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Linking Phenolic Composition with Biological Activities in Three Endemic *Marrubium* Taxa

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Abstract: This study investigates the relationship between phenolic composition and biological activities of three endemic *Marrubium* taxa, namely *Marrubium parviflorum* subsp. *oligodon* (Boiss.) Seybold, *Marrubium cephalanthum* Boiss. & Noë, and *Marrubium globosum* Montbret & Aucher ex Benth. Ethanolic extracts prepared from the aerial parts were analyzed by HPLC-UV and twelve phenolic compounds were quantitatively identified. Among the investigated taxa, *M. cephalanthum* exhibited the highest phenolic content with verbascoside, rutin, and hyperoside as the predominant constituents. This taxon also showed the strongest antioxidant activity in DPPH (IC_{50} = 26.28 µg/mL) and CUPRAC ($A_{0.5}$ = 29.82 µg/mL) assays. In contrast, *M. globosum* demonstrated the highest activity in the ABTS assay (IC_{50} = 3.58 µg/mL) and showed relatively higher cholinesterase inhibitory activity (AChE: $A_{0.5}$ = 65.14 µg/mL; BChE: $A_{0.5}$ = 132.73 µg/mL). Regarding lipoxygenase inhibition, *M. parviflorum* subsp. *oligodon* exhibited the strongest effect (IC_{50} = 106.55 µg/mL), followed by *M. cephalanthum* and *M. globosum*. No considerable inhibitory activity was observed against α-glucosidase or α-amylase under the tested conditions. Overall, the findings indicate that variations in phenolic composition may be associated with differences in biological activities among the investigated taxa. These results contribute to the understanding of the phytochemical–bioactivity relationship in *Marrubium* species and provide a basis for further bioactivity-guided studies.

Keywords: *Marrubium*, phenolic compounds, antioxidant activity, enzyme inhibition, cholinesterase, lipoxygenase.

Üç Endemik *Marrubium* Taksonunda Fenolik Bileşimin Biyolojik Aktiviteler ile İlişkilendirilmesi

Öz: : Bu çalışmada, *Marrubium parviflorum* subsp. *oligodon* (Boiss.) Seybold, *Marrubium cephalanthum* Boiss. & Noë ve *Marrubium globosum* Montbret & Aucher ex Benth. olmak üzere Türkiye'ye endemik üç *Marrubium* taksonunun fenolik bileşimi ile biyolojik aktiviteleri arasındaki ilişki araştırılmıştır. Bitkilerin toprak üstü kısımlarından elde edilen etanol ekstreleri HPLC-UV yöntemi ile analiz edilmiş ve toplamda 12 fenolik bileşik kantitatif olarak belirlenmiştir. İncelenen taksonlar arasında *M. cephalanthum*'un en yüksek fenolik içeriğe sahip olduğu ve başlıca bileşenlerinin verbaskozid, rutin ve hiperozid olduğu saptanmıştır. Bu takson ayrıca DPPH (IC_{50} = 26.28 µg/mL) ve CUPRAC ($A_{0.5}$ = 29.82 µg/mL) analizlerinde en yüksek antioksidan aktiviteyi göstermiştir. Buna karşılık, *M. globosum* ABTS analizinde en yüksek aktiviteyi (IC_{50} = 3.58 µg/mL) sergilemiş ve kolinesteraz inhibisyonu açısından diğer taksonlara göre daha yüksek aktivite göstermiştir (AChE: $A_{0.5}$ = 65.14 µg/mL; BChE: $A_{0.5}$ = 132.73 µg/mL). Lipoksijenaz inhibisyonu bakımından en güçlü etki *M. parviflorum* subsp. *oligodon*'da gözlenmiş (IC_{50} = 106.55 µg/mL), bunu *M. cephalanthum* ve *M. globosum* izlemiştir. Buna karşılık, uygulanan koşullar altında hiçbir taksonda α-glukozidaz ve α-amilaz enzimlerine karşı anlamlı bir inhibisyon gözlenmemiştir. Elde edilen bulgular, fenolik bileşimdeki farklılıkların biyolojik aktivitelerdeki değişimlerle ilişkili olabileceğini göstermektedir. Bu sonuçlar, *Marrubium* türlerinde fitokimyasal bileşim ile biyolojik aktivite arasındaki ilişkinin anlaşılmasına katkı sağlamakta olup, ileri düzey çalışmalar için temel oluşturmaktadır.

Anahtar kelimeler: *Marrubium*, fenolik bileşikler, antioksidan aktivite, enzim inhibisyonu, kolinesteraz, lipoksijenaz.

1. Introduction

Medicinal plants have been utilized for millennia across diverse regions to treat a wide range of diseases, primarily due to their rich bioactive compounds. Their advantages include demonstrated therapeutic efficacy, relatively low incidence of adverse effects, cost-effectiveness, and a

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reduced risk of resistance development compared to synthetic agents (Barkaoui et al., 2017; Avşar et al., 2024; Sarikurkcu et al., 2024). Owing to their diverse phytochemical composition, medicinal plants represent valuable sources for the discovery of novel drug candidates and continue to attract considerable scientific interest. Notably, it has been estimated that approximately 80% of contemporary plant-based medicines are derived from species with a history of traditional use (Derelli Tüfekçi et al., 2024).

The genus *Marrubium* L., belonging to the Lamiaceae family, comprises approximately 40 taxa distributed across temperate regions of North Africa, Europe, and Asia, particularly in the Mediterranean and Irano-Turanian phytogeographic zones. Türkiye, recognized for its remarkable plant biodiversity, constitutes a major center of diversity for this genus, with 16 of the 27 native taxa being endemic (Demiroz Akbulut et al., 2025). In Türkiye, *Marrubium* species are commonly known as "bozcabogum", "küllü bozotu", and "baslı bozot" (Güner et al., 2012). Chemically, members of this genus are characterized by a wide range of bioactive secondary metabolites that include labdane-type diterpenes (notably marrubiin), phenylethanoid glycosides (such as verbascoside and forsythoside B), phenylpropanoids, flavonoids, and lignan derivatives (Demiroz Akbulut et al., 2025; Sarikurkcu et al., 2018). Consequently, *Marrubium* taxa have been reported to exhibit diverse biological activities including anti-inflammatory (Sarikurkcu et al., 2024; Demiroz Akbulut et al., 2025; Kumar et al., 2024; Aćimović et al., 2020; Göger et al., 2019; Ouadghiri et al., 2026; Fathiazad et al., 2017), antidiabetic (Kumar et al., 2024; Aćimović et al., 2020; Göger et al., 2019; Kazemi et al., 2022), antioxidant (Sarikurkcu et al., 2018; Aćimović et al., 2020; Göğer et al., 2019; Ouadghiri et al., 2026), anticholinesterase (Demiroz Akbulut et al., 2025), anticancer (Sarikurkcu et al., 2024), analgesic (Meyre-Silva et al., 2005), antityrosinase (Derelli Tüfekçi et al., 2024), antimicrobial (Mssillou et al., 2021), hypotensive (Bardai et al., 2001), hepatoprotective (Ettaya et al., 2016), gastroprotective(Paula de Oliveira et al., 2011) as well as antiproliferative, antispasmodic, and cardioprotective effects (Aćimović et al., 2020).

