REPUBLIC OF TÜRKİYE

ALTINBAŞ UNIVERSITY

Institute of Graduate Studies

Biomedical Sciences Program

DETERMINATION OF THE ACETYLCHOLINESTERASE ACTIVITY AND ANTIOXIDANT CAPACITY OF *ERICA ARBOREA L.*

Abtisam Hamad Mustafa ALHASADI

Master's Thesis

Supervisor

Asst. Prof. Dr. Yasemin Yücel YÜCEL

İstanbul, 2025

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The thesis titled DETERMINATION OF THE ACETYLCHOLINESTERASE ACTIVITY AND ANTIOXIDANT CAPACITY OF ERICA ARBOREA L., prepared by ABTISAM HAMAD MUSTAF ALHASADI and submitted on 17/06/2025, has been **accepted unanimously** for the degree of Master of Science in Biomedical Sciences.

Asst. Prof. Dr. Yasemin Yücel YÜCEL

Supervisor

Thesis Defense Committee Members:

Asst. Prof. Dr. Yasemin Yücel YÜCEL	Department of Biochemistry, Altınbaş University	.
Asst. Prof. Dr. Ebru ÖZDEMİR NATH	Department of Pharmaceutical Botany, Altınbaş University	
Asst. Prof. Dr. Gül İpek GÜNDOĞAN	Department of Histology and Embryology, Biruni University	

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I hereby declare that all information/data presented in this graduation project has been obtained in full accordance with academic rules and ethical conduct. I also declare all unoriginal materials and conclusions have been cited in the text and all references mentioned in the Reference List have been cited in the text, and vice versa, as required by the abovementioned rules and conduct.

Abtisam Hamad Mustafa ALHASADI

Signature

DEDICATION

To those who instilled in me the love of knowledge and learning, to the spirit of my parents, to my dear brother, my support and pride, to my husband and children, and to everyone who supported me in my academic career, I dedicate the fruits of my efforts as an expression of my sincere thanks and gratitude.

PREFACE

The work described in this thesis/dissertation has been funded by Altınbaş University Office of Scientific Research Projects as part of Project No. TEZP2023-16.

Part of this thesis was presented as an oral presentation at the ISARC 2024—International Science and Art Research Centre Congress, held on December 27, 2024, and the abstract was published in the official congress e-book.

Many thanks to Asst. Prof. Dr. Yasemin Yücel YÜCEL and Res. Asst. Emine ŞEN for helping me with all the steps of my thesis and supporting me with all the assays.

ABSTRACT

DETERMINATION OF THE ACETYLCHOLINESTERASE ACTIVITY AND ANTIOXIDANT CAPACITY OF *ERICA ARBOREA L.*

ALHASADI, Abtisam Hamad Mustafa

M.Sc., Biomedical Sciences Department, Altınbaş University,

Supervisor: Asst. Prof. Dr. Yasemin Yücel YÜCEL

Date: June/2025

Pages: 65

Erica arborea L., also known as "wild heather", is an evergreen shrub that grows between one and seven meters tall. It has dark green, needle-shaped leaves and numerous white, bell-shaped flowers. It exhibits antioxidant activity, as it can produce certain phytochemicals, such as flavonoids and phenolic molecules. It has been used in traditional medicine to promote urination and reduce inflammation. In this context, the current study investigates the plant's acetylcholinesterase inhibitory activity and antioxidant capacity. For this reason, the ethanolic extract was prepared from the dried aerial parts of the plant, and FRAP, DPPH, total flavonoid content, and total phenolic content were determined to identify these activities of the plant. 50, 100, 150, and 200 µg/mL concentrations were tested for the activity assays. The IC_{50} value for DPPH free radical scavenging activity was determined to be 82.09719 µg/mL. At 0.1 mg/mL, the extract showed a gallic acid equivalent phenolic content of 5.72 µg/mL. Acetylcholinesterase inhibitory action was determined employing the Ellman method, with IC_{50} calculated as 0.5285±0.025 mg/mL. The cytotoxic and antitumor effects of *Erica arborea* extract were also evaluated on multiple models (L929, HUVEC, and U87M cells) using the MTT assay. The extract successfully reduced the viability of U87MG cells through selective cytotoxicity, while proliferative responses were observed in both L929 and HUVEC cells. Due to its selective properties, which show low toxicity to non-neuronal cell types, *Erica arborea* can be a suitable option for targeted

cytotoxicity, an admirable source for the production of antioxidant agents, natural source for the treatment of neurodegenerative diseases such as Alzheimer's disease.

Keywords: *Erica Arborea*, Acetylcholinesterase, Antioxidant Activity, Alzheimer's Disease, Cytotoxicity, DPPH, FRAP.

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ABBREVIATIONS

ACh	:	Acetylcholine
AChE	:	Acetylcholinesterase
AD	:	Alzheimer's Disease
BHA	:	Butylated Hydroxyanisole
BHT	:	Butylated Hydroxytoluene
DPPH	:	2,2-Diphenyl-1-picrylhydrazyl
DTNB	:	Dithiol Nitrobenzoate
HPLC	:	High-performance Liquid Chromatography
NFTs	:	Neurofibrillary Tangles
QE	:	Quercetin Equivalent
TFC	:	Total Flavonoid Content
TPC	:	Total Phenolic Content

LIST OF SYMBOLS

μg/mL	:	Microgram Per Milliliter
Aβ	:	Beta-amyloid
$NaNO_2$	:	Sodium Nitrite
λmax	:	Maximum Absorption Wavelength

1. INTRODUCTION

The genus *Erica* is a member of the family *Ericaceae* (McGuire and Kron, 2005), which is a large global family that includes 4,250 species that are classified into 124 genera and is the source of the scientific designation for the heather tree—the *Ericaceae family* (Christenhusz and Byng, 2016; Kuş et al., 2019). "*Arbutus, Calluna*, and *Erica*" are among the most notable examples, with the latter displaying the greatest variety among the climate zones that are classified as Mediterranean. The plant species of the Ericaceae family have been more commonly recognized and accredited, through history, for their medicinal uses. These valuable species' antioxidative effects are mainly due to the abundance of polyphenolic compounds in the plant and their bioactivity (Guvenc & Kendir, 2007). In Turkey, there are many species of Ericaceae, mostly in the coastal parts, and these species constitute 12 genera and up to about 30 species. Commonly a few species are known amongst local populations by some names such as "*funda*" or "*puren*," such as "Erica manipuliflora", "*E. arborea*", "*E. bocquetii*", "*E. spiculifolia*", and "*E. sicula*" (Sun et al., 2024).

The genus "*Erica*" is widely shared in Europe, the Middle East, and Africa and is also very popular in Turkey (McGuire & Kron, 2005). "*Erica arborea L"*, or "tree heath," "*briar root,*" or "*funda*," can be found in many African tropical regions, Asian countries with temperate climates, and numerous regions in Europe, though it is a popularly cultivated species throughout the UK, Australia, and New Zealand (Davis, 1978; GRIN, 2007). This small evergreen tree or bush, growing from 1 to 7 meters, has produced specific and small white bell clusters (Adams, 1996). On a wider note, this species is largely used in traditional plant therapy in almost every region of Turkey and has nourished its biodiversity (Sun et al., 2024).

Outside Turkey, "*E. arborea*", "*E. multiflora*", and "*Arbutus unedo*" are manufactured in Algeria for plant care, anti-inflammatory, wound healing, and so forth (Marquez-Garcia et al., 2009). The fact that Algeria and the rest of the African continent, Europe, and some parts of Asia and the establishment in New Zealand and Australia speak for their ecological versatility and ethnobotanical significance (Yüksel et al., 2021).

"*Erica arborea*" prospers largely in Anatolia, especially in the western and northern parts of this region, and also along the fringes of the Mediterranean and African stretches. The plant with its woody tubers thickly arranged inside the phytophagous cells has gained notoriety

somewhere for quality in nice shape used for making smoking pipes in the northeastern area of Sicily (Kuş & Küçükaydın, 2017). Traditionally, ash leaves are used as diuretic aids, with the reduction in inflammation exalted by the astringent property and antioxidant potency found in the leaves and flowers (La Mantia et al., 2007).

A modern pharmacological study sheds light on potential healing qualities of *Erica* with its biological effect, possibly attributed to the presence of physicochemically related varieties of phyto-molecules, mainly polyphenols and flavonoids. These molecules with high antioxidant activities assist in targeting the oxidative stress in chronic diseases (Amari et al., 2023).

Some plant material, like "*E. arborea*" plant material, holds an antioxidant activity, confirmed through various assays: an array of DPPH radical-scavenging tests and the evaluation of total phenolics (TPC) and total flavonoids (TFC). Furthermore, the cytotoxicity and cell proliferation effect were looked into through MTT assays. ISO emphasizes the employment of HUVEC (human umbilical vein endothelial cells) and U87MG (glioma cell line) as pertinent human cell line-mechanistic models for systemic toxicity. Clinical efficacy with regard to these compounds cannot rule out some diseases such as Alzheimer's. Hence, the reasoning behind the therapeutic potential of isolated compounds from these. A line of activity being pursued is the possibility of achieving AChE inhibitory activity with "*E. arborea*" extracts. AChE inhibition is a recognized treatment for Alzheimer's disease, as this will prolong cholinergic activity and may slow the loss of cognitive function. Furthermore, AChE inhibitors have always been mostly antioxidants by definition, which in turn adds onto their therapeutic interference with disease state-induced oxidative stress. The side effects and other risk factors related to synthetic AChE inhibitors have placed these molecules in the spotlight for plant-derived alternatives. While many plant species have been tested for AChE inhibition and antioxidants, it is difficult to find detailed work on the bioactive constituents of "*E. arborea*" (Amizar et al., 2013).

1.1 STUDY OBJECTIVE

This study aims to reveal the inhibitory activity of the plant *E. arborea* on the AChE enzyme and to show its antioxidant properties. It will also reveal potential preventive treatments for neurodegenerative diseases, such as AD, and analyze cell viability by finding the cytotoxicity of the plant extract and making it a promising candidate in the fields of pharmacology and traditional medicine.

2. GENERAL INFORMATION

2.1 *ERICACEAE FAMILY*

The Ericaceae family is a group of flowering plants often referred to by scientists as the Acanthocephala family, the name of the family to which it belongs. Acidic and potentially infertile growing conditions are the most common environments in which these plants are found. This family is known to include approximately 4,250 species, distributed among 124 different genera. It is the fourteenth family in this sequence and is therefore the richest of all flowering plant genera (Christenhaus and Peng, 2016). Several well-known and important members of this genus include the commercially known *blueberry, cranberry, rhododendrons* (including azaleas), and *huckleberry*, a wide range of common heathers such as *Erica* and *Calluna;* and *Cassiopeia* and *Daboecia*. The Heather family also consists of a large number of members (Stevens, 2001).

The *Ericaceae* family is home to a vast range of species that are differentiated from one another in terms of their morphology. Trees, shrubs, dwarf shrubs, and herbs are all included in this taxonomic group. Stipules are not present on the leaves of these plants, which are normally evergreen, alternating or whorled, simple, and do not have any branches. In addition to being hermaphrodites, the blooms of these plants display a great deal of variation in terms of their morphology, which may range from narrowly tubular to funnelform or extensively urn-shaped. It is a phenomenon called sympetalous marriage that occurs when the petals are repeatedly fused. However, although the corollas of most *Rhododendron flowers* are radially symmetrical (actinomorphic) and urn-shaped, several of the flowers that belong to the *Rhododendron* genus are partly bilaterally symmetrical (*zygomorphic*) (Kron et al., 2002).

Historically, the Ericaceae family has undergone a number of taxonomic revisions, especially in the formation of subfamilies and tribes. Stevens classified the Ericaceae family in a system that recognizes six of such subfamilies: "*Rhododendroideae*", "*Ericoideae*", "*Vaccinioideae*", "*Pyroloideae*", "*Monotropoideae*", and "*Wittsteinioideae*". Among these districts, there are further divisions into families, and to illustrate, "*Rhododendroideae*" is made up of seven distinct tribes, such as "*Bejarieae*", "*Rhodoreae*", and "*Cladothamneae*" (Stevens, 1971).

In 2002, when molecular phylogenetics was greatly advancing, a major taxonomic change occurred. Together with comparative studies on morphological, anatomical, and embryological data, this study proposed the fusion of all the distinct previous families, including *Empetraceae*, *Epacridaceae*, *Monotropaceae*, and Pyrolaceae, with Ericaceae, hence increasing the morphological and geographic diversity of the family (Kron et al., 2002).

Today, the family Ericaceae is a global family, except in Antarctica, frosty areas of the central and high Arctic, much of central Greenland, some parts of lowland tropical regions, and parts of northern and central Australia. Members of this family usually thrive in acidic, nutrient-poor, and shaded environments like peatlands, heathlands, and oak-heath forests-the latter in parts east of North America (Watson & Dallwitz, 2014). Another ecological stratification within this family would be the increase in diversity associated with the method of pollination adopted; for example, many species under "*Vaccinium*" must pollinate by buzz for proper fertilization (Moquet et al., 2017). Some species of "*Rhododendron*" are indigenous to East Asia, while several cultivars found among garden varieties today are hybrids. Economically, the United States dominates the trade in Ericaceae horticultural products, especially blueberries and cranberries, and lowbush blueberries are important for states such as Maine (Kron et al., 2002).

2.2 THE GENUS *ERICA*

The genus *Erica*, which belongs to the *Heatheraceae* family, is a diverse group of approximately 857 species of flowering plants. It is widely recognized for its importance in Mediterranean and South African ecosystems, and the plants in this genus have a variety of ecological and ornamental uses. The English common names "heath" and "heather" are often used to refer to species in *Erica*, as well as related genera like Calluna. While Calluna was formerly classified under *Erica*, it is now distinguished by its scale leaves, which are shorter than those in *Erica*, and its flower *corolla*, which is composed of separate petals rather than fused ones. As a result, *Erica* is sometimes referred to as "winter heather" in contrast to Calluna, also known as "summer heather" or "autumn heather" (Stevens, 2001).

