BİTLİS EREN ÜNİVERSİTESİ
FEN BİLİMLERİ DERGİSİ

ISSN: 2147-3129 / e-ISSN: 2147-3188

**Article Type** : Research Article
**Received** : April 2, 2025
**Revised** : August 22, 2025
**Accepted** : September 23, 2025
**DOI** : 10.17798/bitlisfen.1669087

**Year** : 2025
**Volume** : 14
**Issue** : 3
**Pages** : 1487-1503

# DETERMINATION OF ENZYME INHIBITORY AND ANTIOXIDANT ACTIVITIES OF SELECTED OAK GALLS

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## ABSTRACT

In this study, the enzyme inhibition and antioxidant activities of methanol extracts obtained from galls induced by *Andricus quercustozae* and *Andricus cecconii* on *Quercus brantii* Lindley. and *Andricus quercusramuli* on *Quercus infectoria* Oliver were investigated.

The enzyme inhibition activity was assessed using spectrophotometric methods for acetylcholinesterase (AChE), butyrylcholinesterase (BChE), tyrosinase, and urease inhibition. The total phenolic and flavonoid contents of the extracts were quantified as gallic acid and quercetin equivalents, respectively. Antioxidant activity was evaluated using three distinct assays: DPPH free radical scavenging, ABTS cation radical scavenging, and CUPRAC.

Regarding cholinesterase inhibition, while none of the extracts exhibited activity against AChE, they demonstrated inhibition of BChE, with *A. quercustozae* gall extract showing the highest activity (31.33 ± 1.25%). Similarly, in tyrosinase inhibition, *A. quercustozae* gall extract exhibited the most significant effect, with an inhibition rate of 61.16 ± 0.72%. In urease inhibition, *A. quercustozae* gall extract was the only sample to demonstrate activity (40.98 ± 1.41%), whereas *A. quercusramuli* and *A. cecconii* extracts showed no significant effect.

The total phenolic contents of the extracts were higher than their total flavonoid contents, correlating with their strong antioxidant activities. Some extracts exhibited higher antioxidant activity than standard positive controls, further emphasizing their potential as natural antioxidant sources.

**Keywords:** Gall extracts, Butyrylcholinesterase inhibition, Tyrosinase, Urease, Radical scavenging, *Quercus* sp.

# 1 INTRODUCTION

The genus *Quercus*, belonging to the Fagaceae family, is represented by 531 species worldwide. Among these, 250 species are found in America, 125 in Asia, and the remaining species are distributed across Europe, North Africa, and Micronesia [1]. *Q. infectoria* is a small tree or shrub that can reach a height of up to 10 meters and retains some of its leaves during mild winters. Its natural distribution includes Southern Europe, Türkiye, Syria, and Iran. Similarly, *Q. brantii* is a deciduous tree with a broad, thick-branched canopy, capable of growing up to 10 meters in height. This species thrives at altitudes ranging from 350 to 1700 meters across Iran, the Caucasus, and the Eastern and Southeastern Anatolian regions.

Galls are abnormal plant tissue growths induced by various biological agents, including viruses, bacteria, fungi, nematodes, and arthropods [2]. The formation of galls involves the alteration of plant cellular mechanisms, leading to the development of novel structures with distinct morphological and functional properties. Among gall-inducing organisms, arthropods—such as wasps, midges, aphids, mites, and other insects—trigger gall formation by depositing their eggs on plant tissues. Upon hatching, the larvae feed on the host plant, stimulating the development of galls [3], [4], [5].

These arthropods influence the plant's biochemical pathways, prompting the secretion of secondary metabolites that are not typically produced by the host [6], [7].

It is suggested that these metabolites play a role in defending the plant against external threats [8], [9]. Recent findings indicate that gall formation not only alters plant morphology but also enhances the accumulation of phenolic compounds and flavonoids, thereby strengthening the antioxidant capacity of the host tissues [10].

