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Research Article/Araştırma Makalesi

Investigating the Pharmacological Potential of *Micromeria myrtifolia* Boiss. & Hohen.: Phenolic Profiling and Biological Activity Assessments

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Abstract: *Micromeria myrtifolia* Boiss. & Hohen. is a valuable medicinal plant in Türkiye, recognized for its extensive applications across the country. In this study, plant samples were collected from Muğla, Türkiye and extracts from both the aerial parts and roots were prepared using ethanol. To elucidate their phytochemical composition, a comprehensive LC-MS/MS analysis was conducted. The results revealed that both extracts were quite rich in phenolic compounds. Notably, nicotiflorin was the major constituent in both extracts (12878.31±355.44 µg/g extract in the aerial parts and 47512.4±1311.34 µg/g extract in the roots) along with significant phenolic acids such as rosmarinic acid and caffeic acid as well as flavonoids like hesperidin. Moreover, both extracts demonstrated substantial antioxidant activities compared to the synthetic antioxidant compounds as evaluated by DPPH free radical scavenging, ABTS cation radical scavenging, and CUPRAC activity assays. Both aerial parts and root extracts also exhibited meaningful anti-glucosidase activity with 73.03±0.16% and 47.06±0.41%, respectively, at 2 mg/mL concentration. The root extract also showed moderate butyrylcholinesterase inhibitory activity. This study contributes to the existing body of knowledge with valuable insights regarding the phytochemical profile and biological activities of *M. myrtifolia*, paving the way for future research aimed at exploring its medicinal properties and potential uses in traditional and modern medicine.

Keywords: *Micromeria* Benth., LC-MS/MS, antioxidant, enzyme inhibition.

Micromeria myrtifolia Boiss. & Hohen. Bitkisinin Farmakolojik Potansiyelinin Araştırılması: Fenolik İçeriğinin ve Biyolojik Aktivitelerinin Belirlenmesi

Öz: *Micromeria myrtifolia* Boiss. & Hohen., Türkiye'nin önemli tıbbi bitkilerindendir ve ülke genelinde farklı tıbbi amaçlarla oldukça yaygın olarak kullanılmaktadır. Bu çalışmada, *M. myrtifolia* örnekleri Muğla, Türkiye'den toplanmış hem topraküstü kısımlarından hem de köklerden ayrı ayrı etanol ekstreleri hazırlanmıştır. Ekstrelerin fitokimyasal içeriklerini belirlemek için detaylı bir LC-MS/MS analizi yapılmıştır. Analiz sonuçlarına göre her iki ekstrenin de fenolik bileşikler açısından oldukça zengin olduğu belirlenmiştir. Her iki ekstrede de ana bileşen olarak nikotiflorin öne çıkmış ve topraküstü kısımlarından hazırlanan ekstrede 12878.31±355.44 µg/g, kök ekstresinde ise 47512.4±1311.34 µg/g nikotiflorin tespit edilmiştir. Ayrıca ekstrelerde rozmarinik asit ve kafeik asit gibi önemli fenolik asitler ile hesperidin gibi flavonoitlerin varlığı da belirlenmiştir. Ekstrelerin antioksidan aktiviteleri DPPH serbest radikal giderici, ABTS katyon radikali giderici ve CUPRAC aktivitesi metotları ile değerlendirilmiş, buna göre her iki ekstre de sentetik antioksidan bileşiklere kıyasla anlamlı antioksidan aktiviteler sergilemiştir. Bunun yanı sıra ekstreler enzim inhibisyon aktiviteleri açısından değerlendirildiğinde, ekstrelerin 2 mg/mL konsantrasyonda %73.03±0.16 (topraküstü) ve %47.06±0.41 (kök) oranında anti-glukozidaz aktivite gösterdiği tespit edilmiştir. Kök ekstresi ayrıca orta düzeyde butirilkolinesteraz inhibe edici aktivite de göstermiştir. Bu çalışma, *M. myrtifolia* bitkisinin kimyasal içeriği ve biyolojik aktiviteleri konusunda mevcut literatüre önemli katkılarda bulunmakla birlikte, bitkinin geleneksel ve modern tıp uygulamalarındaki yeni ve potansiyel kullanım alanlarını keşfetmeye yönelik gelecek araştırmalara da zemin hazırlamaktadır.

