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Phytochemical Composition, Biological Activity and Molecular Docking Studies of the Endemic *Marrubium trachyticum* Boiss.

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Abstract: In this study, *Marrubium trachyticum* Boiss., which is an endemic species for Türkiye, belonging to the genus *Marrubium* selected from the family Lamiaceae, was examined. The phytochemical composition of hexane and methanol:chloroform (MeOH/$CHCl_3$) (1:1) extracts of *M. trachyticum* were analyzed quantitatively by GC-MS and LC-MS/MS. Antioxidant activities of the plant extracts and inhibitory activities for various enzymes were determined. In addition, molecular docking studies were performed to understand how the phytochemicals may have an effect on tyrosinase, α-amylase and α-glucosidase enzyme activities. Since there are no studies on the phytochemical composition and bioactivities of *M. trachyticum*, the data obtained from this study will be recorded for the first time. The chemical composition of the extract of *M. trachyticum* was determined by GC/MS and the major compound was the dotriacontane with a percentage of 20.23% followed by nonacosane (19.94%). Phenolic compound analysis of MeOH/$CHCl_3$ extract of *M. trachyticum* plant was carried out quantitatively by LC-MS/MS and rutin (1200 mg kg^{-1} extract), kaempferol-3-O-glucoside (100.2 mg kg^{-1}extract), hesperidin (75.97 mg kg^{-1} extract) were found to be the most abundant main compounds in this plant. While no inhibitory effect of *M. trachyticum* hexane extract was observed on all three enzymes, methanol-chloroform extract showed inhibitory effect on α-glycosidase and tyrosinase enzymes. The *in vitro* and *in silico* results are evaluated, it is seen that compounds such as rutin, hesperidin and isoquercitrin are more abundant in the extract and have the highest potential to inhibit both enzymes. The present study's results indicate that *M. trachyticum* is an excellent source of flavonoid components and other bioactive substances that may be responsible for the observed antioxidant and specific enzyme inhibitory effects.

Keywords: Antioxidant activity, chemical content, enzyme inhibition, molecular docking, *Marrubium trachyticum*

1. Introduction

The Lamiaceae family, which is very rich in medicinal and aromatic properties and the sixth-largest family of Angiosperms, is known as one of the largest and most widespread plant families in terms of distribution worldwide. It is expressed by approximately 250 genera and more than 7000 species and has a cosmopolitan distribution (Mesquita et al., 2019). It grows naturally in temperate regions such as Asia-Europe, Australia, South Africa and especially the Mediterranean region. The Lamiaceae family, which is mostly distributed in Türkiye, are herbaceous, aromatic and small shrub-type plants.

Members of the genus "Marrubium" in the Lamiaceae family are used for beneficial purposes in the world and in Türkiye due to their medicinal and ethnobotanical importance. In addition, Marrubium members are also of great importance because they contain species with economic value and different uses, they are also preferred in beekeeping due to their abundant nectar and abundant flowers, and the leaves of some members are also used as tea (Boulila et al., 2015). Most of the Marrubium species are annual or perennial herbaceous plants, and the use of some species dates back to 2000 years ago and is generally used as a cough suppressant, expectorant, sore throat reliever, in respiratory system diseases, and in the treatment

of indigestion complaints such as feeling of satiety and loss of appetite (Farzaneh et al., 2005).

Plants have been used for many years as folk remedies for the treatment of various diseases and therefore plants are considered indispensable. Due to their bioactive compounds, they are targeted for drug candidate discovery and are actively being investigated all over the world. Of 122 plant-based medicines, 80% were found to be derived from traditionally used plants (Fabricant and Farnsworth, 2001). Although there are about 40 species of the genus Marrubium in the world, only a few species have been chemically studied. According to the studies, the genus Marrubium biochemically contains terpenoids, flavonoids, phenylpropanoids, phytosterols, nitrogenous substances, resins, waxes and minerals. Most of the 23 species endemic to Türkiye have not been biochemically studied (Sarikurkcu et al., 2008). There are also few studies on the essential oil isolated from Marrubium species; *M. duabense*, *M. globosum*, *M. cuneatum*, *M. peregrinum*, *M. deserti*, *M. cylleneum* and *M. incanum*. The studies are mostly related to the chemical characterization and identification of essential oil constituents, while biological activity is mainly related to antimicrobial or antioxidant properties (Sarikurkcu et al., 2008; Laouer et al., 2009; Petrović et al., 2009; Kaurinovic et al., 2010; Chemsa et al., 2016; Golmakani et al., 2016). Although there are no biochemical studies on *M. trachyticum* species, pharmacological studies on the aerial parts of this plant species have identified marrubiin and ladanein from diterpenes and β-sitosterol from fatty acids (Citoglu and Aksit, 2002).

This study on *M. trachyticum*, which is endemic to our country, will elucidate the chemical composition of this plant species and provide information on its bioactivities. In this study, the phytochemical composition, antioxidant and antimicrobial activities and inhibitory activities for various enzymes of hexane and methanol:chloroform (1:1) extracts of *M. trachyticum*, which is endemic to our country, were determined. In addition, molecular docking studies were performed to understand how phytochemicals may have an effect on tyrosinase, amylase and α-glucosidase enzyme activities. There are no studies on the phytochemical composition and bioactivities of *M. trachyticum* Boiss. and the data obtained here are recorded for the first time.