Among the *Erica species*, there is considerable variation in size. Some species, such as *E. arborea* (tree heath) and *E. scoparia* (besom heath), can grow up to 7 meters (23 feet) tall,

while others are much smaller shrubs ranging from 20 to 150 cm (8 to 59 inches). All *Erica species* are evergreen and typically feature needle-like leaves that range from 2 to 15 millimeters (1/8 to 5/8 inches) in length. The flowers are generally small, bell-shaped, and borne in clusters of axillary umbels or spikes at the ends, and they regularly orient outward or downward. *Erica* seeds are quite tiny, and in some cases, they might remain dormant for several decades within the soil, thus adding to the plant's resilience and persistence in the wild (Manning and Paterson-Jones, 2007).

The genus *Erica* divides into several clades. The oldest of these lines includes the European species. The African clade comprises a larger species dimension than that of the European. However, the Cape and Madagascan/Mascarene species constitute the two larger monophyletic groups within Africa. These phylogenetic insights inform evidence that the genus *Erica* has diversified significantly, especially in the Cape Floristic Region, rich in biodiversity, where it radiated into many species with different floral characters (Pirie et al., 2011; Pirie et al., 2016).

Among the most ecologically important of all plants, the church is the *Erica species.* This is found more significantly in the Mediterranean and South African ecosystems. Such soils tend to be acidic, low in nutrients, and pretty much the only storage channels in heathland. Sometimes they provide shelter resources and occasional feeding for the numerous insect species, birds, and small mammals attracted to these habitats. All of these, very importantly, have a value form of ornamentation since a large number of these species, especially those found in the Cape region, are cultivated for their beautiful flowers and as highly used groundcovers in gardens. The genus has also been responsible for honey production, as some members, like *E. arborea,* produce nectar for bees that gives a highly palatable, high-quality honey (Manning and Paterson-Jones, 2007).

Commercially, the wood of some Erica species, such as *E. arborea,* is highly preferred, largely due to the hardness and durability of the wood, making it more useful for the manufacture of small tools as well as wooden items (Oliver, 2000). The genus is also a key component of conservation strategies, with efforts to protect and restore heathland habitats where *Erica* species are dominant (Pirie et al., 2016).

2.3 BOTANICAL DESCRIPTION OF *ERICA ARBOREA*

The genus *Erica* contains about 500 species, most of which are found in southern Africa but also in central and southern Europe and other countries around the Mediterranean. 5 of these species grow naturally in Turkey (Stevens, 1978). In gardens, the *E. arborea L*., also known as the heather tree, is a shrub that typically grows to a height of one to four meters (three to thirteen feet). However, in its natural habitat, particularly in Africa, it can reach a height of seven meters (twenty-three feet). Primary morphological characteristics of the plant include the following (Bartiromo et al., 2013; Gizaw et al., 2013).

The needle-like leaves are dark green and densely packed in whorls of three. With a toothed underside, each leaf is glabrous and linear, measuring approximately 3–6 centimeters (1.2–2.4 inches) in length. Flowers: A large number of bell-shaped flowers, which are typically white but can also be pink or light purple, are produced under the trees. Heather features golden flowers that are borne in pyramidal clusters and measure between 2 and 3 millimeters (0.9 and 1.6 inches) in size (Gizaw et al., 2013).

The pyramidal inflorescences do not appear until November, when the trees are at their peak of blooming. Fruits and Seeds: The capsules are very similar to the white blood cells or pollen of this species. They are four millimeters in length and irregularly thick (often equal but not wider). Each capsule contains nine to eleven seeds that are split into two or three swollen seeds. Once they reach approximately three to four years of age, mature shrubs produce one thousand times more seeds annually than seedlings do (Bartiromo et al., 2013) (Figure 2.1).

Figure 2.1: *Erica arborea L*. (The Heather Garden, 2025).

As follows in Table 2.1, *Erica arborea*, also known as the heather tree, is a hierarchical system that classifies it within the plant kingdom, division Streptophyta, subclass Magnoliidae, order Ericales, family Ericaceae, genus *Erica*, and species *E. arborea*. It belongs to the family Ericaceae, known for its evergreen nature and bell-shaped flowers.

Table 2.1: Scientific Classification of *Erica arborea* (National Center for Biotechnology Information, 2025).

Kingdom	**Plantae**
"Phylum"	"Streptophyta"
"Subclass"	"Magnoliidae"
"Order"	"Ericales"
"Family"	"Ericaceae"
"Genus"	*"Erica"*
"Species"	*"Erica arborea"*

2.3.1 Distribution of the Plant

The humid temperate regions of southern Europe, southwest Asia, the Indian subcontinent, and as far east as Japan are the most common locations for *E. arborea*. Additionally, it can be found in the rainforests that line the northern coast of New Guinea and Australia. In addition to these wonderful locations where this species can be found naturally and in large numbers, this plant can be found spreading westward through the highlands of Ethiopia and the Rwenzori, which are in Uganda. The Cameroon Mountains, which are in the corn swamps, are finally reached by it (Berhe et al., 2025). Additionally, *E. arborea* has become a weed in the southeastern region of Australia, where it has allowed itself to become naturalized (Gil-López et al., 2017). On the other hand, this plant can be found in the countries of Portugal and Spain on the Atlantic coast and in the countries of Turkey and Georgia on the Black Sea coast (Figure 2.2) (Fagúndez, 2006).

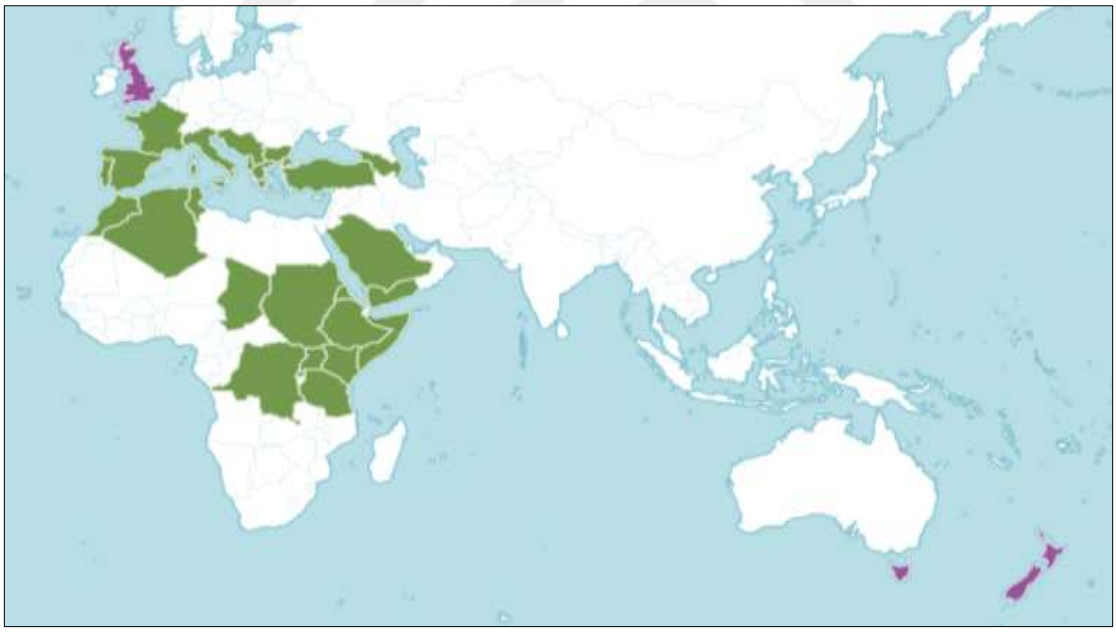

Figure 2.2: Distribution of Erica *arborea L.* (Désamoré et al., 2011).

The native habitat of Erica arborea is found worldwide, in areas such as Southern Europe, North and East Africa, as well as parts of the Middle East. Its natural distribution includes Albania, Algeria, Bulgaria, Chad, Corsica, Eritrea, Ethiopia, France, Greece (including

Crete), Italy (including Sardinia and Sicily), Morocco, Portugal, Rwanda, Saudi Arabia, Somalia, Spain, Sudan, Tanzania, the Caucasus region, Tunisia, and part of Europe in Turkey. It is also found in Uganda, Yemen, the former Yugoslavia, and the former Zaire; these factors reflect its good ecological capacity to adapt to different climates and terrains. Its introduced habitat falls on this list among all other countries to which it has been introduced outside its native habitat. Noteworthy locations include Great Britain, the North and South Islands of New Zealand, and Tasmania, where the distribution pattern gives credence to the adaptability and ability of horticulture in non-native environments where similar conditions apply (Fagúndez, 2006).

2.4 TRADITIONAL USAGE

Heather, also known as *E. arborea*, has been utilized for medicinal purposes by a variety of individuals throughout history. For example, people in ancient Italy used to take a diuretic tea from the infusion of its flowers, that of *E. arborea*. There are areas in the northern part of Africa where the decoction of the flowers has been used to treat kidney stones, with a dosage administered at three cups. It was also used in ancient Turkish medicine by boiling the flower tips (Zengin et al., 2019). The decoction of the flowers was used as a treatment for kidney stones in certain areas of North Africa, where it was given in a dose of three cups. Through the process of boiling the flowering tips, it was also used in ancient Turkish medicine (Amari et al., 2023).

For treating urinary tract infections and kidney stones, this is also effective. Diuretics and urinary tract cleansing are other usages (Pemphrey et al., 2023; Kuzmin et al., 2023). Thus, *Erica arborea* has been documented as a medicinal plant in the Canary Islands, along with its properties of disinfectant, anti-inflammatory, and hypotensive action through the reception of an infusion of flowers (Wondimu et al., 2024).

Insect bites have been effectively treated externally with crushed twigs and leaves of the plant. This was done by crushing a few leaves and twigs of the plant finely. A decoction of the flowers of the *Erica* tree has been used in Algerian traditional medicine as an anti-stress agent and to treat kidney and prostate diseases (Amari et al., 2023). This was one of the plant's many uses in traditional Algerian medicine. Ethiopian people used a decoction of its buds to treat giardiasis. They also used *Erica arborea* to treat wounds by drying and grinding

the leaves, mixing the powder with butter, and applying it to the scars (Wondimu et al., 2024). The plant also possesses antioxidant, analgesic, and anti-inflammatory properties. However, the scent of heather not only permeates the garden but also adds a sense of joy to any type of arrangement. As a result, it is frequently used in landscaping and ornamental plantings (La Mantia et al., 2007).

2.5 ANTIOXIDANT COMPOUNDS IN *ERICA ARBOREA*

It is well known that *E. arborea* is rich in antioxidants. These compounds are mostly classified as phenolic compounds. One of their most notable properties is their ability to eliminate free radicals. These essential compounds are flavonoids, which belong to the polyphenolic family and are known for their powerful antioxidant effects. The active ingredients they contain help eliminate free radicals and protect cells from damage caused by oxidation (Gerber et al., 2023).

2.5.1 Quercetin

Quercetin is one of the major compounds among such compounds (Figure 2.3). It has been associated with a lot of health benefits and also exhibits considerable antioxidant activity (Batiha et al., 2020).

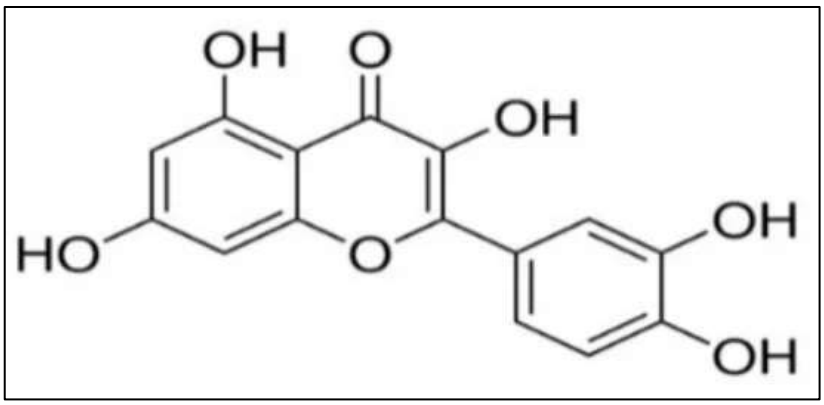

Figure 2.3: Structure of Quercetin (National Center for Biotechnology Information, 2025).

2.5.2 Dihydromyricetin

Dihydromyricetin (Figure 2.4), another flavonoid in the leaves, contributes to the plant's antioxidant capacity. The ethyl acetate extract of *Erica arborea* showed the highest flavonoid content. Flavonoid content values ranged from 65.31 to 67.15 mg of quercetin equivalent (QE) per gram of dry extract (Amari et al., 2023).

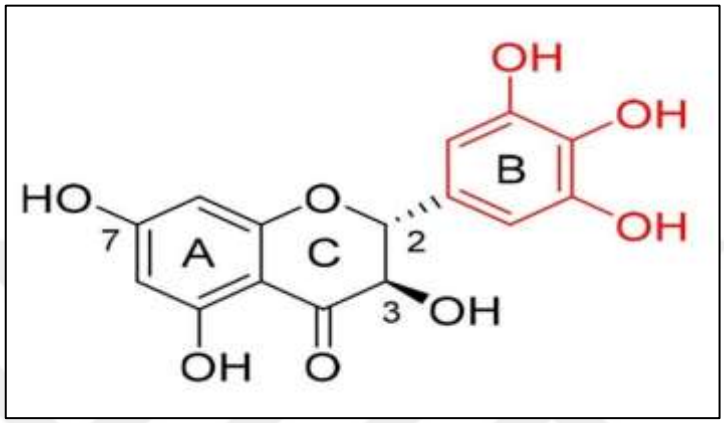

Figure 2.4: Structure of Dihydromyricetin (National Center for Biotechnology Information, 2025).