Anahtar kelimeler: *Micromeria* Benth., LC-MS/MS, antioksidan, enzim inhibisyonu.

1. Introduction

The Anatolian peninsula hosts the richest flora among the Middle Eastern countries. Current estimates place the number of taxa in Anatolia and Thrace (the European part of Türkiye) at around 11.750—about 2.000 more than those recorded by Davis in the Flora of Turkey (Yesilada, 2005). Alongside its diverse flora, the significant cultural diversity of Anatolia, which is shaped by its great historical events, forms the foundation of a vibrant tradition of medicinal practices in the region (Yeşilada, 2002).

Micromeria Benth. genus (Lamiaceae) is widely

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distributed, ranging from South Africa to Western Europe and Asia, and includes a large variety of perennial plants, with 70–90 species of dwarf shrubs and subshrubs. *Micromeria* species frequently grow wild in mountainous, open habitats or rocky areas around the world. In Europe, about twenty-two *Micromeria* species are present, with a significant concentration in the Balkan Peninsula (Mohammadhosseini et al., 2022). In Türkiye, *the Micromeria* genus is represented by nine species and fourteen taxa in Türkiye, nine of which are endemic (Duman & Dirmenci, 2017). Various *Micromeria* species have been reported to be utilized in the traditional medicinal practices of Anatolia for different purposes. For instance, *M. cristata* subsp. *orientalis* decoctions are used to treat ailments such as bronchitis, common colds, diabetes, headache, stomachache, kidney diseases, and prostrate disorders in the Eastern Blacksea Region. Infusions prepared with the aerial parts of *M. juliana* are also used to treat stomachache (Selvi et al., 2022). *M. fruticosa* aerial parts infusions are commonly applied as carminative and against nausea (Salim and Necattin, 2018). *M. graeca* subsp. *graeca* is used in veterinary medicine for skin problems, particularly wounds (Güzel et al., 2015).

Among the *Micromeria* species esteemed for their medicinal properties in Türkiye, *M. myrtifolia* Boiss. Et Hohen is considered as one of the most significant due to its diverse uses in traditional medicine. It possesses numerous local names, including "*kertiş kuyruğu, boğumlu çay, dağ çayı, kırkboğum, yeşil çay*, *Nurettin çayı*, *güvercin otu*, and *topuklu çay*" highlighting its status as a highly valued and widely utilized medicinal plant in Türkiye. Aerial parts of the plant have been used as an infusion for appetitive and carminative purposes. Leaf powder of the plant is applied to treat gallstones and gastrointestinal disorders. Besides, infusions are commonly consumed as medicinal tea for relaxation and pleasure. Infusions are also thought to be beneficial against cold, flu, and sore throat. Flowers and leaves of the plant have been recommended for shortness of breath (Polat & Satil, 2012; Güzel et al., 2015; Sargin, 2015; Kocabaş & Gedik, 2016; Salim & Necattin, 2018; Sargin & Büyükcengiz, 2019; Selvi et al., 2022; Baykan et al., 2023).

Belonging to the Lamiaceae family, *Micromeria* species are renowned for being aromatic plants. Therefore, they are noted for being a rich source of essential oils and the research has been mainly focused on their essential oil and their components. Monoterpenes, sesquiterpenes, and their oxygenated derivatives have been determined as major constituents. The concentrations of these compounds differ based on the subspecies. Along with the volatile secondary metabolites, *Micromeria* species are also characterized by containing high amounts of phenolic compounds. Chlorogenic acid and rosmarinic acid are among the most commonly found phenolic acids. Hesperetin, naringin, and quercetin are the mainly determined flavonoids in different *Micromeria* species (Hamwi & El-Lakany, 2021). Diverse phytochemicals found in *Micromeria* species are responsible for their important biological activities. To this point, different extracts prepared with *Micromeria* species have been shown to exhibit antioxidant, antimicrobial, anti-inflammatory, cytotoxic, analgesic, insecticidal, and antidepressant activities (Küpeli Akkol et al., 2019, Yilmaz et al., 2024). There have also been studies that concentrate specifically on *M. myrtifolia* in this regard. Accordingly, *M. myrtifolia* possesses antioxidant, antidepressant, enzyme inhibitory, antifungal, cytogenetic, and cytotoxic properties (Özcan, 1999; Formisano et al., 2014; Küpeli Akkol et al., 2019; Sarikurkcu et al., 2020a).

