STUDIA UBB CHEMIA, LXVIII, 4, 2023 (p. 71-92)
(RECOMMENDED CITATION)
DOI:10.24193/subbchem.2023.4.06

COMPREHENSIVE ANALYSIS ON *COTINUS COGGYGRIA* SCOP. LEAVES: PHYTOCHEMICAL CHARACTERIZATION, BIOLOGICAL PROFILE AND DOCKING STUDIES

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ABSTRACT. The present work aims to evaluate the antioxidant, cytotoxic, enzyme inhibitory, and anti-inflammatory properties of *Cotinus coggygria* Scop. The leaf extract was analyzed using LC-MS/MS and antioxidant activity was also investigated via several *in vitro* models (DPPH$^{\bullet}$, ABTS$^{\bullet +}$, FRAP, metal chelating, inhibition of *β*-carotene bleaching, and lipid peroxidation). Enzyme inhibition activity was evaluated on *alpha*-amylase and *alpha*-glucosidase. Molecular docking studies were performed to assess the binding efficiency of the identified compounds with *alpha*-glucosidase. The toxicity of the extract was studied using two breast cancer cell lines as well as healthy fibroblast cell lines (L929). Using LPS induced macrophage cell line model, the anti-inflammatory activity was examined by determining NO, IL-1β, IL-6, IL-10, and TNF-*α* levels. The methanol extract significantly affected the *alpha*-glucosidase enzyme, which also exhibited antioxidant activity. Galloyl hexose and methyl trigallate were found to have the highest binding contact energies for the *alpha*-glucosidase enzyme, according to docking analyses. Even at a 31.25 µg/mL concentration, the extract caused 43.46% inhibition in MDA-MB 231, and 48.09% in MCF-7 cell lines. Significant effects on TNF-*α* and IL-6 cytokine levels also proved anti-inflammatory activity. These findings suggest that *C. coggygria* may serve as an efficient *alpha*-glucosidase inhibitor and anti-inflammatory agent.

Keywords: *Cotinus coggygria, enzyme inhibition, docking, anti-inflammatory, antioxidant, cytotoxicity*

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INTRODUCTION

Recently, there has been intense interest in the treatment with herbal medicines in the world in general. Scientists' desire to find drugs with lower side effects due to the possible risks of synthetic drugs has accelerated the studies [1]. In addition, to benefit from the synergistic effects of the chemical structures in its content and to increase the effect, they focus on many herbs and herbal mixtures used in the treatment of diseases among people rather than a single molecule [2]. Many remedies, both written and verbally passed down from generation to generation, have served as crucial guides for scientists conducting such research. In light of this information, studies to determine the preparation and application methods of many plants as medicines, scientifically prove their effects, and identify the components responsible for the effect have gained significant traction [3].

The number of plant species in Turkiye; is around 12.000, including 3000 of them endemic species [4]. Because of Turkiye's strategic location as the union of Eastern and Western cultures, there has been a significant advancement in knowledge of folk remedies [5]. *Cotinus coggygria* Scop., a member of the Anacardiaceae family known as the "Smoke tree", grown in Turkiye, is known to be widely used in oral wound healing among the public [6]. In the literature studies conducted on the *C. coggygria* plant, it has been observed that it has a wound-healing effect on the skin [7,8]. It is known that the plant's leaves and flowers are ground into a paste and used as a blood purifier for skin diseases in Pakistan and as an antiseptic, anti-inflammatory, and anti-hemorrhagic in Serbian traditional medicine [9,10]. In Turkish folk remedies, a decoction of *C. coggygria* leaves is used to treat diabetes [11]. Various studies have been conducted on *C. coggygria* and these usage patterns have been scientifically proven. The plant's antioxidant, antibacterial, antifungal, antiviral, cytotoxic, antigenotoxic, hepatoprotective, and anti-inflammatory effects have been observed [6, 12]. The presence of tannins and various flavonoids was determined by the chemical composition analysis of the plant [13]. It has been reported that the essential oil obtained from the hydrodistillation of leaves collected from young shoots of *C. coggygria* is rich in monoterpenic hydrocarbons [14].

The purpose of this work was to assess the phytochemical and biological capabilities of *C. coggygria* methanol extract. The phytochemical profile of the plant was established through High-performance liquid chromatography-mass spectrometry (LC-MS/MS) profiling and total bioactive ingredients. Compared with previous studies, the antioxidant capacity of the extract was determined using various in vitro bioassays (DPPH$^{\bullet}$, ABTS$^{\bullet+}$, FRAP, *β*-carotene-linoleic acid co-oxidation assay, iron-(II) chelate formation and preventing peroxidation

of the phospholipids) and inhibitory properties against enzymes involved in diabetes pathology, including *alpha*-amylase and *alpha*-glucosidase. Its effects on the *alpha*-glucosidase enzyme were detailed by molecular docking analysis. Its toxicity was investigated using breast cancer cell lines (MDA-MB 231 and MCF-7) as well as healthy fibroblast cell lines (L929). Furthermore, the extract's anti-inflammatory efficacy was assessed by measuring nitric oxide (NO), interleukin-1 *beta* (IL-1β), interleukin-6 (IL-6), interleukin-10 (IL-10), and tumor necrosis factor-*alpha* (TNF-*α*) levels in the lipopolysaccharide (LPS)-induced RAW 264.7 cell line. This study was the first to explore in-depth biological activity evaluation using *C. coggygria* in light of its traditional uses.

RESULTS AND DISCUSSION

Composition Analysis

Total phenol, flavonoid, and flavonol levels were measured spectrophotometrically to identify the secondary metabolite composition of the extract in phenolic structure using the methods presented in the experimental section (Table 1). When the TPC of the plant gathered in Turkiye is compared to the TPC of the species collected in Kosovo and Bulgaria, it is discovered that the TPC of the species collected in Kosovo and Bulgaria is lower, 62.50±2.55 $mg/g_{extract}$ and 114.73±1.14 $mg/g_{extract}$, respectively [15]. The total flavonoid and flavonol content of the plant also contributes to the richness of its chemical composition.

Table1. Extract yield, total phenolic content, flavonoid, and flavonols of *C. coggygria*

Extract	Yield [%]	Total Phenol [$mg_{GAE}/g_{extract}$]	Total Flavonoid [$mg_{CA}/g_{extract}$]	Total Flavonol [$mg_{RE}/g_{extract}$]
C. coggygria	19.42	208.07 ± 0.98	85.73± 2.15	49.82 ± 3.01

Values are the mean ± SD (n = 3).

Based on the formation of molecular ion peaks and base peaks, secondary metabolites present in 70% methanol extract were evaluated using the LC-MS/MS system. Table 2 lists the compounds found in the extract. Gallic acid, methyl gallate, quinic acid, galloyl hexose, methyl digallate, quercetin rhamnoside, myricetin rhamnoside, methyl trigallate, and pentagalloyl hexoside were all detected in the LC/MS/MS studies. In the literature, it has been

determined that *C. coggygria* is rich in tannins, as well as essential oil and various flavonoid content [13]. In an analysis of the plant's chemical composition; gallic acid and its derivatives such as methylgallate, methyl ester of gallic acid, and pentagalloyl glucose (pentahydroxy gallic acid and pentagalloyl glucose, which is the glucose ester) were found [8]. In the analysis made by HPLC; compounds such as sulfuretin, fisetin, and fustin have been identified [13]. Other flavonoid compounds are: disulfuretin, sulfurein, taxifolin, 4'7-dihydroxyflavanonol, liquiritigenin, biauron, myricetin, kaempferol, quercetin, 4',5,7-trihydroxy flavanone, and isoliquiritigenin [16].

