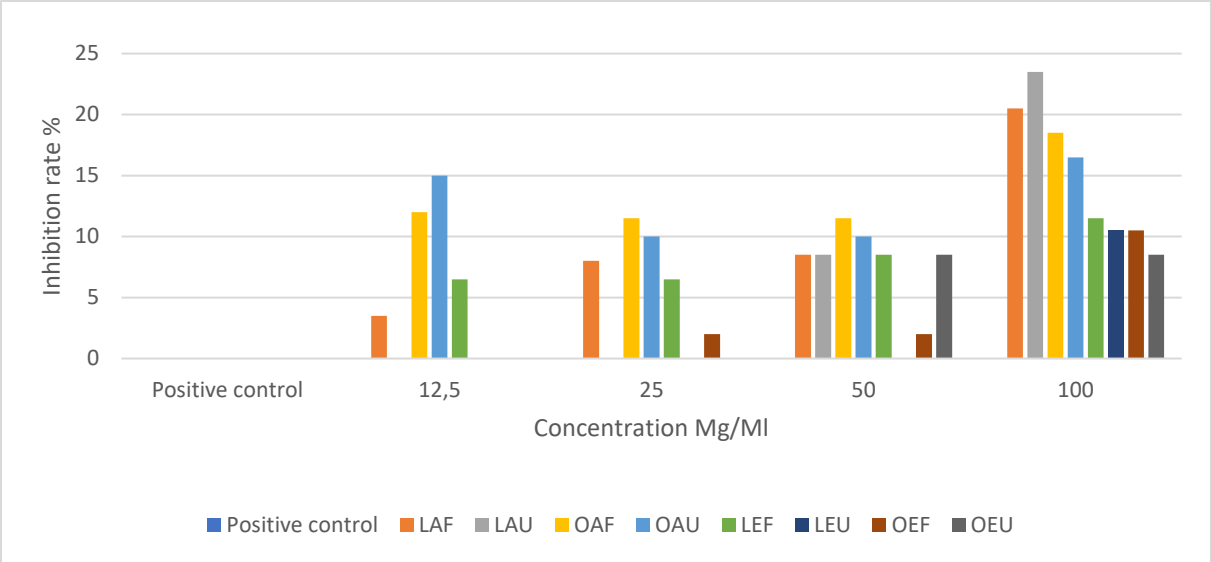


Figure 29: Rate of inhibition of *F. oxysporum f. sp lycopersici* by the aqueous and ethanolic extracts of the leaves and acorns of *Querqus spp*.

Literature reports have highlighted significant antifungal activity in certain species of the genus *Quercus* **(Sarwar et al., 2015).** Research by (**Ahmed and Salih 2019**) indicates that both ethanolic and aqueous extracts of *Q. infectoria* acorns exhibited antifungal activity against *Candida albicans*. Moreover, studies by **(Moshfeghy *et al*.,2018)** have demonstrated that the hydro-alcoholic extract of *Quercus brantii* acorns shows efficacy against vaginal candidiasis, comparable to clotrimazole vaginal cream. Additionally, the alcoholic extract of *Q. brantii* leaves exhibits polysaccharide antifungal activity against *Candida albicans* and *Planococcus citri*. Furthermore, **(Tahmouzi.,2014)** has shown that the leaves of *Q. brantii* possess antibacterial and antifungal properties.

Despite these findings, antifungal tests related to *Quercus spp.* have yielded varied results. While some studies support their antifungal efficacy, others contradict these findings. Factors contributing to this disparity include the type of extract, harvest time, oak species, microbial strain type, and measurement methods **(Burlacu et al., 2020).**

III.8.*In-vitro* Anti-inflammatory activity

III.8.1 Inhibition of BSA denaturation assay:

Table 7 presents the results of the *in vitro* anti-inflammatory activity of *Quercus* extracts, evaluated by the percentage inhibition of bovine serum albumin (BSA) denaturation.

Table 7: Percentage inhibition of BSA denaturation of different extracts of leaves and acorns of *Quercus spp.*

Extracts	Percentage of inhibition (%)
Lau	73,38±2,49
LAF	54,46±0,5
LEu	68,25±3,17
LEF	64,08±2,17
OAF	63,49±1,59
OEu	44,44±1,58
OEF	61,9±3,17
OAu	31,75±1,58

