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Investigation of the Potential Antioxidant and Antidiabetic Effects of *Cotinus coggygria* Scop.

Cotinus coggygria Scop.'un Potansiyel Antioksidan ve Antidiyabetik Etkilerinin İncelenmesi

Alperen CANPOLAT[1]
Hafize YUCA[2]
Bilge AYDIN[3]
Zühal GÜVENALP[2]

[1] Ağrı İbrahim Çeçen University, Faculty of Pharmacy,Department of Pharmacology, Ağrı, Türkiye
[2] Atatürk University, Faculty of Pharmacy, Department of Pharmacognosy, Erzurum, Türkiye
[3] Erzincan Binali Yıldırım University, Faculty of Pharmacy, Department of Pharmacognosy, Erzincan, Türkiye

ÖZ

Amaç: *Cotinus* coggygria Scop. türünün farklı zamanlarda toplanan farklı kısımlarının (yeşil renkli yapraklar, kırmızı renkli yapraklar, ince dallar ve gövde iç kısmı) toplam fenolik madde, toplam flavonoit madde, antioksidan (DPPH$^{\bullet}$ ve ABTS$^{\bullet+}$ süpürücü etki) ve α-glukozidaz inhibisyon etkileri açısından değerlendirilmesi ve karşılaştırılması bu çalışmanın amacını oluşturmaktadır.

Yöntemler: Ekstrelerin toplam fenolik madde miktarı Folin ve Denis tarafından geliştirilen, Singleton tarafından modifiye edilen yöntem kullanılarak tayin edilmiştir. Toplam flavonoit madde miktarı ise Lar'kina ve arkadaşları tarafından geliştirilen yönteme göre tayin edilmiştir. Ekstrelerin antioksidan kapasitelerinin test edilmesi amacıyla DPPH$^{\bullet}$ ve ABTS$^{\bullet+}$ süpürücü kapasite tayini deneyleri; antidiyabetik etkilerinin test edilmesi amacıyla α-glukozidaz enzim inhibisyonu deneyleri yapılmıştır.

Bulgular: Toplam fenolik madde, toplam flavonoit madde bakımından en yüksek değerlere gövde ekstresinin sahip olduğu bulunmuştur. Antioksidan aktivite deneylerinde de toplam fenolik madde ve toplam flavonoit madde miktar tayini deneylerinin sonuçlarıyla benzer şekilde en yüksek etkiye gövde ekstresinin sahip olduğu bulunmuştur. Bununla beraber diğer bütün ekstrelerin de özellikle ABTS$^{\bullet+}$'ye karşı yüksek antioksidan etki gösterdiği gözlenmiştir. Ekstrelerin antidiyabetik etkilerinin incelenmesi amacıyla yapılan α-glukozidaz inhibisyonu deneylerinde ise başta kırmızı ve yeşil yaprak olmak üzere diğer ekstrelerin de antidiyabetik etkiye sahip olduğu bulunmuştur.

Sonuç: Halk arasında çeşitli tıbbi etkilerinden dolayı kullanılan *C. coggygria* türünün farklı kısımlarından elde edilen ekstrelerin antioksidan ve antidiyabetik etkileri bilimsel olarak değerlendirilmiş, kanıtlanmış ve karşılaştırılmıştır.

Anahtar Kelimeler: α-glukozidaz inhibisyonu, ABTS$^{\bullet+}$, *Cotinus coggygria* Scop., DPPH$^{\bullet}$, Toplam fenolik içerik, Toplam flavonoit içerik

ABSTRACT

Objective: The aim of this study is to evaluate and compare the total phenolic content, total flavonoid content, antioxidant activities (DPPH$^{\bullet}$ and ABTS$^{\bullet+}$ scavenging effects), and α-glucosidase inhibition effects of different parts of *Cotinus* coggygria Scop. (green leaves, red leaves, branches, and the heartwood) collected at different times.

Methods: The total phenolic content of the extracts was determined using the method developed by Folin and Denis and modified by Singleton. The total flavonoid content was determined according to the method developed by Lar'kina and colleagues. To test the antioxidant capacities of the extracts, DPPH$^{\bullet}$ and ABTS$^{\bullet+}$ scavenging capacity assays were conducted; and to test their antidiabetic effects, α-glucosidase enzyme inhibition assay was performed.

Results: It was found that the heartwood extract had the highest values in terms of total phenolic content and total flavonoid content. In the antioxidant activity assays, similar to the results of the total phenolic and total flavonoid content determinations, the heartwood extract also showed the highest content. However, it was observed that all other extracts also exhibited high antioxidant activity, especially against the ABTS$^{\bullet+}$. In the α-glucosidase inhibition assay conducted to examine the antidiabetic effects of the extracts, it was found that in addition to red and green leaves, other extracts also possessed antidiabetic activity.

Conclusion: The antioxidant and antidiabetic effects of extracts obtained from different parts of *C. coggygria*, a species used in traditional medicine for its various therapeutic effects, have been scientifically evaluated, proven, and compared.

Keywords: α-glucosidase inhibition, ABTS$^{\bullet+}$, *Cotinus coggygria* Scop., DPPH$^{\bullet}$, Total flavonoid content, Total phenolic content

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Sorumlu Yazar/Corresponding author: Alperen Canpolat
E-mail: alperencanpolat@yahoo.com

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Introduction

Plants have been used by people since ancient times to eliminate health problems and treat diseases due to their various effects. Although these effects of plants were discovered through trial and error, scientific studies on plants support and prove the accuracy of the effects of most of them, which are used among the public. Nowadays, with these developments over time, there is a significant increase in the importance of herbal treatment (Matic, 2013).