2. Materials and Methods

2.1. Plant material and extraction

Marrubium trachyticum was collected from Yapraklı, Çankırı, Türkiye (BŞ8099, 700-900 m, gypsum steppe, 27.05.2022) and it has been deposited at the Herbarium of the Çankırı Karatekin University. The aerial parts of the plant were dried in a place away from sunlight and airflow. The aerial parts of the ground and dried plant were first extracted with hexane solvent using the mesaration method. Then it was extracted with methanol:chloroform ($MeOH/CHCl_3$, 1:1 v/v) solvent system. The extraction process was performed in 3 replicates and similar extracts were combined. The solvents of the extracts were removed in a rotary vacuum evaporator at 30 °C. Extract samples were stored at 4 °C until use.

2.2. Phytochemical composition analysis of extracts

After the extraction of the aerial parts of the plant with hexane solvent was completed, the fatty acid composition of the hexane extract was determined by gas chromatography (GC) analysis. The hexane extract was esterified to form fatty acid methyl esters (FAME) before analysis by GC/MS (Tüfekçi et al., 2018). For the esterification procedure, 50-100 mg of the extract was dissolved in hexane and 4 mL of potassium hydroxide (KOH) solution (prepared in 2 M methanol) was added. After vigorous shaking, the supernatant containing FAME was transferred to a flask and diluted with hexane for GC analyses. Fatty acid composition analysis was performed on an Agilent 7000 GC/MS Triple Quadrapol 7890 GC system. The analysis was performed in split mode at a ratio of 20:1 and the injection volume was set to 1 mL. Helium (as carrier gas) H_2 and dry air were used at a flow rate of 40 and 450 (mL min^{-1}), respectively. HP-5MS column with a length of 30 m x 0.25 mm x 0.25 µm and filled with (5% phenyl)-methylpolysiloxane was used. Injector and detector temperatures were set at 250 °C and 280 °C, respectively. The initial temperature was set to 50 °C and the column was kept at oven temperature for 2 minutes. The temperature was then increased by 3 °C per minute to 140 °C. Without waiting at this temperature, the temperature was increased by 4 °C per minute to 210 °C (held for 10 minutes) and finally the temperature was increased by 4 °C per minute to 270 °C (held for 30 minutes). A total of 104.5 minutes was programmed. Determination of FAME peaks was performed by comparing the retention times of each peak with authentic standards (Supelco 37 Component Fatty acid Mix, Sigma-Aldrich).

The 1.260 Infinity II liquid chromatography system (Agilent) coupled with a 6460 Triple Quad mass spectrometer was used for quantitative and qualitative analyses. Poroshell 120 EC-C18 (100 mm × 4.6 mm I.D., 2.7 µm) column was used for

chromatographic separation (Bursal et al., 2021). Water mobile phase A containing 0.1% formic acid and 5 mM ammonium formate and methanol mobile phase B containing 0.1% formic acid and 5 mM ammonium formate were used. The flow rate was 0.4 ml min^{-1} and a gradient program of 15% for 1-12 min, 50% for 12-30 min, 90% for 30-32 min and 10% for 32-35 min was applied in the mobile phase B, respectively. Column temperature was maintained at 40 °C and injection volume was 4 µL.

2.3. Antioxidant tests

The Folin-Ciocalteu technique was used to examine the total phenolics content (TPC) in a 96-well microplate (Sarikurkcu et al., 2018). In each well of a 96-well microplate, 12.5 mL of diluted Folin-Ciocalteu reagent (1:9) was combined with 25 mL extract and 187.5 mL ultrapure water. 25 mL of 20% (w v^{-1}) sodium carbonate were added to the mixture. With a microplate reader, the absorbance was measured at 760 nm. The findings were presented as milligrams of gallic acid (standard) equivalents (mg GAE g^{-1} extract). The aluminum chloride colorimetric technique was used to determine the total flavonoids content (TFC) (Sarikurkcu et al., 2018). The extract was placed in each well of a 96-well microplate together with 25 mL of methanol, 10 mL of 5% $NaNO_2$, and 100 mL of ultrapure water. 15% of aluminum chloride was added to the mixture (15 mL). After adding 50 mL of ultrapure water and 100 mL of 1 M NaOH after five minutes, the absorbance was measured at 510 nm. The findings were reported as milligrams of rutin equivalent per gram of extract (mg RE g^{-1} extract) after rutin was used to plot a standard curve.