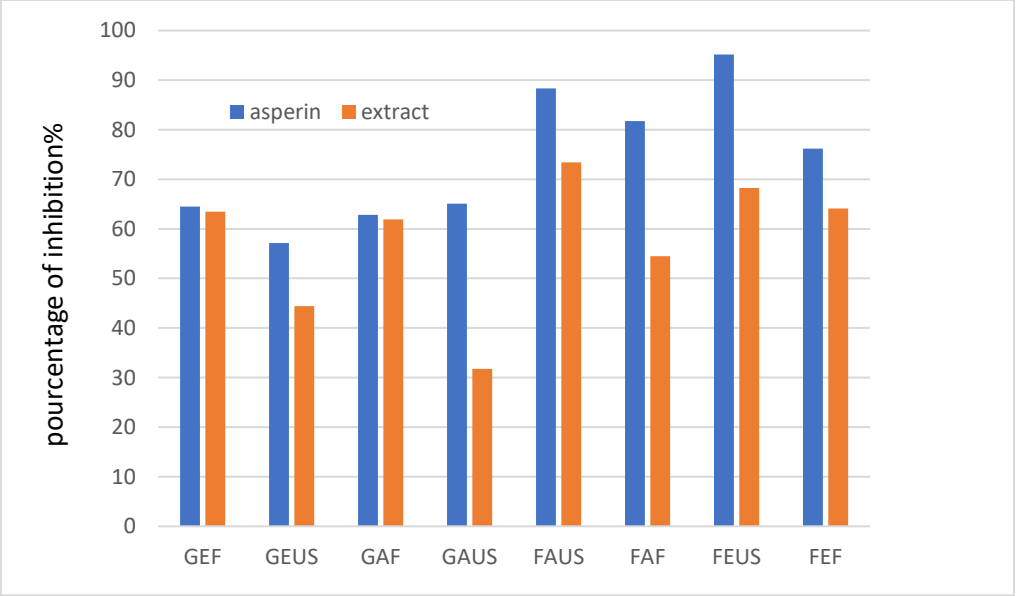


Figure 30: histogram showing concentration and inhibition percentage by linear analysis of *in-vitro* anti-inflammatory activity using the bovine serum albumin protein denaturation method of ethanolic extract and Aspirine. Mean±SE of three trials.

The findings indicate that the BSA denaturation inhibition rates of the *Quercus* extracts are comparable to that of the standard anti-inflammatory agent, aspirin. Notably, the aqueous leaf extract exhibited the highest inhibition at a concentration of 1000 µg/mL, with a rate of 73.37%±2.49. This was followed by the ethanolic leaf extract, which showed inhibition rates

ranging from 68.25% to 64.08%. The acorn extracts displayed lower inhibition rates, ranging from 63.5%± 1.59 to 31.75%± 1.58.

Despite these results, aspirin demonstrated a superior inhibitory effect on BSA denaturation, with a percentage inhibition of 95.15% at the same concentration.

Protein denaturation occurs when proteins lose their three-dimensional structure due to physical or chemical agents, such as strong acids or bases, concentrated inorganic salts, pH changes, organic solvents, and heat. Most proteins lose their biological function when denatured and denatured proteins can trigger inflammatory reactions. Denaturation involves alterations in electrostatic, hydrogen, hydrophobic, and disulfide bonds that maintain protein structures. (BSA) is particularly susceptible to temperature increases, leading to reduced solubility.

The inhibitory activity of BSA denaturation may be attributed to the presence of various bioactive compounds, such as polyphenols, in the extracts. Previous studies have suggested that the inhibitory effect of these extracts could be due to the interaction of specific components with protein linkage sites rich in tyrosine, threonine, and lysine. Furthermore, therapeutic molecules might reactivate receptors rich in tyrosine motifs and threonine, which regulate biological signal transduction pathways, contributing to their overall biological action **(Bekkouche & Seghaier, 2020).**

III.8.2. Evaluation of hemolytic activity

The proportion of hemolysis was determined by preparing a 10% RBC solution using aqueous and ethanolic extracts, followed by incubation of the reaction mixture for 30 minutes at 37°C. Hemolysis percentages were calculated using distilled water as the positive control, which induced 100% hemolysis.

The results displayed in the hemolysis percentage histogram **(Figure 31)** indicate varying levels of hemolysis for aqueous extracts. For LAF, the percentages were 2.48%, 18.40%, and 94.52%, and for LAU, they were 11.94%, 13.93%, and 55.22% at concentrations of 250, 500, and 1000 µg/ml, respectively. Similarly, OAF exhibited hemolysis percentages of 6.22%, 39.8%, and 42.87%, while OAU showed 10.9%, 17.41%, and 75.62% at the same concentrations.