Scientific studies on gall extracts have identified their bioactive components and elucidated the underlying biological mechanisms. The predominant phytochemicals found in galls include triterpenes, gallic acid, ethyl gallate, catechin, epicatechin, tannic acid, and resin. Gall tissues are exposed to higher levels of oxidative stress compared to normal plant tissues. The elevated production of polyphenols, anthocyanins, flavonoids, and tannins is considered an adaptive response to mitigate oxidative stress [11]. In particular, *Quercus infectoria* galls have recently been reported to exhibit strong antioxidant activities in DPPH, ABTS, and FRAP assays, supporting their potential as natural sources of bioactive agents [12].

Alzheimer disease (AD) is the most common form of dementia, characterized by cognitive decline and severe behavioral disorders. It is a progressive neurodegenerative brain

disorder that ultimately leads to death [13]. Currently, four drugs are available for AD treatment: galantamine, rivastigmine, donepezil, and memantine. The first three act as acetylcholinesterase (AChE) inhibitors. However, there is still no effective treatment for either curing or preventing AD. Given the increasing prevalence of AD in the population, the urgent development of disease-modifying agents remains a critical necessity. Recent studies have emphasized the potential of natural AChE inhibitors derived from plant polyphenols as promising candidates for AD therapy [14].

Tyrosinase is a copper-containing enzyme that plays a key role in the biosynthesis of melanin, which determines skin and hair pigmentation. The inhibition of tyrosinase has clinical applications in treating skin disorders associated with melanin hyperpigmentation. Additionally, tyrosinase inhibitors are widely used in cosmetics due to their skin-lightening effects. Although several synthetic inhibitors, such as kojic acid, have been developed to suppress tyrosinase activity, their long-term toxic effects have led to an increasing focus on identifying natural alternatives [15]. Recent findings have shown that *Q. infectoria* extracts exhibit up to 76% tyrosinase inhibition, confirming their potential as safe natural inhibitors for cosmetic and therapeutic use [16].

Urease activity serves as an important biochemical marker in both agriculture and medicine, particularly in viral diagnostics. Research has focused on urease inhibitors as potential therapeutic agents for diseases in which urease plays a role [17]. Bacteria, fungi, yeasts, and plants synthesize urease to obtain the nitrogen necessary for growth and development. Additionally, urease is considered a virulence factor in various pathogenic bacteria. One of the most extensively studied bacterial ureases is that of *Helicobacter pylori*, which is implicated in gastritis, peptic ulcer disease, and gastric cancer [18], [19]. The development of urease inhibitors is crucial for treating infections caused by urease-producing pathogens in humans and animals [20]. In recent years, increasing attention has been given to investigating the inhibitory effects of various plant extracts on urease activity [21].

With the increasing shift towards the utilization of natural resources, the search for natural antioxidant compounds has accelerated, prompting scientists to focus on the development of plant-derived pharmaceuticals. Additionally, natural compounds with enzyme inhibitory potential have garnered significant attention in the pharmaceutical industry. Enzyme inhibitors are incorporated into a wide range of therapeutic drugs used for the treatment of infectious, metabolic, cardiovascular, neurological diseases, and cancer [22]. For instance,

flavonoid derivatives isolated from *Quercus mongolica* pollen demonstrated significant antioxidant and tyrosinase inhibitory activities, surpassing some synthetic standards [23].

Lipid oxidation is a significant concern in the food industry due to the formation of undesirable food components and potentially toxic compounds. One of the primary strategies to prevent food oxidation is the use of antioxidants. However, several synthetic antioxidants commonly utilized in the food industry have been found to pose adverse health effects. Synthetic antioxidants such as butylated hydroxyanisole (BHA) and butylated hydroxytoluene (BHT) have been associated with carcinogenicity and liver damage in laboratory animals. These alterations are believed to contribute to the development of cancer, atherosclerosis, and cardiovascular diseases [24]. Consequently, there is a growing interest in natural antioxidants, particularly those derived from plants. The antioxidant properties of *Quercus* species have attracted increasing attention due to their role in preventing or mitigating oxidative damages caused by free radicals in the body [25]. Recent investigations also support the potential application of *Quercus* extracts in reducing oxidative rancidity in food products, highlighting their industrial significance [26].

This study aims to investigate the enzyme inhibition and antioxidant activities of methanol extracts obtained from galls induced by *Andricus quercusramuli*, *Andricus quercustozae*, and *Andricus ceconii* on *Quercus infectoria* and *Quercus brantii* L. species in the Siirt region of Türkiye.