2.5.3 Phenolic Acids

These plant substances also contain phenolic acids, which are moderate antioxidants. They can act against oxidative stress and inhibit the oxidation of lipids (Figure 2.5) (Nazemiyeh et al., 2008).

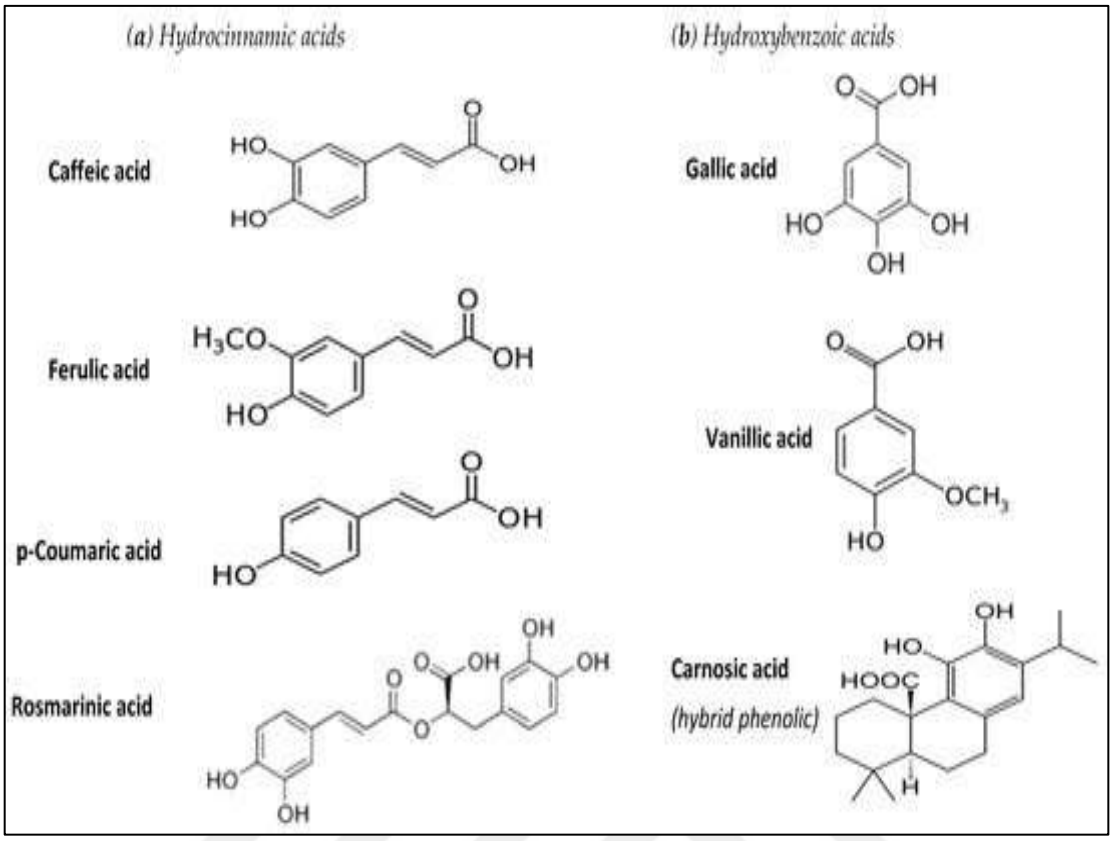

Figure 2.5: Structure of Phenolic Compound (Kiokias and Oreopoulou, 2021).

2.5.4 Tannins

Tannins found in *E. arborea* are found to be separate types of polyphenolic compounds from the other types of polyphenolic compounds. This plant shows antioxidant activities, which can be attributed to the presence of these tannins in the plant body, though there are possible health benefits from this particular plant, including anti-inflammatory, antibacterial, and anti-cancer properties. All of these notions probably give their contributions to the probable health benefits of the plant together (Figure 2.6) (Köroğlu et al., 2019).

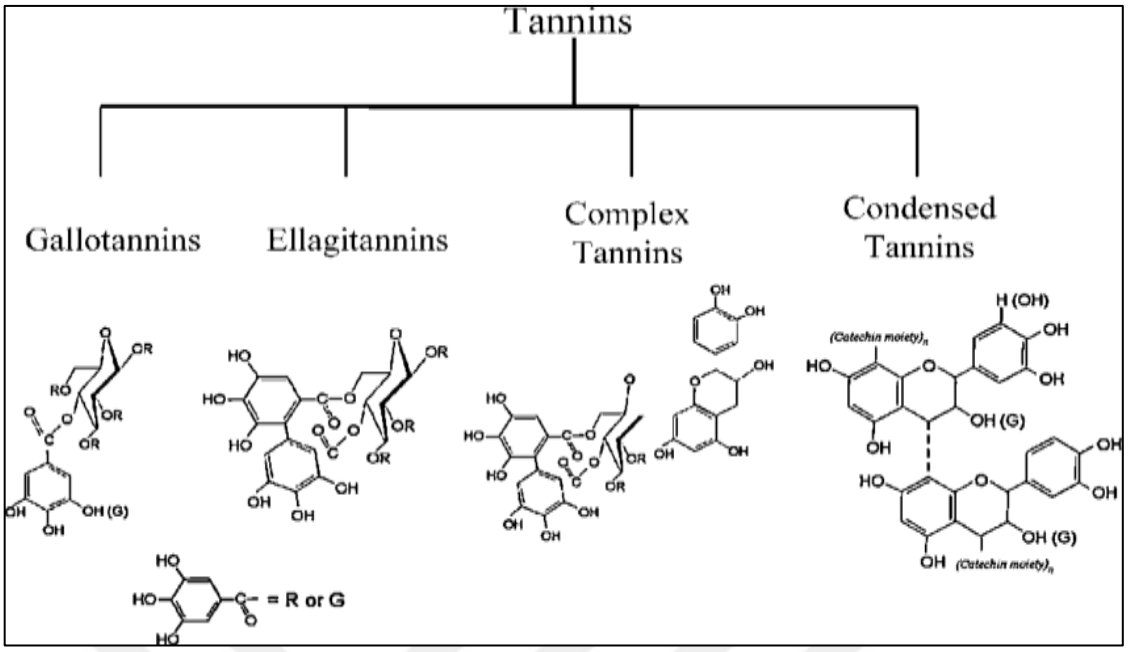

Figure 2.6: Structure of Tannins (Khanbabaee and Van Ree, 2001).

2.6 BIOLOGICAL ACTIVITIES OF *ERICA ARBOREA L.*

The antioxidant activity of *E. arborea* has been extensively studied, with the focus of such studies on various extracts derived from this plant: ethanolic, methanolic, and aqueous extracts. Common tests to evaluate the antioxidant capacity of such extracts are the ABTS and DPPH assays, which are simply a set of assays that measure the free radical scavenging capacity of the extracts. *E. arborea* extracts are reported to have strong antioxidant activity; however, some authors suggest that other species of the genus *Erica* may have more potent antioxidant activities (Amari et al., 2003).

The antimicrobial properties of *Erica arborea* have been the subject of considerable interest. Plant extracts have shown activity against a wide range of bacterial strains, although their action against yeast organisms remains more limited. Certain methods are frequently used to assess these antimicrobial properties (Kıvçak et al., 2013). Therefore, *E. arborea* could also be a potential candidate for a natural antimicrobial. The fact that the *E. arborea* extracts were shown to possess anti-inflammatory properties and interact with inflammatory pathways indicates that there is also the potential for further research into the treatment of chronic inflammatory disorders. Along with their antioxidant and antibacterial properties,

their diuretic property has brought them into the framework of traditional medicine for treating kidney stones and urinary infections. The analgesic effect of the *Erica arborea* also contributes to pain relief in various conditions (Ay et al., 2007).

2.6.1 Antioxidant Activity

Oxidative stress plays a critical role in triggering cellular damage and programmed cell death (apoptosis). This condition arises primarily due to the accumulation of free radicals—highly reactive molecules characterized by unpaired electrons—that can disrupt normal cellular functions. A growing body of evidence links oxidative stress to the onset and progression of various pathological disorders, including those affecting the cardiovascular and nervous systems. In efforts to counteract oxidative damage, synthetic antioxidants such as butylated hydroxytoluene (BHT) and butylated hydroxyanisole (BHA) have been widely employed for decades (Jomova et al., 2023).

Plants, however, have attracted increasing attention because they are non-toxic and effective in traditional medicine. Many phytochemicals, including phenolic compounds, flavonoids, and alkaloids, are reported to have antioxidant activities (Krishnamurthy et al., 2024). Such phytochemicals exist in plants such as *E. arborea*. These scavengers scavenge free radicals and prevent the oxidation of biomolecules, which are considered as naturally occurring antioxidants that protect against diseases associated with oxidative stress (Chaudhary et al., 2023). Research carried out on *E. arborea* shows the presence of phytochemicals such as polyphenols, flavonoids, and alkaloids (Chaudhary et al., 2023; Lewis et al., 2011).

In vitro studies with enzymes such as ABTS, FRAP, and DPPH show that extracts obtained from the aerial parts of *E. arborea* possess a higher degree of antioxidant capabilities. These phytochemicals may have significant roles to play in oxidative damage protection as well as in therapeutics (Chaudhary et al., 2023; Lewis et al., 2011; Martemucci et al., 2022).

2.6.2 Antimicrobial Activity

Several investigations have been conducted to explore the antibacterial potential of Erica arborea, with a special emphasis on the flower extracts. These showed a significant antibacterial effect, especially with Salmonella gallica, Bacillus cereus, Pseudomonas aeruginosa, and Escherichia coli. The activity of both leaf and flower hydroethanolic extracts

is active against a variety of Gram-positive and Gram-negative bacteria, also attributed to the presence of bioactive phytochemicals such as epicatechin, palmitic acid, and kaempferol-3-O-glucoside, which contain antimicrobial properties.

Of additional interest, recent advances in nanotechnology have identified an interest in E. arborea for use in green synthesis of zinc oxide nanoparticles. Notably, these biologically synthesized nanoparticles displayed substantially greater antibacterial effectiveness than those seen with standard unmodified zinc oxide, suggesting promise as an eco-friendly, alternative natural synthetic source of antibiotics (Amari et al., 2023; Kokak & Meydan, 2023).

In addition, nanoparticles derived from E. arborea were shown to deliver antimicrobial activity against a wide panel of pathogens such as Staphylococcus aureus and Candida albicans. This strengthens the view that nanomaterials of plant origin can serve as a sustainable alternative to conventional nanoparticle synthesis processes, which are mostly performed using energy-exhaustive physical or chemically hazardous methods (Gunduz et al., 2024).

2.6.3 Enzyme Inhibitory Activity

It has been proposed that *E. arborea* could also be a source of AChE inhibitors, of interest in treating various neurological disorders like AD. Aqueous extracts from various parts of the plant inhibit AChE and butyrylcholinesterase. The aerial parts have been reported to inhibit both enzymes at a concentration of 200 μg/mL, with a maximum inhibition of 70.10% (Elaloui et al., 2019). These differential activities are most likely due to the phenolic compounds contained in the extracts. Furthermore, it is interesting that related species such as *E. australis and E. manipulative Flora* have shown similar inhibition, suggesting that these plants merit further study for their therapeutic applicability in neurodegenerative diseases (Orhan et al., 2004; Elaloui et al., 2019; Krishnamurthy et al., 2024). To have a complete understanding of the therapeutic potential of *E. arborea*, further studies are warranted to characterize and identify the biologically active compounds in the plant.

2.7 ACETYLCHOLINESTERASE

The enzyme acetylcholinesterase is critical to proper nervous system function, particularly at cholinergic synapses. Acetylcholinesterase quickly hydrolyzes the cholinergic neurotransmitter acetylcholine to acetate and choline, which stops excessive activation of postsynaptic receptors. This rapid breakdown is essential to promptly terminate nerve impulse transmission for precise regulation of excitatory signaling within the nervous system. The enzyme works with high efficiency and can flower up to 25,000 acetylcholines per second. This high catalytic functioning is due to certain distinctive structural features: serine, histidine, and glutamate function as a catalytic triad, and there are aromatic residues located within the binding pocket that stabilize the interaction with the substrate (Patočka et al., 2004).

Inhibition of acetylcholinesterase is a well-documented treatment strategy for cholinergic deficits associated with AD and myasthenia gravis in clinical settings. Medications like edrophonium and tacrine serve as inhibitors of AChE to increase synaptic levels of acetylcholine and enhance cholinergic neurotransmission, thereby ameliorating cognitive and neuromuscular symptoms associated with these disease states (Tougu, 2001).

In contexts beyond pure neurology, AChE has been associated with a whole new generation of scientific use as a biological marker for environmental and occupational health applications. Inhibitory action on AChE is a potential indication of exposure to certain toxicants, such as organophosphate pesticides and carbamates, which cause impairment of the neural function. Measurement of AChE activity thus becomes an expedient, powerful tool to determine whether serious health risks, such as neurotoxicity and chemical exposure, exist within either clinical or environmental settings (Jameson et al., 2007).

An emerging line of research is the involvement of the various splice variants of AChE, hinting that such forms might exist to serve non-enzymatic functions, especially during developmental neurotoxicity. This could ultimately furnish the scientific community with not only biological pathways opening for neurodegenerative diseases or developmental disorders but also new ways of treating them. Therefore, both the regulation and inhibition of AChE remain uppermost concerns regarding present-day biomedical research that holds

implications for the treatment of these diseases and environmental health monitoring (Jameson et al., 2007).