Table 2. LC-MS/MS analysis of compounds in the *C. coggygria* leaf extract

MA	[M-H] m/z	Compounds	% amount*	Ref.
170	169, 125	Gallic acid	< 1	[17]
184	183, 124	Methyl gallate	29.51	[17]
192	191, 173	Quinic acid	9.27	[17]
332	331, 191, 169	Galloyl hexose	1.63	[18]
336	335, 183	Methyl digallate	24.80	[18]
448	447, 301	Quercetin rhamnoside	< 1	[17]
464	463, 316	Myrcetin rhamnoside	3.51	[17]
470	469, 183	Methyl trigallate	4.69	[18]
940	939, 787, 617, 469	Pentagalloyl hexoside	2.13	[17]

***** Percentages are relative values in the total ion spectrum obtained by LC-MS/MS;
MA: molecular weight.

Antioxidant Activity

FRAP assay

The extract's ability to reduce iron (III) was not as efficient as the positive controls, which included AA, BHT, BHA, RA, and GA. The reduction power of the extract was found to be significantly lower ($p<0.001$) compared to other standards (Table 3). The extract's ability to convert iron(III) to iron(II) is considered hydrogen donor capacity and is very crucial in the initial phase of radical chain reactions [19]. As stated in Simić et al. (2008), the ethyl acetate fraction of acetone extract displayed significant ferric-reducing ability with a 5.0 mmol Fe^{2+}/g extract value [20]. Similarly, in another study, the reducing ability of the acetone extract's ethyl acetate fraction was reported as 10.7 mmol Fe^{2+} /g extract [12]. The reason for not making a direct comparison with the data obtained from the study is calculating the results as equivalent to ascorbic acid (AscAE).

DPPH• (1,1-diphenyl-2-picrylhydrazyl) scavenging effect

In the experimental environment studied at physiological pH, the extract was studied at different concentrations and the percentage of inhibition against DPPH radical increased as the concentration increased. However, it was shown that the extract's scavenging ability was inferior to that of the positive controls used, which included AA, BHT, BHA, GA, and RA, it was significantly lower ($p<0.001$). Methanol extracts of *C. coggygria* prepared from leaves and flowers have high antioxidant activity when tested with DPPH• according to Savikin et al. (2009) [21]. According to the results, the IC_{50} value of the flower extract is 2.6 μg/mL and the leaves extract IC_{50} value is 3.8 μg/mL. In another study, DPPH• scavenging properties were identified for acetone extract and its fractions including chloroform, ethyl acetate, and water. Acetone extract had a more noticeable impact than AA and a comparable result to GA. In comparison to AA and GA, the ethyl acetate fraction had higher DPPH• quenching capacity [12].

ABTS•+ (2,2'-azino-bis (3-ethylbenzathiazoline-6- sulfonic acid) scavenging effect

It was observed that AA, BHT, BHA, and GA were more potent than the extract in scavenging the radical and even the extract had a statistically less ($p<0.001$) effect than them (Table 3). It was found to be slightly higher than rosmarinic acid at a low concentration (0.1 mg/mL). Sukhikh et al.(2021) determined the TEAC value of *C. coggygria* ethanol extract against ABTS•+ and it was reported as 0.46 ± 0.02 mM Trolox equivalent/g fresh mass [22]. The scavenging ability of 0.5 mL essential oil of the *C. coggygria* was also found to be 55.43±0.4 in the research of Shagun et al. (2016) [23]. The plant's capacity to scavenge radicals was proven by the data gathered from several studies.

β-carotene / linoleic acid co-oxidation inhibitory effect

One of the most popular techniques for assessing the level of oxidation of unsaturated fatty acids in food and cell walls is the *β*-carotene/ linoleic acid bleaching test. Results were given as Antioxidant activity coefficient (AAC) in Table 3. The plant's 70% methanol extract showed higher activity than gallic acid and lower activity than synthetic oxidants such as BHA and BHT ($p<0.001$). The *β*-carotene bleaching potential of the plant extract has not been measured before but in the study conducted with its essential oil, it was stated that the oil has the capacity to inhibit bleaching by 56.4 ± 1.88% [24].

Ascorbate-Fe (III) -catalyzed phospholipid peroxidation inhibition

Biologically significant molecules called phospholipids that are high in polyunsaturated fatty acids are vulnerable to hydroxyl radical ($^{\bullet}OH$) destruction. The principle of the experiment is based on measuring the $^{\bullet}OH$ sweeping effect by inhibiting the formation of TBA-reactive compounds after the catalysis of phospholipid liposomes prepared from the bovine brain with ascorbate-Fe(III) at physiological pH [25]. A study examining the effects of methanol extracts made from the plant's flowers and leaves on lipid peroxidation expressed the IC_{50} values as 31.9 ± 5.1 µg/mL and 35.8±3.9 µg/mL, respectively [19]. Our result was found to be compatible with the literature with a value of 0.09 ± 0.01 mg/mL (Table 3).

Table 3. Antioxidant activity results of *C. coggygria* and standards

Samples	GA	AA	BHA	BHT	RA	*C. coggygria*	*p*
Reduction Power (FRAP)							
AscAE(mmol/g)	4.08±0.13*	5.72±0.10*	1.93±0.06*	2.26±0.05*	3.10±0.03*	0.85±0.01	<0.001
DPPH Radical Scavenging							
IC_{50} (mg/mL)	0.02±0.01*	0.13±0.01*	0.12±0.01*	0.07±0.01*	0.02±0.01*	0.19±0.01	<0.001
ABTS Radical Scavenging							
0.1 mg/mL	2.09±0.02*	1.18±0.01*	0.86±0.01*	0.55±0.01*	0.37±0.01*	0.50±0.03	<0.001
0.2 mg/mL	2.49±0.01*	1.91±0.01*	1.87±0.01*	1.45±0.01*	1.32±0.01*	1.04±0.01	<0.001
***β*-carotene Bleaching**							
AAC	639.13±0.88*	--	987.55±1.33*	933.23±1.25*	661.42±1.10*	813.59±4.06	<0.001
Ascorbate-Fe (III) -catalyzed Phospholipid Peroxidation Inhibition							
IC_{50} (mg/mL)	0.16±0.06	0.90±1.29*	0.02±0.01	0.09±0.01	0.19±0.23	0.09±0.01	<0.001

*Indicates the difference with *C. coggygria* extract for the respective row. Values are the mean ± SD (n = 3), data is given as mean values with ± 95% confidence interval.

The ferrous ion chelating activity

Iron chelating action is dependent on the quantitative production of complexes of Ferrozine with Fe^{+2}. The ferrozine reagent, a potent chelator, competes with metal-binding chemicals in the environment to bind Fe^{+2} ions. The development of the Fe^{+2}/ferrozine complex is avoided by strong chelating power. Divalent transition metal ions play a crucial part in hydroperoxide decomposition reactions for instance catalyzing the oxidative process, formation

of hydroxyl radicals, and Fenton chemistry. These reactions can be terminated by chelation and deactivation of iron [26]. The extract had an activity of greater than 10 mg/mL, according to research on the extract's effect on the chelation of iron (II). Nićiforović et al. (2010), discovered that the *C. coggygria* methanol extract has a metal chelating capability of more than 10 mg/mL, which is consistent with our findings [27]. The low metal chelating capacity may be associated with the low flavonol content of the plant.