## 2 MATERIAL AND METHODS

### 2.1 Plant Material and Extraction Method

Gall samples formed by *Andricus quercusramuli, Andricus quercustozae,* and *Andricus ceconii* on *Quercus infectoria* and *Quercus brantii L.* were collected from Nergizli (Türkiye, Siirt province, Şirvan) in October 2016 by entomologist Dr. Erdem Seven and species identification made by Dr. Musa Tataroğlu. Upon being transported to the laboratory, the samples (Figure 1.) were cleaned of larval and insect residues, subsequently dried, and ground into powder form. The samples were macerated with methanol and the solvent was subsequently removed using a rotary evaporator to obtain the extracts.



Sexual galls of *A. quercusramuli* on *Quercus infectoria*

Asexual galls *of A. quercustozae* on *Quercus brantii*

Sexual galls of *A. pseudocecconii* on *Quercus brantii*

***Figure 1. Morphological appearance of the oak cynipid galls (Cynipidae, Cynipini)***

## 2.2 The Enzyme Inhibition Activity

### 2.2.1 Acetylcholinesterase and Butyrylcholinesterase Inhibition Activity

The acetylcholinesterase (AChE) and butyrylcholinesterase (BChE) inhibition activities were assessed using the spectrophotometric method described by Ellman et al. (1961), with modifications by Kıvrak et al. (2009) [27], [28]. In this assay, AChE and BChE enzymes were utilized alongside their respective substrates, acetylthiocholine iodide (AcI) and butyrylthiocholine iodide (BcI). The enzymatic reaction was monitored based on the formation of a yellow-colored complex with 5,5'-dithiobis-(2-nitrobenzoic acid) (DTNB). Ethanol was used as the control, while galantamine served as the positive control.

The enzyme inhibition assay was performed by mixing 130 µL of pH 8 buffer solution, 10 µL of sample extract, 20 µL of AChE or BChE enzyme solution, 20 µL of DTNB, and 20 µL of AcI or BcI substrate. The reaction was recorded spectrophotometrically at 412 nm.

The anticholinesterase activity was calculated as the percentage of inhibition relative to the control using the following equation:

$$I\% = (A_{control} - A_{sample}/A_{control}) \times 100$$

### 2.2.2 Tyrosinase Enzyme Inhibition Activity

Tyrosinase inhibitors are typically evaluated in the presence of either a monophenolic substrate, such as tyrosine, or a diphenolic substrate, such as L-DOPA. The tyrosinase inhibition activity was determined using the spectrophotometric method described by Hearing (1987), with kojic acid serving as the positive control [29].

For the assay, the tyrosinase enzyme solution was prepared by dissolving 1 mg of enzyme in 250 µL of pH 6.8 buffer and dividing it into 50 µL aliquots. Prior to use, the solution was diluted to a final volume of 2000–3000 µL. The L-DOPA solution was prepared by dissolving 8.4 mg of L-DOPA in 5 ml of distilled water.

The reaction mixture consisted of 150 µL of pH 6.8 buffer solution, 10 µL of sample or control solution (prepared from a 4000 µg/ml stock solution), 20 µL of tyrosinase enzyme solution, and 20 µL of L-DOPA solution. The enzymatic reaction was monitored spectrophotometrically at 475 nm.

### 2.2.3 Acetylcholinesterase and Butyrylcholinesterase Inhibition Activity

The urease inhibition activity was determined using the method described by Mobley and Hausinger (1989) [30]. In this assay, extracts were incubated with urease enzyme, followed by the addition of urea as a substrate. The reaction product, ammonia, was quantified spectrophotometrically. Thiourea was used as a positive control.

The reaction mixture consisted of the sample solution, urease enzyme, and urea substrate prepared in phosphate buffer (pH 8.2). The reaction was performed by sequentially adding 10 µL of sample solution (from a 4000 µg/ml stock), 25 µL of urease enzyme (prepared in phosphate buffer, pH 8.2), and 50 µL of urea substrate solution. An initial absorbance reading was taken, followed by the addition of 45 µL of phenol reagent and 70 µL of alkaline reagent. The reaction was then monitored spectrophotometrically at 630 nm.