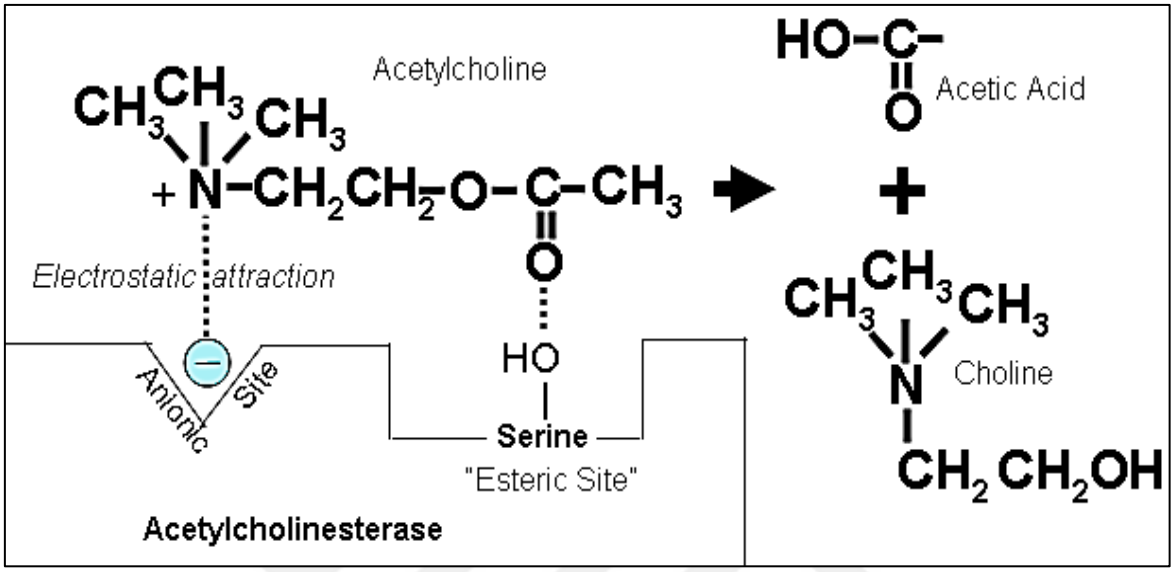

Figure 2.7: Acetylcholinesterase Reaction (Umar and Aisami, 2020).

2.7.1 Acetylcholinesterase Inhibition

Acetylcholinesterase (AChE) is a crucial enzyme to maintain the balance of neurotransmission in the nervous system by catalyzing hydrolysis reactions of acetylcholine to choline and acetate. This is crucial for preventing overstimulation at the synapses to allow coordinated and controlled muscle movements and modulation of sensory inputs such as tactile sensation. AChE assumes an even greater value at the neuromuscular junction, where its enzyme activity allows for proper transmission of signals from motor neurons to muscle fibers. Dissolution of this process has been associated with neurodegeneration, including Alzheimer's disease, where cholinergic signaling deficits have become a major landmark (Mukherjee et al., 2007).

Pharmacological inhibition of AChE has become the mainstay for alleviating cognitive decline in Alzheimer's dementia. By inhibiting the activity of AChE, the drugs increase acetylcholine levels in the brain, enhancing cholinergic neurotransmission that is directed toward memory retention and cognitive function. Such a mechanism is critical in delaying

the degeneration of cholinergic neurons—a recognized feature of the pathophysiology of Alzheimer's (Ibach & Haen, 2004).

In recent years, plant-derived substances have acquired growing interest as potential AChE inhibitors for the therapy of incipient neurodegenerative diseases. Certain plant extracts are indeed very potent, showing more than 80% inhibition of AChE activity in important in vivo tests. These findings regarding such phytochemicals classify them as important candidates in the search for natural therapeutics against Alzheimer's and other ailments resulting from cholinergic dysfunction (Mukherjee et al., 2007). Modulating AChE activity may be one of the pathways through which these natural compounds raise synaptic acetylcholine levels to boost the neural communication and possibly slow down the disease progression (Can et al., 2023).

In addition to being a molecule of neurotransmission, AChE is thought to participate in various neuronal processes, including the transport of choline, synthesis of acetyl-CoA, and regulation of acetylcholine storage and release from synaptic vesicles. After the exocytosis of acetylcholine into the synaptic cleft, it binds to nicotinic and muscarinic receptors to accomplish signal transduction. The signal is then terminated by AChE via the rapid degradation of acetylcholine, followed by the recycling of choline for reuse in neurotransmitter synthesis. Clinically available drugs, including donepezil, rivastigmine, and galantamine, work through the inhibition of AChE and restore neurotransmitter balance in Alzheimer's patients, resulting in cognitive improvements. However, AChE can also be disrupted by toxicants, namely pesticides, by inhibiting the enzyme and bringing about a pathological accumulation of acetylcholine, leading to severe neurotoxic effects. Thus, both therapeutic and toxicological implications of AChE inhibition must be understood for the successful management of diseases alongside general health safety (Ibach & Haen, 2004; Can et al., 2023).

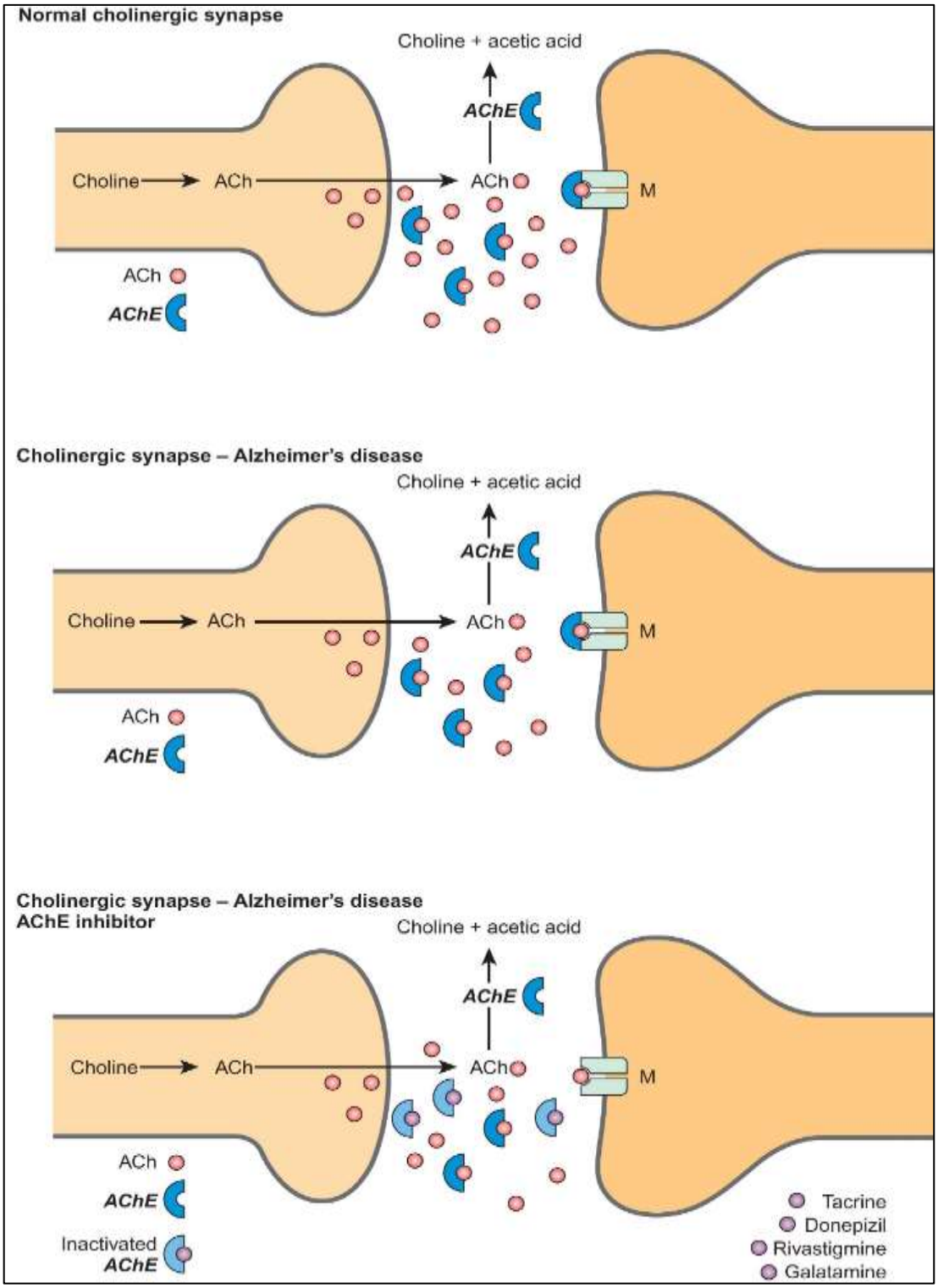

Figure 2.8: Acetylcholinesterase Inhibition (Neupsykey, 2018).

2.7.2 Alzheimer's Disease

Alzheimer's disease (AD) is a neurodegenerative disorder that is progressive and cannot be reversed. It was first described by Alois Alzheimer, a German psychiatrist, in the year 1907 (Alzheimer, 2010). In the beginning, the prevalence of AD was relatively low; however, over the past few decades, it has become more prevalent, particularly among the elderly population. AD is characterized by a gradual decline in cognitive abilities, beginning with memory deficits and eventually affecting all intellectual functions. This disease ultimately leads to complete dependence on basic daily functions and death at an earlier age (Masters et al., 2015). The pathological characteristics of AD include the formation of extracellular amyloid plaques, intracellular neurofibrillary tangles (NFTs) composed of hyperphosphorylated tau proteins, atrophied neurons, degenerating synapses, and reactive glial cells. The complex nature of the disease is the basis of two major hypotheses for its etiology: the cholinergic hypothesis, relating to deficits in cholinergic neurotransmission, and the amyloid hypothesis, relating to beta-amyloid (Aβ) plaque in the progression of AD.

Several risk factors for AD are known. These include aging, genetic predisposition, traumatic brain injury, vascular problems, inflammation, and environmental exposures. These factors, in turn, contribute to the accumulation of Aβ proteins and NFTs. Damage to the cholinergic system has been identified as one of the central molecular mechanisms in the pathophysiology of AD. Thus, enhancing cholinergic activity may create beneficial outcomes for improving cognitive function among victims of the disease (Jabir et al., 2018).

Cholinesterase inhibitors (ChEIs) are a pharmacological approach for the potential treatment of AD, reducing cognitive decline as well as psychological and behavioral symptoms. ChEIs, in effect, enhance cholinergic activity. They include the following: Donepezil, galantamine, and rivastigmine are the most widely used agents in clinical practice (Masters et al., 2015).

Alzheimer's accounts for sixty to eighty percent of all dementia cases. Due to the increased prevalence of this disease, especially amongst the aging population, there is an acute need for the development of novel and effective therapies. Even though synthetic drugs have been the focus for a long time, naturally occurring compounds, particularly polyphenols, are beginning to be regarded as promising alternatives. These compounds are relatively obtainable, cheaper, and mostly less toxic compared to synthetic drugs. In recent years,

increasing attention has been given to investigating the potential of polyphenol drugs, either alone or in combination, for the treatment of AD. These natural substances have exhibited promise in the area of cholinesterase enzyme targeting and thus may unveil an avenue for AD treatment associated with off-late side effects (Jabir et al., 2018).

2.8 ELLMAN PHOTOMETRIC METHOD

The general consensus is that AChE activity measurement by the Ellmann photometric method is presumably the most reliable. This method is predicated on the enzymatic breakdown of acetylcholine, which results in the formation of thiocholine as a by-product. After this, thiocholine reacts with dithiol nitrobenzoate (DTNB), resulting in the formation of a yellow product due to the transfer of electrons to sulfur. Thus, absorbance at 412 nm becomes a quantitative measure of AChE activity. Because of its reliability and high accuracy, the method is the most widely used for the measurement of enzyme activity. However, the Ellmann method is limited in some respects, including low substrate specificity and sensitivity to pH changes, which can adversely affect the accuracy of measurements in certain conditions.

The present study used the Ellmann photometric method to analyze AChE inhibition. A thorough understanding of the mechanisms underlying neurotoxicity and the interaction of pesticides and other agents with the cholinergic system is required (Oliveira Costa et al., 2018). This method yielded substantial information about the effect of different substances on AChE activity, shown in Figure 2.9.

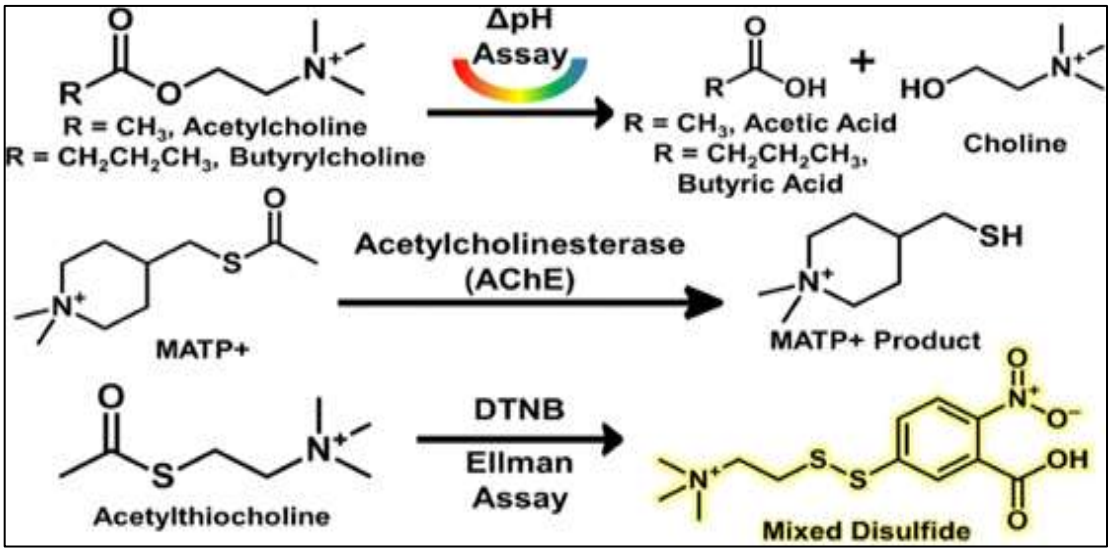

Figure 2.9: Ellman Photometric Method (Smith et al., 2021).