The antioxidants found in plants play a crucial role in herbal medicine because they mitigate oxidative stress caused by free radicals, thereby preventing cellular damage. Free radicals can attack biomolecules in the body, leading to damage in DNA, lipids, and proteins, which can increase the risk of various chronic diseases, especially cardiovascular diseases and cancer. Antioxidants neutralize these free radicals, helping to maintain cellular integrity and strengthen the body's defense mechanisms. Therefore, plant-derived antioxidants have attracted significant interest in the health sector for their protective and therapeutic properties (Dias, 2021).

Similarly, Diabetes mellitus (DM) is a significant metabolic disorder characterized by hyperglycemia, and medicinal plants can also play an important role in treating this condition. The inhibition of enzymes such as α-amylase and α-glucosidase, which play a critical role in the digestion of dietary starch, is one of the therapeutic approaches used in the management of DM. Due to the side effects of antidiabetic drugs that inhibit carbohydrate-hydrolyzing enzymes, there is increasing interest in the potential therapeutic uses of medicinal plants (Özbek et al., 2019).

Figure 1. Red leaves, green leaves, branches and heartwood of *C. coggygria*

Cotinus genus is one of the plants used for their beneficial effects. There are 8 species of the genus *Cotinus* and only *Cotinus* coggygria Scop. (Anacardiaceae) grows in Türkiye (Davis,1982; Bizim Bitkiler, 2024). *C. coggygria* is popularly known as 'Smoke tree', 'Dyer sumac' or 'Tetra' (Deniz, 2020). Various parts of *C. coggygria* are used for different purposes among the public. Researches have proven that various drugs and extracts obtained from *C. coggygria* are effective in terms of pharmacological activity (Figure 1).

The leaves of *C. coggygria* have been used by the Balkan and Anatolian people for many years due to their wound healing effects. The infusion prepared from the leaves of *C. coggygria* is used in the treatment of throat and intestinal infections, gastric and duodenal ulcers, diarrhea and DM. As a result of different biological activity studies, it has been observed that the leaves of *C. coggygria* have anti-inflammatory, antidiarrheal and antioxidant effects (Georgieva, 2015; Saeed, 2016).

One of the compounds responsible for various pharmacological effects in plants is antioxidants. Antioxidants have a protective effect against cancer and immune system diseases by significantly reducing the negative effects of free radicals. Vitamins A, C, E, phenolic compounds and flavonoids are examples of natural antioxidants. Therefore, evaluating the total phenolic compound amount and total flavonoid amount of a plant may give us an idea about the antioxidant effectiveness of a plant. When evaluating antioxidant activity, the scavenging effects of $ABTS^{\bullet+}$ [2,2'-azinobis(3-ethylbenzothiazoline-6-sulfonicacid)] and $DPPH^{\bullet}$ (2,2-diphenyl 1-picrylhydrazyl) are used. There are some antioxidant activity studies using one or more of the following: total phenolic substance determination, total flavonoid substance determination, $ABTS^{\bullet+}$ and $DPPH^{\bullet}$ scavenging capacity assays in extracts prepared from the leaves, branches and young shoots of *C. coggygria* (Matic, 2013; Marcetic, 2013). There are also many studies comparing the antioxidant activity of the *C. coggygria* with other plant species. As a common result of these studies, it has been found that the antioxidant potential of *C. coggygria* is higher than other species (Niciforovic et al., 2010; Karagöz et al., 2015).

There are 11 known flavonoids isolated from heartwood of *C. coggygria*. Fustin, fisetin, sulfuretin, quercetin, 3',4',5,7-tetrahydroxyflavanol (taxifolin), 4',7-dihydroxy flavanol, 3',4',7-trihydroxyflavanone (butin), 4',7- dihydroxyflavanone (liquiritigenin), trans-2',3,4,4'- tetrahydroxychalcone (butein), 4',5,7-trihydroxyflavanone, trans-2',4,4'-trihydroxychalcone (isoliquiritigenin), have been isolated and identified by NMR in a previous research (Valianou et al., 2009).

DM, a metabolic disorder characterized by elevated blood sugar levels, is another condition in which plants are utilized for their therapeutic properties. In the treatment of DM, the inhibition of the α-glucosidase is crucial. Studies on the α-glucosidase inhibitory effects of extracts obtained from the leaves and branches of *C. coggygria*, commonly used as an antidiabetic among the public, can be shown as evidence that the species has antidiabetic activity. In a previous antidiabetic activity research, c and 1,2,3,4,6-penta-O-galloyl-β-glucose have been isolated from the leaves of *C. coggygria*. The compound 1,2,3,4,6-penta-O-galloyl-β-glucose has been found to exhibit significant inhibition of alpha-glucosidase (Özbek et al., 2019; Cha et al., 2009). 1,2,3,4,6-penta-O-galloyl-β-glucose and gallic acid have also been isolated from branch extracts of *C. coggygria* (Matic, 2013).

However, there is no study on which part of the plant has higher antioxidant activity, which part is richer in total phenolic and total flavonoids, and which part shows higher antihyperglycemic effects through α-glucosidase inhibition. In addition, no research has been found on the inner part of the heartwood of the plant in terms of antioxidant activity and antidiabetic effects. Based on this lack of information in the literature, it was aimed to evaluate and compare different parts of *C. coggygria* collected at different times (green leaves, red leaves, thin branches and heartwood) in terms of total phenolic content, total flavonoid content, antioxidant effects ($ABTS^{\bullet+}$ and $DPPH^{\bullet}$ scavenging effect) and α-glucosidase inhibition.