The current study begins with the preparation of ethanol extracts from both the aerial parts and roots of *M. myrtifolia* collected from Köyceğiz-Muğla/Türkiye. We aim to provide a comprehensive analysis of the chemical constituents through liquid chromatography-mass spectrometry, while also investigating the total phenolic and flavonoid content, as well as the diverse biological activities of the studied extracts, including antioxidant, anticholinesterase, antityrosinase, antiurease, and anti-glucosidase properties. By elucidating the multifaceted benefits of these extracts, we hope to highlight the potential of *M. myrtifolia* as a valuable source of natural bioactive compounds, thereby contributing to the growing body of knowledge surrounding its therapeutic applications and traditional medicinal uses in Türkiye.

2. Material and Method

2.1. Plant Material

Micromeria myrtifolia Boiss. & Hohen. aerial parts and roots were collected during the flowering phase by Assoc. Prof. Yeter Yeşil (Istanbul University, Faculty of Pharmacy, Pharmaceutical Botany Department) and Prof. Dr. Mehmet Boğa (Dicle University, Faculty of Pharmacy, Analytical Chemistry Department) in June 2014, approximately 100 meters past the Namnam Bridge, on the left side of the Köyceğiz-Muğla road. The plant was identified by Assoc. Prof. Yeter Yeşil and samples have been preserved in the Herbarium of Istanbul University Faculty of Pharmacy (ISTE No: 116045).

2.2. Preparation of the Extracts

The aerial and root parts of *M. myrtifolia* were shade-dried and subsequently pulverized. Dry weights were determined to be 22.20 g for the aerial parts and 32.30 g for the root parts. The powdered samples were then subjected to maceration with ethanol (96%) at room temperature for three consecutive 24-hour periods. Solvent removal was performed using a rotary evaporator (Heidolph, Germany), yielding the extracts in dry form. The extraction yield (%) was calculated according to the following equation:

Yield (%) (w/w) = (Amount of extract / Amount of dry plant material) x 100

2.3. Chemicals and Instruments

The chemical composition of the extracts was analyzed via LC-MS/MS (Shimadzu, Kyoto, Japan). *In vitro* biological activity assays were performed using a Shimadzu UV spectrophotometer and a BioTek PowerWave XS microplate reader (USA). Compounds for LC-MS/MS analysis and biological assays were obtained from Merck (Germany), Sigma (Germany), and Fluka (Germany). All solvents were of analytical grade.

2.4. LC-MS/MS Analysis

The LC-MS/MS analysis of the extracts was conducted by a previously validated protocol (Yilmaz et al., 2018). Quantification of 37 phenolic constituents was achieved

via a Nexera UHPLC system (Shimadzu) coupled with a tandem mass spectrometer. Data acquisition and processing were performed using Shimadzu Lab Solutions software, leveraging the capabilities of the LC-ESI-MS/MS platform for detailed compound profiling.

2.5. Total Phenolic and Flavonoid Contents of the Extracts

The total phenolic content of the extracts was determined as micrograms of pyrocatechol equivalents (PEs) using the method developed by Slinkard and Singleton (1977). Details: Volumes of 0, 1, 2, 3, 4, 5, 6, 7, and 8 µL of 100 ppm pyrocatechol were adjusted with water to achieve a final volume of 184 µL. Following this, 4 µL of Folin-Ciocalteu reagent and 12 µL of 2% Na_2CO_3 were added to each mixture. After a 2-hour incubation period, absorbance was measured at 760 nm. Total flavonoid contents were expressed as micrograms of quercetin equivalents (QEs), calculated by the method described by Moreno et al. (2000). Details: Volumes of 0, 1, 2, 3, 4, 5, 6, 7, and 8 µL of 1000 ppm quercetin were combined with 80% ethanol to bring the total volume to 192 µL. Following this, 4 µL of 1 M potassium acetate was added, and after 1 minute, 4 µL of 10% aluminium nitrate was incorporated. After a 40-minute incubation period, absorbance readings were taken at 415 nm.