Historically, the *Marrubium* genus has occupied an important place in traditional pharmacopoeias. *Marrubium vulgare* L., in particular, has been widely used as an expectorant and antispasmodic agent in the treatment of respiratory conditions such as bronchial asthma, chronic bronchitis, and non-productive cough as well as in the management of gastrointestinal disorders (Aćimović et al., 2020; Lodhi et al., 2017). In addition, ethnobotanical studies conducted in Morocco, Algeria, and Mexico have documented its use in the traditional treatment of diabetes and inflammatory diseases, highlighting its therapeutic versatility in folk medicine (Aitbaba et al., 2025; Rodríguez Villanueva et al., 2017; El-Mernissi et al., 2025; Tahraoui et al., 2007).

Despite this ethnopharmacological importance, scientific data on the phytochemical composition and biological activities of several endemic Turkish *Marrubium* taxa remain limited. In particular, comparative studies integrating phytochemical characterization with multiple enzyme inhibitory and antioxidant assays are still scarce. Therefore, further investigation of these endemic taxa is warranted to better understand their potential pharmacological relevance.

Acetylcholinesterase (AChE) and butyrylcholinesterase (BChE) are serine hydrolase enzymes responsible for the hydrolysis of the neurotransmitter acetylcholine at cholinergic synapses. Inhibition of these enzymes results in elevated acetylcholine levels within the synaptic cleft, thereby enhancing cholinergic neurotransmission. This mechanism holds clinical significance in the treatment of neurodegenerative disorders, particularly Alzheimer's disease and other forms of dementia (Demiroz Akbulut et al., 2025). Lipoxygenases (LOXs) are iron-containing enzymes that are widely expressed in human tissues and serve as key mediators of inflammatory signaling. Under conditions of oxidative stress, excessive production of reactive oxygen species (ROS) can trigger inflammatory cascades, leading to cytokine release and subsequent upregulation of LOX activity. The resulting overproduction of LOX-derived lipid mediators — including leukotrienes and prostaglandins — has been implicated in the pathogenesis of a broad spectrum of chronic diseases, among them are cancer, stroke, cardiovascular disorders, and neurodegenerative conditions. Given their central role in eicosanoid biosynthesis and disease progression, LOXs have emerged as attractive therapeutic targets and their inhibition is widely regarded as a promising strategy for the prevention and management of inflammation-associated pathologies (Lončarić et al., 2021). Among the in vitro methods commonly employed for antioxidant activity assessment, the 2,2-diphenyl-1-picrylhydrazyl (DPPH), 2,2'-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) (ABTS), and cupric reducing antioxidant capacity (CUPRAC) assays are the most widely applied. The DPPH and ABTS methods quantify the capacity of a compound to neutralize stable free radicals, whereas CUPRAC evaluates the ability of antioxidants to reduce the cupric ion–neocuproine complex, thereby providing a complementary measure of electron transfer-based reducing capacity (Apak et al., 2010; Ersoy et al., 2020). In the context of carbohydrate metabolism, α-glucosidase and α-amylase represent two critical digestive enzymes that govern the hydrolysis of dietary carbohydrates and the subsequent rise in postprandial blood glucose levels. Inhibition of these enzymes delays carbohydrate absorption and attenuates glycemic response, making them well-established targets in the pharmacological management of type 2 diabetes mellitus (Schmidt et al., 2012).

In this context, the present study aims to evaluate the phytochemical composition and biological activities of three endemic taxa, namely *Marrubium parviflorum* subsp. *oligodon*, *Marrubium cephalanthum*, and *Marrubium globosum*. The inhibitory effects of these taxa on key enzymes associated with metabolic, neurodegenerative, and inflammatory processes—α-glucosidase, α-amylase, AChE, BChE, and LOX—were investigated. In addition, their antioxidant capacities were assessed using DPPH, ABTS, and CUPRAC assays. While limited antioxidant data exist for some of these taxa, comprehensive comparative evaluations remain insufficient in the literature.

Based on the phenolic-rich composition of *Marrubium* species and their reported neuroprotective and anti-inflammatory properties, we hypothesized that these endemic taxa may exhibit cholinesterase and lipoxygenase inhibitory activities relevant to neurodegenerative and inflammatory disorders. Furthermore, the phenolic profiles of the ethanol extracts were characterized using HPLC-UV analysis. This integrated approach aims to contribute to the scientific understanding of these endemic taxa and to provide a foundation for future bioactivity-guided studies.

2. Material and Method

2.1. Plant material

Aerial parts of *Marrubium cephalanthum* Boiss. & Noë (Antalya-Akseki), *Marrubium parviflorum* subsp. *oligodon* (Boiss.) Seybold (Yozgat-Sorgun) and *Marrubium globosum* Montbret & Aucher ex Benth. (Niğde-Çamardı) were collected from Türkiye in July 2024. The plant materials were identified by Prof. Dr. Serpil Demirci Kayıran (Department of Pharmaceutical Botany, Faculty of Pharmacy, Cukurova University). Voucher specimens have been deposited in the Herbarium of the Faculty of Pharmacy, Cukurova University, Adana, Türkiye (CUEF No: 1961, 1945, and 2039, respectively).

Herbarium specimens of the three endemic *Marrubium* taxa examined in this study are given in Figure 1.

2.2. Extraction

The dried plant materials (10 g) were extracted with 200 mL of 96% ethanol using a shaking incubator at room temperature (25 °C) for 24 h. The extraction procedure was repeated three times with fresh solvent to ensure exhaustive extraction. The combined extracts were filtered and concentrated under reduced pressure at 40 °C using a rotary evaporator. The resulting extracts were lyophilized and stored at −20 °C until further analysis.

Figure 1. Herbarium specimens of the three endemic *Marrubium* taxa investigated in the present study. (A) *M. globosum* Montbret & Aucher ex Benth. (Niğde-Çamardı); (B) *M. parviflorum* subsp. *oligodon* (Boiss.) Seybold (Yozgat-Sorgun); (C) *M. cephalanthum* Boiss. & Noë (Antalya-Akseki). Voucher specimens are deposited in the Herbarium of the Faculty of Pharmacy, Cukurova University (CUEF No: 2039, 1945, and 1961, respectively).

2.3. HPLC Analysis

Phenolic compound analysis was performed using a high-performance liquid chromatography (HPLC) system (Shimadzu model 20A, Kyoto, Japan) equipped with a quaternary pump (LC-2010), UV detector, and autosampler. Chromatographic separation was carried out on an ACE C18 reversed-phase column (250 × 4.6 mm i.d., 5 µm particle size) maintained at 25 °C.

The mobile phase consisted of acetonitrile (solvent A) and 0.2% (v/v) aqueous acetic acid (solvent B) delivered at a flow rate of 1.0 mL/min. The gradient elution program was as follows: 3% A (0–8 min), 3–15% A (8–20 min), 15–25% A (20–40 min), 25–30% A (40–45 min), 30–90% A (45–55 min) followed by re-equilibration to initial conditions up to 60 min (Al-Zaban et al., 2021).

Sample aliquots (20 µL) were injected and chromatograms were recorded at 280 and 330 nm. Phenolic compounds were identified by comparing their retention times and UV spectral characteristics with those of authentic reference standards (Sigma-Aldrich) and quantification was performed using external calibration curves.

The calibration curves exhibited good linearity within the tested concentration ranges ($r^2 > 0.998$). It should be noted that the analysis was limited to the selected phenolic standards and does not represent a comprehensive characterization of all constituents present in the extracts.