2.9 ANTIOXIDANT CAPACITY MEASUREMENT

Antioxidant capacity assessment involves the use of different approaches in ascertaining the ability of lots of substances, like foodstuffs, plant extracts, or chemical compounds, to inhibit oxidation or neutralise free radicals. These methods include testing the antioxidant capacity of a substance. However, antioxidant assays are necessary to appreciate the health benefits that may accrue from antioxidants concerning the prevention of oxidative-stress-related diseases. It is as follows:

2.9.1 DPPH (2,2-diphenyl-1-picrylhydrazyl) Assay

The compound 2,2-diphenyl-1-picrylhydrazyl (DPPH) is a widely used stable free radical in antioxidant activity studies due to its property of accepting hydrogen atoms from antioxidant molecules and causing a measurable color change (Guija-Poma et al., 2015). This method is known as the radical scavenging assay and is predominantly used to evaluate the antioxidant potential of various individual compounds, plant extracts, and food samples. One approach that is, however, tedious and time-consuming, especially in the interaction study of multiple antioxidants, requires that one set up various binary mixtures of the different antioxidants of varying concentrations for testing. These procedures can cause extremely massive datasets with high complexity for data analysis (Silva et al., 2024).

2.9.2 ABTS (2,2'-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid)) Assay

Comparable to the DPPH test, this ABTS test evaluates the degree to which ABTS is eliminated from the body. This test, which involves the creation of an ABTS radical cation, which is characterised by a blue-green pigment, therefore provides a colorimetric assessment of antioxidant activity. This test is carried out in order to. As antiradical activity decreases, the reduced colour is measured using spectrophotometric technology (Shraim et al., 2021).

2.9.3 FRAP (Ferric Reducing Antioxidant Power) Assay

This is a method and a means of measuring the ability of antioxidants to quench free radicals resulting from oxidative stress (Ay et al., 2007).

2.9.4 Total Phenolic Content (TPC) Assay

Even though it cannot be directly correlated to antioxidant action, this test of TPC at the very least provides a good estimation of the concentration of the latter, frequently seen as the one responsible for the efficacy of the antioxidant treatment. Further, the Folin-Ciocalteu reagent is used in this assay, and results are given in terms of gallic acid equivalents. The Folin-Ciocalteu assay for TPC quantification appears to have gained considerable popularity (Arituluk et al., 2016).

The importance of this assay is due to its use in assessing the total antioxidant capacity because higher concentrations of phenolics have been correlated with greater antioxidant potential. The method that started out as a simple procedure has undergone considerable development, and various protocols and methods have been established for various situations. The relationship between the absorbance measured and total phenolic content (TPC) expressed as gallic acid equivalent is direct. The thorough consideration of a number of factors is important for the accurate generation of data. Some of the factors are maximum absorption wavelength (λmax), time of color development, and volumetric ratio of Na_2CO_3 to Folin-Ciocalteu reagent (Arituluk et al., 2016).

2.9.5 Total Flavonoid Content Assay

The extracts are precipitated with aluminium chloride in alkaline solutions so that total flavonoids can be determined. This is done in the process of determination. The presence of

flavonoids in the aluminum chloride complex gives a bright yellow fluorescence visible under UV photodocumentation. The total flavonoid content (TFC) is expressed in terms of QE mg/g of dry extract. After solvent extraction, the most widely applied method is colorimetric analysis for TFC of plant materials. The aluminium chloride colorimetric method, employing Al (III) as a complexing agent, is a widely used method to determine TFC in plant extracts. The method of analysis described here is for determining the quantity of flavanol derivatives in pharmaceutical preparations (Christ and Muller, 1960).

This method is based on the use of flavonoids and aluminium (III) to make chelates. The excellent ability of flavonoids to bind metal ions such as Al (III) rests on the high number of oxygen and hydroxyl functionalities that they possess. Flavonoids are usually complex in a 1:1 ratio, though that can change depending on the experimental conditions and perhaps the presence or absence of effects of pH. Many different modifications of the basic method have been implemented over the years (Subedi et al., 2014).

One of these modifications was the addition of sodium nitrite (NaNO2) prior to the introduction of aluminum chloride. Within the immediate vicinity of aromatic diols, sodium nitrite acts as a selective nitrating agent, resulting in the formation of a flavonoid-nitroxyl derivative. This derivative is distinguished by the appearance of a new absorption band at a wavelength of 500 nm. In addition, conducting the Al (III)-flavonoid complexation in the presence of acetate salts was an additional adaptation that was carried out. Those who advocate for this method assert that the quantification of TFC through the utilisation of $AlCl_3$ is only feasible if the absorbance of the metal chelates derived from the individual flavonoids that are present in the sample demonstrates quantitatively analogous absorption at a particular wavelength. The empirical application of the method demonstrated that yellow Al (III)-flavonoid complexes were produced when $AlCl_3$ was introduced in the absence of $NaNO_2$, and the absorbance of these complexes was measured at specific wavelengths within the range of 410-440 nm. The TFC was discovered through the utilisation of calibration curves that were based on a reference flavonoid standard that was investigated under the same experimental conditions and at the same wavelength. Among the flavonoid standards that are utilised frequently are quercetin, catechin, and rutin, among others. In this investigation, the quantitative evaluation of total flavonoids was carried out by precipitating extracts with aluminum chloride in an alkaline medium. This was done in order to acquire

the results of the investigation. A brilliant yellow fluorescence is observed in flavonoids when they are subjected to ultraviolet spectroscopy in the presence of aluminum chloride. Quantification of the TFC was performed using the QE milligrams per gram of dry extract weight (Subedi et al., 2014).

3. MATERIAL AND METHOD

3.1 COLLECTION OF PLANT MATERIAL

The aerial and floral parts of *Erica arborea* were collected from Gemlik, Bursa. The plant sample was identified by Assoc. Prof. Dr. Ebru Özdemir Nath from the Department of Pharmaceutical Botany, Faculty of Pharmacy, Altınbaş University, Istanbul/Türkiye. A herbarium specimen was deposited at the Herbarium of Altınbaş University Faculty of Pharmacy (HERA) with the herbarium number HERA (1110), as shown in Figure 3.1.

Figure 3.1: Dried Sample of *Erica arborea L.*

3.2 PREPARATION OF PLANT EXTRACTS

After the samples had been dried, they were cut into pieces, put into a grinder, and then ground into a powder. After taking 20 grams of the ground plant, 200 mL of ethanol was added to the mixture. The sample of the plant was extracted by macerating it for twenty-four hours while shaking it occasionally. A rotary evaporator, specifically a Heidolph Hei-VAP Advantage rotary evaporator, was used to evaporate the solvent until it reached a dried state. Following the procedure as shown in Figure 3.2, the extract was stored at a temperature of +4°C until the biological activity research was carried out.

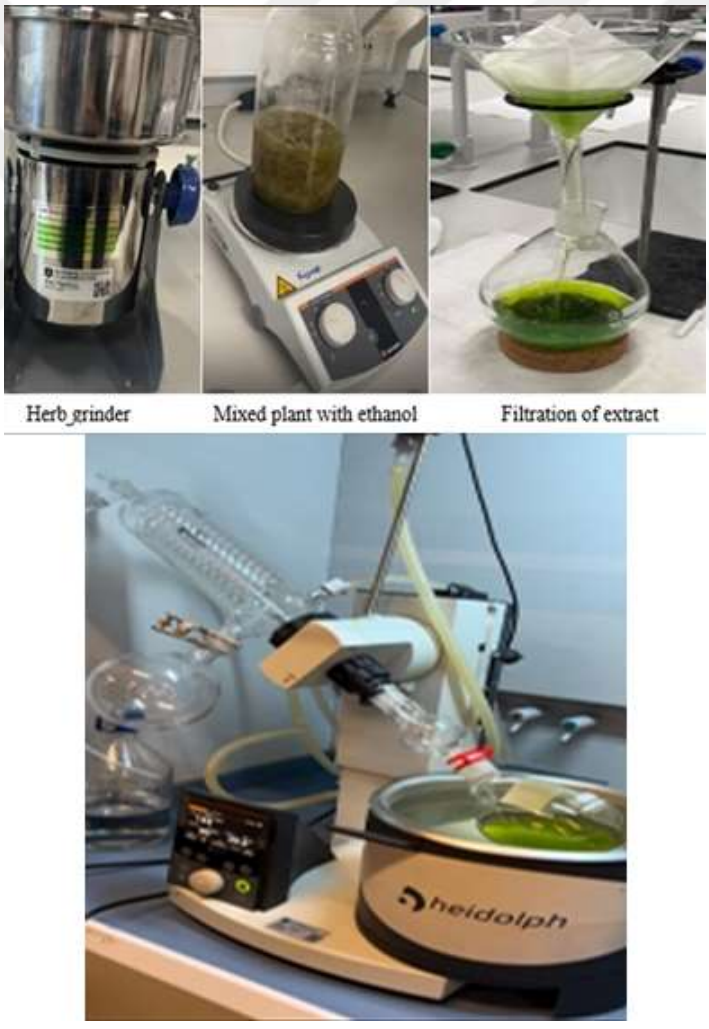

Figure 3.2: Rotary Evaporator Steps to Prepare the Plant Extract.

Then, the final extract was placed in a bottle labelled with the name of the plant, as shown in Figure 3.3.

Figure 3.3: Plant name label on the final extract.

3.3 BIOLOGICAL ACTIVITIES

3.3.1 DPPH (2,2-diphenyl-1-picrylhydrazyl) Radical Scavenging Assay

DPPH stands for 2,2-diphenyl-1-picrylhydrazyl, a stable free radical commonly used to test antioxidant activity. Therefore, any color change (due to loss of absorbance) shows a decrease in activity. The ability of antioxidants to reduce DPPH is assessed by a color shift from purple to yellow, which can be measured by absorbance measurement (Subedi et al., 2014). In this study, free radical scavenging activity was evaluated using the DPPH assay described by Brand-Williams (1999), with some minor modifications (Brand-Williams, 1999). These modifications included the preparation of a 0.1 mM DPPH solution in methanol (Sigma-Aldrich). The first preparation of DPPH consisted of 3.9 mg in 10 mL of methanol. After ten to fifteen minutes, it was placed in a dark bottle and placed in a water bath. Following that, a cylinder was filled with 10 mL of the initial solution, and then 90 mL of methanol was added to it. Following that, the mixture was thoroughly combined. After this, 240 µL of DPPH solution was added to 10 µL of plant extract solutions in ethanol at various concentrations (50 to 250 µg) in a 96-well plate. The concentrations of the plant extract solutions were varied accordingly. Once the reaction mixture had been stirred, the samples

were placed in an incubator at room temperature and kept in the dark for thirty minutes. At a wavelength of 517 nm, the absorbance was measured after the incubation period had been completed, and the inhibitory concentration (IC_{50}) was determined, which is a measure of the sample's activity in scavenging DPPH radicals. To construct a calibration curve, butylhydroxytoluene (BHT) was utilised as the reference compound. The absorbance of various BHT solutions with known concentrations was measured for standardization purposes to create the curve. The absorbance for the sample was carried out in a similar manner for the prepared solutions. The antioxidant activity of the extract was determined, and the figures below in Figure 3.4 show the percentage of DPPH radical scavenging activity. This was computed by using the following formula:

$$Percentage\ inthibition = \frac{(A\ control - A\ sample)}{A\ Control} x\ 100 \quad (3.1)$$

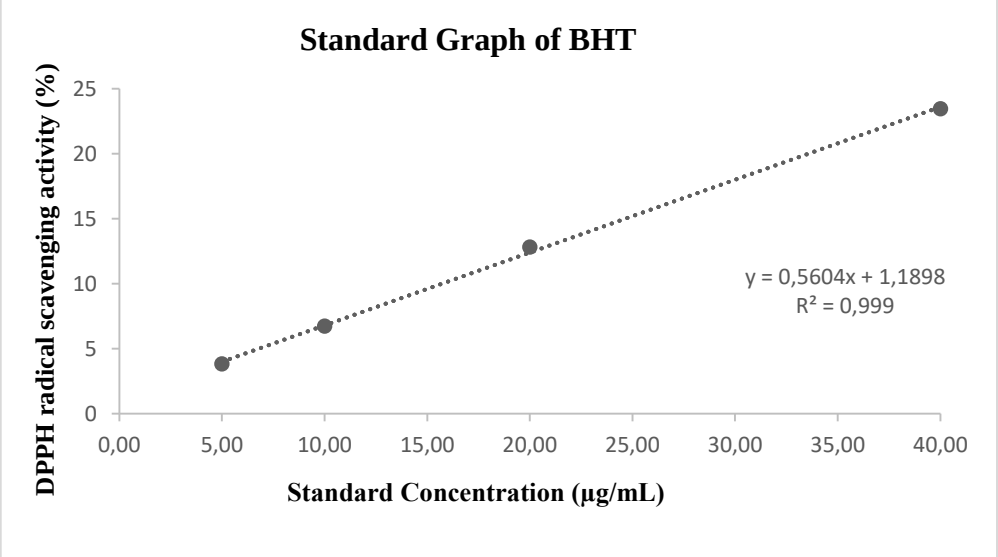

Figure 3.4: Standard Graph DPPH.