Conversely, the ethanolic extracts showed hemolysis percentages for LEF of 4.97%, 8.95%, and 68.65%, and for LEU of 5.97%, 12.93%, and 19.9%, respectively. Additionally,

OEF displayed hemolysis percentages of 12.93%, 12.93%, and 46.76%, and OEU exhibited 12.43%, 14.42%, and 19.92% hemolysis at the same concentrations.

When compared to Aspirin at concentrations of 250 and 500 µg/ml, both aqueous and ethanolic extracts demonstrated low hemolytic activity. The aqueous extract of leaves by the maceration method (LAF) showed the highest hemolysis percentage (94.52%) at 1000 µg/ml, whereas the ethanolic extract of leaves by the maceration method (LEF) exhibited 68.65% hemolysis at the same concentration.

However, ethanolic solutions obtained through ultrasonic extraction, specifically LEU and OEU, exhibited significantly lower toxicity levels (19.9% and 19.92%, respectively) compared to Aspirin (97.51%) at equivalent concentrations of 250 and 500 µg/mL.

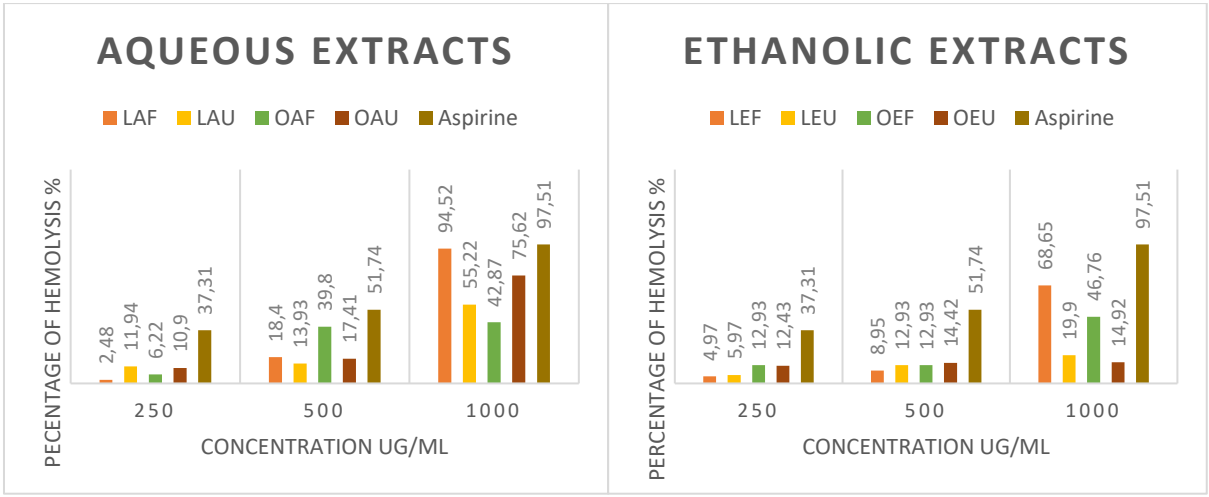


Figure 31: Histogram showing the percentage of hemolysis at different concentrations of aqueous and ethanolic extracts of *Quercus spp.*

Hemolysis refers to the irreversible breakdown of red blood cells, resulting in the release of their hemoglobin content and causing the sample to appear red. External factors contributing to hemolysis include abnormalities in the immune system's response and adverse effects from certain medications, such as non-steroidal anti-inflammatory drugs **(Beaumont & Hergaux, 2005; Mintzer & Billet, 2009).**

According to (**Houcher *et al.*,2001)**, human erythrocytes exposed to oxidizing chemicals like acetylsalicylic acid (ASA), a precursor to aspirin, only undergo hemolysis at

significant doses. Aspirin's oxidizing activity can lead to the generation of free radicals, which then attack the cell membrane, resulting in hemolysis.

III.8.3.Stabilization of the red blood cell membrane

The test, based on guidelines from **(Ganesh *et al*.,2013; Djolu RD *et al*.,2023),** evaluates the ability of these extracts to stabilize erythrocytes after hemolysis induced by a hypotonic solution at high temperatures. Gallic acid was used alongside the extracts as a reference anti-inflammatory compound.