2.4. α-Glucosidase Inhibitory Activity

α-Glucosidase inhibitory activity was evaluated according to Schmidt et al. (Schmidt et al., 2012) with minor modifications. The reaction mixture (200 µL total volume) consisted of α-glucosidase enzyme (0.05 U/mL), phosphate buffer (0.1 M, pH 7.5), test sample at different concentrations, and 4-nitrophenyl-β-D-glucopyranoside (PNPG) substrate (1.0 mM).

After pre-incubation for 10 min at 28 °C, the reaction was monitored kinetically at 405 nm for 35 min using a microplate reader. Acarbose was used as the positive control, while a reaction mixture without inhibitor served as the negative control.

The percentage of inhibition was calculated based on the slopes of the reaction curves and IC_{50} values were determined by nonlinear regression analysis using GraphPad Prism software.

2.5. α-Amylase Inhibitory Activity

α-Amylase inhibitory activity was evaluated according to Okutan et al. (Okutan et al., 2014; Şahin et al., 2023) with minor modifications. The reaction mixture (200 µL total volume) consisted of α-amylase enzyme (0.05 U/mL), phosphate buffer (0.1 M, pH 6.0), test sample at different concentrations, and 2-chloro-4-nitrophenyl-α-maltotrioside (CNP-G3) substrate (1.0 mM). After pre-incubation for 10 min at 37 °C, the reaction was monitored at 405 nm for 30 min using a microplate reader. Acarbose was used as the positive control, while a reaction mixture without inhibitor served as the negative control. The percentage of inhibition was calculated based on the slopes of the reaction curves and IC_{50} values were determined by nonlinear regression analysis using GraphPad Prism software. All measurements were performed in triplicate.

2.6. Lipoxygenase Inhibitory Activity

Lipoxygenase (15-LOX) inhibitory activity was evaluated according to the previously described methods (del Carmen Pinto et al., 2007; Erhabor et al., 2020) with minor modifications. The reaction mixture consisted of lipoxygenase enzyme (0.2 U/mL), test sample at different concentrations, and linoleic acid (140 µM) as substrate in Tris–HCl buffer (pH 7.4). After incubation at 25 °C for 10 min, the reaction was terminated using ferrous oxidation–xylenol orange (FOX) reagent and the absorbance was measured at 560 nm using a microplate reader. Quercetin was used as the positive control, while a reaction mixture without inhibitor served as the negative control. The percentage of inhibition was calculated based on absorbance differences between the sample and control wells and IC_{50} values were determined by nonlinear regression analysis using GraphPad Prism software. All measurements were performed in triplicate.

2.7. Cholinesterase Inhibitory Activities

AChE and BChE inhibitory activities were evaluated using Ellman's microplate method (Ellman et al., 1961). The reaction mixture consisted of 130 µL of sodium phosphate buffer (pH 8.0), 20 µL of enzyme solution, 10 µL of 5,5'-dithiobis(2-nitrobenzoic acid) (DTNB), 10 µL of test sample (prepared at 4 mM stock concentration), and 10 µL of substrate (acetylthiocholine or butyrylthiocholine). After pre-incubation for 15 min at 25 °C, the reaction was monitored at 412 nm using a microplate reader. Galantamine was used as the positive control, while a reaction mixture without inhibitor served as the negative control. The percentage of inhibition was calculated based on the absorbance changes and IC_{50} values were determined by nonlinear regression analysis using GraphPad Prism software. All measurements were performed in triplicate.

2.8. Antioxidant Potential Assays

The antioxidant activities of the extracts were evaluated using DPPH, ABTS, and CUPRAC assays according to Ersoy et al. (Ersoy et al., 2020) with minor modifications. 160 µL of 0.1 mM DPPH solution, prepared in methanol, was combined with 40 µL of methanolic sample solutions at varying concentrations. The resulting mixtures were incubated in the dark at room temperature for 30 minutes after which the absorbance was measured at 517 nm.

To synthesize the ABTS cation radical, 7 mM solution of ABTS was initially prepared in water and then combined with 2.45 mM $K_2S_2O_8$. This mixture was stored in the dark at ambient temperature for a period ranging from 12 to 16 hours. The resulting solution was subsequently diluted with ethanol until its absorbance at 734 nm was adjusted to 0.700 ± 0.025. Thereafter, 160 µL of the $ABTS^{\bullet+}$ solution was mixed with 40 µL of sample solutions at different concentrations. The absorbance change was recorded a 734 nm following a 6-minute incubation period.

In the CUPRAC assay, 61 µL of a 1.0×10^{-2} M $CuCl_2$ solution, 61 µL of 1 M NH_4OAc buffer at pH 7.0, and 61 µL of a 7.5×10^{-3} M neocuproine solution were combined. Various concentrations of sample solutions were then added and the total volume was brought up to 250 mL with distilled water. After incubating for 1 hour, the absorbance was recorded at 450 nm.

Butylated hydroxyanisole (BHA) and butylated hydroxytoluene (BHT) were used as positive controls. The antioxidant activity was expressed as IC_{50} (for DPPH and ABTS) and $A_{0.5}$ (for CUPRAC) values, calculated by nonlinear regression analysis using GraphPad Prism software. All experiments were performed in triplicate.

2.9. Data Analysis

Inhibitory activities were expressed as IC_{50} values, calculated by nonlinear regression analysis using GraphPad Prism (version 8.0.1). All assays were conducted in triplicate and data are presented as mean ± SD. Statistical comparisons among the three taxa were performed using Student's t-test ($p < 0.05$) and pairwise comparison results for all biological activities are summarized in Table 1.

The overall experimental design and workflow of the study are summarized in Figure 2.

3. Results and Discussion

3.1. Extraction Yields of the *Marrubium* Taxa

The extraction yields were determined for three *Marrubium* taxa: *Marrubium parviflorum* subsp. *oligodon* (14.46%), *Marrubium cephalanthum* (11.27%), and *Marrubium globosum* (10.86%). These values represent the dry weight yield of each species obtained through the extraction process.

3.2. HPLC Analysis

The phenolic composition of the ethanolic extracts obtained from the investigated *Marrubium* taxa was quantitatively analyzed by HPLC and a total of twelve phenolic compounds were detected. The calibration curves for all reference standards showed good linearity

(r^2 = 0.9985–0.9999), indicating the suitability of the method for quantitative analysis. The results are summarized in Table 2.

Table 1. Pairwise statistical comparisons of biological activities among *Marrubium* taxa

Comparison	15-LOX	DPPH	ABTS	CUPRAC	AChE	BChE
M. globosum vs *M. parviflorum* subsp. *oligodon*	16.54 ***	-35.27 ***	-17.49 ***	-5.55 **	-32.10 ***	-48.35 ***
M. globosum vs *M. cephalanthum*	12.25 ***	42.20 ***	-111.09 ***	17.32 ***	-35.30 ***	-2.04 *ns*
M. parviflorum subsp. *oligodon* vs *M. cephalanthum*	-5.30 **	85.50 ***	-16.29 ***	30.34 ***	-1.32 *ns*	30.13 ***

t-statistics are shown for each assay; significance levels are indicated as superscripts: *** $p < 0.001$; ** $p < 0.01$; * $p < 0.05$; *ns*: not significant ($p > 0.05$). All comparisons: df = 4, n = 3 per group.