Methods

Plant Material

C. coggygria was collected from the Erzurum-Oltu crossroads in October 2020 and identified by Assist. Prof. Songül Karakaya (Department of Pharmacognosy, Faculty of Pharmacy, Atatürk University, Erzurum, Türkiye). After the aboveground parts were separated, the leaves, heartwood and branches of the plant were separated from each other. The bark of the body has been peeled off. The peeled heartwood, branches, green and red leaves were dried in the shade and then powdered in a grinder. The plant sample is kept in the Atatürk University Biodiversity Application and Research Center Herbarium (Herbarium No: AUEF 1004).

Extraction

50 g each of heartwood, green leaves, red leaves and branches were dried in the shade and powdered. They were placed in flasks and macerated with 500 mL of methanol overnight and extracted the next day in a mantle heater under a reflux cooler for 3 hours. The extracts were filtered and the filtrates were concentrated to dryness in a rotary evaporator at 40 °C and 120 rpm. As a result of the extraction processes, 13.06 g of extract was obtained from green leaves. The yield in this process was 26.12%. 11.35 g (% yield = 22.70) was extracted from red leaves, 6.07 g (% yield = 12.14) was extracted from branches, and 5.37g (% yield = 10.74) was extracted from heartwood.

Determination of Total Phenolic Content

Total phenolic content of methanol extracts prepared from red leaves, green leaves, heartwood and branches of *C. coggygria* were determined using the method developed by Folin and Denis (Folin. And Denis, 1912), modified by Slinkart and Singleton (Slinkart and Singleton, 1977). Gallic acid was used as the standard phenolic compound. Gallic acid solutions were prepared at concentrations of 100, 200, 400, 500 and 600 µg/mL, and 1 ml of each solution was taken and made up to 23 mL with distilled water. After completion, 0.5 mL of Folin-Ciocâlteu Reagent (FCR) was added and after a 3 minute waiting period, 1.5 mL of 2% sodium carbonate (Na_2CO_3) solution was added. After mixing on a magnetic stirrer at room temperature for 2 hours, absorbances were recorded at 760 nm against a blank consisting of distilled water. As a result of the experiment, the concentration-absorbance standard graph was obtained.

To prepare samples of plant parts, stock solutions of each extract were prepared at a concentration of 1 mg/mL. 1 mL of each of these stock solutions was taken and made up to 23 mL with distilled water. After completion, 0.5 ml of FCR and 1.5 mL of 2% Na_2CO_3 were added after 3 minutes, respectively. The samples were mixed in a magnetic stirrer for 2 hours at room temperature. At the end of this period, the absorbance of the samples was recorded at 760 nm against the blank consisting of distilled water. Measurements were repeated 3 times. Gallic acid equivalents corresponding to the absorbance values of the samples were found with the help of the equation obtained from the standard graph, and the results were given as gallic acid equivalent (GAE).

Determination of Total Flavonoid Content

Total flavonoid contents of methanol extracts prepared from red leaves, green leaves, heartwood and branches of *C. coggygria* were determined according to the method developed by Lar'kina et al. (Lar'kina, 2009).

30 mL of 70% ethanol was added to 1 g of powdered different plant parts and heated under reflux at 60 °C for 1 hour. The process was repeated three times. The extracts were filtered and placed in a 100 mL volumetric flask and the volumes were made up to 100 mL with ethanol (solution A).

Sample solution: 2 mL of solution A, 4 mL of 10% ethanolic $AlCl_3$ and 0.1 mL of diluted HCl were taken into the volumetric flask and the volume was completed to 50 mL with 95% ethanol. After 20 min, absorbances were measured against reference solutions at 410 nm.

Reference solution: 2 ml of solution A, 0.1 mL diluted HCl was taken into a volumetric flask and the volume was completed to 50 mL with 95% ethanol.

Rutin sample solution: 0.05 g rutin was weighed and taken into a volumetric flask. 10 mL of 95% ethanol was added and dissolved by heating in an 80°C water bath. After complete dissolution, the volume was made up to 50 mL with 95% ethanol (solution A).

Rutin test solution: 1 mL of solution A was taken into a volumetric flask and 4 ml of 10% $AlCl_3$ solution was added, and the volume was completed to 50 mL with 95% ethanol. Absorbance was measured at 410 nm. Measurements were repeated 3 times and averages were taken. Calculations were made according to the equation below.

$$X = (D \times MR \times 100 \times 50 \times 100 \times 100) / DR \times M \times 2 \times 50 \times 50 \times 100$$

D: Absorbance of sample solution

DR: Absorbance of Rutin test solution

M: Weight of raw material (g)

MR: Weight of Rutin (g)

X: Total amount of flavonoids calculated based on Rutin (%)

Antioxidant Activity Assay: DPPH• Scavenging Capacity

The DPPH• scavenging capacities of extracts obtained from different parts of the of *C. coggygria* were tested according to the Blois method (Blois, 1958). 1 mM DPPH• solution was used as free radical. Stock solutions of the extracts at different concentrations (20-1000 µg/mL) were prepared and 210 µL of stock solution and 70 µL of DPPH• solution were transferred to each microplate well. The samples were shaken for approximately 1 minute and incubated for 30 minutes at room temperature and in the dark. The absorbance of all samples against the ethanol blank was recorded at 517 nm.

For the control sample, 210 µL ethanol and 70 µL DPPH• solution were used, and α-tocopherol and trolox were used as standard antioxidants. Percentage of inhibition values were calculated with the formula given below.