## 2.3 Determination of Antioxidant Activities of the Extracts

The antioxidant activities of the extracts were evaluated using three different methods: the DPPH free radical scavenging assay, the ABTS cation radical scavenging assay, and the CUPRAC assay, which measures the cupric ion reducing antioxidant capacity. Additionally, the total phenolic and flavonoid contents of the extracts were determined using appropriate analytical methods. BHT, BHA, and ascorbic acid were used as positive controls. Each sample was tested in triplicate.

### 2.3.1 DPPH Free Radical Scavenging Activity

The DPPH method measures the ability of antioxidant compounds to scavenge the stable free radical 2,2-diphenyl-1-picrylhydrazyl (DPPH). The absorbance of the DPPH radical at 517 nm was recorded using a UV-visible spectrophotometer. A decrease in absorbance indicates higher antioxidant activity and is accompanied by a color change from deep purple to yellow. This

method is widely used due to its simplicity, cost-effectiveness, and reliability in assessing total antioxidant capacity.

The DPPH radical scavenging activity was determined using the method developed by Blois (1958) [31]. The assay was performed by mixing 1 ml of the extract or positive control solution, prepared at different concentrations, with 4 ml of DPPH (0.1 mM) solution. The reaction was measured spectrophotometrically at 517 nm. The activity was evaluated based on the $IC_{50}$ value (µg/ml).

### 2.3.2 ABTS Cation Radical Scavenging Activity

ABTS [2,2'-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid)] is a chromogenic redox radical that is both stable and soluble in aqueous and organic solvents. This versatility allows its use in assessing both hydrophilic and hydrophobic antioxidant activities through a colorimetric reduction assay. In the original method, the ABTS radical cation ($ABTS^{\bullet+}$) is generated through the interaction between ferryl myoglobin, produced via the reaction of methyl myoglobin with hydrogen peroxide [32]. The $ABTS^{\bullet+}$ radical solution exhibits a blue-green color with characteristic absorbance maxima at 414, 645, 734, and 815 nm [33].

The ABTS cation radical scavenging activity of the extracts was determined using the method described by Re et al. (1999) [33]. For the assay, 1 ml of the extract or positive control solution at varying concentrations was mixed with 4 ml of ABTS solution. The absorbance was measured spectrophotometrically at 734 nm. The activity was evaluated based on the $IC_{50}$ value (µg/ml).

### 2.3.3 CUPRAC (Cupric Ion Reducing Antioxidant Capacity)

The CUPRAC (Cupric Ion Reducing Antioxidant Capacity) method, developed by Apak et al., is a widely used assay for determining total antioxidant capacity (TAC). It utilizes the Cu(II)-neocuproine (Nc) reagent, which is stable, cost-effective, and applicable to both hydrophilic and lipophilic antioxidants. In this redox reaction, phenolic hydroxyl groups are converted into their corresponding quinones, leading to the reduction of Cu(II) to Cu(I) and the formation of a Cu(I)-Nc complex with maximum absorbance at 450 nm [34].

The assay was performed by mixing 1 mL of $CuCl_2$, 1 ml of neocuproine, 1 ml of ammonium acetate buffer, and 1 ml of extract or positive control solution at varying concentrations. The absorbance was recorded spectrophotometrically at 450 nm. The activity was evaluated based on the $A_{0.5}$ value.

### 2.3.4 Determination of Total Phenolic and Flavonoid Content of Extracts

The total phenolic content of the extracts was determined using the Folin-Ciocalteu phenol reagent (FCR) and expressed as gallic acid equivalents [35].

The assay was performed by mixing different volumes of gallic acid solution or extract with distilled water to a final volume of 4.7 ml, followed by the addition of 0.1 ml of FCR and 0.3 ml of $Na_2CO_3$ solution. The absorbance was measured spectrophotometrically at 760 nm.

The total phenolic content of the extracts was calculated using the standard calibration curve generated from gallic acid absorbance values, following the equation:

Absorbance=0.0399 × gallic acid (µg) + 0.4642 (R2=0.9912)

The total flavonoid content of the extracts was determined using the aluminum nitrate method and expressed as quercetin equivalents [36].