With some adjustments, the 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging activity was investigated within the context of antioxidant activity research (Demirtas et al., 2017). A 190 µL DPPH solution (0.004%) was combined with 10 mL of the extract. The absorbance at 517 nm was measured following a 30-minute incubation period at room temperature. With a few changes, copper ion-reducing activity (CUPRAC) was carried out (Irawan et al., 2022). To create the CUPRAC reagent, 10 mM Copper (II) chloride ($CuCl_2$), 7.5 mM neocuproin, and 1 M ammonium acetate (NH_4Ac) (pH= 7) were combined at a ratio of 10:1:1:1 (v/v/v). Trolox (mg TE per g extract) and EDTA (mg EDTAE per g extract) were used as standards for radical scavenging, reducing activity and metal chelating activities, respectively. For 30 minutes at room temperature, the wells were filled with the extract (25 mL) and 175 mL of reagent. At 450 nm, absorbance was measured. The technique of measuring the total antioxidant activity of the samples involved some changes to the phosphomolybdenum method. The reagent solution for the procedure was made up of 1:1:1:1 (v/v/v) of 0.6 M H_2SO_4, 28 mM $Na_2HPO_4.12H_2O$, and 4 mM ammonium molybdate. After adding 12.5 mL of extract and 125 mL of reagent solution, the samples were combined and incubated for 90 minutes at 95 °C. At 695 nm, absorbance was observed. The technique of measuring the total antioxidant activity of the samples involved some changes to the phosphomolybdenum method. The ratio of the reagent solution to be employed in the procedure was 1:1:1:1 (v/v/v) and included 0.6 M H_2SO_4, 28 mM $Na_2HPO_4.12H_2O$, and 4 mM ammonium molybdate. After adding 12.5 mL of extract and 125 mL of reagent solution, the samples were combined and incubated for 90 minutes at 95 °C. 695 nm was used to quantify absorbance (Sarikurkcu et al., 2008).

2.4. Enzyme inhibition tests

L-3,4-dihydroxyphenylalanine (L-DOPA) was used as the substrate in the dopachrome technique to measure the tyrosinase inhibitory activity (Sarikurkcu et al., 2020). After combining 25 mL of extract with 40 mL of tyrosinase solution and 100 mL of sodium phosphate buffer (pH= 6.8), the mixture was incubated for 15 minutes at 25 °C. The mixture was mixed with 40 mL of L-DOPA and incubated for 10 minutes at 25 °C. At 492 nm, the absorbance was measured. We also looked at scutellarin's ability to decrease tyrosinase activity. Following a 1 mg mL^{-1} dimethyl sulfoxide (DMSO) dissolution, it was diluted 10 times with clean water. Through the use of the Caraway-Somogyi iodine/potassium iodide (IKI) technique, the α-amylase inhibitory activity was found. A 50 mL α-amylase solution prepared in a buffer (sodium phosphate, pH= 6.9) was combined with 25 mL of the extract. For ten minutes, the mixture was incubated at 37 °C. To initiate the reaction, 50 mL of starch solution was added to each well and then incubated at 37 °C for 10 min. Next, 25 mL of 1 M HCl was added to halt the reaction, and 100 mL of potassium iodide solution was added. At 630 nm, the absorbance was measured. Using paranitrophenyl-α-D-glucopyranoside (pNPG) as the substrate, the inhibitory activity of α-glucosidase was ascertained (Uysal et al., 2021). The extract (50 mL) was combined with 50 mL glutathione, 50 mL pNPG, and 50 mL of an α-glucosidase solution made in a buffer (pH= 6.8). After 15 minutes of incubation at 37 °C, the mixture was stopped by adding 50 mL of sodium carbonate. At 400 nm, the absorbance was measured. The IC50 value was ascertained and the enzyme's % inhibition was computed.

2.5. Molecular docking studies

Phytochemicals included in the extract that are crucial for enzyme interactions were identified using molecular docking experiments. The structure data file (SDF) containing the three-dimensional structures of chemicals were obtained from the PubChem database (Anonymous, 2023). The Protein Data Bank (PDB) (Anonymous, 2024) provided the crystal structures of the enzymes used in the docking investigations. The codes for the enzymes were α-glucosidase (PDB code: 3A4A) and tyrosinase (PDB code: 2Y9X). The Molegro Virtual Docker program was used to import these structures (Maiti and Bidinger, 1981). Protein structural flaws were examined; erroneous residues were optimized and rearranged both within and among themselves and with nearby residues with which they interacted. Using the BIOVIA Discovery Studio program, the molecular interactions between enzymes and phytochemicals that have a greater affinity for binding to proteins were examined.