Table 2. Quantitative HPLC analysis of phenolic compounds (mg/g extract) in three *Marrubium* taxa

Compound	Retention time (min)	Calibration equation values	Linear regression (r2)	*Marrubium globosum*	*Marrubium parviflorum* subsp. *oligodon*	*Marrubium cephalanthum*
Chlorogenic acid	19.82	Y = (1.21005e-007)X – (0.0098786)	0,9993924	0.058±0.001	0.012±0.001	0.140±0.003
Apigenin	48.01	Y = (8.96784e-007)X + (0.039119)	0.9988569	0.027±0.001	0.046±0.001	0.031±0.007
Isorhamnetin	49.49	Y = (4.243587e-007)X – (7.707421e-003)	0.9991452	0.001±0.001	Nd	0.005±0.001
Catechin	19.35	Y= (6.333737e-007)X + (7.863926e-003)	0.9999746	Nd	Nd	Nd
Caffeic acid	20.20	Y = (4.08579e-008)X + (0.00920376)	0.9988046	0.019±0.01	0.011±0.00	0.015±0.00
Hyperoside	29.44	Y = (1.47085e-007)X + (0.0152048)	0.9989594	0.102±0.00	0.078±0.002	0.215±0.00
Isoquercitrin	29.90	Y = (1.64743e-007)X + (-0.000356428)	0.9999916	Nd	0.012±0.001	Nd
Kaempferol	48.76	Y = (1.0749e-007)X + (0.00116731)	0.9990873	0.125±0.002	0.002±0.00	0.003±0.003
Naringin	45.56	Y = (4.76803e-008)X + (0.000143725)	0.9997916	0.001±0.00	0.007±0.00	0.014±0.003
Quercetin	42.48	Y = (1.08874e-007)X + (0.00894193)	0.9985216	0.012±0.00	0.011±0.00	0.01±0.00
Quercitrin	33.14	Y = (1.57436e-007)X + (-0.0026778)	0.9996901	Nd	0.005±0.001	0.028±0.00
Rutin	29.09	Y = (2.70032e-007)X + (-0.00285908)	0.9999442	0.160±0.002	0.085±0.005	0.377±0.005
Verbascoside	28.82	Y = (4.71717e-007)X + (-0.00449292)	0.9997209	0.28±0.001	0.13±0.006	0.658±0.008

Nd: not detected

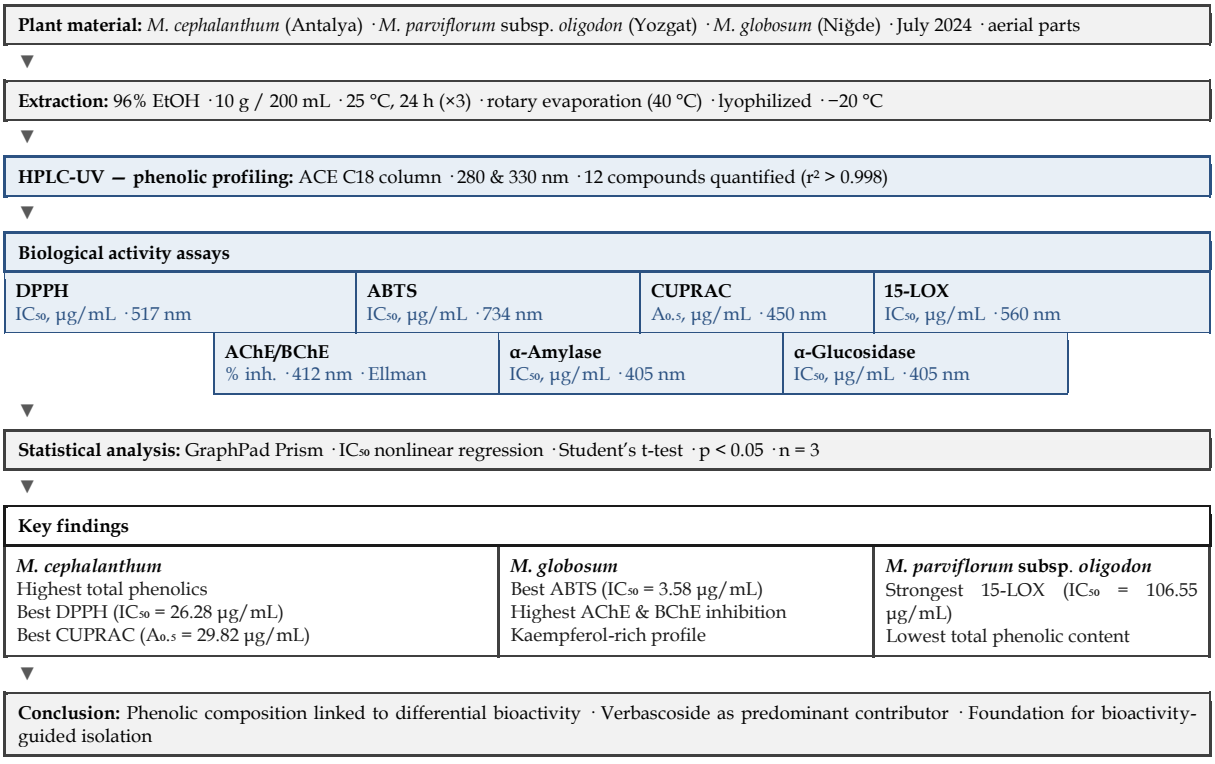

Figure 2. Experimental workflow for the phytochemical and biological evaluation of three endemic *Marrubium* taxa.

Among the studied taxa, *Marrubium cephalanthum* exhibited the highest overall phenolic content. The major constituents identified in this extract were verbascoside (0.658 mg/g), rutin (0.377 mg/g), and hyperoside (0.215 mg/g), while other compounds such as chlorogenic acid, apigenin, kaempferol, quercetin, and quercitrin were present at lower concentrations. The relatively higher abundance of these phenolic compounds suggests a more pronounced accumulation of secondary metabolites in this species.

Marrubium globosum displayed a comparable but quantitatively lower phenolic composition. In this extract, verbascoside (0.28 mg/g), rutin (0.160 mg/g), kaempferol (0.125 mg/g), and hyperoside (0.102 mg/g) were identified as the predominant compounds. In contrast, isoquercitrin and quercitrin were not detected and catechin was absent in all investigated taxa, indicating differences in flavonoid distribution among species.

In comparison, *Marrubium parviflorum* subsp. *oligodon* showed the lowest levels of phenolic constituents. The dominant compounds were verbascoside (0.13 mg/g), rutin (0.085 mg/g), and hyperoside (0.078 mg/g), while isoquercitrin and quercitrin were detected only in trace amounts. Isorhamnetin and catechin were not detected in this extract.

Verbascoside was the predominant phenolic compound in all three taxa. Given its well-documented antioxidant, anti-inflammatory, and neuroprotective properties (Marčetić et al., 2025), verbascoside is considered the primary contributor to the biological activities reported in the present study.

Overall, verbascoside, rutin, hyperoside, apigenin, quercetin, and chlorogenic acid were identified as common constituents across all three taxa; however, their relative abundances varied significantly. These quantitative differences may be attributed to species-specific metabolic profiles and environmental factors and are likely to be reflected in the variation observed in biological activities. It should also be acknowledged that the overall pharmacological profile of each taxon is probably shaped by synergistic interactions among multiple constituents rather than by a single dominant compound.