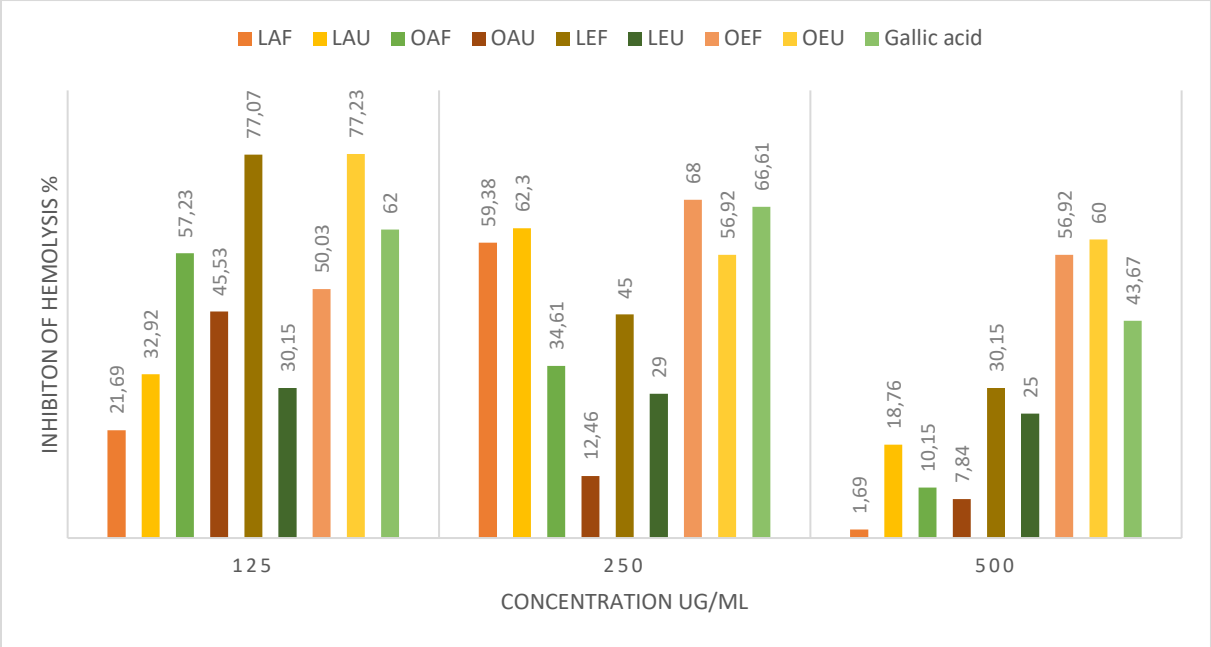


Figure 32: Histogram showing the percentage of inhibition percentage at different concentrations of aqueous and ethanolic extracts of *Quercus spp.*

Our calculations of hemolysis inhibition percentage revealed that the ethanolic ultrasonic extract of acorns and the macerated leaf extract exhibited the highest inhibition rates at 125µg/ml, with 77.23% and 77.07%, respectively, followed by Gallic acid with 62%.

Interestingly, some extracts showed increased inhibition at 250µg/mL, surpassing Gallic acid. Specifically, the ethanolic acorn extract using the maceration method ranked the highest with 68%, followed by aqueous leaf extracts using ultrasound and maceration methods with 62.3% and 59.38%, respectively. However, other extracts, such as OAF, OAU, LEF, and LEU, demonstrated decreasing inhibition rates with increasing concentrations (125, 250, 500µg/mL).

At 500µg/mL, all extracts except for ethanolic acorn extracts using ultrasound (60%) and maceration methods (56.92%) showed decreased activity. Notably, irrespective of the extraction technique, ethanol solutions, especially ethanolic oak extracts, exhibited the strongest anti-hemolysis activity across all doses.

Additionally, *Quercus ilex* extract at concentrations of 50 and 75µg/mL demonstrated significant anti-hemolytic effects **(Meziti *et al*., 2019)**.

This observed effect in our plant's acorns may be attributed to their composition, particularly their richness in components like vitamin E. According to **(Rabhi *et al*., 2016),** acorn fruits has been found to contain vitamin E. Previous studies by (**Houcher *et al*., 2001)** and **(Leger.,2000)** have underscored vitamin E's antioxidant properties, which safeguard erythrocytes from destruction, lipid peroxidation, and neutralize free radicals.

Furthermore, when red blood cells are exposed to heat or other toxins, their membranes may lyse, leading to hemolysis and hemoglobin oxidation. To evaluate this phenomenon, stability tests using hypotonic solutions and increased temperatures can be performed, comparing them to control red blood cells **(Chou, 1997; Reshma *et al*., 2014; Oyedapo *et al*., 2015).**

Conclusion

Conclusion

Over the past few decades, there has been a growing interest in plant-derived extracts, supported by scientific evidence indicating their potential to play a significant role in preventing and treating various diseases, many of which involve oxidative stress as a common underlying factor.