DPPH• radical scavenging capacity (%) = [(AC- AS) / AC] × 100

AS: Absorbance found after adding sample to DPPH• solution

AC: Absorbance of the control sample containing DPPH• and ethanol

Antioxidant Activity Assay: $ABTS^{\bullet+}$ Scavenging Capacity

ABTS cation radical scavenging capacity determination was determined according to the study conducted by Re et al. (Re, 1999). 2 mM $ABTS^{\bullet+}$ solution was prepared. $ABTS^{\bullet+}$ was obtained by adding 2.45 mM potassium persulfate solution to this solution in a 1:1 ratio. 140 µL of the stock solutions of the extracts at different concentrations (20-1000 µg/mL) and 100 µL of the $ABTS^{\bullet+}$ solution were transferred to the wells of 96-well microplate and shaken for 1 minute.

140 µL of $ABTS^{\bullet+}$ solution and 100 µL of phosphate buffer (0.1 M, pH = 7.4) were used as control samples. The absorbance of the control sample at 734 nm should be 0.700 ± 0.025. At the end of the 30 minute incubation period, absorbances were recorded at 734 nm against the blank consisting of buffer. Trolox was used as the standard compound. $ABTS^{\bullet+}$ scavenging capacities of the extracts were calculated as percentages according to the equation below.

$ABTS^{\bullet+}$ scavenging capacity (%) = [(AC- AS) / AC] × 100

AS: Absorbance value found after adding samples to $ABTS^{\bullet+}$ solution

AC: Absorbance value of the control sample containing only $ABTS^{\bullet+}$ and buffer solution

Antidiabetic Activity Assay: α-Glucosidase Inhibition Assay

α-Glucosidase inhibitory effect determination was applied to the Bachhawat et al. (2011) method with some modifications (Bachhawat, 2011). In a 96-well microplate, 50 µL of phosphate buffer (50 mM, pH 6.9), 10 µL of α-glucosidase (1 unit/mL) and 20 µL of plant extract (concentration range of 1-5000 µg/mL) were mixed and heated at 37 °C. It was kept for 5 minutes. 20 µL of 3 mM p-nitrophenyl-α-D-glucopyranoside (pNPG) was added to the mixture as substrate and incubated at 37 °C for 30 minutes. The reaction was completed by adding 50 µL of 0.1 M sodium carbonate. All solutions were prepared in buffer system and acarbose was used as a positive control. The amount of yellow p-nitrophenol (pNP) formed was measured at 405 nm. Each determination was repeated 3 times and the results were calculated according to the equation below.

% Inhibition = [(Acontrol – Asample) / Acontrol] x 100

Results

In this study, methanol extracts prepared from different parts of *C. coggygria* (green leaves, red leaves, thin branches, and heartwood) were evaluated and compared in terms of total phenolic content, total flavonoid content, antioxidant activity ($ABTS^{\bullet+}$ and DPPH• scavenging capacities), and α-glucosidase inhibition. At the end of the extraction process, 13.06 g of extract was obtained from 50 g of powdered green leaves (%yield= 26.12). 11.35 g (% yield = 22.70) was extracted from 50 g red leaves, 6.07 g (% yield = 12.14) was extracted from 50 g branches, and 5.37 (% yield = 10.74) was extracted from 50 g heartwood.

Determination of Total Phenolic Content

Total phenolic contents of methanol extracts prepared from red leaves, green leaves, heartwood and branches of *C. coggygria* were determined by the FCR Method (Slinkart & Singleton, 1977). Gallic acid was used as the standard phenolic compound and the standard graph to be used in the calculations was prepared. Total phenolic compound contents of the extracts were calculated as GAE.

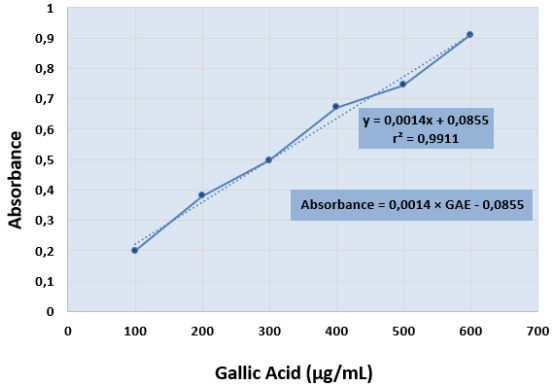

Figure 2. Standard graph of gallic acid

Table 1. Total phenolic contents of extracts

Extract	Total Phenolic Content (mg GAE/g dry extract)
Red leaf	142.50
Green leaf	65.50
Heartwood	297.93
Branches	105.86

It was determined that the heartwood extract, with a total phenolic content of 297.93 mg GAE/g dry extract, has a significantly higher phenolic content compared to the other extracts. The heartwood extract is followed by the red leaves with 142.50 mg GAE/g dry extract, the branches with 105.86 mg GAE/g dry extract, and the green leaves with 65.55 mg GAE/g dry extract, respectively (Table 1).

Determination of Total Flavonoid Content

In terms of total flavonoid content, it was concluded that the heartwood extract has the highest value at 6.32%, followed by the red leaf extract at 5.21%. These two extracts are followed by the green leaf extract at 4.23% and the branch extract at 1.59% (Table 2).

Antioxidant Activity Assay: DPPH• Scavenging Capacity

The DPPH• scavenging capacity of α-tocopherol, used as a standard antioxidant, reached its highest value at a concentration of 60 µg/ml. DPPH• scavenging capacity of trolox, another standart antioxidant, reached its highest value at a concentration of 45 µg/ml. In line with these data, the concentration range to be studied was determined as 10-100 µg/mL.

Inhibition (%) values, IC_{50} values, inhibition (%) graphs against concentration of DPPH• scavenging capacity of methanol extracts prepared from red leaves, green leaves, heartwood and branches of *C. coggygria* and standard antioxidant compounds at 45 µg/ml and 60 µg/ml concentrations were obtained (Table 3, Figure 4).