3. Results and Discussion

3.1. Phytochemical composition of *M. trachyticum*

The content analysis of the hexane extract of the aerial part of *M. trachyticum* was performed by GC-MS. The GC-MS chromatogram is given in Figure 1 and the result of the analysis is given in Table 1. The chemical composition of the extract of *M. trachyticum* was determined by GC/MS and revealed the presence of 47 compounds with a total of 100% (Table 1). The major compound was dotriacontane with a percentage of 20.23% followed by nonacosane (19.94%), and other compounds such as palmitic acid methyl ester with the lowest percentage achieved 9.47%. The GC chromatogram obtained from the studies indicates that the primary derivative of saturated fatty acid methyl ester (with an area of more than 8%) is hexadecanoic acid methyl ester (palmitic acid methyl ester). On the other hand, the primary unsaturated fatty acid methyl esters were 9,12,15-octadecatrienoic acid methyl ester (Z,Z,Z,Z) (linolenic acid methyl ester) (36.99%) and 9,12-octadecadienoic acid (Z,Z) methyl ester (18.75%), both of which were also reported for their diverse activities (Swantara et al., 2019). Phytol is one of the most prevalent compounds (10.01% of the total area). The pharmacological effects of phytols include anxiolytic, cytotoxic, antioxidant, autophagy and apoptosis triggering, antinociceptive, anti-inflammatory, immunological modulating, and antibacterial properties (Islam et al., 2018). As a result of GC-MS analysis of methanol extracts of *M. vulgare*, it was determined that 5-Methoxy-2-nitrobenzoic acid had the highest abundance, followed by hexadecanoic acid and its methyl ester (Ullah and Alqahtani, 2022). The methanol extract of *M. anisodan* was characterized by GC/MS and fatty acids such as dodecanoic acid (1.036%) and pentadecanoic acid (1.55%), alkaloids such as alpha-pyrrolidone (2.21%) and cyclic isoprenoids such as cyclotetradecane (2.32%) were determined (Mohammadi et al., 2019).

Phenolic compound analysis of the MeOH/$CHCl_3$ extract from the aerial part of the *M. trachyticum* plant was carried out quantitatively by LC-MS/MS according to the multiple reaction monitoring (MRM) method with 48 standards (Table 2). The analysis showed the analysis of negative ions, as the analysis of polyphenols is better in negative ionization mode. In this study, the content analysis of any extract of this plant by LC-MS/MS is reported for the first time. According to the results, flavonoids such as isoquercitrin, rutin, hesperidin, kaempferol-3-*O* glucoside, fisetin, luteolin and diosgenin were determined from *M. trachyticum* extract. In addition, the presence of phenolic and organic acids such as trans-ferulic acid, salicylic acid, coumaric acid, vanillin, caffeine, caffeic acid, hydroxybenzaldehyde and chlorogenic acid were characterized. Rutin (1200 mg kg^{-1} extract), kaempferol-3-*O*-glucoside

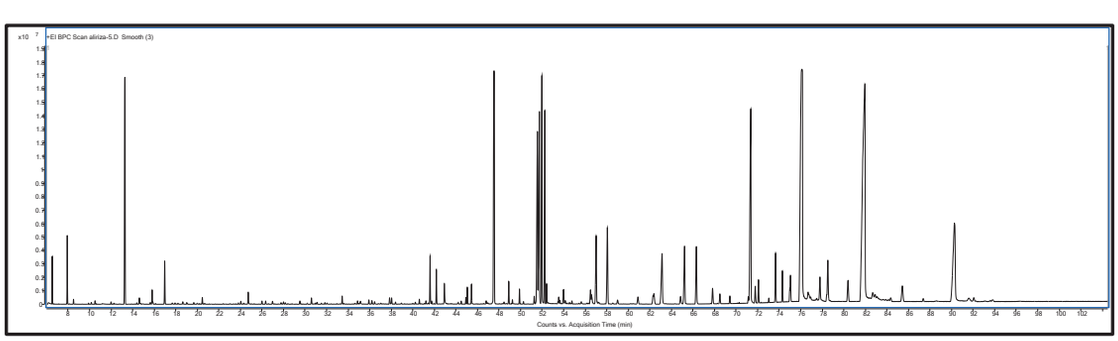

Figure 1. GC-MS chromatographic profile of the hexane extract of *M. trachyticum*

Table 1. Chemical compositions of *M. trachyticum* hexane extract

RT	Compounds	% Area
26.00	2.6.11-Trimethyldodecane	0.06
26.98	2-Undecanol	0.06
30.60	β-Bourbonene	0.13
33.45	Lauric acid. methyl ester	0.15
35.92	Undecanoic acid. 10-methyl-. methyl ester	0.08
36.21	Dihydroactinidiolide	0.07
37.85	Spathulenol	0.12
38.03	Caryophyllene oxide	0.11
40.23	Eudesma-4(15).7-dien-1β -ol	0.03
41.22	ent-Germacra-4(15).5.10(14)-trien-1β-ol	0.07
41.60	2-Hexadecanol	0.88
42.19	Myristic acid. methyl ester	0.63
44.93	Pentadecanoic acid. methyl ester	0.11
45.45	Hexahydrofarnesyl acetone	0.37
47.54	Palmitic acid methyl ester (PAME)	9.47
49.25	Eicosane	0.07
49.90	Heptadecanoic acid. methyl ester	0.27
51.27	1-Octadecanol	0.18
51.57	Linoleic acid. methyl ester	6.82
51.65	Linolenic acid. methyl ester	0.42
51.98	Phytol	6.04
52.25	Stearic acid. methyl ester	3.80
54.77	Nonadecanoic acid. methyl ester	0.05
57.02	Tricosane	2.06
58.06	Arachidic acid methyl ester	2.45
59.00	4.8.12.16-Tetramethylheptadecan-4-olide	0.13
62.30	Heneicosanoic acid. methyl ester	0.25
64.85	Behenic alcohol	0.28
65.22	Pentacosane	2.01
66.31	Behenic acid. methyl ester	1.82
68.50	Hexacosane	0.27
69.43	Tricosanoic acid. methyl ester	0.18
71.13	1-Heptacosene	0.16
71.37	Heptacosane	7.47
72.09	Lignoceric acid methyl ester	0.53
73.67	Octacosane	1.18
74.30	Squalene	0.67
76.12	Nonacosane	19.74
77.79	Isopropyl hexacosyl ether	0.75
78.52	Triacontane	1.34
80.39	Hentriacontane	0.80
81.96	Dotriacontane	20.23
85.43	Tetratriacontane	0.76
90.27	γ-Sitosterol	6.42
Total		99.49

RT: Retantion time (minute)

(100.2 mg kg^{-1} extract), and hesperidin (75.97 mg kg^{-1} extract) were found to be the most abundant main compounds in this plant (Table 2).