Cytotoxic activity

The extract was evaluated for its toxic effects against various breast cancer lines including MDA- MB 231 and MCF-7, as well as healthy mouse fibroblast cells (L929), and the results are given in Figure 1. Significant cytotoxicity ($p<0.001$) of the extract was observed against all three cell lines at the highest concentration applied, 2000 µg/mL. Given the vast distribution in the human body, it is naturally very difficult to obtain this concentration, where the highest effect is observed. While there was no significant decrease in L929 cell viability at 31.25 µg/mL concentration, MDA-MB 231 and MCF-7 cell viability were found to be 56.53% and 51.9%, respectively ($p<0.05$).

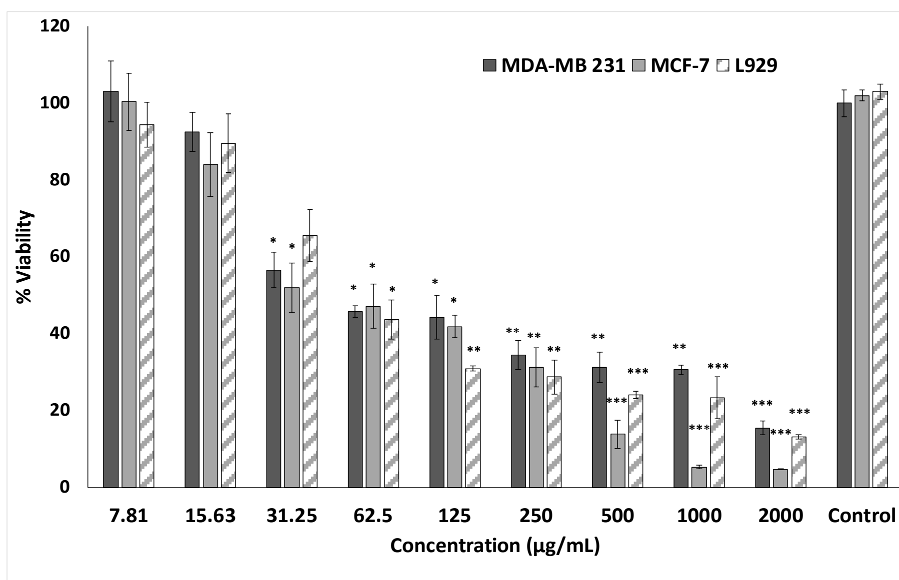

Figure 1. Cytotoxic activity on MDA-MB231, MCF-, and L929 cell lines. Each value presented as mean ± SD (n = 3), * indicate $p<0.05$; ** indicate $p<0.01$; *** indicate $p<0.001$ (Dunnet test).

However, at lower doses, the extract had no discernible effects on viability (Figure 1). In a recent study, it has been proven that *C. coggygria* ethanol extract causes apoptosis and S-phase cell cycle arrest in MCF-7 cells, inhibits colony formation, causes DNA damage, and changes cellular thermodynamic parameters [28]. Another investigation found that the *C. coggygria* ethyl acetate extract had an IC_{50} value of 67.63±3.67 µg/mL in the triple-negative breast cancer line (MDA-MB 231), and these data were found to be congruent with our findings [29].

Enzyme inhibitory-docking analysis

At 1 mg/mL concentration, *C. coggygria* methanol extract had no impact on the *α*-amylase enzyme. The extract demonstrated a noticeable inhibitory impact on the *α*-glucosidase enzyme (Figure 2). In the concentration range of 7.81-1000 µg/mL, inhibition was found to be the same as acarbose ($p>0.05$). At 1.95 and 3.91 µg/mL concentrations, the percentage of inhibition was found to be 13.92% and 63.55%, respectively. According to a study by Özbek et al. (2019), it was stated that the ethyl acetate fraction, in which *C. coggygria* did not show any effect against *α*-amylase, exhibited an IC_{50} value of 8.2 µg/mL in inhibition of the α-glucosidase enzyme [11]. The fact that *C. coggygria* is used in the treatment of diabetes among the public has been confirmed by the results of the experiments. Therefore, to understand which compounds exert this effect, docking analysis was put forward for the first time with this study.

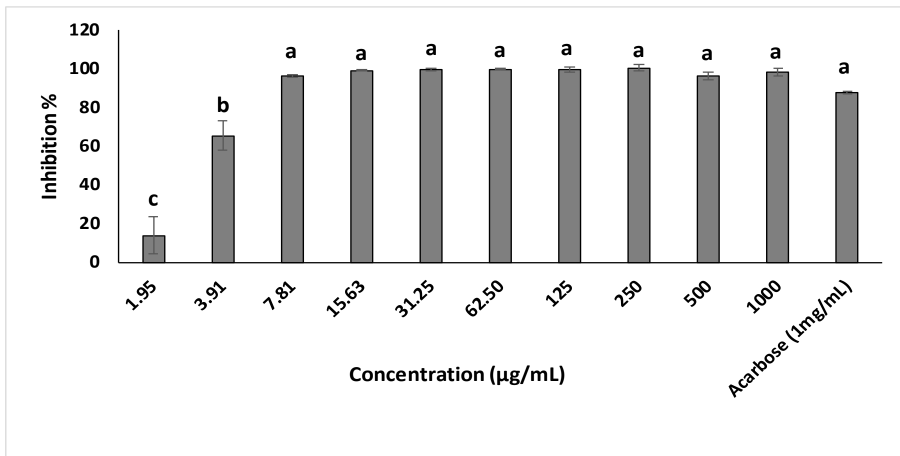

Figure 2. Inhibition effect of *C. coggygria* on α-glucosidase enzyme. Results were expressed as the mean of triplicates ± SD (n = 3).[a-c] identical lowercase letters indicate statistical differences ($p > 0.05$).

Utilizing molecular docking studies to predict the activity state of natural compounds on macromolecules such as enzymes, proteins, and RNA is a rational approach for drug research [30]. Molecular docking analyzes were undertaken to assess the interactions of the compounds obtained from *C. coggygria* by LC-MS/MS analysis on the *alpha*-glucosidase enzyme. Compounds and standard substance acarbose were docked with Glide SP. As given in Table 4, the docking interaction energy of the compounds was between -8.238 and -4.154, while acarbose formed -4.508 kcal/mol interaction energy. *Alpha*-glucosidase and protein-ligand interactions of all compounds were analyzed for H bond, hydrophobic, π-π stacked, polar, negatively charged, and positively charged interaction patterns. The binding poses and schematic protein-ligand interaction diagrams of galloyl hexose and methyl trigallate compounds with the highest docking interaction energy are given in Figure 3. Galloyl hexose formed nine H bonds with active site residues Asp68 (1.90 Å), His111 (Å), Gln181 (2.75 Å), Asn241 (1.66 and 1.95 Å) Glu276 (1.87 Å), Asp349 (1.78 and 2.13 Å) and Arg439 (2.03 Å). Methyl trigallate, on the other hand, formed four H bonds with residuals Asn241 (1.62Å), Ser308 (2.20 Å), and Asp349 (1.49 and 1.65 Å), and π-π stacking interactions with Phe157 (4.25 Å). The standard compound acarbose formed two H bonds with Glu304 (1.92 Å) and Ser308 (2.26 Å). The number and length of the H bond can provide information about the interaction of a compound with the target enzyme. Galloyl hexose followed by methyl trigallate formed highly potent interactions. Detailed protein-ligand interaction types and energies of other compounds gallic acid, quinic acid, quercetin 3-*O*-rhamnoside, methyl gallate, digallic acid methyl, and myricetin 3-rhamnoside are given in Table 4.