The assay was performed by mixing different volumes of quercetin solution or extract with 80% ethanol to a final volume of 4800 µL, followed by the addition of 100 µL of 1 M potassium acetate solution and 100 µL of 10% aluminum nitrate solution. The absorbance was recorded spectrophotometrically at 415 nm.

The total flavonoid content of the extracts was calculated using the standard calibration curve generated from quercetin absorbance values, following the equation:

Absorbance=0.1079 × quercetin (µg) − 0.0509 (R2=0.9984)

## 3 RESULTS AND DISCUSSION

### 3.1 Yield

The yield of the extracts was determined as 11.56% from *A. quercustozae* asexual galls, 5.66% from *A. cecconii* sexual galls, and 5.20% from *A. quercusramuli* sexual galls. Among the three, *A. quercustozae* demonstrated the highest yield, indicating a more efficient extraction of bioactive compounds.

### 3.2 Enzyme Inhibition Activity Results

The enzyme inhibition activities of *A. quercusramuli*, *A. quercustozae*, and *A. cecconii* gall extracts were evaluated against acetylcholinesterase (AChE), butyrylcholinesterase (BChE), tyrosinase, and urease at a concentration of 200 µg/ml (Table 1).

Cholinesterase inhibition plays a crucial role in neurodegenerative disease management, particularly in Alzheimer's disease, by increasing acetylcholine levels in the brain. Consequently, inhibiting cholinesterase enzymes can enhance neurotransmission, making plant-derived inhibitors valuable candidates for natural therapeutic applications [37], [38].

None of the extracts exhibited inhibitory activity against AChE, confirming that these galls do not influence this enzyme. However, *A. quercustozae* gall extract exhibited the highest BChE inhibition (31.33 ± 1.25%), indicating moderate potential as a cholinesterase inhibitor. In contrast, both *A. quercusramuli* and *A. cecconii* galls showed significantly lower inhibition (7.42 ± 0.26% and 8.58 ± 0.52%, respectively). The reference inhibitor, galantamine, displayed 81.33 ± 1.04% inhibition, significantly higher than the tested extracts, suggesting that while these gall extracts may contribute to BChE inhibition, their potency remains lower than synthetic inhibitors. This aligns with the findings of Fahimirad et al. (2023), who reported BChE inhibition of 35.4% for *Quercus brantii* galls, reinforcing the potential of gall-derived compounds as natural cholinesterase inhibitors [39]. Similarly, Kamarudin et al. (2021) observed moderate BChE inhibition in *Quercus* gall extracts, further supporting their neuroprotective potential [40]. However, the inhibition observed remains significantly lower than that of synthetic inhibitors such as galantamine (81.33 ± 1.04%), indicating that while gall extracts may contribute to enzyme inhibition, further fractionation and compound isolation are required to enhance their effectiveness.

***Table 1. Enzyme inhibition activity results***

| | Enzyme inhibition[a] (%) | | | |
|---|---|---|---|---|
| **Samples** | **AChE** | **BChE** | **Tyrosinase** | **Urease** |
| ***A.quercusramuli*** | NA | 7.42 ±0 .26 | 51.78 ± 0.61 | NA |
| ***A.quercustozae*** | NA | 31.33 ± 1.25 | 61.16 ± 0.72 | 40.98 ± 1.41 |
| ***A. cecconii*** | NA | 8.58 ± 0.52 | 55.86 ± 0.28 | NA |
| **Galanthamine[b]** | 82.85 ± 0.21 | 81.33±1.04 | - | - |
| **Kojic acid[b]** | - | - | 95.05 ± 0.37 | - |
| **Thiourea[b]** | - | - | - | 98.37 ± 0.40 |

*a: The concentrations were 200 µg/ml. B:Positive controls. NA: Not active. The results are presented as mean ± standard deviation.*

Tyrosinase plays a pivotal role in melanin biosynthesis and enzymatic browning, making tyrosinase inhibitors crucial for cosmetic, food, and medicinal industries. Due to its significant role in various biological processes, the identification, synthesis, and characterization of new tyrosinase inhibitors have gained attention in fields such as food, cosmetics, and medicine.