Representative HPLC-UV chromatograms of the ethanolic extracts of the three investigated taxa are presented in Figure 3.

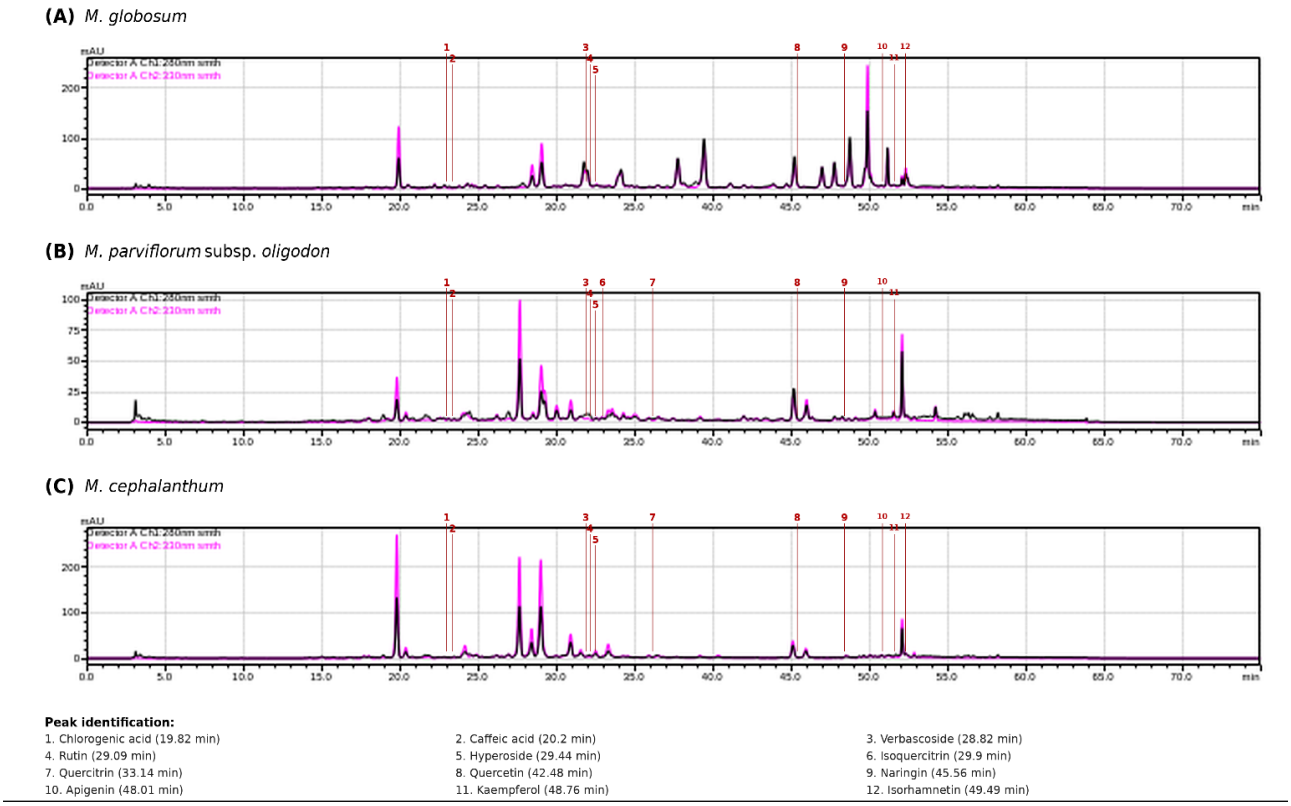

Figure 3. Representative HPLC-UV chromatograms of (A) *M. globosum*, (B) *M. parviflorum* subsp. *oligodon*, and (C) *M. cephalanthum*.

3.3. Enzyme Inhibitory Activity

3.3.1 α-Amylase and α-Glucosidase Enzyme Inhibitory Activities

The inhibitory effects of *Marrubium globosum*, *M. parviflorum* subsp. *oligodon*, and *M. cephalanthum* on α-glucosidase and α-amylase were evaluated to assess their potential impact on carbohydrate-digesting enzymes. The in vitro results indicated that none of the tested extracts exhibited notable inhibitory activity within the studied concentration range. The IC_{50} values exceeding 2000 µg/mL for α-glucosidase and 1000 µg/mL for α-amylase indicate that the samples exhibit weak inhibitory activity against both enzymes under the applied experimental conditions. In contrast, the positive control acarbose showed strong inhibition, with IC_{50} values of 672.93 ± 9.62 µg/mL for α-glucosidase and 0.03248 ± 8.49 × 10^{-5} µg/mL for α-amylase (Table 3).

These findings indicate that the investigated extracts have limited inhibitory activity against carbohydrate-hydrolyzing enzymes. However, this does not necessarily

exclude their potential involvement in antidiabetic effects as other mechanisms such as antioxidant activity or modulation of glucose metabolism were not assessed in the present study.

Table 3. α-Amylase and α-glucosidase inhibitory activities of *Marrubium* extracts (IC_{50}, µg/mL)

Sample	Anti-Glycosidase IC_{50} µg/mL	α-Amylase IC_{50} µg/mL
Marrubium globosum	>2000	>1000
Marrubium parviflorum subsp. oligodon	>2000	>1000
Marrubium cephalanthum	>2000	>1000
Acarbose	672.93 ± 96	0.032 ± 8.49E-05

Results are expressed as mean ± SD (n = 3). Statistical significance was set at $p < 0.05$.

Previous studies on *Marrubium* species have reported variable enzyme inhibitory activities depending on species, extract type, and phytochemical composition. For instance, the methanolic extract of *Marrubium radiatum* demonstrated significant inhibition of both α-amylase and α-glucosidase (IC_{50} = 61.1 and 68.8 µg/mL, respectively) (Loizzo et al., 2008). Similarly, the ethyl acetate fraction of *M. astracanicum* showed considerable α-glucosidase inhibition, and bioactivity-guided fractionation identified apigenin derivatives as potent inhibitors (Kazemi et al., 2022). In addition, extracts of *M. astracanicum* subsp. *macrodon* and *M. sivasense* have been reported to exhibit notable enzyme inhibitory or hypoglycemic effects (Avşar et al., 2024; Göger et al., 2019).

The reduced activity observed in this study compared with previous reports may be attributed to variations in phytochemical composition, particularly the relative abundance of flavonoids, such as apigenin and kaempferol derivatives, which are associated with enzyme inhibition.

Moreover, variations in extraction solvents, plant origin, and assay conditions may also contribute to discrepancies in activity profiles.

Overall, the results highlight a limited enzyme inhibitory potential for the investigated taxa under the tested conditions, while emphasizing the importance of phytochemical diversity and methodological factors in determining biological activity.

3.3.2. LOX Enzyme Inhibitory Activity

To the best of our knowledge, there are no detailed reports on the 15-lipoxygenase (15-LOX) activities of *Marrubium parviflorum* subsp. *oligodon, Marrubium cephalanthum* and *Marrubium globosum*. Therefore, the present study provides a comparative evaluation of their in vitro LOX inhibitory potential.