Several of these constituents have earlier been reported for *Marrubium* species. In the ethyl acetate extract of *M. vulgare* from Tunisia, the main chemicals found were quercetin-3-D-galactoside, apigenin, luteolin-7-*O*-*β*-*D*-glucoside, and rosmarinic acid, according to Boulila et al. (2015). Additionally, luteolin, apigenin, luteolin-O-glucoside, and apigenin-O-glucoside have all been found in Algerian *M. vulgare*, according to Amessis-Ouchemoukh et al. (2014). In Algerian *M. deserti*, Zaabat et al. (2011) found apigenin and several 7-*O*-substituted derivatives. According to Karioti et al. (2007), quercetin, apigenin, luteolin glucoside, coumaric acid, ferulic acid, and other bioactive components were present in the Greek extract of *M. velutinum*.

3.2. Antioxidant effects

The results of *M. trachyticum* extracts in terms of TPC and TFC are presented in Table 3. Methanol/chloroform extract was richer in phenolic

Table 2. LC-MS/MS analysis result of methanol/chloroform extract of the aerial part of *M. trachyticum* plant

RT	Compounds	Compounds / plant extract (mg kg^{-1})
1.375	Shikimic acid	nd
3.229	Gallic acid	nd
5.303	Protocatechuic acid	nd
6.721	Epigallocatechin	nd
6.802	Catechin	nd
7.295	Chlorogenic acid	6.20
7.548	Hydroxybenzaldeyde	7.52
7.649	Vanillic acid	nd
7.718	Caffeic acid	13.20
8.296	Syringic acid	nd
8.310	Caffein	0. 54
8.529	Vanillin	7.82
9.323	o-coumaric acid	12.11
9.644	Salicylic acid	46.73
9.686	Taxifolin	nd
9.803	Resveratrol	nd
9.850	Polydatine	nd
10.025	Trans-ferulic acid	51.45
10.447	Sinapic acid	nd
11.482	p-coumaric acid	nd
11.494	Coumarin	nd
11.003	Protocatehuic ethyl ester	nd
11.671	Hesperidin	75.97
11.703	Isoquercitrin	70.25
11.671	Rutin	1200
12.324	Quarcetin-3-*O*-ksilozid	nd
13.038	Kaempferol-3-*O*-glucoside	100.2
13.040	Fisetin	2.77
13.485	Baicalin	nd
14.145	Chrysin	nd
14.272	Daidzein	nd
14.121	Trans-cinnamic acid	nd
14.804	Quercetin	nd
14.974	Naringenin	nd
15.865	Hesperetin	nd
15.828	Morin	nd
16.363	Kaempferol	nd
17.093	Baicalein	nd
17.496	Luteolin	12.68
17.876	Biochanin A	nd
18.203	Capcaicin	nd
18.716	Dihydrocapcaicin	nd
23.432	Diosgenin	2.65

RT: Retantion time, nd: Not detected

and flavonoid contents than hexane extract (30.23 mg GAE g^{-1} and 25.73 mg RE g^{-1}, respectively). Since hexane is more effective in the uptake of non-polar compounds, lower phenolic and flavonoid content is an expected result (7.78 mg GAE g^{-1} and 3.93 mg RE g^{-1}, respectively). The radical scavenging effects of the extracts were investigated by the DPPH method and their antioxidant properties were determined by CUPRAC method and metal chelating methods. Accordingly, MeOH/$CHCl_3$ (1:1) extract was found to be more effective than hexane extract in DPPH, CUPRAC and metal chelating antioxidant tests (22.76 mg TE g^{-1}, 398.48 mg TE g^{-1}, 8.91 mg EDTAE g^{-1}, respectively). In general, it is known that the antioxidant effects of plants increase in a correlated manner with phenolic and flavonoid compounds. These compounds have been shown to have significant antioxidant ability in the past (Tsvetkov et al., 2016). There is growing evidence in the literature that polyphenols can reduce oxidative damage in a number of ways. It is commonly recognized that increased antioxidant activity in plant extracts is correlated with their phenol content (Zhang and Tsao, 2016).