In the tyrosinase inhibition assay, all three extracts exhibited notable activity, reinforcing their potential as natural depigmentation agents. *A. quercustozae* galls displayed the highest inhibition (61.16 ± 0.72%), followed by *A. cecconii* galls (55.86 ± 0.28%) and *A. quercusramuli* galls (51.78 ± 0.61%). While the extracts showed promising activity, they remained lower than the reference inhibitor, kojic acid (95.05 ± 0.37%), indicating the need for further optimization. These findings are in agreement with those of Choudhry and Akhtar (2023), who reported 76% tyrosinase inhibition for *Quercus infectoria* gall extracts [41], as well as Nair et al. (2020), who observed moderate tyrosinase inhibition in *Quercus brantii* [42]. Compared to these studies, *A. quercustozae* galls showed promising activity, though it remained lower than that of the standard inhibitor kojic acid (95.05 ± 0.37%). These variations may stem from differences in phenolic composition, extraction methods, and plant species, highlighting the importance of further analysis to identify the most active compounds responsible for this effect.

Urease plays a critical role in pathogenic bacterial infections such as *Helicobacter pylori*-associated ulcers, and its inhibition has significant implications in medical and agricultural applications [43], [44].

For urease inhibition, *A. quercustozae* galls were the only extract that exhibited measurable activity (40.98 ± 1.41%), while *A. quercusramuli* galls and *A. cecconii* galls showed no inhibition. Thiourea, the positive control, demonstrated a near-complete inhibition (98.37 ± 0.40%). This aligns with the findings of Fahimirad et al. (2023), who reported 45.2% urease inhibition for *Quercus* gall extracts, supporting their potential as natural antibacterial agents [39]. However, compared to thiourea (98.37 ± 0.40%), the inhibition observed remains moderate, suggesting that further purification of the active compounds could enhance their bioactivity. The moderate activity of *A. quercustozae* galls suggests potential for further development as a natural urease inhibitor.

Overall, *A. quercustozae* galls demonstrated the strongest enzyme inhibition, particularly in BChE, tyrosinase, and urease assays, positioning it as a promising candidate for enzyme-targeted applications.

## 3.3 Antioxidant Activity Results

Table 2 presents total phenolic and flavonoid content, and antioxidant activity of *A. quercusramuli*, *A. quercustozae*, and *A. cecconii* galls extracts. The data are expressed in terms of phenolic and flavonoid content (as gallic acid equivalents and quercetin equivalents per mg of extract), and $IC_{50}$ or $A_{0.5}$ values for DPPH, ABTS, and CUPRAC assays.

Among them, *A. quercustozae* galls exhibited the highest total phenolic content (438.2 ± 0.8 µg GAE/mg extract) and flavonoid content (49.2 ± 0.6 µg QE/mg extract), followed by *A. cecconii* galls (176.5 ± 0.8 µg GAE/mg extract, 22.8 ± 0.6 µg QE/mg), while *A. quercusramuli* galls contained significantly lower levels (51.5 ± 0.1 µg GAE/mg, 12.0 ± 0.0 µg QE/mg).

In terms of antioxidant activity, *A. quercustozae* galls demonstrated the strongest radical scavenging effect in the DPPH assay ($IC_{50}$ = 5.0 ± 0.7 µg/ml), closely followed by *A. cecconii* galls ($IC_{50}$ = 25.8 ± 0.3 µg/ml), while *A. quercusramuli* galls exhibited significantly weaker activity ($IC_{50}$ = 211.6 ± 0.2 µg/ml). Similarly, in the ABTS assay, *A. quercustozae* galls displayed the lowest $IC_{50}$ value (1.0 ± 0.4 µg/ml), indicating the highest scavenging potential, followed by *A. cecconii* galls (5.1 ± 0.5 µg/ml) and *A. quercusramuli* galls (38.3 ± 1 µg/ml). In the CUPRAC assay, *A. quercustozae* galls again exhibited the lowest $A_{0.5}$ value (3.8 ± 0.3 µg/ml), demonstrating superior reducing capacity compared to *A. cecconii* galls (12.0 ± 0.8 µg/ml) and *A. quercusramuli* galls (91.1 ± 0.2 µg/ml).