To calculate the total phenolic and flavonoid contents of the extract, the equations below were used:

Absorbance = 0.0409 pyrocatechol (µg) + 0.0495 (R^2 = 0.9975)

Absorbance = 0.0347 quercetin (µg) + 0.1174 (R^2 = 0.9951)

The calibration curves for pyrocatechol and quercetin to calculate the total phenolic and flavonoid contents are given in Fig. 1 and Fig. 2.

2.6. Antioxidant Activity Assays

The antioxidant activity of the ethanol extracts from the aerial and root parts of *M. myrtifolia* was assessed using the DPPH free radical scavenging, ABTS cation radical scavenging, and CUPRAC activity methods. Absorbance measurements were performed with a UV-Vis spectrophotometer (PG Instruments T80+ UV/VIS Spectrometer, UK) and an ELISA reader (BioTek EON Microplate Reader, USA).

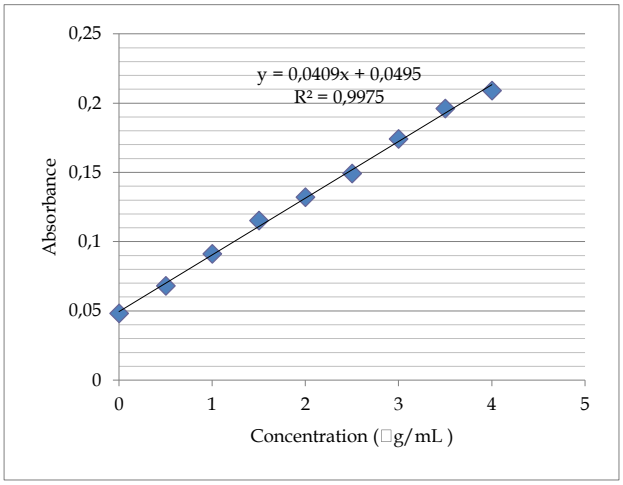

Figure 1. Pyrocatechol Calibration Curve for Total Phenolic Content

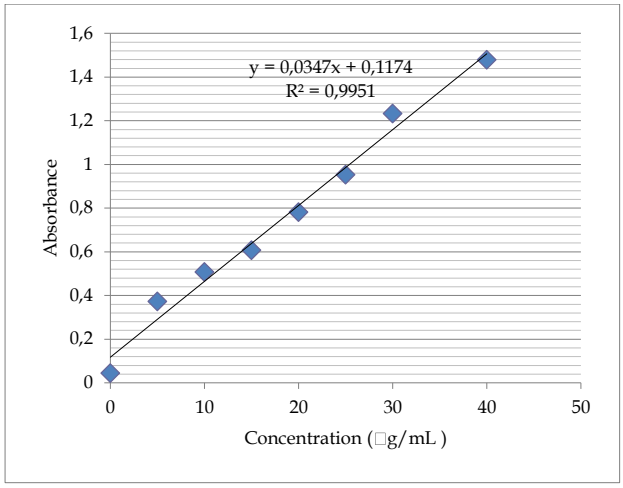

Figure 2. Quercetin Calibration Curve for Total Flavonoid Content

2.6.1. DPPH Free Radical Scavenging Activity

The free radical scavenging activity of the aerial and root parts of *M. myrtifolia* against the 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical was evaluated using the method designed by Blois (1958) with modifications (Eroğlu Özkan et al., 2022). Stock solutions of the extracts were prepared at a concentration of 1000 µg/mL. Butylated hydroxytoluene (BHT), butylated hydroxyanisole (BHA), and α-tocopherol served as standards. Details: Volumes of 2, 5, 10, and 20 µL of the stock solution were prepared. The volumes were adjusted to 40 µL using ethanol, then 160 µL of 0.1 mM DPPH was added. After a 30-minute incubation at room temperature in the dark, the absorbance was measured at 517 nm.

The following equation was used to calculate the DPPH free radical scavenging potential:

DPPH scavenging effect (Inhibition %) = $(A_{control} - A_{sample})/A_{control}$ x 100

$A_{control}$ = Absorbance of the control

A_{sample} = Absorbance of the samples

2.6.2. ABTS Cation Radical Scavenging Activity

The cation radical scavenging activity of the aerial and root parts of *M. myrtifolia* was assessed using 2,2'-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) by applying the method designed by Re et al. (1999) with modifications (Ersoy et al., 2022). Stock solutions of the extracts were prepared at a concentration of 1000 µg/mL. BHT, BHA, and α-Toc were used as standards. Details: Volumes of 2, 5, 10, and 20 µL of the stock solution were prepared in triplicate. The total volume was adjusted to 40 µL with ethanol, then 160 µL of 7 mM ABTS was added. After a 6-minute incubation in the dark, the change in absorbance was measured at 734 nm.