Table 3. Total phenolic content, TFC and antioxidant effects of *M. trachyticum* extracts

Test	MeOH/$CHCl_3$	Hexane
TPC (mg GAE g^{-1})	30.23	7.78
TFC (mg RE g^{-1})	25.73	3.93
CUPRAC (mg TE g^{-1})	398.48	45.54
DPPH (mg TE g^{-1})	22.76	11.81
MC (mg EDTAE g^{-1})	8.91	6.57

MC: Metal chelation

According to previous studies; 87.12 mg GAE mg^{-1} of extract was the total phenolic compound level in *M. vulgare*, according to Akther et al. (2013). According to Elberry et al. (2015), *M. vulgare* contains 15.53 mg quercetin (QE) g^{-1} dry weight of flavonoids. According to Sarikurkcu et al. (2020), *M. parviflorum*'s overall level of phenolic compounds ranged from 22 to 38 mg GAE g^{-1} extract, while the amount of flavonoids varied from 2 to 19.5 mg QE g^{-1} extract. Numerous researchers have connected these variations to the solvent's composition, sample properties, and analytical methods (Ballesteros-Vivas et al., 2019; Venkatachalam et al., 2020).

3.3. Enzyme activity results and molecular docking studies

The effects of *M. trachyticum* extracts on enzyme activities are shown in Table 4 and Figure 2. While no inhibitory effect of hexane extract was observed on all three enzymes, methanol-chloroform extract showed an inhibitory effect on α-glycosidase and tyrosinase enzymes. The extract was shown to has a significant α-glucosidase inhibitory action and may have applications in the treatment of diabetes. Inhibition of the tyrosinase enzyme reduces or prevents melanin production. Consequently, plant extracts exhibiting tyrosinase inhibition are commonly utilized in skincare and cosmetic products for the treatment and prevention of conditions such as hyperpigmentation, sunspots, and various skin tone irregularities. Such products typically aid in diminishing spots, correcting skin

Table 4. Enzyme activity results of *M. trachyticum* extracts

Extracts	α- amylase	α-glycosidase	Tyrosinase
MeOH/$CHCl_3$	--	408 µg mL^{-1}	856 µg mL^{-1}
Hexane	--	--	--

tone, and generally evening out complexion (Tüfekçi et al., 2023). As the usage of goods made from various plant parts has risen, one of the most effective methods for treating health issues is now determining the enzyme-inhibitory aptitude of plant extracts. Pharmaceuticals are used to treat a variety of illnesses, including skin conditions, by inhibiting tyrosinase (Kim and Uyama, 2005). Numerous researchers have proposed that biomolecules derived from plant extracts have inhibitory effects on tyrosinase and α-amylase (Zhang et al., 2017; Yuan et al., 2018; Tlili et al., 2019; Özyazıcı et al., 2023). To the best of our knowledge, Karioti et al. (2007) and Sarikurkcu et al. (2020) have reported on the tyrosinase inhibitor activity for *Marrubium* species (*M. velutinum*, *M. cylleneum*, *M. lutescens*). There are numerous promising studies on the phytochemical properties and biological activities of various *Marrubium* species (Karioti et al., 2007; Argyropoulou et al., 2012; Hamedeyazdan et al., 2014; Dehbashi et al., 2015; Namjoyan et al., 2015; Saad et al., 2016). The hypoglycemic effect of *M. vulgare* extract was clinically documented in patients with type II non-controlled diabetes mellitus (Herrera-Arellano et al., 2004). It was reported, that *M. radiatum* extract exerted the strongest activity against α-amylase and α-glucosidase (Loizzo et al., 2008).

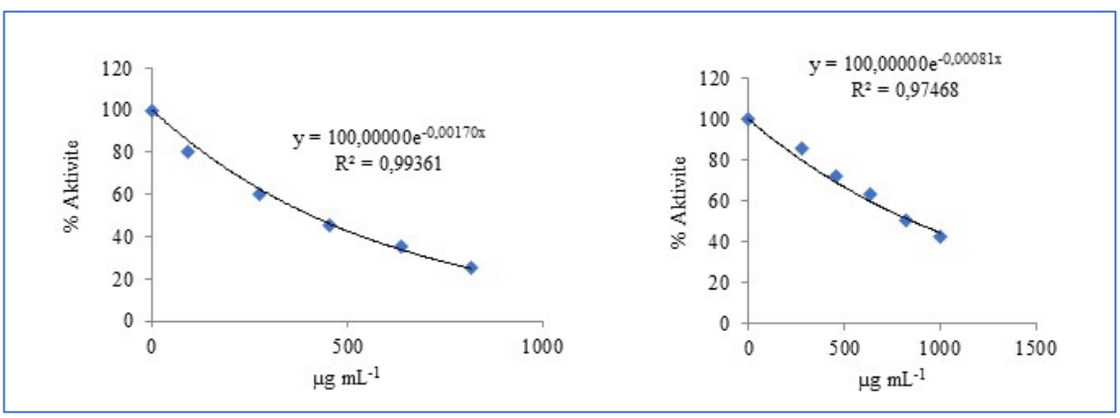

Figure 2. The inhibition effect of extracts on α-glycosidase and tyrosinase enzyme activity