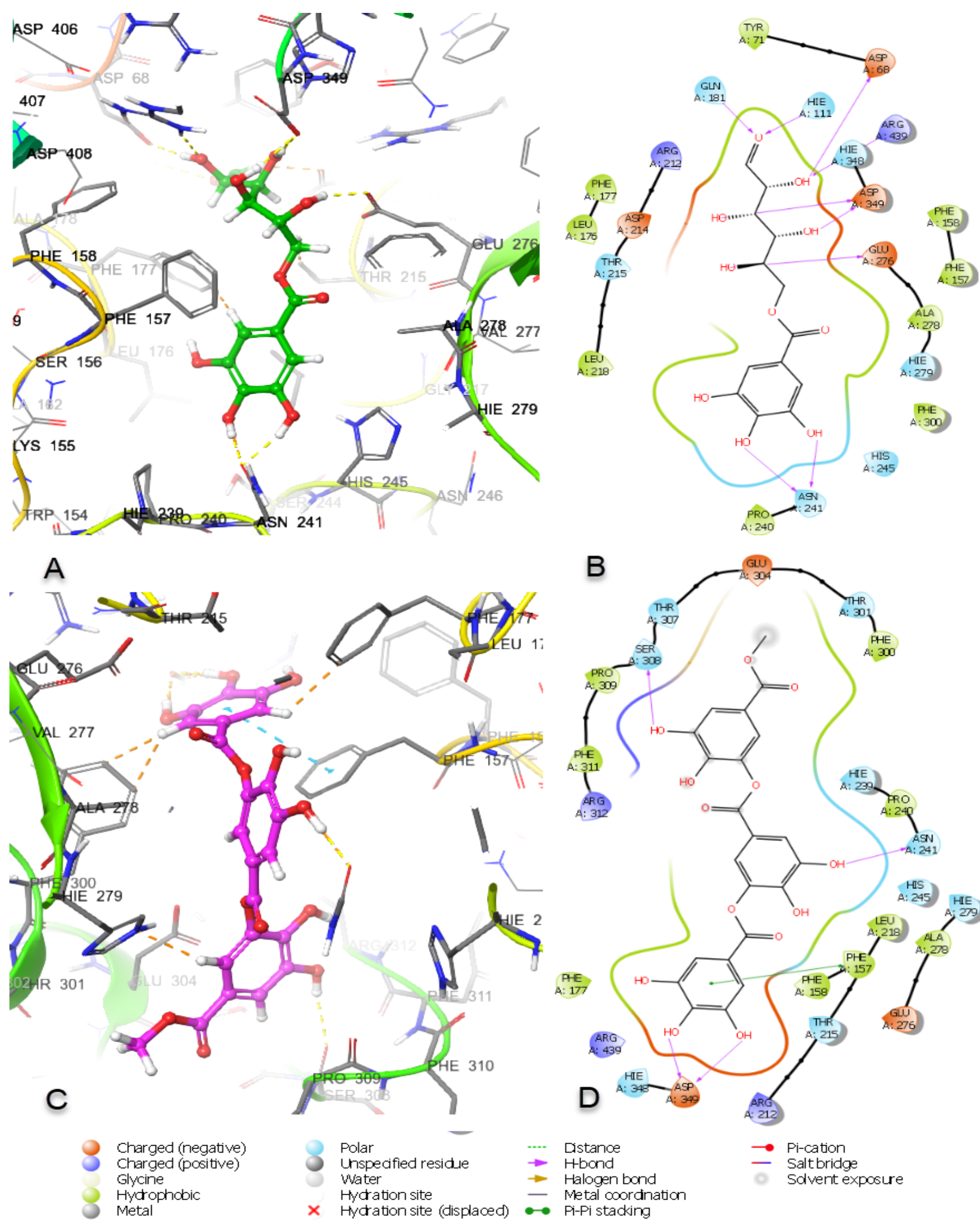

Figure 3. Glide SP molecular docking results of compounds galloyl hexose and methyl trigallate with alpha-glucosidase. (A) Binding pose and, (B) schematic interaction diagram of galloyl hexose (C) 3D interaction, and (D) 2D protein-ligand interaction of methyl trigallate in the *alpha*-glucosidase active site.

Table 4. Protein-ligand interaction energies (kcal/mol) and interaction types obtained from molecular docking study against *alpha*-glucosidase enzyme of compounds identified by LC-MS/MS analysis from *C. coggygria*

	Docking Scores		**Protein-ligand interaction types**					
Compounds (PubChem ID)	glide gscore	glide emodel	H bond	Hydrophobic	π-π stacked	Polar	Negative Charged	Positive Charged
Galloyl hexose (128839)	-8.2	-81.9	Asp68 (1.90 Å), His111 (Å), Gln181 (2.75 Å), Asn241 (1.66 and 1.95 Å) Glu276 (1.87 Å), Asp349 (1.78 and 2.13 Å), Arg439 (2.03 Å)	Tyr71, Phe157, Phe158, Leu176, Phe177, Leu218, Pro240, Ala278, Phe300	NI	His111, Gln181, Thr215, Asn241, His245, His348	Asp68, Asp214, Glu276, Asp349	Arg212, Arg439
Methyl trigallate (156096784)	-7.1	-67.7	Asn241 (1.62Å), Ser308 (2.20 Å), Asp349 (1.49 and 1.65 Å)	Phe157, Phe158, Leu176, Phe177, Leu218, Pro240, Ala278, Phe300, Pro309, Phe311	Phe157 (4.25 Å)	Thr215, His239, Asn241, His245, His279, Thr301, Thr307, Ser308, His348,	Glu276, Glu304, Asp349,	Arg212, Arg312, Arg439
Gallic acid (370)	-5.1	-39.3	Lys155 (2.01 and 2.32 Å), Phe157 (2.01 Å), His239 (2.14 Å), Glu304 (1.73 Å)	Phe157, Pro240, phe300	Phe157 (4.09 Å)	His239, asn241, his279, asn412	Glu304	Lys155, arg312
Quinic acid (6508)	-5.1	-43.2	Asp214 (1.65 and 1.87 Å), Asp349 (1.93 Å),	Tyr71, Val108, Phe157, Phe158, Leu176, Phe177, Phe300	NI	His111, Gln181, Thr215, His348	Asp68, Asp214, Glu276, Asp349	Arg212, Arg312, Arg439
Quercetin 3-O-rhamnoside (5353915)	-4.5	-43.2	Ser308 (2.60 Å), Pro309 (1.86 Å)	Phe157, Phe177, Leu218, Ala278, Phe300, Pro309, Phe310	His279 (1.93 Å)	Thr215, His239, Asn241, His245, His279, Thr307, Ser308	Glu304	NI
Methyl gallate (7428)	-4.4	-30.5	Asp68 (1.69 Å), Asp214 (1.78 Å)	Tyr71, Val108, Phe157, Phe158, Phe177, Phe300	NI	His111, Gln181, Thr215, His348, Gln350	Asp68, Asp214, Glu276, Asp349	Arg439, Arg443
Digallic acid methyl 10131824	-4.3	-40.1	Asn241 (2.31 Å), Glu276 (2.53 Å)	Phe157, Phe158, Phe177, Ala278, Phe300, Tyr313	NI	Thr215, Asn241, His245, His279, Gln350		Arg312, Arg439
Myricetin 3-rhamnoside (5352000)	-4.2	-46.9	Glu304 (1.98 Å), Pro309 (2.78 Å)	Phe157, Leu218, Ala278, Phe300, Pro309, Phe300	Hie279 (4.91 Å)	Asn241, His245, Hie279, Thr301	Glu304	NI
Acarbose (41774)	-4.5	-26.5	Glu304 (1.92 Å), Ser308 (2.26 Å)	Phe157, Phe158, Phe177, Leu218, Pro240, Ala278, Phe300, Pro309, Phe311	NI	Thr215, Hie239, Asn241, His245, Hie279, Thr307, Ser308, Hie348, Gln350	Asp214, Glu276, Glu304, Asp349	Arg212, Arg312, Arg439

Anti-inflammatory activity

To determine the non-toxic dose before the anti-inflammatory activity study, toxicity tests were performed on the macrophage cell line by the MTT method. Concentrations that did not reduce viability were determined as 31.25 and 62.5 μg/mL (Figure 4 A).