***Table 2. Antioxidant activity, total phenolic and flavonoid content of gall extracts***

| | | | $IC_{50}$ (µg/ml) | | |
|---|---|---|---|---|---|
| **Samples** | **Phenolic content** | **Flavonoid content** | **DPPH Free Radical** | **ABTS Cation Radical** | **CUPRAC $A_{0.5}$ (µg/ml)** |
| ***A. quercusramuli galls*** | 51.5 ± 0.1 | 12.0 ± 0.0 | 211.6 ± 0.2 | 38.3 ± 1 | 91.1 ± 0.2 |
| ***A. quercustozae galls*** | 438.2 ± 0.8 | 49.2 ± 0.6 | 5.0 ± 0.7 | 1.0 ± 0.4 | 3.8 ± 0.3 |
| ***A. cecconii galls*** | 176.5 ±0.8 | 22.8 ± 0.6 | 25.8 ± 0.3 | 5.1 ± 0.5 | 12.0 ± 0.8 |
| **Ascorbic acid** | - | - | 6.1 ± 0.6 | 1.9 ± 0.9 | 5.2 ± 0.9 |
| **BHA** | - | - | 4.9 ± 0.7 | 52.1 ± 0.4 | 38.2 ± 1 |
| **BHT** | - | - | 934.9 ± 1 | 2.5 ± 0.8 | 2.7 ± 0.7 |

Compared to standard antioxidants, *A. quercustozae* galls displayed an $IC_{50}$ value comparable to ascorbic acid (6.1 ± 0.6 µg/ml) and BHA (4.9 ± 0.7 µg/ml) in the DPPH assay, while in the ABTS assay, its activity was significantly stronger than that of BHT (2.5 ± 0.8 µg/ml). In the CUPRAC assay, *A. quercustozae* galls also exhibited high reducing capacity, outperforming BHT (2.7 ± 0.7 µg/ml).

These findings align with existing research demonstrating the strong correlation between polyphenolic content and antioxidant capacity in medicinal plants [45], [46]. Several studies have reported a direct association between total phenolic content and radical scavenging ability, further supporting the robust antioxidant properties of *A. quercustozae* galls [47], [48].

The strong antioxidant properties of *A. quercustozae* galls are directly linked to its high polyphenolic content, which was found to be 438.2 ± 0.8 µg GAE/mg extract, the highest among the tested samples. This aligns with the study by Azmaz et al. (2020), who reported 479.56 mg GAE/g for *A. quercustozae*, confirming that this species is a rich source of bioactive phenolics [49]. The total flavonoid content (49.2 ± 0.6 µg QE/mg extract) also supports its strong radical scavenging activity, as flavonoids are known to contribute to antioxidant mechanisms.

Antioxidant activity, as determined by free radical scavenging assays, showed that *A. quercustozae* galls exhibited the strongest activity across all the tests. The DPPH assay revealed an $IC_{50}$ of 5.0 ± 0.7 µg/ml, which is lower (indicating stronger activity) than the values reported by Mohammadzadeh et al. (2021) for *Quercus infectoria* (7.8 µg/ml) [50]. Similarly, in the ABTS assay, *A. quercustozae* galls demonstrated an $IC_{50}$ of 1.0 ± 0.4 µg/ml, significantly stronger than the 7.3 µg/ml reported by Nair et al. (2020) for *Quercus brantii*, confirming its superior ability to neutralize free radicals [42]. The CUPRAC assay, which measures reducing power, further supported these findings, with *A. quercustozae* galls exhibiting an $A_{0.5}$ value of 3.8 ± 0.3 µg/ml, outperforming *A. cecconii* (12.0 ± 0.8 µg/ml) and *A. quercusramuli* galls (91.1 ± 0.2 µg/ml). These results align with Limsuwan et al. (2020), who reported similar reducing capacities for *Quercus brantii* galls (192.8 mg TE/g), reinforcing the strong metal-reducing potential of *A. Quercustozae* [51].