3.3.2 FRAP (Ferric Reducing Antioxidant Power) Assay

Using the FRAP assay method, as described by Benzi and Stren (1996) and with slight modifications according to Piatto (2005), the antioxidant capacity of the plant extract was

determined (Benzi and Stren, 1996; Piatto, 2005). The assay measures the ability of antioxidants to reduce ferric ions (Fe^{3+}) to ferrous ions (Fe^{2+}) and subsequently form a colored complex with a specialized reagent. This method is based on the premise that antioxidant capacity is linearly correlated to color enhancement, which can then be measured to provide an estimate of the extract's antioxidant capacity. In the experimental protocol, negligible amounts of sodium acetate trihydrate (31 g was accurately weighed) were transferred to a beaker in order to prepare a 300 mmol/L acetate buffer solution with pH 3.6 (Sigma-Aldrich, Germany). Subsequently, 800 mL of distilled water was introduced into the beaker, and the resultant mixture was agitated until the sodium acetate was entirely dissolved. Following the complete dissolution of sodium acetate, 1.6 mL of glacial acetic acid (Eso Laboratories, Germany) was meticulously added dropwise over an interval of several minutes, with the pH being continuously monitored throughout the addition process. Acetic acid was incorporated until the pH of the solution attained the value of 3.6. Upon achieving the specified pH, the solution was carefully transferred to a graduated cylinder, ensuring thorough homogenization, and the final volume was calibrated to 100 mL using distilled water. Ten microliters of the extract or standard solution were pipetted into Eppendorf tubes, and then 300 μL of FRAP reagent (with or without TPTZ) was added. The mixture was incubated for 15 min at 37°C in an incubator. After incubation, it was left at room temperature for another 5 min. 250 μL of the mixture was then transferred from the Eppendorf tube to the well plate. The absorbance of the reaction was measured at a wavelength of 593 nm using a Synergy H1 microplate reader (BioTek). All samples or standards were tested at least three times, as shown in Figure 3.5.

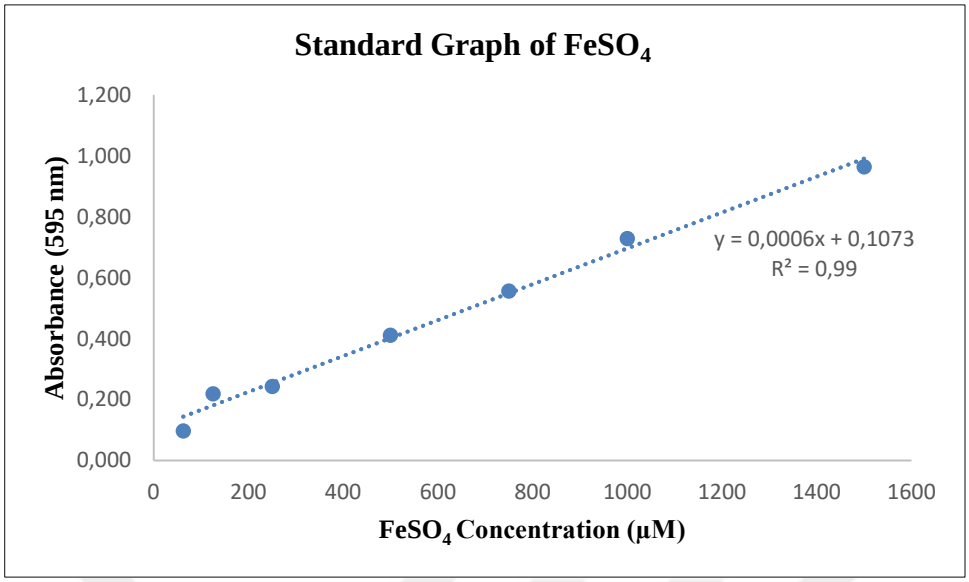

Figure 3.5: Standard Graph FRAP

3.3.3 Determination of Phenol Content

The total phenolic content (TPC) of the plant extract was determined using the Folin-Ciocalteu colorimetric method with slight modifications based on protocols previously described (Sinkard & Singleton, 1977; Sakanaka et al., 2005). A standard stock solution of gallic acid (5 mg/mL) was prepared by dissolving 52 mg of gallic acid (Sigma-Aldrich) in 10.4 mL of distilled water. From this stock, dilutions (5.0, 0.25, and 0.125 mg/mL) were made approximately seven times in Eppendorf tubes to form the calibration curve. For the assay, each well of a microplate contained 225 µL of distilled water and 5 µL of Folin-Ciocalteu reagent (Sigma-Aldrich). The addition of 5 µL of either gallic acid standard or plant extract sample to each well was followed. The sample was vortexed well and allowed to stand for 3 minutes. The colorimetric reaction was then initiated with the addition of 15 µL of sodium carbonate (Na_2CO_3) 2% solution (Sigma-Aldrich), thus providing alkaline conditions under which the redox reaction occurs between phenolic compounds and the Folin reagent. The microplate was incubated in the dark at room temperature for 2 hours in order to allow the color to fully develop. Absorbance readings were recorded at 760 nm using a spectrophotometer. From a standard calibration curve, the phenolic content of each sample was defined and expressed as gallic acid equivalents (GAE) following the equation derived from the gallic acid standard curve (Munteanu & Apetrei, 2021).

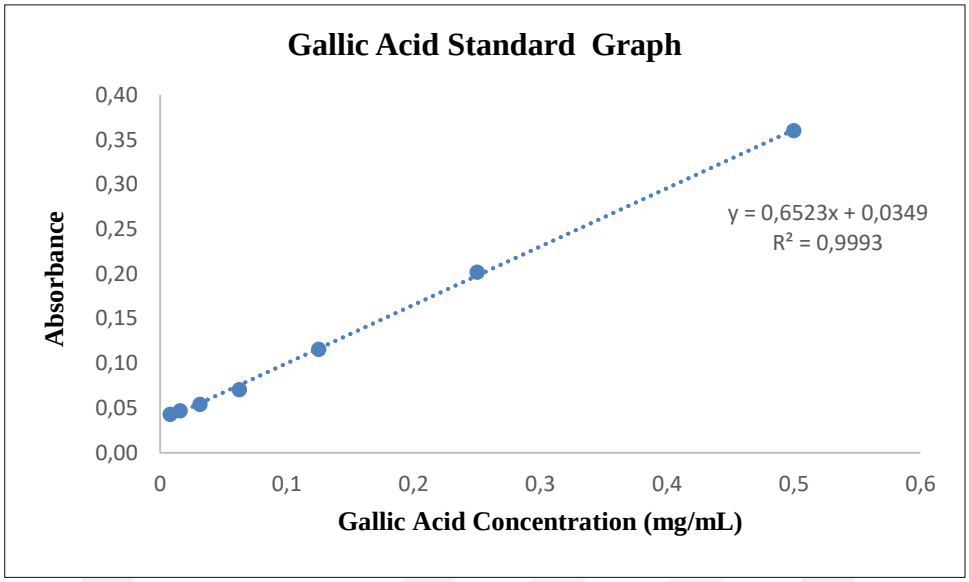

Figure 3.6: Standard Graph TPC.

3.3.4 Determination of Flavonoid Content

The TFC of the plant extract was determined by comparing it with the standard catechin. Experiments in this research were based on some references Slinkard and Singleton (1977) mentioned, with some modifications (Slinkard and Singleton, 1977). Total flavonoids were found quantitatively by precipitating the plant extract in an alkaline medium, where an intense yellow color was observed for flavonoids in the presence of aluminum chloride. Experiments were conducted by adding 7.5 µL of sodium nitrate ($NaNO_2$) to a 96-well plate having 125 µL of distilled water. Then, 25 µmol of ethanol was added to the blank sample, along with 25 µL of the standard quercetin solution. The quercetin solution was prepared by adding 10 mg of quercetin (Sigma-Aldrich) in a dark tube with 2 mL of methanol and placing it in a water bath after mixing it well, then diluting it serially to obtain different concentrations, for example, adding 500 µg of methanol in each Eppendorf tube, about seven tubes; then we added 500 µg of stock solution to the first, then we took 500 µg from it and added it to the second tube, and so on until the last concentration, and the concentrations were as (0.05 to 0.3) mg/mL. In addition to 25 µL of the plant extract. The samples were incubated for 5 minutes at room temperature. Next, 15 µL of aluminum chloride ($AlCl_3$) 10% (Sigma-Aldrich) was added to the plate and left to stand for another five minutes. Then, 27.5 µL of distilled water and 50 µM of sodium hydroxide solution (1M) were added, and

after mixing well, the absorbance was measured at 510 nm, and the results were expressed in μg/mL, using quercetin equivalents as the standard, as shown in Figure 3.7.

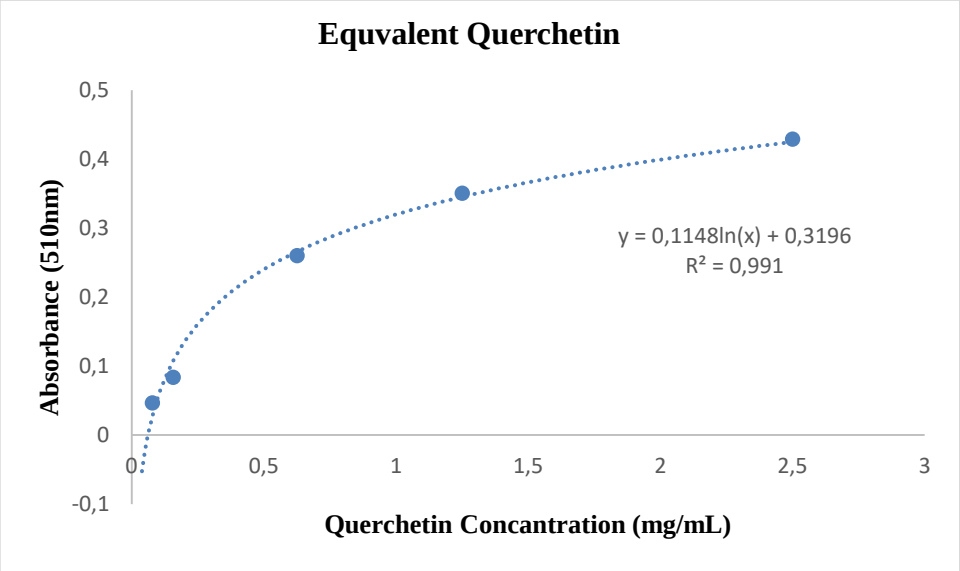

Figure 3.7: Standard Graph TFVC.

3.4 ACETYLCHOLINE ESTERASE INHIBITION ASSAY

The inhibitory effect of the methanolic extract of *Erica arborea* on AChE, previously described by Ellman et al. (1961), was used. The test solution included 1000 μL of Tris-HCl buffer pH 8.0, 240 μL of 5,5-dithiobis-(2-nitrobenzoic acid) (DTNB), 192 μL of acetylthiocholine iodide (ATC), 1200 μL of 100 mM Tris-HCl buffer pH 8.0, and 20 μL of extract solution (at concentrations of 1.0, 0.5, and 1.5 mg/mL) in methanol. The reactions were started by adding 6-10 μL AChE (electric eel) to the mixture and mixing well. During 2 min at 412 nm by spectrophotometer (Carry 60 Single Beam Spectrophotometer, Agilent Technologies, USA), the enzyme activity, in the form of the change in absorbance (ΔA) /time, was obtained from the equation.

$$\frac{\Delta A}{\Delta t} = E \tag{3.2}$$

In addition, according to the activity resulting from the blank sample, the blank enzyme activity was calculated and measured without adding the enzyme to determine the background absorbance. The computed value should be between 0.11 and 0.15. In addition,

it should be noted that the experiment should be conducted at a proper temperature to ensure the stability of the enzyme; the solutions should be prepared fresh, the inhibitor should be added before starting the reaction, and its effect on the enzyme activity should be measured for exact results.

3.5 CELL CULTURE AND MTT ASSAY

The consequences of the tested material were determined using the MTT assay in L929 (mouse fibroblast), HUVEC (human umbilical vein endothelial), and U87MG (human glioblastoma) cell lines. In suitable growth media supported with adjustment supplements such as 10% fetal bovine serum and 1% penicillin and streptomycin and maintained at 37°C in a humidified atmosphere containing 5% CO_2, the cells were cultured. The cells were implanted in 96-well plates at a density of 0.01×10^6 cells per well and left to adhere for 24 hours for the MTT assay. After that treatment with different concentrations of test samples for 24, 48, and 72 hours. 20 mL of MTT contained (5 mg/mL in PBS) was added for each well and incubated for 4 hours at 37°C, where the supernatant was removed after incubation and the formazan crystal formed was dissolved in 100 mL of dimethyl sulfoxide (DMSO); then, by using a microplate reader, measure the absorbance at 570 nm.

To calculate the cell viability as a percentage compared to the negative control group (untreated cells), which was expressed to represent 100% viability, hydrogen peroxide was used as a positive control to induce the cytotoxicity needed to observe the cell death in this group.

In triplicate, all experiments were performed, and the results were manifested as ± standard deviation (SD) (Gündoğan and Özdemir Nath, 2021; Özdemir Nath, Gündoğan, and Kartal, 2023).