The following equation was used to calculate the ABTS cation radical scavenging potential:

ABTS cation radical scavenging effect (Inhibition %) = $(A_{control} - A_{sample})/A_{control}$ x 100

$A_{control}$ = Absorbance of the control

A_{sample} = Absorbance of the samples

2.6.3. CUPRAC Activity

The CUPRAC activity of the aerial and root parts of *M. myrtifolia* was assessed using the method developed by Apak et al. (2004). BHT, BHA, and α-Toc were used as standards. Details: Volumes of 2.5 µL, 6.25 µL, 12.5 µL, and 25 µL of the stock solution were prepared. The total volumes were adjusted to 67 µL with distilled water, followed by the addition of 61 µL of 0.01 M $Cu(II)Cl_2$, 61 µL of 0.1 M NH_4CH_3COO, and 61 µL of 0.1 M neocuproine. After a 1-hour incubation, the change in absorbance was measured at 450 nm.

2.7. Enzyme Inhibitory Activity Assays

The following equation was used to calculate the enzyme inhibition % in all tests:

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

$A_{control}$ = Absorbance of the control

A_{sample} = Absorbance of the samples

2.7.1. Acetylcholinesterase and Butyrylcholinesterase Inhibitory Activities

For the assessment of acetylcholinesterase and butyrylcholinesterase inhibitory activities of the aerial and root parts of *M. myrtifolia*, a rapid colorimetric method known as Ellman (Ellman et al., 1961) with modifications (Ersoy et al., 2020) was employed. This method is based on the measurement of absorbance of the yellow-colored 5-thio-2-nitrobenzoate anion formed by the reaction of thiocholine released from acetylcholine hydrolysis with 5,5'-dithiobis-(2-nitrobenzoic acid) (DTNB). After preparing stock solutions of the extracts at 4000 µg/mL, experiments were conducted three times. Galantamine was used as the standard. Details: The assay procedure began by adding 150 µL of 100 mM sodium phosphate buffer (pH 8) to a reaction vessel, followed by the addition of 10 µL of the sample (4000 µg/mL) or control. Next, 20 µL of the enzymes were added and the mixture was incubated at 25°C for 15 minutes. Following this initial incubation, 10 µL of 5 mM DTNB was introduced, along with 10 µL of 7.1 mM acetylthiocholine iodide (0.79 mM butyrylthiocholine iodide). The mixture was then incubated at 25°C for another 10 minutes before the absorbance was measured at 412 nm.

2.7.2. Tyrosinase Inhibitory Activity

To evaluate tyrosinase inhibition activity, tyrosinase enzyme was utilized as the enzyme and L-DOPA acted as the substrate. In the microplate wells, 10 µL of the sample, 150 µL of phosphate buffer (pH 6.8) and 25 µL of the enzyme solution prepared in the buffer were combined. This mixture was stirred for 3 minutes and then incubated at 37°C for 10 minutes. Following this incubation, 20 µL of L-DOPA was added as the substrate. After an additional 10 minutes at 37°C, the change in absorbance was measured at 475 nm. Kojic acid was used as the standard. This method was first reported by Masamoto and Kubo (1980).

2.7.3. Urease Inhibitory Activity

To measure urease inhibition activity, urease enzyme was used along with urea as the substrate. In the microplate wells, 10 µL of the sample, 25 µL of the enzyme mixed in phosphate buffer (pH = 8) and 50 µL of urea were added together. The mixture was then incubated at 30°C for 15 minutes. Afterward, 45 µL of phenol reagent and 70 µL of alkaline reagent were added. After another 20 minutes, the microplate was placed in an ELISA reader and the absorbance was measured at 630 nm. The details of this procedure were given by Weatherburn (1967).