Table 2. Total flavonoid contents of extracts

Extract	Total Flavonoid Contents as Rutin Equilavent (mg RE/g dry extract)
Heartwood	6.32
Red leaf	5.21
Green leaf	4.23
Branches	1.59

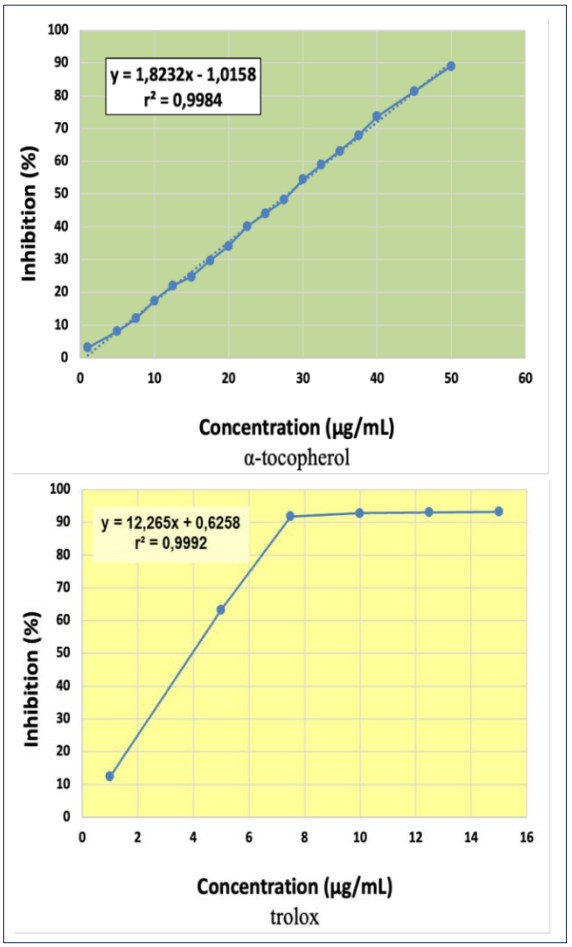

Figure 3. Inhibition (%) - concentration graphs of α-tocopherol and trolox

Based on the obtained data, the DPPH• scavenging capacities of the extracts at concentrations of 45 µg/mL and 60 µg/mL were compared with standard antioxidant compounds, trolox and α-tocopherol. At the concentration of 45 µg/mL, trolox showed 94.26% inhibition and α-tocopherol showed 81.30% inhibition. The heartwood extract demonstrated 80.88% inhibition, having the highest effect among the extracts and an effect close to that of the standard compounds. The heartwood extract was followed by the red leaf extract with 46.47%, the branch extract with 42.16%, and the green leaf extract with 31.86%. At the concentration of 60 µg/mL, trolox exhibited 94.32% inhibition, while α-tocopherol exhibited 90.65% inhibition. The heartwood extract demonstrated 84.07% inhibition, again showing the highest effect among the extracts and an effect close to that of the standard compounds. The heartwood extract was followed by the red leaf extract with 56.45%, the branch extract with 48.58%, and the green leaf extract with 40.39%.

The results of the DPPH• scavenging capacity determination experiments on extracts derived from *C. coggygria* showed that the stem extract (IC_{50} 14.68 µg/mL) was found to be more effective than the standard antioxidant compound α-tocopherol

(IC_{50}= 27.98 µg/mL), but not trolox (IC_{50} 4.03 µg/mL). Other extracts were found to be less effective than the standards (Table 4).

Table 3. Inhibition (%) values of DPPH• scavenging capacity of extracts and standard compounds

Extracts and standarts	Inhibition (%) 45 µg/mL	Inhibition (%) 60 µg/mL
Red leaf	46.47	56.45
Green leaf	31.86	40.39
Heartwood	80.88	84.07
Branches	42.16	48.58
Trolox	94.26	94.32
***α*-Tocopherol**	81.30	90.65

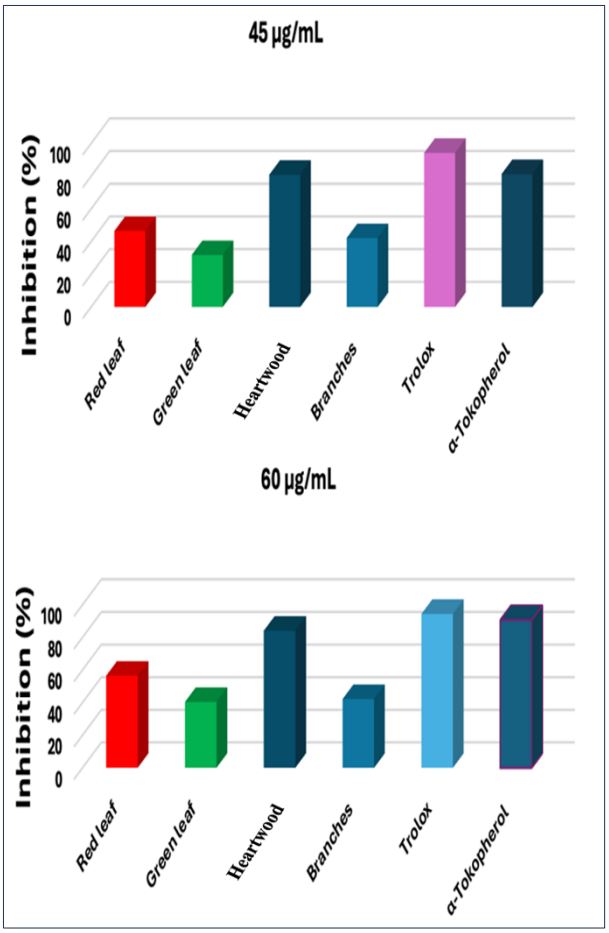

Figure 4. Inhibition (%) graph of DPPH• scavenging capacity of extracts and standard compounds at 45 and 60 µg/mL concentrations