Our analysis reveals that the biological properties of oak leaves and acorns from their diverse and rich phytochemical profile, featuring phenolic acids, flavonoids, hydrolyzable tannins, terpenes, saponins, and other phenolic constituents. *In vitro* experiments have shown strong antioxidant capacity, anti-thermo denaturation effects, as well as antifungal and anti-hemolytic activities activities associated with these compounds.

Further biological, *in vitro*, and clinical research is needed to develop *Quercus* extracts into appropriate pharmaceutical forms and utilize them for therapeutic or cosmetic purposes. Based on our preliminary study which is considered as a first study of this plant, oak leaves and acorns emerge as promising candidates for medical use.

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Appendixes

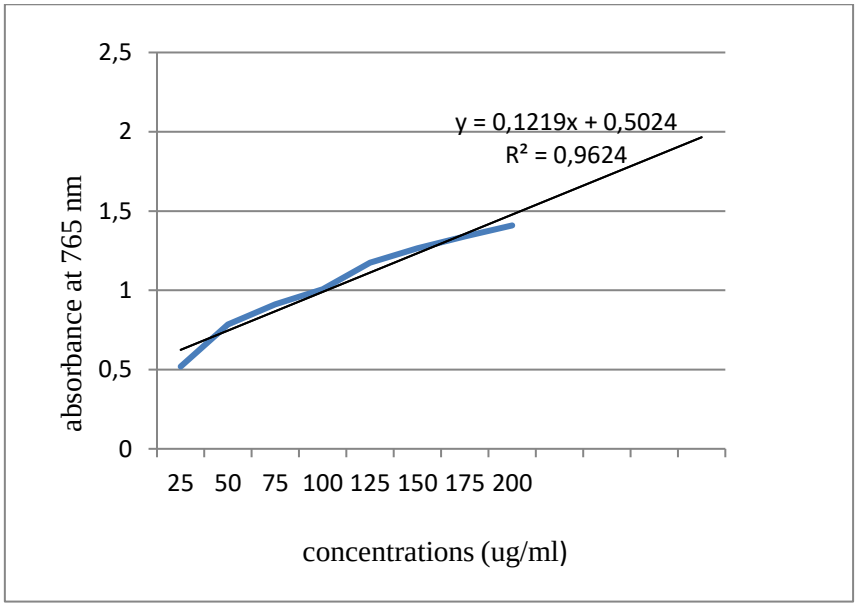
Appendix N° 01: Phosphate buffer preparation (300ml)

Sodium phosphate monobasic: (NaH2PO4): 0.54g

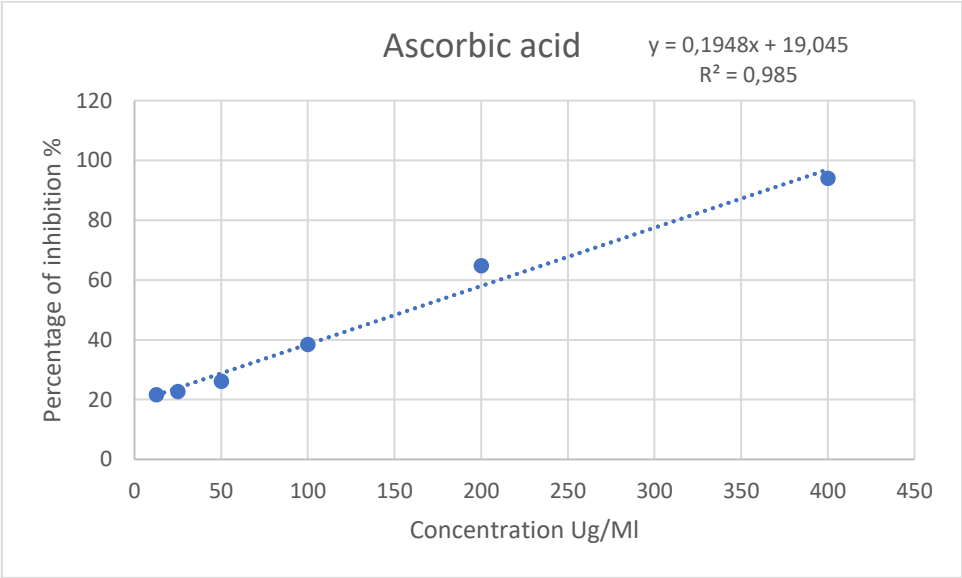
Sodium phosphate dibasic (Na2HPO4): 3.45g

Sodium chloride (NaCl): 26,25g

Appendex N° 02: Calibration curve of gallic acid.



Appendex N° 03: Standard curve of ascorbic acid.



Appendex N° 04: Quercetin calibration curve for the flavonoids assay.