2.7.4. α-Glucosidase Inhibitory Activity

The α-glucosidase inhibition activity was assessed following the method described by Schmidt et al. (2012). Details: In each well, 90 µL of 0.1 M phosphate buffer (pH 7.5; 0.02% NaN_3), 10 µL of the sample and 80 µL of the enzyme solution (with a final concentration of 0.05 U/mL in the well) were added. The mixture was incubated at 28°C for 10 minutes. Subsequently, 20 µL of PNPG (with a final concentration of 1.0 mM in the well) was added, and the mixture was incubated for an additional 35 minutes. Absorbance was measured at 405 nm, with acarbose used as the standard.

2.8. Statistical Analysis

The biological activity results are presented as the mean and standard deviation of three parallel measurements. The results were evaluated within a 95% confidence interval according to the Student t-test, with a significance threshold of $p < 0.05$. Linear regression analysis, using the least squares method, was performed to assess the slope, intercept, and correlation coefficients.

3. Results and Discussion

The LC-MS/MS analysis of ethanol extracts from the aerial parts and roots of *M. myrtifolia* was conducted following the method optimized and validated by Yilmaz et al. (2018). Table 1 presents the analytical parameters and results of these analyses and Figs. 3, 4, and 5 show the chromatograms for the standard compounds and studied extracts. Both extracts were found to be quite rich in terms of biologically active phytochemicals.

In *M. myrtifolia* aerial parts extract, 18 different secondary metabolites were determined. The most abundant constituent was revealed as nicotiflorin with 12878.31±355.44 µg/g extract. Other major components were found to be quinic acid (8171.29±67.00 µg/g extract), rosmarinic acid (6620.59±472.05 µg/g extract), naringenin (2532.19±131.93 µg/g extract), malic acid (917.44±10.37 µg/g extract). Moreover, hesperidin (570.28±14.94 µg/g extract), caffeic acid (580.79±20.56 µg/g extract), fumaric acid (564.63±7.00 µg/g extract), rhoifolin (544.73±51.26 µg/g extract), apigenin (235.19±15.29 µg/g extract), protocatechuic acid (195.83±8.05 µg/g extract), vanillin (80.08±2.24 µg/g extract), isoquercitrin (69.07±0.92 µg/g extract), salicylic acid (35.44±1.17 µg/g extract), fisetin (18.2±0.27 µg/g extract), hesperetin (17.75±1.00 µg/g extract), quercetin (8.19±0.44 µg/g extract), and *p*-coumaric acid (0.65±0.03 µg/g extract).

In *M. myrtifolia* root extract, 16 different components were identified. Similar to the aerial parts extract, nicotiflorin was the primary constituent with a concentration of 47512.4±1311.34 µg/g extract. Further major compounds were determined as quinic acid (1762.88±14.46 µg/g extract), rosmarinic acid (1527.81±108.93 µg/g extract),

rhoifolin (1067.04±100.41 µg/g extract), and hesperidin (726.54±19.04) µg/g extract). Malic acid (594.28±6.72 µg/g extract), fumaric acid (527.90±6.55 µg/g extract), caffeic acid (199.72±7.07 µg/g extract), vanillin (140.52±3.93 µg/g extract), protocatechuic acid (59.67±2.45 µg/g extract), *p*-coumaric acid (49.62±2.56 µg/g extract), apigenin (25.52±1.66 µg/g extract), hesperetin (23.24±1.31 µg/g extract), naringenin (15.36±0.80 µg/g extract), salicylic acid (12.93±0.43 µg/g extract), and isoquercitrin (8.63±0.11 µg/g extract).