Inflammation is increased and maintained by pro-inflammatory cytokines, which are primarily produced by activated macrophages in the regulation of inflammatory responses. These are IL-1β, IL-6, and TNF-*α* common proinflammatory cytokines [31]. Human cytokine synthesis inhibitory factor (IL-10) is a cytokine that reduces inflammation [32]. NO is a temporary free radical formed by the reaction of the nitrogen atom in the guanido group of the arginine amino acid with molecular oxygen [33]. The results of the extract's anti-inflammatory effect are shown in Figure 4.

There was an increase in nitric oxide levels in the LPS-treated control well in comparison to the untreated control. The NO amount considerably ($p<0.001$) elevated in the LPS group. In the co-treatment and pre-treatment groups to which the extract was applied, both concentrations caused a significant reduction ($p<0.05$). 62.5 μg/mL concentrated extract decreased the NO amount from 70.36 μM to 25.56 μM in the pre-treatment group (Figure 4B).

In the study, IL-1β, one of the pro-inflammatory cytokines, showed a noticeable boost in the LPS group as opposed to the untreated control group ($p<0.05$). In the pre-treatment group, 62.5 μg/mL concentration of the extract exhibited a substantial decrease in the quantity of IL-1β compared to the LPS group, and the amount of IL-1β was found to be 143.03 pg/mL (Figure 4C). There was no discernible distinction in the amounts of IL-1β in either concentration in the co-treatment group as compared to the values in the LPS group.

It is worth mentioning that IL-6 is another pro-inflammatory cytokine and is associated with the regulation of immune responses. Therefore, the reduction of its levels could be an indication of the extract's potential for inflammation. There was an increase in the amount of IL-6 in LPS-inflamed wells compared to non-inflamed control wells. The results showed that the co-treated group's IL-6 level dropped to 113.30 pg/mL at a dose of 62.5 μg/mL and was statistically equivalent to the untreated control group ($p>0.05$). No discernible effect on inflammation was noticed in the pretreatment group at 31.25 μg/mL concentration (Figure 4D).

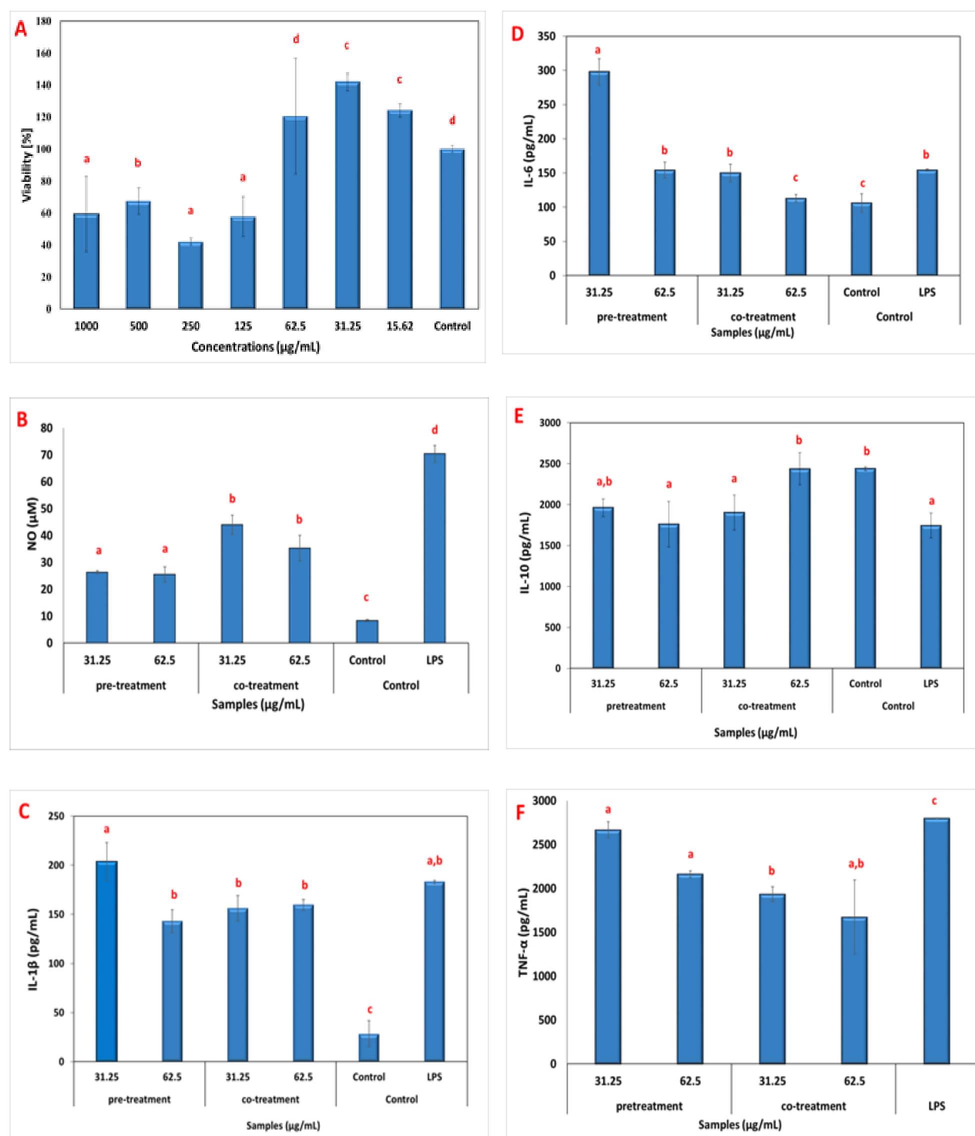

Figure 4. Anti-inflammatory activity of the *C. coggygria*. A: Toxicity profile of the extract; B: Amount of NO; C: Amount of IL-1β; D: Amount of IL-6; E: Amount of IL-10; F: Amount of TNF-*α*. Values are the mean ± SD (n = 3). [a–d] identical lowercase letters indicate statistical differences (p > 0.05).

A significantly decreased amount of IL-10 was detected in the LPS group ($p<0.001$). Besides, while no major changes were observed in IL-10 levels in the pre-treatment group, it was determined that the values measured in the wells treated with high-dose extract in the co-treatment group approached the control values with a significant ($p<0.05$) increase (Figure 4E).

The results exhibited that the LPS group had a substantial increase in TNF-*α* levels. On the other hand, in the co-treatment group, the TNF-*α* levels decreased significantly to 1675.43 pg/mL at a dose of 62.5 μg/mL, indicating the potential of the extract on inflammation (Figure 4F). In contrast, no considerable difference was observed between the LPS and extract groups in the pre-treatment group, suggesting that the extract had no prophylactic effect on TNF-*α* production.

There are few research evaluating the *in vivo* anti-inflammatory activity of *C. coggygria*, despite the fact that there is no study in the literature revealing the anti-inflammatory activity of *C. coggygria* extracts on the RAW 264.7 cell line. In the study by Marčetić et al. (2013) it was reported that the ethyl acetate fraction of *C. coggygria* had the potential in minimizing inflammation in a carrageenan-induced rat paw edema model and the group administered 100 mg/kg was evaluated to be more active than indomethacin ($p < 0.01$) [12]. Additionally, the more recent investigation by Şen et al. (2023) exemplified that *C. coggygria* extract had a positive result in decreasing the amounts of well-known cytokines (TNF-*α*, IL-1β, IL-6, and TGF-1β levels) in an acetic acid-induced rat colitis model. The findings from these *in vivo* models were supported by current research results on the RAW 264.7 cell line, which revealed the anti-inflammatory potential of *C. coggygria* extracts *in vitro*. These findings suggest that *C. coggygria* extracts may have the potential as a natural anti-inflammatory agent [34].