Antioxidant Activity Assay: $ABTS^{\bullet+}$ Scavenging Capacity

Trolox and α-tocopherol were used as positive controls. The concentration range to be tested was determined as a result of studies conducted on standard antioxidant samples. The $ABTS^{\bullet+}$ scavenging capacity of α-tocopherol reached its highest activity at a concentration of 40 µg/mL, and trolox reached its highest activity at a concentration of 32.50 µg/mL. For this reason, the concentration range to be studied was determined as 1-50 µg/mL. Inhibition (%) values, IC_{50} values, inhibition (%)-concentration graphs of $ABTS^{\bullet+}$ scavenging capacity of extracts and standards at 32.5 µg/ml and 40 µg/ml concentrations were obtained (Table 5, Figure 5 and 6).

Table 4. DPPH• radical scavenging capacity IC_{50} values of extracts and standard compounds

Extracts and standarts	IC_{50} (µg/mL)
Heartwood	14.68
Red leaf	50.31
Branches	72.35
Green leaf	76.91
Trolox	4.03
***α*-Tocopherol**	27.98

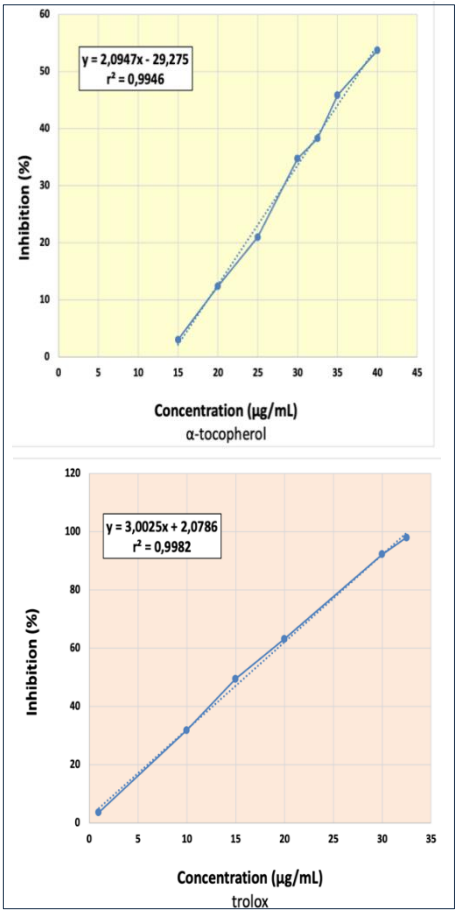

Figure 5. Inhibition (%) - concentration graphs of α-tocopherol and trolox

At the concentration of 32.50 µg/mL, trolox showed 98.01% inhibition and α-tocopherol showed 38.22% inhibition. The heartwood extract exhibited 99.21% inhibition, demonstrating the highest effect among the extracts and being more effective than the standard compounds. The heartwood extract was followed by the red leaf extract with 71.65%, the thin branch extract with 57.66%, and the green leaf extract with 49.76%. This indicates that while the heartwood extract was more effective than both standard compounds, the other extracts were more effective than α-tocopherol but did not match the effectiveness of trolox. At the concentration of 40 µg/mL, trolox showed 98.81% inhibition and α-tocopherol showed 53.69% inhibition. The heartwood extract exhibited 99.10% inhibition, again having the highest effect among the extracts and being more effective than the standard compounds. The heartwood extract was followed by the red leaf extract with 86.32%, the thin branch extract with 74.86%, and the green leaf extract with 66.94%. This indicates that while the heartwood extract was more effective than both standard compounds, the other extracts were more effective than α-tocopherol but did not reach the effectiveness of trolox.

Table 5. Inhibition (%) values of $ABTS^{\bullet+}$ scavenging capacity of extracts and standard compounds

Extracts and standarts	Inhibition (%) 32.5 µg/mL	Inhibition (%) 40 µg/mL
Red leaf	71.65	86.32
Green leaf	49.76	66.94
Heartwood	99.21	99.10
Branches	57.66	74.86
Trolox	98.01	98.81
***α*-Tocopherol**	38.22	53.69

It has been found that the heartwood extract (IC_{50}=11.24 µg/mL) obtained from *C. coggygria* has a higher $ABTS^{\bullet+}$ scavenging capacity than the standard compounds trolox (IC_{50}=15.96 µg/mL) and α-tocopherol (IC_{50}=37.85 µg/mL), and all other extracts have a higher capacity than α-tocopherol but lower capacity than trolox (Table 6).

It was observed that the results of the total phenolic content, total flavonoid content, and antioxidant activity ($ABTS^{\bullet+}$ and $DPPH^{\bullet}$ scavenging capacities) experiments are consistent with each other. In these experiments, the heartwood extract exhibited the highest values, followed by the red leaf extract as the second most effective extract. It was determined that the green leaf extract and the thin branch extract had similar levels of antioxidant activity.

Antidiabetic Activity Assay: α-Glucosidase Inhibition Assay

The α-glucosidase inhibition capacities of methanol extracts obtained from various parts of the plant were examined and compared with each other. With enzyme inhibition studies conducted on acarbose, which is a standard enzyme inhibitor, the concentration range to be tested has been determined 5-200 µg/mL for heartwood extract, 5-50 µg/mL for red leaf extract, and 5-150 µg/mL for green leaf and branch extracts.