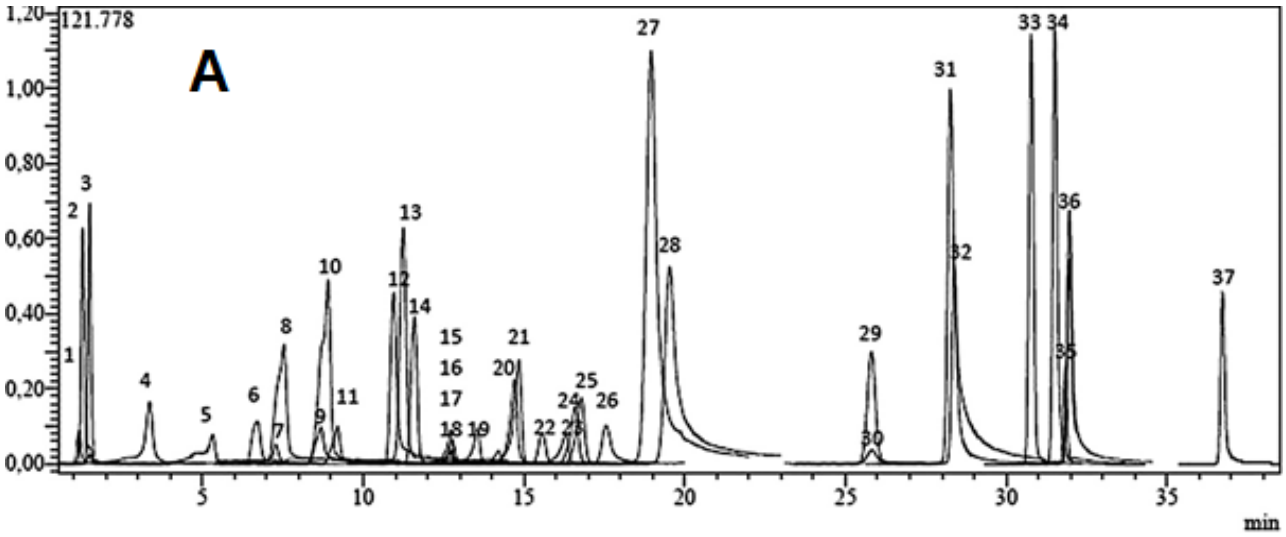

Figure 3. LC-MS/MS Chromatogram of the Standard Compounds

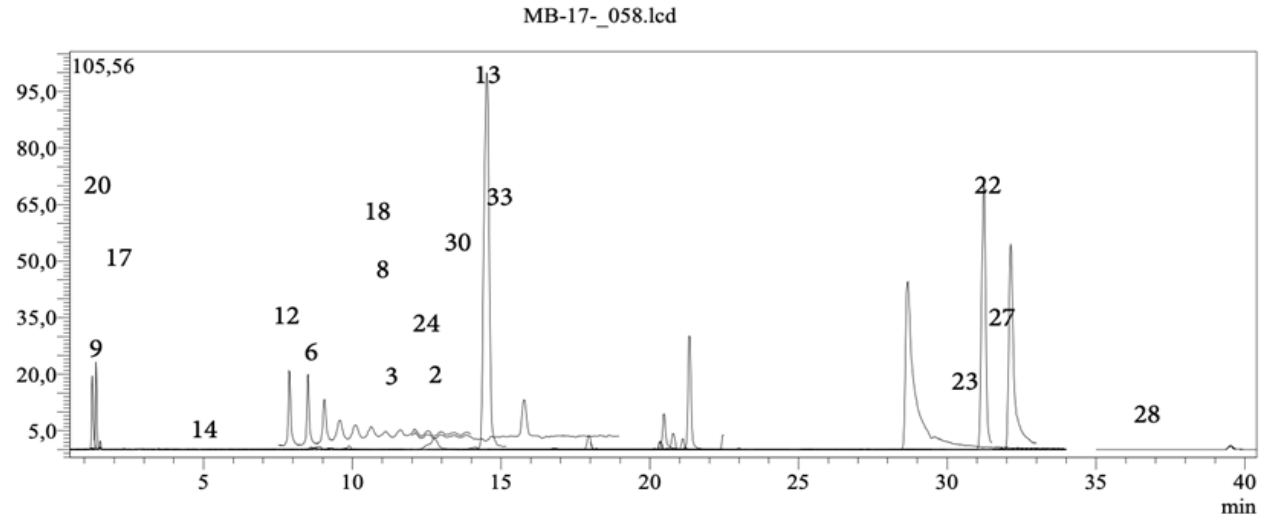

Figure 4. LC-MS/MS Chromatogram of *M. myrtifolia* Aerial Parts Extract

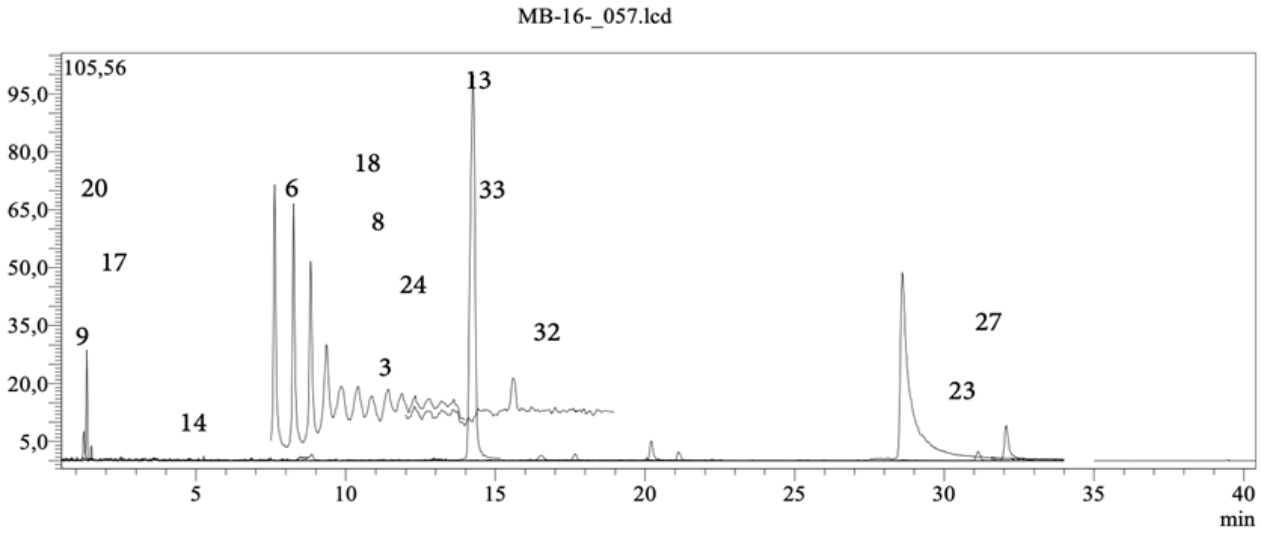

Figure 5. LC-MS/MS Chromatogram of *M. myrtifolia* Roots Extract

Table 1. LC-MS/MS Results of the Studied Extracts with Analytical Parameters

	Compounds	RT[a]	Parent ion (m/z)	Daughter Ions	Ion. Mode	Quantification(µg analyte/g extract)[b]	
						M. myrtifolia aerial parts	*M. myrtifolia* roots
1	**Coumarin**	17.40	147.05	91.0-103.2	Pos	ND	ND
2	**Hesperidin**	12.67	610.90	303.1-465.1	Pos	570.28±14.94	726.54±19.04
3	**p-coumaric acid**	11.53	162.95	119.25-93.25	Neg	0.65±0.03	49.62±2.56
4	**o-coumaric acid**	15.45	162.95	119.35-93.25	Neg	ND	ND
5	**Gallic acid**	3.00	168.85	125.2-79.2	Neg	ND	ND