CONCLUSIONS

This work highlights the chemical composition, antioxidant, cytotoxic, anti-diabetic, and anti-inflammatory activities of *Cotinus coggygria* leaves methanol extract. It has been proven that *C. coggygria* leaves contain a significant amount of biologically active chemicals, particularly phenolic compounds. The methanol extract displayed antioxidant activity and had a strong effect on the alpha-glucosidase enzyme. Docking analyses showed that the compounds with the highest binding interaction energies for the alpha-glucosidase enzyme were galloyl hexose and methyl trigallate, thus consistent with the use of the plant. The fact that the extract exhibited significant inhibition of viability to both breast cancer cell lines even at a concentration

of 31.25 µg/mL indicates that research should continue in this direction. Considering the results, 62.5 µg/mL *C. coggygria* extract had a significant effect on TNF-*α* and IL-6 cytokine levels. A significant effect on IL-1β levels was observed in the pre-treatment group, again at a dose of 62.5 µg/mL. The effect of the extract on IL-10 cytokine level was determined as the highest at 31.25 µg/mL dose. In light of these results, it can be said that the plant has an anti-inflammatory effect. With this research, it is emphasized that this plant has very serious biological activities, but standardized extract preparation studies should be the next goal on the way from the plant to the drug.

EXPERIMENTAL SECTION

Plant material and extraction

C. coggygria Scop. utilized in the experiments were collected from the Muğla-Fethiye (Mediterranean Region, Turkiye), during the flowering season of the plant. Identification of the plant was performed by Professor Müberra Koşar. The herbarium sample of the plant (GNK 1001) is kept in the Erciyes University Faculty of Pharmacy.

150 g of dried herbal material was roughly ground and subjected to three 24-hour macerations using 70% methanol as the solvent in a shaking water bath. After filtering, the obtained extracts were concentrated in a rotavapor (37 °C) under a vacuum. After being lyophilized, the entire extract was kept at -18ºC until analysis.

Composition analysis

Total phenol, flavonoid and flavonol content determination

The total content of phenol (TPC) present in the extract was defined as equivalent to gallic acid by the Folin-Ciocalteu method, the total content of flavonoid (TFC) equivalent to catechin was defined by the method used by Zhishen et al. (1999), and the total content of flavonol equivalent to the rutin was defined by Miliauskas et al. (2004)'s method [35- 37].

High-pressure liquid chromatography-mass spectrometry (LC-MS/MS) analysis

The Shimadzu LC/MS-8040 LC-MS/MS system was used as the detector, and the ESI negative ion acquisition technique was employed for analysis. The mobile phase consisted of a 50:50 mixture of solvent A and

solvent B, with a flow rate of 0.2 mL/min. Solvent A was 1% acetic acid in the water, and solvent B was 1% acetic acid in methanol. The sample was injected at a volume of 1 µL, and elution was performed using aqueous methanol. The fundamental peaks and molecular ion peaks were analyzed.

Antioxidant activity

Determination of ferric reduction power (FRAP)

The extracts and standards were mixed with 0.2 M phosphate buffer (pH 6.6) and 1% potassium hexacyanoferrate solution to determine the activities that will be assessed in the FRAP experiment. This mixture was incubated at 50°C for 30 minutes before 2.5 mL of 10% trichloroacetic acid (TCA) was added. 2.5 mL was then removed from the upper portions of the centrifuged samples. This portion was mixed with 2.5 mL of water and 0.5 mL of 0.1% $FeCl_3$. Standards included Butylated hydroxytoluene (BHT), Butylated hydroxyanisole (BHA), rosmarinic acid (RA), ascorbic acid (AA), and gallic acid (GA). Absorbance measurement was performed at 700 nm. To evaluate the ferric-reducing ability, the results were calculated as equivalent to ascorbic acid [38].

DPPH • scavenging effect

To determine the DPPH radical scavenging activities of the extracts, standards including BHT, BHA, GA, AA, and RA were combined with Tris-HCl buffer (50 nM, pH 7.4) and 0.1 mM methanol. After 30 minutes of incubation in a dark environment at room temperature, the absorbances were measured at 517 nm. The activity of the extracts was compared to the standards to calculate percent inhibition, which was determined using the following equation. Nonlinear regression curves via Sigma Plot 2001 version 7.0 (SPSS Inc., Chicago IL) were used to calculate IC_{50} values, as described by Gyamfi et al. (1999) [39].

$$\% \text{ Inhibition} = [(\text{Abscontrol} - \text{Absample}) / \text{Abscontrol}] \times 100 \quad \text{Eq.1}$$

ABTS•+ scavenging effect

The ABTS•+ scavenging effects of the extracts and standards were evaluated using an ethanol solution of ABTS•+. The ABTS•+ solution was prepared by combining a 7 mM aqueous solution of ABTS with 2.45 mM $K_2S_2O_8$ and incubating it in a dark environment for 16 hours. The absorbance of the ABTS•+ solution was adjusted to 0.700 ±0.024 at 734 nm using ethanol. To assess the kinetics of the reaction, 990 µL of the radical solution was mixed with 10 µL of the sample, and the absorbance was measured at

734 nm at 1-minute intervals for 30 minutes. The Trolox equivalent antioxidant capacity (TEAC) value was calculated by determining the percent inhibition and comparing it to the concentration of Trolox. The mean values of three parallel experiments were calculated, as described by Re et al. (1998) [40].

Determination of β-carotene / linoleic acid co-oxidation inhibitory effect

Tween 20 and linoleic acid, each weighing 120 mg, were mixed with 1mg/mL of *β* -carotene in 1.2 mL of chloroform. After the chloroform of the mixture was removed via the rotavapor, distilled water was added in small amounts in a total volume of 300 mL with stirring. This prepared emulsion was combined with the extract and standards at a concentration of 1 mg/mL. To carry out autoxidation, it was kept in a water bath at 50 °C, and samples were taken from the tubes every 15 minutes for 2 hours. Measurements of these samples taken at certain time intervals were made at 470 nm and the degree of fading was determined Antioxidant activity percentages (AA%) were calculated using Equation 2 [41].

$$AA\% = [1-(Abs^{0}\ sample - Abs^{120} sample) / (Abs^{0}\ control - Abs^{120}\ control)] x100 \qquad \text{Eq 2.}$$

Ascorbate-Fe (III) -catalyzed phospholipid peroxidation inhibition

Commercially available Bovine brain extract (Folch VII) was sonicated in an ice bath and mixed with 10 mM phosphate buffer (PBS, pH 7.4) to obtain phospholipid liposome (5 mg/mL). 0.2 mL of this prepared liposome was taken and combined with 0.5 mL of PBS buffer, 0.1 mL of 1 mM $FeCl_3$, and 0.1 mL of extract/standards. 0.1 mL of 1 mM ascorbate solution was added to accelerate peroxidation. After this mixture was incubated at 37ºC for 60 minutes, 50 µL of 2% BHT, 1 mL of 2.8% TCA, and 1 mL of 1% TBA (in 0.05 M NaOH) were added. Each sample was extracted with 2 mL of *n*-butanol and the resulting (TBA) 2-MDA chromogens were allowed to switch to the *n*-butanol phase. To measure the degree of peroxidation, absorbances were recorded at 532 nm [42].