When the *in vitro* and *in silico* results are evaluated, it is seen that compounds such as rutin, hesperidin and isoquercitrin are more abundant in the extract and have the highest potential to inhibit both enzymes (Figure 3 and 4). The results revealed that hesperidin, rutin and isoquercitrin have the most binding affinity against two enzymes. When *in vitro* enzyme inhibition and docking results are evaluated together, compounds such as hesperidin, rutin and isoquercitrin are found in excess in the extract and have the highest potential to inhibit the α-glycosidase and tyrosinase enzymes. Their interaction types and attachment positions are shown in the figures. For the enzyme tyrosinase; isoquercitrin, hydrogen bonding interaction with amino acids Arg268, Met280, Gly281, Ser282 and Val283, π-π interaction with amino acids His85, His244 and Ser282, and π-alkyl interaction with amino acids Val248, Val283 and Ala286 in the active site, hesperidin, hydrogen bonding interaction with His85, Asn260, Gly281 and Val283 in the active site. It has been observed that rutin has π-π interaction with His244 and π-alkyl interaction with Val248, Val283, hydrogen bond interaction with His85, Asn260, Met280 and Ser282 amino acids in the active site of the protein, π-π interaction with His244 amino acid and π-alkyl interaction with Val248, Val283 amino acids. For the α-glucosidase enzyme, hesperidin in the active site of α-glucosidase is docked to the receptor by five hydrogen bonds, one π- π interaction and six π-alkyl interactions. Hydrogen bonds are formed with the amino acids Gln279, Ser241, Glu277, Asp215 and Asp69. π- π interaction is formed with Tyr158 and π-alkyl interaction is formed with His351, Tyr72, Arg315, Lys156, Leu177 and Tyr158 amino acids. Rutin forms hydrogen bonds with Glu411, His280, Asp307, Gln353, Gln279, Asp352, Glu277, Asp215 and Asp69, π- π interactions with Tyr158 and Phe178, and π- alkyl interactions with Val216 and Arg315 in the active site of the protein. Isoquercitrin has hydrogen bonding interaction with Gln279, His280, Asp307, Gln353, Asp352, Glu277, Asp69 and Glu411 in the active site; π-π interaction with His85, Tyr72; π-alkyl interaction with Val216 and Arg315; π-sigma interaction with Phe303; π-anion interaction with Glu411 and Asp352.

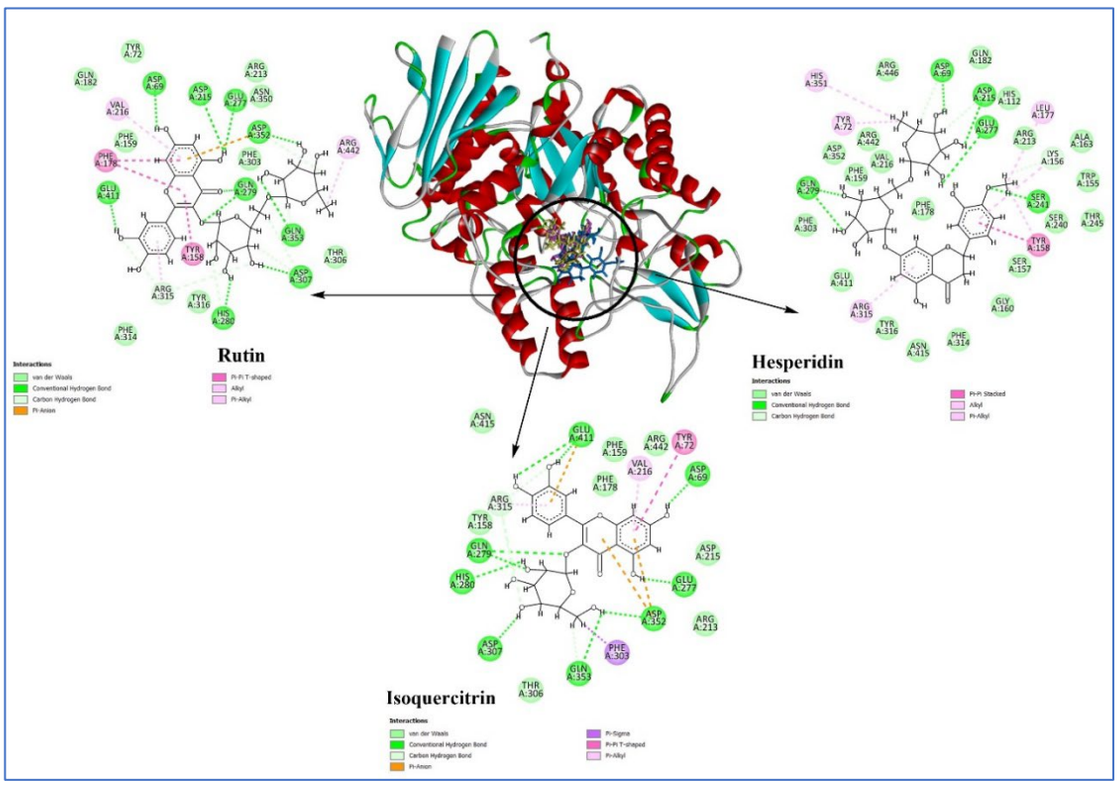

Figure 3. Representation of docked ligand-protein complex and interaction of hesperidin, rutin and isoquercitrin with amino acid residues of α-glycosidase