The 15-LOX inhibitory activities of the investigated taxa are presented in Table 4 and Figure 4. Among the tested species, *Marrubium parviflorum* subsp. *oligodon* exhibited the strongest inhibitory effect, with an IC_{50} value of 106.55 ± 2.33 µg/mL, followed by *Marrubium cephalanthum* (IC_{50} = 118.10 ± 2.97 µg/mL) and *Marrubium globosum* (IC_{50} = 159.23 ± 5.01 µg/mL). The reference compound quercetin showed a markedly stronger activity (IC_{50} = 23.37 ± 3.17 µg/mL) indicating that the extracts possessed lower inhibitory potency compared to the standard.

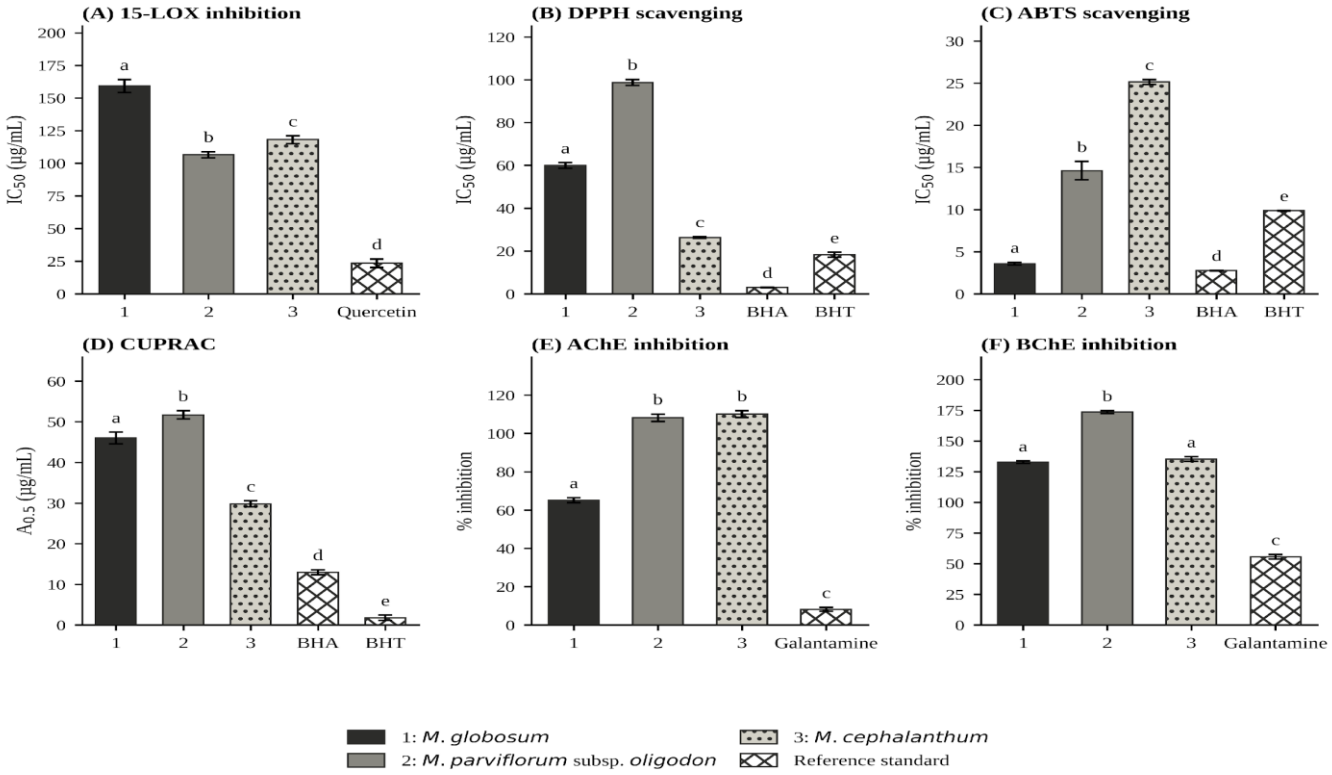

Figure 4. Biological activities of ethanolic extracts of three endemic *Marrubium* taxa

(A) 15-LOX inhibition; (B) DPPH scavenging; (C) ABTS scavenging; (D) CUPRAC reducing capacity; (E) AChE inhibition; (F) BChE inhibition. 1: *M. globosum*; 2: *M. parviflorum* subsp. *oligodon*; 3: *M. cephalanthum*. Quercetin, BHA, BHT, and galantamine were used as reference standards. Values are means ± SD (n = 3); different letters indicate significant differences ($p < 0.05$, Student's t-test).

Table 4. Antioxidant and enzyme inhibitory activities of *Marrubium* taxa

Sample	15-LOX†	DPPH Free Radical†	ABTS Cation Radical†	CUPRAC‡	AChE§	BChE§
	IC_{50} (µg/mL)	IC_{50} (µg/mL)	IC_{50} (µg/mL)	$A_{0.5}$ (µg/mL)	% inhibition	% inhibition
M. globosum	159.23 ± 5.00[a]	59.96 ± 1.30[a]	3.58 ± 0.17[a]	46.01 ± 1.45[a]	65.14 ± 1.33[a]	132.73 ± 1.01[a]
M. parviflorum subsp. *oligodon*	106.55 ± 2.33[b]	98.71 ± 1.39[b]	14.62 ± 1.08[b]	51.69 ± 1.02[b]	108.12 ± 1.90[b]	173.60 ± 1.06[b]
M. cephalanthum	118.10 ± 2.97[c]	26.28 ± 0.47[c]	25.14 ± 0.29[c]	29.82 ± 0.72[c]	110.10 ± 1.76[b]	135.30 ± 1.93[a]
Quercetin¶	23.37 ± 3.17[d]	—	—	—	—	—
BHA¶	—	2.94 ± 0.06[d]	2.74 ± 0.03[d]	12.97 ± 0.62[d]	—	—
BHT¶	—	18.23 ± 1.18[e]	9.85 ± 0.04[e]	1.78 ± 0.67[e]	—	—
Galantamine¶	—	—	—	—	8.21 ± 1.00[c]	55.72 ± 1.84[c]

† Values reported as IC_{50} (µg/mL)

‡ Values reported as $A_{0.5}$ (µg/mL)

§ Enzyme inhibition values expressed as % inhibition at a sample concentration of 200 µg/mL.

¶ Standard (reference) compound.

Values are means ± S.D. (n = 3). Different superscript lowercase letters ([a], [b], [c]...) within the same column indicate statistically significant differences among groups (Student's t-test, $p < 0.05$). Entries marked — were not tested in that assay.

In the literature, marrubiin has been reported as a moderate LOX inhibitor (IC_{50} = 173 µg/mL) (Radulović et al., 2024). In this context, the inhibitory activity observed for *M. parviflorum* subsp. *oligodon* and *M. cephalanthum* appears to be comparable or slightly stronger than that of marrubiin, while *M. globosum* exhibited a similar level of activity. On the other hand, some *Marrubium* taxa, such as *M. cuneatum*, have been reported to show no LOX inhibitory activity (İçen et al., 2025) that highlights the variability within the genus.