The ferrous ion chelating activity

200 µL extract solution, 100 µL 2.0 mM aqueous $FeCl_2$, and 900 µL methanol were mixed. After the reaction mixture was incubated for 5 minutes, the reaction was accelerated with 400 µL 5.0 mM ferrozine solution. After waiting for 10 minutes, the absorbance was measured at 562 nm [43]. The iron chelate activity was calculated according to Eq 1. using the control absorbance (Ac) and sample absorbance (As) and given as equivalent to Na_2EDTA (mgNa_2EDTA/sample).

Cytotoxic activity

Human breast adenocarcinoma cell lines, MCF-7, and MDA-MB-231 cell lines were grown in DMEM containing 10% FBS (fetal bovine serum), and 1% penicillin-streptomycin. The L929 (mouse fibroblast) cell line was grown in an EMEM medium containing 1% penicillin/streptomycin mixture solution and 10% horse serum at 37 °C in 5% CO_2 and 95% air. All cell lines were purchased from the American Type Culture Collection (Manassas, VA, USA).

The 3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide (MTT) colorimetric method, which is often used to assess toxicity, was employed to assess the effectiveness of *C. coggyria* extract on MCF-7, MDA-MB-231, and L929 cell lines. After counting the number of cells grown in culture the cells were dispensed into a 96-well microplate at 1 x 10^4 cells / well. Following 24 hours, the cells that had adhered to the plate's base were removed from the supernatant, and 100 µL of media with an extract concentration of 7.81–2000 g/mL was added. After a 24-hour incubation period, 100 µL of MTT (5 mg/mL in PBS) solution was dispensed into each emptied well. The wells were drained after 2 hours in the incubator, and 100 µL of dimethyl sulfoxide (DMSO) was dispensed into each well. ELISA (Biotek Synergy HT) was used to measure absorbance at 570 nm [43]. The analyses were performed in triplicate.

Enzyme inhibitory-docking analysis

α-Glucosidase inhibition assay

The procedure described by Liu et al. (2003) was followed for conducting the assay for the inhibitory activity of the -glucosidase enzyme [44]. A mixture of 2 U/mL *α*-glucosidase (50 µL) solution, phosphate buffer (1000 µL), and extract/acarbose (200 µL) was combined. Following a 10-minute incubation period at 37 °C, 50 µL of *p*-nitrophenyl-D-glucopyranoside (5 mM, pNPG) was added, and the mixture was then incubated for an additional 20 minutes at the same temperature Then, the reaction was stopped by adding 2000 µL of sodium carbonate solution prepared at 0.2 M concentration and 4700 µL of distilled water. Absorbances were measured at 405 nm with a spectrophotometer. Eq 1 was used to calculate inhibition %.

α-Amylase inhibition assay

To evaluate the inhibitory effects of the extracts on the α-amylase enzyme, a modified Sigma-Aldrich technique was used. The assay mixture consisted of 40 µL of extract or acarbose samples, 160 µL of phosphate buffer (pH 6.9) with 20 mM sodium chloride, and 200 µL of α-amylase enzyme solution (EC3.2.1.1, tip VI, Sigma; 20 unit/mL), mixed at specific dose intervals. After incubating for 5 minutes, a 0.5% w/v prepared starch

solution was added as a substrate in a volume of 400 µL and incubated for 3 more minutes at 25°C. The reaction was stopped by adding 200 µL of the dinitro salicylic acid reagent (96 mM 3,5-dinitro salicylic acid, 5.31 M sodium potassium tartrate in 2 M NaOH) and placing the tubes in a water bath at 85°C for 15 minutes. After the incubation period, the absorbance at 540 nm was measured using a spectrophotometer after the addition of 4000 µL of distilled water. Inhibition% was calculated using Eq 1.

Molecular docking

Molecular docking work was carried out with Schrödinger Maestro version 2021.2 version with protein preparation, ligand preparation, creation of active site grid box files, and ligand docking steps, respectively [45]. For the three-dimensional structure of α-glucosidase, PDB ID: 5NN8 was selected and retrieved in the 'Protein Preparation Wizard' [46,47]. The water molecule and other heteroatoms were removed, and hydrogens were added. H bond assignment was set to PROPKA:7.0, using the water molecule orientation, and minimized using OPLS4 force fields. Compounds obtained from *C. coggygria* by LC-MS/MS analysis galloyl hexose (PubChem ID: 128839), methyl trigallate (PubChem ID: 156096784), gallic acid (PubChem ID: 370), quinic acid (PubChem ID: 6508), quercetin 3-O-rhamnoside (PubChem ID: 5353915), methyl gallate (PubChem ID: 7428), digallic acid methyl (PubChem ID: 10131824), myricetin 3-rhamnoside (PubChem ID: 5352000) and acarbose (PubChem ID: 41774)) structure was downloaded in 3D SDF file format and prepared with 'LigPrep' module using OPLS4 force fields. Active site coordinates x: -6.658, y: -45.820, z: 84.129, and 25*25*25 Å3 were created with the 'Receptor Grid Generation' module. Ligand docking was performed with the Glide SP docking modüle [48]. Two- and three-dimensional visualization and protein-ligand analyses were performed with the Maestro interface and' Ligand Interaction' module.

Anti-inflammatory activity

Determination of non-toxic dose in RAW 264.7 cells

The non-toxic dose of the extract in the RAW 264.7 cell line was determined by the MTT method. The toxicity of the extract has been studied in the concentration range of 7.81 -1000 µg/mL.

Determination of IL-1β, IL 10, IL6, TNF-α, and NO amounts

Two groups, pre-treatment, and co-treatment, were designed to measure the anti-inflammatory activity. RAW 264.7 cells for both groups were distributed in a $1x10^6$ number to a six-well plate. At the end of the 24 hours given for the

cells distributed in the pretreatment group to adhere, the extracts were added to the wells at a concentration of 62.5 µg/mL and 31.25 µg/mL and left for 6 hours. Then inflammation was induced by incubating with LPS at a concentration of 1 µg/mL for 24 hours. In the co-treatment group, extract at concentrations of 31.25 µg/mL and 62.5 µg/mL and LPS (1 µg/mL) were added to the wells at the same time and incubated for 24 hours [49].

The supernatant obtained from the wells was used to investigate IL-1β, IL-6, IL-10, and TNF-*α* cytokine levels, and NO amount after centrifugation. The amount of NO was measured using Griess reagent, and the amount of cytokines was measured using commercial kits.

Statistical analysis

After performing ANOVA, Tukey, and Dunnett's tests were used to determine the significant differences between means. In this study, a significance level of $p < 0.05$ was defined as statistically significant between groups.

ACKNOWLEDGMENTS

This work is granted by Erciyes University Research Council under Grant [TDK-2015-5501].

REFERENCES

1. D.J.Newman; G.M. Cragg; *J Nat Prod.,* **2020**, *83(3)*, 770–803.
2. X. Zhou; S.W. Seto; D. Chang; H. Kiat; V. Razmovski-Naumovski; K. Chan; A. Bensoussan; *Front Pharmacol*., **2016**, *7*, 201.
3. H. Yuan; Q. Ma; L. Ye; G. Piao; *Molecules*, **2016**, *21*(5), 559.
4. C. Türe; H. Böcük; *J Nat Conserv.,* **2010**, *18(4)*, 296-303.
5. S. Uğurlu; *Turkish Stud*., **2011**, *6(4)*, 317-327.
6. S. Matić; S. Stanić; M. Mihailović; D. Bogojević; *Saudi J Biol Sci*., **2016**, *23(4)*,452–461.
7. H. Aksoy; M. Sancar; A. Sen; B. Okuyan; L. Bitis; F. Uras; D. Akakin; O. Cevik; S. Kultur; F.V. İzzettin; *Nat Prod Res.*, **2016**, *30(4)*, 452–455.
8. B. Ertas; B. Okuyan; A. Şen; F. Ercan; H. Önel; F. Göğer; G. Şener; *J Res Pharm*., **2022**, *26(3)*, 554-564.
9. O. Tzakou; I. Bazos; A. Yannitsaros; *Flavour Fragr J.,* **2005**, *20(5)*, 531-533.
10. M.S. Amjad; M. Arshad; R. Qureshi; *Asian Pac J Trop Biomed.*, **2015**, *5(3)*, 234-241.