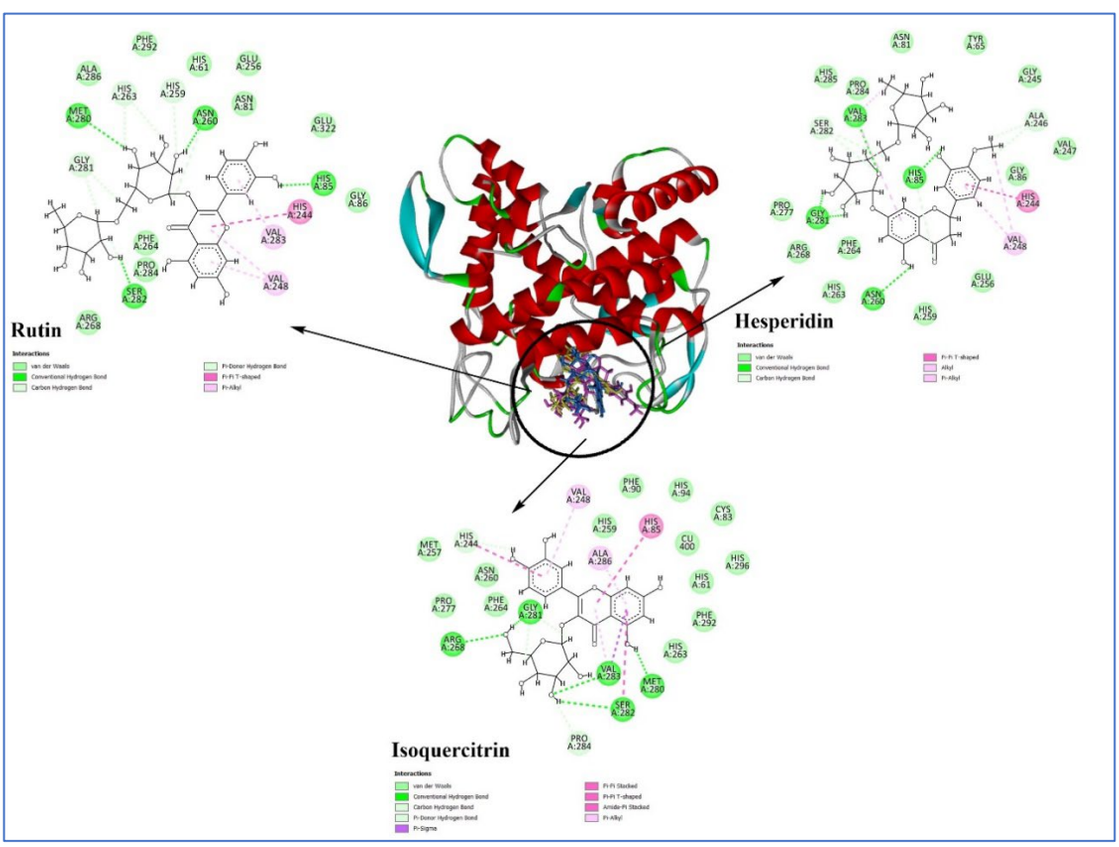

Figure 4. Representation of docked ligand-protein complex and interaction of hesperidin, rutin and isoquercitrin with amino acid residues of tyrosinase

4. Conclusions

In this study, the phytochemical composition, antioxidant and antimicrobial activities and inhibitory activities for various enzymes of hexane and methanol:chloroform (1:1) extracts of *M. trachyticum*, which is endemic to Türkiye, were investigated. *M. trachyticum* is a good source of flavonoid components and other bioactive compounds that may be in charge of the observed antioxidant and certain enzyme inhibitory actions, according to the results acquired from the current study. Important phytochemicals that may have a major role in tyrosinase and α-glucosidase inhibitory actions have been found using molecular docking investigations. Based on the outcomes of molecular docking and enzyme inhibition investigations, the extract containing significant amounts of rutin, hesperidin, and isoquercitrin has demonstrated its efficacy as a tyrosinase inhibitor. This characteristic allows the extract to be utilized in the cosmetics sector as an alternative skin-whitening agent. The extract was shown to have a significant a-glucosidase inhibitory activity and may have applications in the treatment of diabetes. These intriguing findings motivate more research aimed at separating powerful phytochemicals with varying polarity from *M. trachyticum*.

Ethical Statement

The authors declare that ethical approval is not required for this research.

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Declaration of Author Contributions

Conceptualization, Material, Methodology, Investigation, Data Curation, Formal Analysis, Visualization, Supervision, Project Administration, Writing-Original Draft Preparation, Writing-Review & Editing, *E. DERELLİ TÜFEKÇİ*; Material, Methodology, Investigation, Formal Analysis, Funding Acquisition, *A. GÜRGİÇ*; Formal Analysis, Writing-Original Draft Preparation, *E. EKİNCİ*; Material, Methodology, Investigation, Data Curation, Formal Analysis, Writing-Review & Editing, *A.R. TÜFEKÇİ*. All authors declare that they have seen/read and approved the final version of the article ready for publication.

Declaration of Conflicts of Interest

All authors declare that there is no conflict of interest related to this article.

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