Molecular docking studies on *Marrubium vulgare* L. have revealed that flavonoid compounds, particularly luteolin and quercetin, exhibit high binding affinity and favorable interaction profiles with cyclooxygenase enzymes, suggesting that these constituents hold significant potential as natural COX inhibitors (Kadri et al., 2025). Furthermore, flavonoids present in *M. vulgare* L. extracts have been shown to exert inhibitory effects on enzymes and signaling pathways involved in the regulation of the inflammatory response while modulating calcium homeostasis by reducing intracellular calcium levels and suppressing the release of pro-inflammatory mediators (Aitbaba et al., 2025).

The observation that *M. parviflorum* subsp. *oligodon*, despite possessing the lowest total phenolic content among the three investigated taxa, exhibits the strongest LOX inhibitory activity suggests that the structural specificity of particular constituents toward the LOX active site may be a more decisive factor than the overall phenolic abundance. The relatively high LOX inhibitory activity observed in this taxon may be attributed to the presence of isoquercitrin, which was not detected in the other two taxa, and the comparatively elevated levels of apigenin.

Indeed, apigenin has been reported to upregulate COX-1 expression while simultaneously suppressing COX-2 and 5-LOX expression (Abdel Fattah et al., 2025), suggesting a compound-specific regulatory mechanism that may contribute to the observed activity. Nevertheless, further studies incorporating bioactivity-guided fractionation and molecular docking analyses are warranted to validate these hypotheses and elucidate the precise contributions of individual phenolic constituents to the 15-LOX inhibitory activity of *M. parviflorum* subsp. *oligodon*.

Overall, the results indicate that the investigated taxa exhibit moderate LOX inhibitory activity that may contribute to their reported anti-inflammatory properties.

3.3.3. Cholinesterase Inhibitory Activities

The cholinesterase inhibitory activities of the ethanolic extracts of *Marrubium globosum*, *Marrubium parviflorum* subsp. *oligodon*, and *Marrubium cephalanthum* were evaluated against AChE and BChE. To the best of our knowledge, comparative data on these taxa are limited. Galantamine was used as the reference compound with IC_{50} values of 8.21 ± 1.00 µg/mL for AChE and 55.72 ± 1.84 µg/mL for BChE (Table 4 and Fig. 4).

Among the tested extracts, *M. globosum* exhibited the strongest inhibitory activity against both AChE and BChE, followed by *M. cephalanthum* and *M. parviflorum* subsp. *oligodon*. Although all extracts displayed weaker inhibition relative to galantamine, the observed differences in inhibitory potential among the taxa appear to be rooted in qualitative differences in phenolic composition rather than total phenolic content alone.

A key structural factor underlying the superior activity of *M. globosum* is its relatively higher kaempferol content. Unlike the dominant constituents of *M. cephalanthum* — verbascoside, rutin, and hyperoside — which are all present in glycosylated forms, kaempferol occurs predominantly as an aglycone. This structural distinction carries considerable mechanistic implications as kaempferol acts as a reversible mixed-mode inhibitor of AChE by binding concurrently to both the catalytic active site (Trp86, Glu202, and Tyr337) and the peripheral anionic site (Tyr72, Asp74, and Tyr124) through hydrogen bonds

and hydrophobic interactions (Shi et al., 2023). In contrast, glycosylation at the C-3 and C-7 positions of flavonoids has been shown to significantly reduce cholinesterase inhibitory potency as the sugar moieties introduce steric hindrance that obstructs access to the enzyme active site. Accordingly, rutin, hyperoside, and verbascoside — despite their abundance in *M. cephalanthum* — are structurally restricted in their ability to interact effectively with the active sites of both AChE and BChE. It is therefore proposed that the aglycone character and dual-site binding capability of kaempferol confer a disproportionate advantage in cholinesterase inhibition that is not reflected in total phenolic content measurements (Li et al., 2023).

Verbascoside has been the subject of considerable mechanistic investigation with respect to its neuroprotective activity. Verbascoside plays a neuroprotective role in Alzheimer's disease and proteomic analysis has demonstrated that its neuroprotective effect correlates closely with its anti-inflammatory effect particularly through the blockade of the NF-κB-p65 signaling pathway (Chen et al., 2022)

These findings are in line with previously reported cholinesterase inhibitory activities within the *Marrubium* genus. The acetone extract of *M. vulgare* showed strong inhibition of both enzymes (Orhan et al., 2010), while methanolic extracts of *M. alysson* and *M. deserti* demonstrated moderate inhibitory effects (Edziri et al., 2018; Chemsa et al., 2016). Among Turkish endemic taxa, *M. peregrinum* was reported to exhibit relatively higher cholinesterase inhibitory activity (Demiroz Akbulut et al., 2025). The activities recorded in the present study are broadly consistent with these reports; though, direct comparisons remain limited by differences in extraction solvent, phytochemical composition, geographical origin, and experimental conditions.

Overall, the results indicate that the investigated *Marrubium* taxa possess moderate cholinesterase inhibitory activity with potential relevance to neurodegenerative disorders. Further bioactivity-guided studies and validation in more advanced biological models are nonetheless required to identify the active constituents and to confirm the pharmacological significance of these findings.

3.3.4. Antioxidant Potential Assays

The antioxidant activities of the three *Marrubium* taxa were evaluated using DPPH, ABTS, and CUPRAC assays and the results are presented in Table 4 and Figure 4. All extracts exhibited concentration-dependent antioxidant activity with notable variations depending on the assay and species.

Marrubium cephalanthum showed the strongest activity in the DPPH assay (IC_{50} = 26.28 ± 0.47 µg/mL) followed by *M. globosum* and *M. parviflorum* subsp. *oligodon*. In contrast, *M. globosum* exhibited the highest activity in the ABTS assay (IC_{50} = 3.58 ± 0.17 µg/mL), while *M. cephalanthum* demonstrated the greatest reducing capacity in the CUPRAC assay ($A_{0.5}$ = 29.82 ± 0.72 µg/mL). Despite these differences, all extracts showed lower activity compared to the reference standards (BHA and BHT).

The variation in activity among assays is consistent with their different reaction mechanisms as DPPH is primarily based on hydrogen atom transfer, whereas ABTS and CUPRAC involve electron transfer processes (İçen et al., 2025). Such assay-dependent variability has been widely reported in the literature.

The relatively higher antioxidant activity of *M. cephalanthum* may be associated with its higher phenolic content, particularly verbascoside, rutin, and hyperoside. These compounds are known to contribute to antioxidant activity through radical scavenging and metal chelation mechanisms. Verbascoside, a phenylethanoid glycoside, is recognized for its potent hydrogen-donating capabilities and its direct interaction with DPPH free radicals (Sarikurkcu et al., 2018). However, it should be noted that the observed biological effects are likely the result of synergistic interactions among multiple constituents rather than a single compound.

The stronger ABTS radical scavenging activity of *M. globosum* compared to its moderate DPPH activity can be attributed to its phenolic composition, particularly its relatively high kaempferol content.