4. RESULTS AND DISCUSSION

4.1 DPPH ANALYSIS

DPPH testing is a fundamental methodology for assessing the antioxidant capacity of plant extracts and various compounds. Due to its simplicity and rapid results, this method is particularly suitable for the initial assessment of antioxidant activity. IC_{50} values provide a numerical representation of the antioxidant activity of samples, enabling comparisons between different extracts and standard antioxidants such as BHT. A lower IC_{50} value correlates with greater antioxidant activity in the sample (Brand-Williams, 1999).

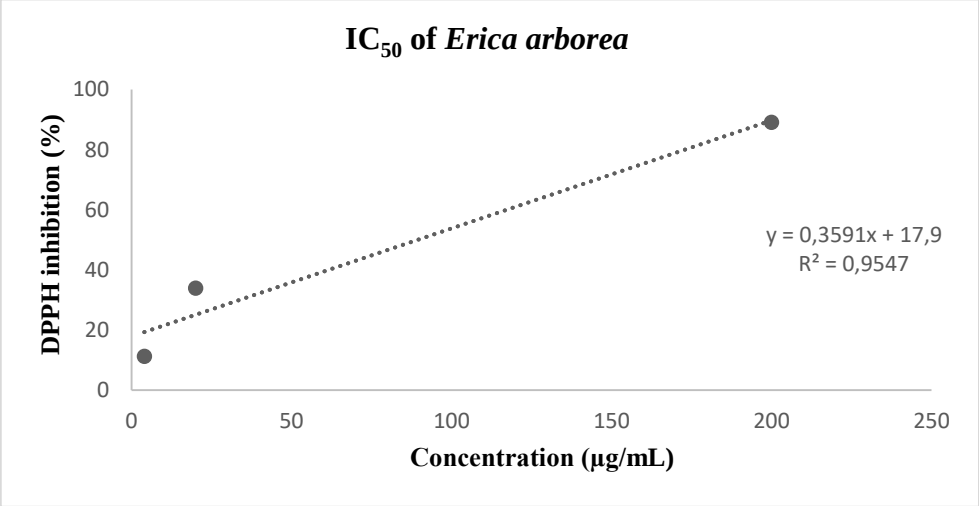

Figure 4.1: IC_{50} of *Erica arborea* for DPPH.

The figure shows the dose-dependent antioxidant activity of *Erica arborea* extract, measured using a DPPH (2,2-diphenyl-1-picrylhydrazyl) free radical scavenging assay (Figure 4.1). The y-axis stands for the percentage of DPPH inhibition, and the x-axis represents the extract concentration (µg/mL). The IC_{50} value was 82.09719 µg/mL. This value is considered moderate, showing antioxidant activity, albeit not significant. Comparing the extract to the synthetic antioxidant BHT, it was found that each gram of extract had 78.35125 mg of BHT-equivalent antioxidants, which also shows significant antioxidant activity. There was a linear correlation, which was positive, though stronger than average, and was found at the end of the investigation to be 0.5548. That indicates concentration is related well to free radical scavenging activity.

Thus, R^2 of this high value confirms the reality of the prediction made by the models that antioxidant effects and behavior of the extract are dependent on the dosage concentration.

4.2 TPC ANALYSIS

Heather's ability to inhibit AChE and its antioxidant potential were evaluated through various tests, focusing on total phenolic content (TPC) as an indicator of its antioxidant ability. The total phenolic content (TPC) data for *Erica arborea* extracts at different concentrations. Values are expressed in absorbance units, with the highest values being the most concentrated extracts.

Table 4.1: TPC Analysis.

Plant concentration	GAE (µg/mL)	GAE (mg/g extract)
0.1 mg/mL	5.72 ± 0.022	57.18

Table 4.1 shows the results of a total phenolic content (TPC) assay performed on *Erica arborea* extract, using the Folin-Ciocalteau method and expressed in gallic acid equivalents (GAE). The assay measures the total amount of phenolic compounds present, molecules that primarily contribute to antioxidant activity due to their ability to donate electrons and neutralize free radicals. At a plant extract concentration of 0.1 mg/mL, the total phenolic content of *Erica arborea* extract was determined to be 5.72 µg/mL of gallic acid equivalents (µg/mL GAE), equivalent to 57.18 mg of gallic acid equivalents per gram of extract (mg/g GAE). This value shows that Areca arborea extract is rich in phenolic compounds capable of preventing cellular oxidation by reacting with free radicals. These results show that *Erica arborea* may be a valuable source of natural antioxidants, and its bioactive compounds could be used in the development of natural antioxidants and therapeutic agents for various diseases associated with oxidative stress.

4.3 TOTAL FLAVONOID CONTENT (TFC) ANALYSIS OF *ERICA ARBOREA L.*

The study aimed to evaluate the TFC of the extracts of *Erica arborea L*. as micrograms per milliliter (µg/mL QE), on the basis of colour analysis of the extracts by the aluminium chloride method.

The effect of concentrations of the extract from *Erica arborea* (mg/mL) on TFC was studied in terms of quercetin equivalent (QE, mg/mL). The results showed a clear relationship between plant concentration and the number of extracted flavonoids. It was observed that the flavonoid content was very low at lower concentrations, ranging between 0.05 and 0.1 mg/mL, and began to increase with increasing concentration. The flavonoid content reached approximately 1.34 mg/mL at a concentration of 0.50 mg/mL of the plant extract, showing a clear increase in flavonoid production with increasing concentration. These results show that there is a direct relationship between the concentration of the *Erica arborea* extract and the flavonoid content, which explains to us that there is a biological activity of the plant that appears at a certain concentration and increases with increasing concentration.

4.4 FRAP ANALYSIS OF *ERICA ARBOREA* L.

In this study, the antioxidant ability of *Erica arborea* was evaluated across different concentrations using the FRAP method. The results were calculated in terms of Fe^{2+} concentration (µmol/L) and then normalized to the Eppendorf volume (using different concentrations of extract), as shown in Figure 4.2.

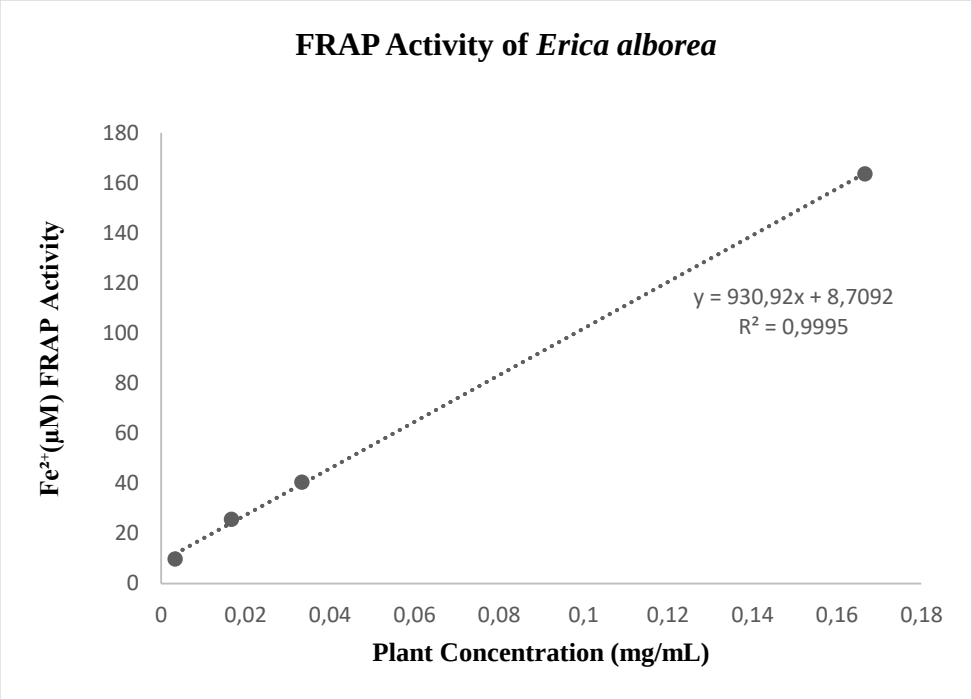

Figure 4.2: FRAP Activity.

The graph illustrates the correlation between the antioxidant capacity, or FRAP activity, measured in μM Fe^2, and the extract concentration, measured in mg/ml. $R^2 = 0.9910$ is the value that the equation yields. A strong correlation between the two variables is indicated by a high value. Moreover, there is a clear and strong linear relationship. From 160 μM at 0.17 mg/mL, the FRAP activity increases with the concentration of the *Erica arborea* extract. This suggests that there are a lot of active compounds in the extract, which can donate electrons and change iron (III) into iron (II), increasing the extract's antioxidant potential. The graph proves that the values rise steadily over time, giving us the ability to forecast how the extract will function and boosting confidence when consuming it.

4.5 ACETYLCHOLINESTERASE (ACHE) INHIBITION ASSAY ANALYSIS

In this study, various concentrations of *Erica* extract were evaluated to determine their inhibitory effect. A crude solution at a concentration of 10 mg/mL was prepared, and serial dilutions were performed. Based on the results, the IC_{50} value was calculated to be 0.5285 ± 0.025 mg/mL, indicating moderate bioactivity of the extract.

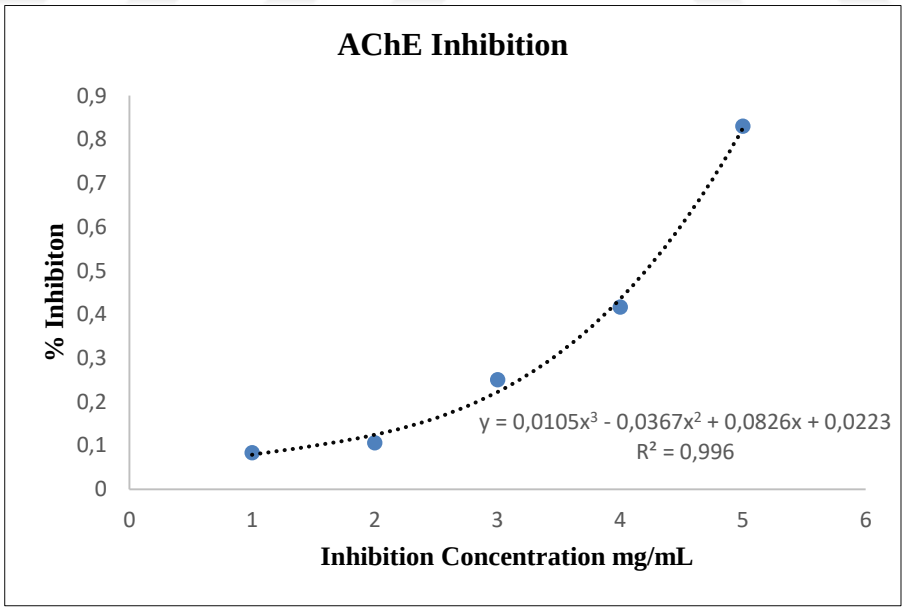

Figure 4.3: Inhibition Graph of AChE.

4.6 CELL CULTURE AND MTT ASSAY

Extract of *E. arborea* exerted a potent cytotoxic effect against U87MG cells at 24 h but had a lower action against L929 and HUVEC cells, showing the selectivity for neural-derived cells. Such selective cytotoxicity was still present at 48 h, for which, however, fibroblasts and endothelial cells kept higher rates of viability. The cell viability was marginally improved in L929 and HUVEC cells, while U87MG cells were still displaying significantly decreased viability at 72 h. As per these results, *E. arborea* and its extracts have bioactive molecules that have selective cytotoxicity towards neuron cells, which might be used in preclinical studies for the treatment of neurodegenerative diseases or brain cancer, with minimal, at the same time, no toxicity in non-neuronal cell populations (Figure 4.4, 4.5, and 4.6).

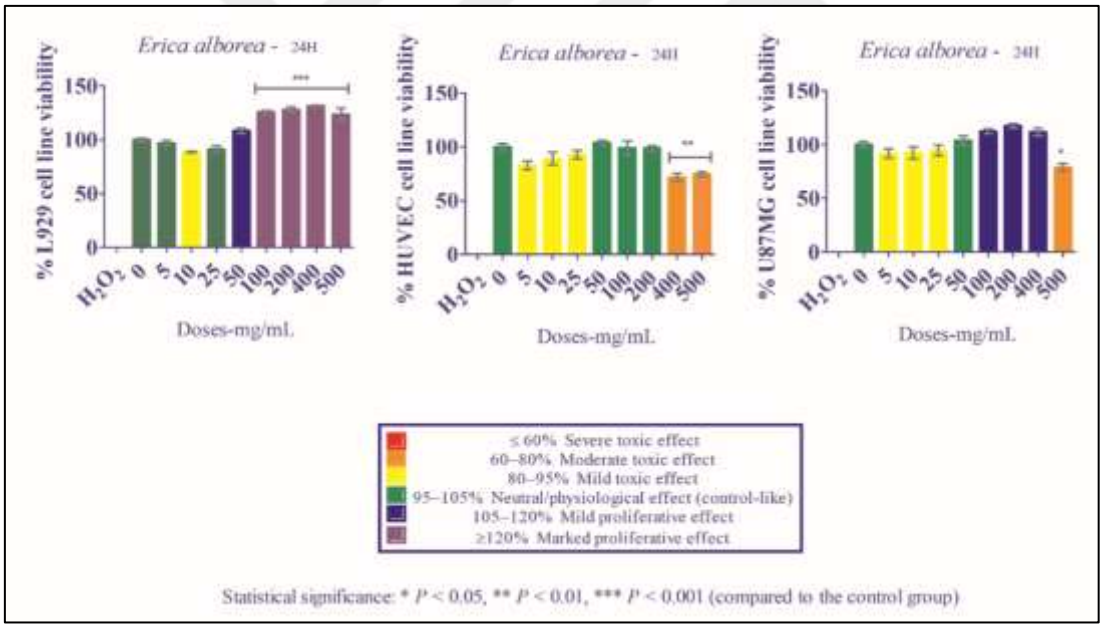

Figure 4.4: MTT Assay Results – 24 H.