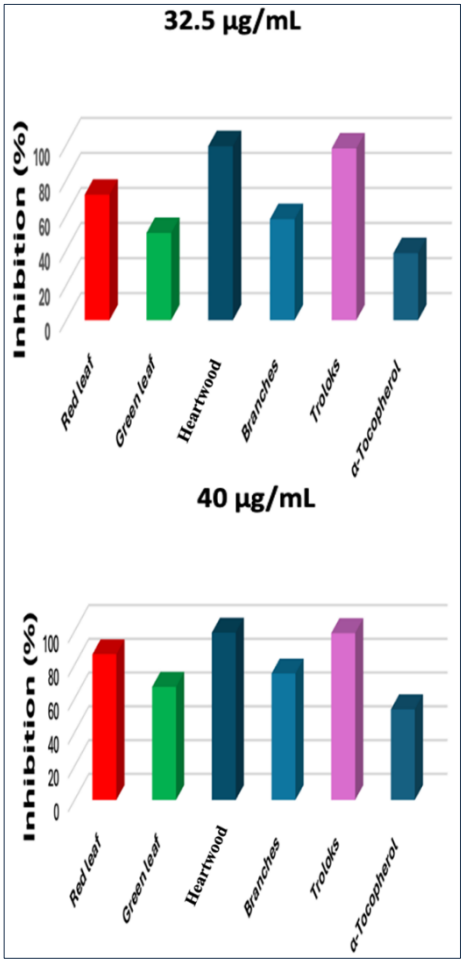

Figure 6. Inhibition (%) graph of $ABTS^{\bullet+}$ scavenging capacity of extracts and standard compounds at 32.5 and 40 µg/mL concentrations

Table 6. $ABTS^{\bullet+}$ scavenging capacity as IC_{50} values of extracts and standard compounds

Extracts and standarts	IC_{50} (µg/mL)
Red leaf	24.18
Green leaf	33.33
Heartwood	11.24
Branches	30.64
Trolox	15.96
***α*-Tocopherol**	37.85

To determine the antidiabetic activities of the extracts obtained from *C. coggygria*, the α-glucosidase inhibitory effect assay was applied with some modifications to the method by Bachhawat et al. (2011) According to the obtained data, at concentrations of 50 µg/mL and 100 µg/mL, the α-glucosidase inhibition activities of the extracts were compared with the

standard compound acarbose.

At the concentration of 50 µg/mL, acarbose was ineffective; however, the extract prepared from red leaves demonstrated 72.94% enzyme inhibition. Following the red leaf extract, the green leaf extract showed 49.02% inhibition, the branch extract showed 40.72% inhibition, and the heartwood extract showed 10.40% inhibition. It was observed that the red leaf extract reached 100% inhibition at concentrations lower than 100 µg/mL, indicating a very high inhibition capacity. Therefore, the red leaf extract was tested only within the concentration range of 5-50 µg/mL.

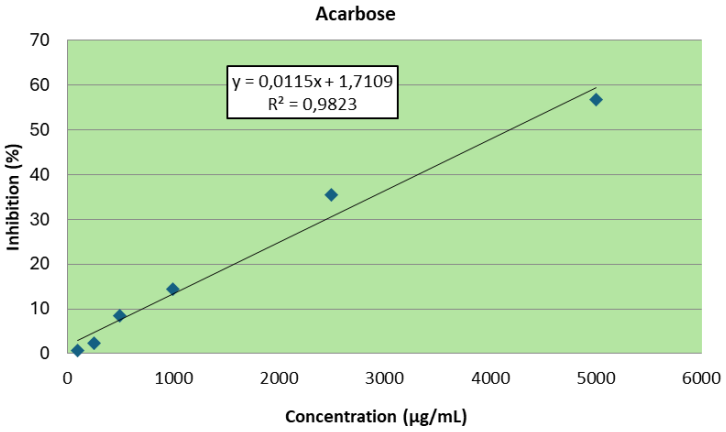

Figure 7. α-Glucosidase inhibition (%) - concentration graph of acarbose

Table 7. α-Glucosidase inhibition values of extracts and acarbose

Extracts and standart	Inhibition (%) 50 µg/mL	Inhibition (%) 100 µg/mL
Red leaf	72.94	>95.00
Green leaf	49.02	87.65
Branches	40.72	80.02
Heartwood	10.40	43.94
Acarbose	0.00	0.65

At the concentration of 100 µg/mL, acarbose showed 0.65% enzyme inhibition, while the extract prepared from green leaves demonstrated 87.65% inhibition. Following the green leaf extract, the branch extract showed 80.02% inhibition, and the heartwood extract showed 43.94% inhibition (Table 7, Figure 7 and 8).

It has been found through experiments that all extracts obtained from *C. coggygria* have α-glucosidase enzyme inhibition capacities that are much higher than the standard compound acarbose (IC_{50}=4199.05 µg/mL), and the extract with the highest enzyme inhibition capacity of the plant is the red leaf extract (IC_{50}=30.44 µg/mL). In conclusion, the α-glucosidase enzyme inhibition activity experiment revealed that the extracts prepared from the red leaves of the plant are much more effective in terms of antidiabetic activity compared to acarbose and the other extracts (Table 8).