Kaempferol and its glycosylated derivatives are well known for their strong electron-donating properties and since the ABTS assay is primarily driven by electron transfer reactions, these compounds are expected to perform well under such conditions. In contrast, the DPPH assay relies more heavily on hydrogen atom transfer (HAT), a mechanism that is particularly sensitive to how easily phenolic hydroxyl groups can physically reach and interact with the radical. When kaempferol is present in its glycosylated form, the sugar moieties attached to the molecule create steric hindrance around the hydroxyl groups making it harder for them to donate a hydrogen atom to the bulky DPPH radical. This explains why DPPH activity appears lower despite the same compounds being present. A similar pattern has previously been reported for flavonol glycosides where DPPH and ABTS results diverge precisely because of this structural difference (Apak et al., 2010). In addition, verbascoside likely plays a role here as well. Its catechol group is known to be a highly efficient ABTS scavenger and even at relatively low concentrations, it may contribute more strongly to ABTS activity than its quantity alone would suggest. Taken together, the phenolic profile of *M. globosum* appears particularly well suited to electron transfer-based scavenging which explains why its ABTS activity stands out while its DPPH activity remains at a moderate level.

In comparison with previous studies, the antioxidant activity of *M. cephalanthum* falls within the range reported for *Marrubium* species (Mssillou et al., 2021; Hellal et al., 2020; Boulila et al., 2015; Menaiaia et al., 2021; Chedia et al., 2014) while the lower activity observed for *M. parviflorum* subsp. *oligodon* is consistent with its relatively lower phenolic content. Overall, these findings support the role of phenolic compounds in the antioxidant properties of *Marrubium* taxa while highlighting the importance of compositional and methodological factors in determining activity profiles.

To further elucidate the structure-activity relationships, scatter plots with linear regression lines were constructed to compare the total phenolic content and key individual compounds (verbascoside, rutin, and

hyperoside) with antioxidant activities (Fig. 5). A robust and statistically significant negative correlation was found between verbascoside content and CUPRAC-reducing capacity (R^2 = 0.999, p = 0.017), establishing verbascoside as the main factor influencing electron transfer-based antioxidant activity in these taxa. Although strong, the trends for total phenolic content versus DPPH IC_{50} (R^2 = 0.965) and CUPRAC $A_{0.5}$ (R^2 = 0.985) were not statistically significant, likely due to the small number of taxa (n = 3). The weak correlations noted for ABTS IC_{50} (R^2 = 0.375–0.469) align with the idea that ABTS activity in *M. globosum* is determined by compound-specific structural characteristics, particularly the electron-donating ability of kaempferol aglycone and the catechol moiety of verbascoside, rather than the overall phenolic content.

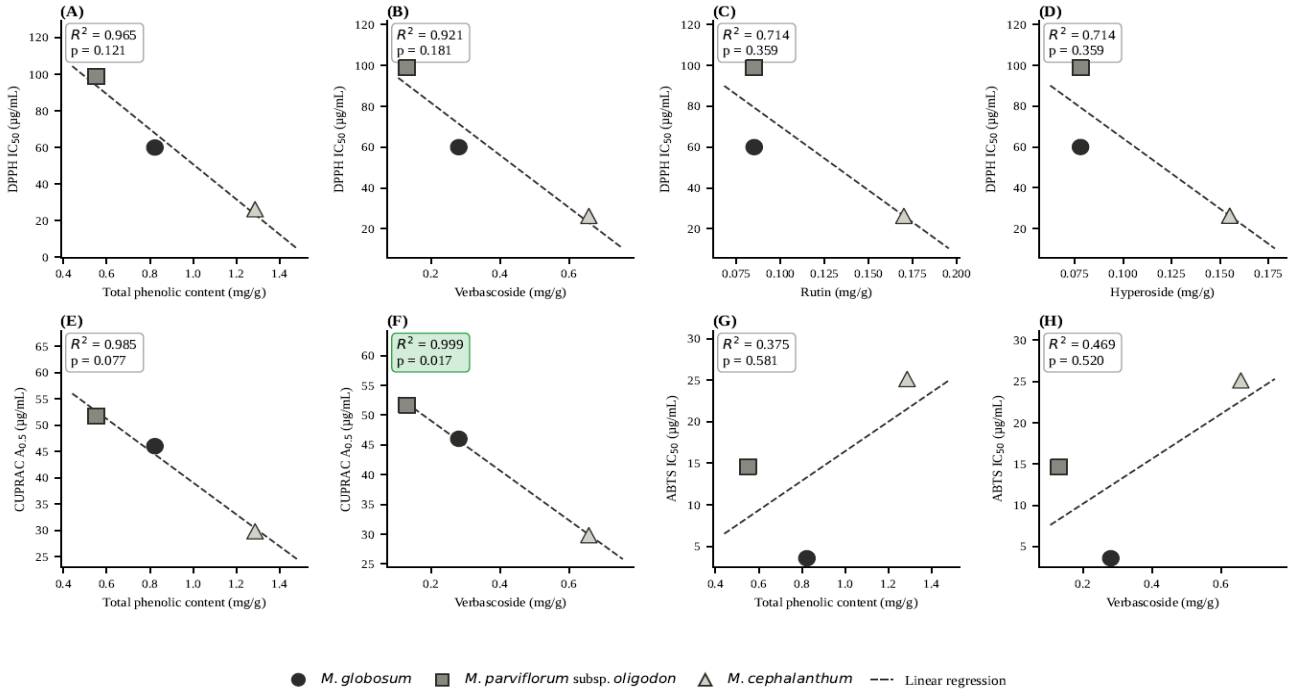

Figure 5. Structure-activity relationship between phenolic composition and antioxidant activities in three endemic *Marrubium* taxa.

R^2 and p values are indicated for each regression. The highlighted panel (F) denotes a statistically significant correlation ($p < 0.05$). Note that the limited number of data points (n = 3) restricts the statistical power of these analyses.

4. Conclusions

This study presents a comparative phytochemical and biological assessment of three endemic Turkish *Marrubium* taxa —*M. cephalanthum*, *M. globosum*, and *M. parviflorum* subsp. *oligodon* — through HPLC-based phenolic profiling combined with antioxidant and enzyme inhibitory assays. Twelve phenolic compounds were quantitatively identified across the taxa, with verbascoside consistently representing the dominant constituent. *M. cephalanthum*, which contained the highest phenolic content, showed the strongest DPPH and CUPRAC activities consistent with its elevated concentrations of verbascoside, rutin, and hyperoside. *M. globosum*, despite its lower overall phenolic content, outperformed the other taxa in both ABTS scavenging and cholinesterase inhibition — an outcome that appears to reflect the structural advantages of its kaempferol aglycone in accessing enzyme active sites. The strongest 15-LOX inhibitory activity was recorded for *M. parviflorum* subsp. *oligodon*, a finding tentatively linked to the catechol moiety of verbascoside and the iron-chelating properties of rutin and hyperoside. No meaningful inhibition of α-glucosidase or α-amylase was detected under the conditions tested.

Taken together, the results suggest that biological activity in these taxa is shaped more by the identity and proportions of specific phenolic compounds than by total content, with verbascoside, kaempferol, rutin, and hyperoside appearing to act through complementary mechanisms. These findings point to the potential pharmacological relevance of the investigated taxa in the context of oxidative stress and neurodegenerative conditions. To build on these results, future work should examine the cholinesterase inhibitory properties of *M. globosum* in in vivo Alzheimer's models, pursue bioactivity-guided isolation of LOX-active fractions from *M. parviflorum* subsp. *oligodon*, and validate the anti-inflammatory potential of *M. cephalanthum* in relevant in vivo systems. Investigating possible synergistic interactions among the key phenolic constituents would also be a valuable next step.

Ethics committee approval: Ethics committee approval is not required for this study.

Conflict of interest: The authors declare that there is no conflict of interest.

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