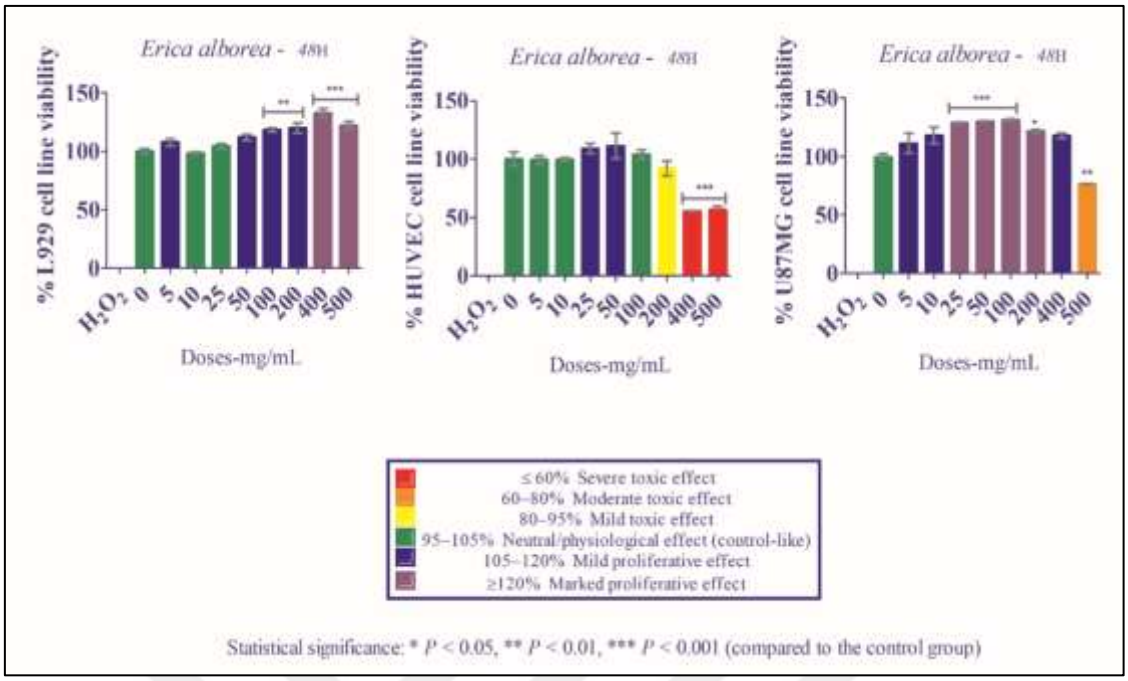

Figure 4.5: MTT Assay Results – 48 H.

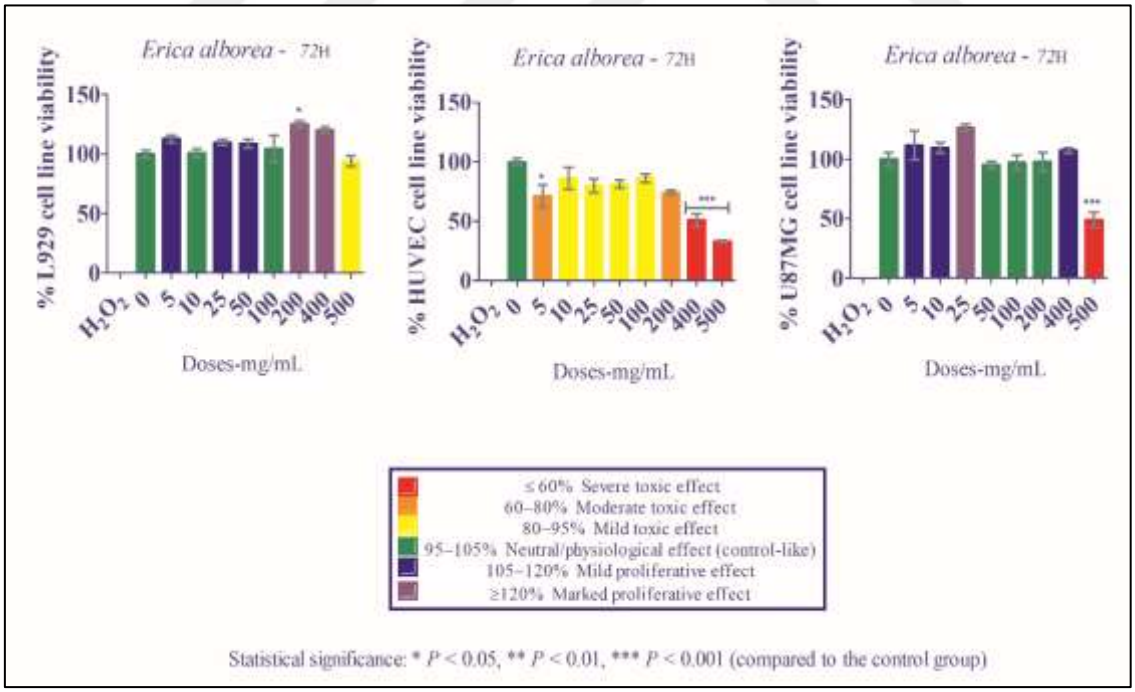

Figure 4.6: MTT Assay Results – 72 H.

4.7 STATISTICAL ANALYSIS

The various test samples were first analyzed for any statistically significant differences; ANOVA was used, followed by Tukey's multiple range test for post hoc comparisons. The level of significance was set at "p" < 0.05. To establish the correlation matrix, the relationship between the measured biological activities and concentrations of bioactive compounds was analyzed. On three antioxidant assays, DPPH, total phenolic content (TPC), and ferric reducing antioxidant power (FRAP), the effects of different concentrations of *Erica arborea L.* extracts were measured. For the final multivariate analysis, unsupervised principal component analysis (PCA) and partial least squares discriminant analysis (PLS-DA) were performed to cluster and discriminate samples on the basis of biological activities. Hierarchical clustering was conducted using Ward's method on the correlation coefficients and the Euclidean similarity measure to calculate the distance between all samples. All statistical analyses were performed using R software version 3.5.1, while one-way ANOVA followed by Tukey's post hoc tests on cell viability data were performed in GraphPad Prism version 8. The significance level was set at "p" < 0.05, reflecting the meaningful differences between experimental groups (GraphPad Software, 8).

4.8 DISCUSSION

In the present study, *Erica arborea* extract exhibited a dose-dependent antioxidant effect, as evidenced by the DPPH radical scavenging assay. The activity increased proportionally with concentration, revealing a significant linear correlation. These findings confirm the extract's effective radical neutralization potential and reinforce previous research that attributes such activity to bioactive compounds in *E. arborea*. Notably, Amezouar et al. (2013) highlighted the antioxidant and anti-inflammatory potential of this plant, linking it to its phytochemical profile. Similarly, Pavlović et al. (2009) assessed antioxidant properties in ethanol extracts from five Ericaceae species, including *E. arborea*, and reported comparable strong antioxidant performance using the DPPH method, supporting the present study's conclusions (Amezouar et al., 2013; Pavlović et al., 2009)

The total phenolic content (TPC) of *E. arborea* extract was quantified using the Folin–Ciocalteu method and expressed in gallic acid equivalents (GAE). At a concentration of 0.1 mg/mL, the TPC was measured at 5.72 µg/mL GAE, translating to 57.18 mg GAE/g of dry

extract. These results suggest that the extract is abundant in phenolic compounds, which are recognized for their capacity to mitigate oxidative damage by scavenging free radicals. This is in line with earlier studies that emphasized the phytochemical richness of *E. arborea*, particularly its high levels of phenolic acids and flavonoids, which contribute significantly to its antioxidant and anti-inflammatory properties (Nunes and Carvalho, 2013; Nunes et al., 2014).

In addition to phenolics, the total flavonoid content (TFC) of *E. arborea* was also assessed, showing a direct, linear relationship between concentration and flavonoid presence. This correlation highlights the extract's promise as a potent natural source of bioactive flavonoids. These findings align with those of Amari et al. (2023), who investigated the phytochemical constituents of multiple sub-extracts from *E. arborea* flowers and leaves. Their research confirmed a rich flavonoid profile and emphasized the antioxidant potential of these compounds (Amari et al., 2023).

The antioxidant efficacy of *E. arborea* was further confirmed through the FRAP (ferric reducing antioxidant power) assay, which evaluates a compound's ability to donate electrons and reduce ferric ions (Fe^{3+}) to ferrous ions (Fe^{2+}). The extract demonstrated considerable electron-donating capacity, reinforcing its strong antioxidant potential. This observation supports previous findings that identified *E. arborea* as a source of reducing agents and antioxidant molecules (Uysal et al., 2017; Zengin et al., 2019).

The study also investigated the inhibitory effects of the extract on acetylcholinesterase (AChE), an enzyme involved in cholinergic neurotransmission. Moderate inhibition was observed, suggesting the presence of neuroactive compounds capable of modulating cholinergic signaling. This mirrors the findings of Yüksel et al. (2021), who explored the AChE inhibitory properties of *Erica manipuliflora* extracts and confirmed the presence of naturally derived compounds with potential therapeutic applications, particularly for neurodegenerative disorders (Yüksel et al., 2021).

Lastly, preliminary evaluations revealed that *E. arborea* and its derivatives might exhibit selective cytotoxicity toward neuronal cells, suggesting potential application in neurotherapeutics or brain cancer research. These properties are likely attributed to the plant's diverse phytochemicals. Similar results were observed in earlier investigations by

Edda Ikra et al. (2019) and Amari et al. (2023), who identified potent bioactive constituents in *E. arborea* linked to various pharmacological activities (Edda Ikra et al., 2019; Amari et al., 2023).

5. CONCLUSION

The study reveals that *Erica arborea L.* has significant antioxidant and AChE inhibitory activities, indicating its potential as a bioactive compound source for therapeutic development. The DPPH assay showed moderate antioxidant activity, with an IC_{50} value of 1.82 mg/mL. The extract also showed a significant total phenolic content of 285.80 µg gallic acid equivalent at the highest concentration, indicating the role of phenolic compounds in its bioactivity. Neuroscience has opened up doors by allowing the use of AChE inhibition. An example of a neuroprotective agent backed by these facts is *Erica arborea*, which fuels the hope of using it in neurodegenerative diseases, such as Alzheimer's. These findings support traditional medicinal uses and authenticate the modern pharmacological relevance of the plant.

The full-on study scrutinized both the antioxidant and neuroprotective potency of *Erica arborea L.* using an entire multidisciplinary approach of several biochemical assays—DPPH radical scavenging, TPC, TFVC, FRAP, and AChE inhibition evaluation. These assays consistently reflected a concentration-dependent increase in bioactivity profiles across tests, with the most apparent effect with the 5% extract, which gave rise to the best antioxidant and enzyme inhibitions.

Among these assays, the DPPH emphasized the extract's ability to scavenge free radicals effectively. At 5% concentration, however, the extract demonstrated an important level of effectiveness in scavenging the free radicals, indicating good antioxidant activity. Similarly, both TPC and TFVC analyses showed that higher concentrations would result in containing greater amounts of the phenolic and flavonoid compounds, which have been known to contribute to antioxidant potential. TPC values were also notably congruent with trends in antioxidant activity, affirming the contribution of phenolic compounds to the bioactive profile of *E. arborea*:

Interestingly, the 0.1% concentration is one that one would expect to have little or no activity, high in flavonoids. This may indicate that there are effects of strong single flavonoids or other synergistic compounds even at low concentrations, as further phytochemical investigation may reveal.

Again, the FRAP assay confirmed the strong reducing capacity of this extract, where the maximum Fe^{2+} value was attained with the 5% extract. The AChE inhibition assay revealed a clear dose-response relationship, highlighting *E. arborea's* potential role in neuroprotective strategies, particularly in the context of diseases like Alzheimer's, where acetylcholine breakdown is implicated.

Multivariate analyses, including PCA and PLS-DA, supported these findings by identifying significant correlations between extract concentration, bioactive compound levels, and biological activity.

The results obtained in this study suggest that the extract from *Erica arborea* has selective cytotoxic activity by exerting its most powerful effects on U87MG cells while leaving fibroblast and endothelial cells almost unaffected. The proposed mechanism for this selectivity would allow directed targeting of neural-derived tumor cells or regulation of aberrant cell proliferation within the central nervous system. Before such avenues are considered, the neurotoxicity of the extract must be vetted to avoid complications in neurodegenerative models. Altogether, *E. arborea* holds potential as a bioactive source for selective applications in neuro-oncology.

5.1 RECOMMENDATIONS

a. Phytochemical Profiling: Future research should isolate and identify specific phenolic and flavonoid compounds in *E. arborea*, especially from the 0.1% extract, which had an unexpectedly high TFVC. High-resolution methods like LC-MS/MS or NMR can show their bioactive constituents.

b. In Vivo Validation: After promising in vitro results, in vivo studies should assess the extract's pharmacokinetics, bioavailability, and potential toxicity, especially at higher concentrations.

c. Mechanistic Studies: To better understand antioxidant and neuroprotective pathways, studies of cellular and molecular mechanisms (e.g., modulation of oxidative stress pathways or inhibition of cholinesterase activity) are recommended.

d. Formulation for Therapeutic Use: *Erica arborea* extracts' bioactive richness could lead to standardised nutraceuticals or functional food supplements. Dosage, formulation stability, and regulations must be considered.

e. Ethnobotanical Integration: The findings strongly support Mediterranean medicine's use of *Erica arborea.* Integrating ethnopharmacological data with scientific findings can expand therapeutic uses and preserve traditional knowledge.

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