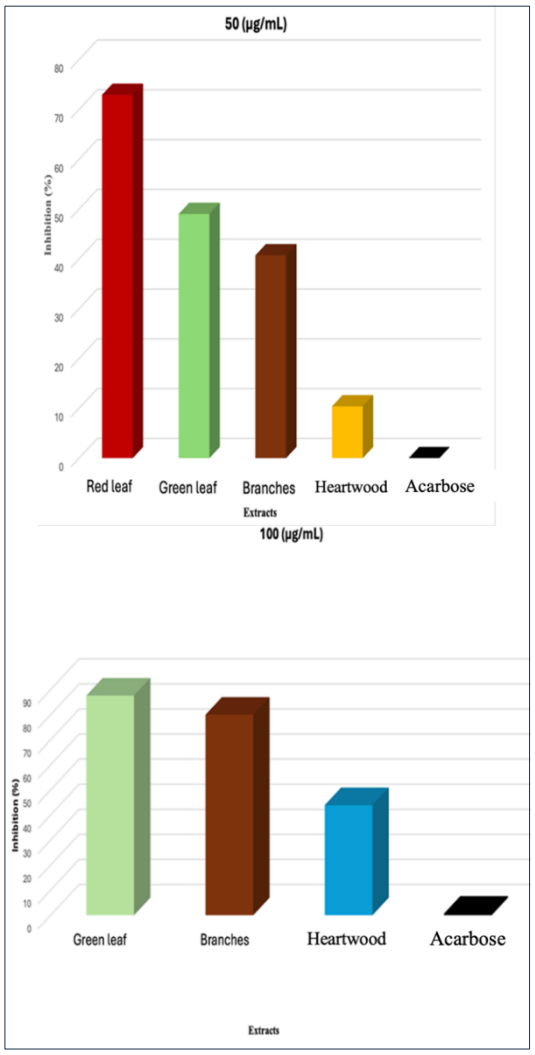

Figure 8. α-Glucosidase inhibition (%) graph of extracts and acarbose at 50 and 100 µg/mL concentration

Table 8. α-Glucosidase inhibition capacity IC_{50} values of extracts and acarbose

Extracts and standart	IC_{50} (µg/mL)
Red leaf	30.44
Green leaf	62.49
Branches	68.19
Heartwood	105.97
Acarbose	4199.05

Discussion

One of the most important reason for the use of *C. coggygria* for medical purposes is its antioxidant and antihyperglycemic effects. At the same time, as a result of α-glucosidase inhibition studies conducted on extracts prepared from the leaves and branches of *C. coggygria*, it is seen that the

plant exhibits antihyperglycemic effects by significantly inhibiting the enzyme. Our study, conducted to determine which part of C. coggyria exhibits greater activity, given its antioxidant and antihyperglycemic activities demonstrated in previous research, has yielded results that are in alignment with the published literature.

In our study, it was found that extracts prepared from various parts of *C. coggygria*, known to be rich in total phenolic content and flavonoid content, exhibit differences in terms of phenolic and flavonoid content, with the heartwood extract showing the highest values. In $DPPH^{\bullet}$ and $ABTS^{\bullet+}$ scavenging capacity studies, similar results were obtained. Considering the IC_{50} values, it was observed that all extracts are effective, but the heartwood extract showed the strongest antioxidant activity. Based on similar findings obtained from our research that provide insights into the plant's potential antioxidant effects, it suggests that the heartwood of the plant contains higher quantities of flavonoids—such as fustin, fisetin, sulfuretin, and quercetin—as well as other phenolic compounds with antioxidant properties, compared to other parts of the plant. It is hoped that these extracts, especially the heartwood extract, may provide benefits against free radicals that are implicated in serious diseases such as cancer. Hence, it is important to conduct further research and clinical trials on the potential uses of *C. coggygria* extracts in the field of health. We believe that conducting isolation studies on extracts to identify the main compounds responsible for the observed effects is essential, and that more detailed research on these compounds should be pursued.

The antihyperglycemic effect of *C. coggygria*, which is used as an antidiabetic among the public, has been proven by α-glucosidase inhibition studies (Özbek et al., 2019). In our study, the α-glucosidase inhibition values of extracts prepared from C. coggyria were compared with the standard substance acarbose. It was concluded that all extracts had much higher enzyme inhibition capacity than acarbose. Among the extracts, the highest enzyme inhibition was performed by the red leaf extract. When the results we obtained are considered and integrated with the outcomes of previous studies, it allows us to infer that the quantities of primarily 1,2,3,4,6-penta-O-galloyl-β-glucose, along with gallocatechin, methyl gallate, myricetin-3-O-α-rhamnoside, and myricetin-3-O-β-galactoside previously isolated from the plant, should be greater in the red leaves compared to other parts of the plant. To obtain clearer and more detailed results, further research on this topic is necessary.

On the whole, this carried out study may contribute to our understanding of the positive effects of plant-derived antioxidants on human health and the development of natural remedies. In addition to antioxidant activity, the presence of antidiabetic activity in all extracts prepared from various parts of *C. coggygria* makes this species quite valuable.

Conclusion

It was concluded that the heartwood extract, which was found to have the highest values in terms of total phenolic content and total flavonoid content, is more effective against the $ABTS^{\bullet+}$ than trolox and α-tocopherol, and exhibits antioxidant activity comparable to these standard compounds against the $DPPH^{\bullet}$. The other extracts, especially the red leaf extract, also showed higher antioxidant activity against the $ABTS^{\bullet+}$ compared to α-tocopherol.

All prepared extracts demonstrated high α-glucosidase inhibition even at low concentrations where acarbose was ineffective. It was observed that the extracts prepared from leaves had higher enzyme inhibition than the other extracts. Among the leaf extracts, the red leaf extract showed higher enzyme inhibition than the green leaf extract, leading to the conclusion that the red leaf extract is the most effective extract in terms of antidiabetic activity.

The research on the pharmacological activities of different parts of the *C. coggygria* which is commonly grown in Türkiye could provide valuable insights for future studies focused on product development. This could open up possibilities for utilizing the plant for economic, commercial, and social benefits.

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Hakem Değerlendirmesi: Dış bağımsız.
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