11. H. Özbek; H. Yuca; S. Gözcü; B. Dursunoğlu; N. Özvenver; Z. Güvenalp; C. Kazaz; M. Önal; L.Ö. Demirezer; *Fabad J Pharm Sci*., **2019**, *44(2)*, 127-132.
12. M. Marčetić; D. Božić; M. Milenković; N. Malešević; S. Radulović; N. Kovačević; *Phytother Res.*, **2013**, *27(11)*, 1658–1663.
13. D.S. Antal; S. Schwaiger; E.P. Ellmerer-Müller; H. Stuppner; *Planta Med.*, **2010**, *76(15)*, 1765-1772.
14. M. Novaković; I. Vučković, P. Janaćković; M. Sokovik; A. Filipovic, V. Tesevic; S. Milosavljevic; *J Serb Chem Soc*., **2007**, *72(11)*, 1045-1051.
15. S. Matic; S. Stanic; S. Solujic; T. Milosevic; N. Niciforovic; *Period Biol.*, **2011**, *113*, 87–92.
16. H.E. Westenburg; K.J. Lee; S.K. Lee; H.H.S. Fong; R.B.V. Breemen; J.M. Pezzuto, D. Kinghorn; *J. Nat. Prod.,* **2000**, *6*, 1696–1698.
17. K. Rendeková; S. Fialová; L. Jánošová; P. Mučaji; L. Slobodníková; *Molecules*, **2016**, *21(1)*, 50.
18. L. Regazzoni; E. Arlandini; D. Garzon; *J Pharm Biomed Anal*., **2013**, *72,*202-207.
19. H.D. Dorman; M. Kosar; K. Kahlos; Y. Holm; R. Hiltunen; *J Agric Food Chem.*, **2003**, *51(16)*, 4563-4569.
20. M. Simić; D. Vučićević; M. Milenković; N. Kovačević; *Planta Med.*, **2008**, *74*, PA63.
21. K. Šavikin, G. Zdunić; T. Janković; T. Stanojković; Z. Juranić; N. Menković; *Nat Prod Res.*, **2009**, *23*, 1731–1739.
22. S. Sukhikh; S. Noskova; A. Pungin; S. Ivanova; L. Skrypnik; E. Chupakhin; O. Babich; *Plants.*, **2021**,*10*, 1224.
23. S. Shagun; B. Sujata; S. Manjul; *Int J Pharmacog Phytochem Res.*, **2016**, *8(7)*, 1183-1186.
24. S.C. Joshi; C.S. Mathela; *J Nat Prod Plant* Resour., **2014**, *4(3)*, 39-43.
25. M. Koşar; H.J.D. Dorman; R. Hiltunen; *Food Chem.*, **2005**, *91(3)*, 525-533.
26. B. Halliwell; *Adv Pharmacol.*, **1996**, *38*, 3-20.
27. N. Nićiforović; V. Mihailović; P. Mašković; S. Solujić; A. Stojković; D. Pavlović Muratspahić; *Food Chem Toxicol.*, **2010,** *48(11)*, 3125-3130.
28. Z.I. Gospodinova; I. Zupkó; N. Bózsity; V.I. Manova; M.S. Georgieva; S.J. Todinova; S.G. Taneva; I. Ocsovszki; M.E. Krasteva; *Z Naturforsch C J Biosci.*, **2020**, *76(3-4)*, 129-140.
29. I. Iliev; I. Ivanov; K. Todorova; D. Tasheva; M. Dimitrova; *Acta Morphol.*, **2021**, *28(1-2)*, 13-18.
30. J.A. Junejo; K. Zaman; M. Rudrapal; I. Celik; E.I. Attah; *S Afr J Bot.*, **2021**, *143*, 164-175.
31. C.A. Dinarello; *Chest.*, **2000**, *118(2)*, 503-508.
32. T.R. Mosmann; K.W. Moore; *Immunol Today.*, **1991**, *12(3)*, A49-53.
33. M. Rizk; M.B. Witte; A. Barbul; *World J Surg.*, **2004**, *28(3)*, 301-306.
34. A. Şen; B. Ertaş; Ö. Çevik; A. Yıldırım; D.G. Kayalı; D. Akakın; L. Bitiş; G. Şener; *Appl Biochem Biotechnol.*, **2023**, 10.1007/s12010-023-04474-1.
35. V.L. Singleton; R. Orthofer; R.M. Lamuela-Raventós; *Meth Enzymol.*, **1999**, *299,* 152–178.

36. J. Zhishen; T. Mengcheng; W. Jianming; *Food Chem.*, **1999**, *64(4)*, 555-559.
37. G. Miliauskas; P.R. Venskutonis; T.A. Van Beek; *Food Chem*., **2004**, *85(2)*, 231-237.
38. G. Şeker Karatoprak; Ç. Yücel Aşık; A. Çakır; E. Köngül Şafak; *Int J Environ Health Res*, 2021, *31(8)*, 991–1000.
39. M.A. Gyamfi; M. Yonamine; Y. Aniya; *Gen Pharmacol -Vasc S.*, **1999**, *32(6)*, 661-667.
40. R. Re; N. Pellegrini; A. Proteggente; A. Pannala; M. Yang; C. Rice-Evans; *Free Radic Biol Med*., **1999**, *26(9)*, 1231-1237.
41. B.D. Oomah; G. Mazza; *J Agric Food Chem*., **1996**, *44*, 1746–1750.
42. O.I. Aruoma; J.P. Spencer; D. Warren; *Food Chem.*, **1997**, *60(2)*, 149-156.
43. J.k. Taher Al-Isawi; A.M. Mohammed; D.T.A. Al-Heetimi; *Studia UBB Chemia,* **2023**, *67(2)*, 131-144.
44. S. Liu; D. Li; B. Huang; Y. Chen; X. Lu; Y. Wang; *J Ethnopharmacol.*, **2003**, *149*, 263–269.
45. D.S. Ghallab; E. Shawky; A.M. Metwally; I. Celik; R.S. Ibrahim; MM. Mohyeldin; *RSC Adv*., **2022**, *12(5)*, 2843-2872.
46. K. Karrouchi; I. Celik; S. Fettach; T. Karthick; K. Bougrin; S. Radi; M. El Abbes Faozi; M. Ansar; R. Renjith; *J Mol Struct.*, **2022**, *1265*, 133363.
47. V. Roig-Zamboni; B. Cobucci-Ponzano; R. Iacono; M.C. Ferrara; S. Germany; Y. Bourne; G. Parenti; M. Moracci; G. Sulzenbacher; *Nat Commun.*, **2017**, *8*, 1111.
48. R.A. Friesner; J.L. Banks; R.B. Murphy; T.A. Halgren; J.J. Klicic; D.T. Mainz; M.P. Repasky; E.H. Knoll; M. Shelley; J.K. Perry; *J Med Chem.*, **2004**, *47(7)*, 1739-1749.
49. G. Şeker Karatoprak; S. İlgün; M. Koşar; *Chem Biodivers*., **2017**, *14(9)*, e1700150.