These findings confirm that *A. quercustozae* galls are a highly potent antioxidant source, with its DPPH, ABTS, and CUPRAC activity surpassing that of many previously studied *Quercus* gall extracts. Compared to synthetic antioxidants, *A. quercustozae* galls exhibited DPPH activity comparable to ascorbic acid (6.1 ± 0.6 µg/ml) and BHA (4.9 ± 0.7 µg/ml), while its ABTS scavenging ability exceeded that of BHT (2.5 ± 0.8 µg/ml), reinforcing its potential as a natural antioxidant alternative.

The variations in activity among different studies are likely influenced by differences in extraction methods, plant species, and environmental conditions affecting phytochemical composition. Several studies, including those by Kovacevic (2000) and Rakic et al. (2007), have reported a direct correlation between total phenolic content and antioxidant capacity, further supporting the strong antioxidant properties observed in *A. Quercustozae* [45], [46]. Additionally, Shirzad et al. (2011) and Rahnama et al. (2014) emphasized the importance of polyphenolic compounds in free radical scavenging, which is consistent with our results [47], [48].

# 4 CONCLUSION AND SUGGESTIONS

This study comprehensively evaluated the enzyme inhibition and antioxidant activities of *Andricus quercusramuli* galls, *Andricus quercustozae* galls, and *Andricus cecconii* galls extracts. The results highlight *A. quercustozae* galls as the most promising species due to its high phenolic and flavonoid content, which strongly correlates with its superior antioxidant capacity. Additionally, it exhibited moderate inhibition against BChE, tyrosinase, and urease, suggesting its potential in applications related to neuroprotection, cosmetics, and antibacterial treatments.

Overall, the findings of our study indicate that *A. quercustozae* gall extract possesses a high phenolic content and strong antioxidant potential, with enzyme inhibition activities that are generally in line with those reported in the literature. When compared with *Quercus* gall extracts from previous studies, our results demonstrate that *A. quercustozae* galls exhibits greater radical scavenging activity in ABTS and DPPH assays, as well as a higher phenolic content in most cases. However, tyrosinase inhibition was slightly lower than in some studies, indicating that its enzymatic activity may vary depending on specific phytochemical compositions. These differences are likely influenced by variations in extraction techniques, solvent types, plant species, and environmental factors affecting phytochemical composition. Future studies should focus on standardizing extraction methods and further exploring the bioactive properties of *A. quercustozae* gall extracts for potential pharmaceutical and industrial applications.

Despite these promising results, several limitations of the present study should be acknowledged. First, the bioactivity assessments were limited to in vitro assays, which may not fully represent in vivo biological effects. Second, only methanol extracts were tested, leaving the potential activities of other solvent fractions unexplored. The study also focused on a restricted set of enzymes (BChE, AChE, tyrosinase, and urease), whereas additional biological targets relevant to neurodegenerative and infectious diseases were not included. Furthermore, the plant material was collected from a single geographical region (Siirt, Türkiye), which may not capture the full phytochemical diversity of these species in different habitats. Finally, no isolation or structural characterization of individual compounds was conducted, which limits the ability to directly link specific metabolites to the observed activities. Addressing these limitations in future research will provide a more comprehensive understanding of the pharmacological and industrial potential of Quercus galls.

## Acknowledgements

This study is based on the master's thesis of Merve Doğan Abdioğlu and was supported by the Scientific Research Committee of Batman University under project number .18.001. The authors would like to express their gratitude to Dr. Erdem Seven for his assistance in sample collection and to Dr. Musa Tataroğlu for his valuable contribution to the identification of the samples.

## Conflict of Interest Statement

There is no conflict of interest between the authors.

## Statement of Research and Publication Ethics

The study is complied with research and publication ethics.

## Artificial Intelligence (AI) Contribution Statement

This manuscript was entirely written, edited, analyzed, and prepared without the assistance of any artificial intelligence (AI) tools. All content, including text, data analysis, and figures, was solely generated by the authors.

## Contributions of the Authors

Merve Doğan Abdioğlu conducted the conceptualization, experimental studies, data collection, and preparation of the original draft of the manuscript. Nesrin Haşimi supervised the research, contributed to the methodology, provided guidance during data interpretation, and critically revised the manuscript. Mehmet Boğa contributed to the experimental analyses and assisted in refining the final version of the paper. All authors read and approved the final manuscript.

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