6	**Caffeic acid**	8.80	178.95	135.2-134.3	Neg	580.79±20.56	199.72±7.07
7	**Vanillic acid**	8.57	166.90	152.25-108.25	Neg	ND	ND
8	**Salicylic acid**	11.16	136.95	93.3-65.3	Neg	35.44±1.17	12.93±0.43
9	**Quinic acid**	1.13	190.95	85.3-93.3	Neg	8171.29±67.00	1762.88±14.46
10	**4-OH-Benzoic acid**	7.39	136.95	93.3-65.3	Neg	ND	ND
11	**tr-Ferulic acid**	12.62	192.95	178.3	Neg	ND	ND
12	**Chlorogenic acid**	7.13	353.15	191.2	Neg	ND	ND
13	**Rosmarinic acid**	14.54	359.00	161.2-197.2	Neg	6620.59±472.05	1527.81±108.93
14	**Protocatechuic acid**	4.93	152.95	108.3	Neg	195.83±8.05	59.67±2.45
15	**Cinnamic acid**	25.61	147.00	103.15-77.3	Neg	ND	ND
16	**Sinapinic acid**	12.66	222.95	208.3-149.2	Neg	ND	ND
17	**Fumaric acid**	1.48	115.00	71.4	Neg	564.63±7.00	527.90±6.55
18	**Vanillin**	10.87	151.00	136.3-92.2	Neg	80.08±2.24	140.52±3.93
19	**Pyrocatechol**	6.48	109.00	108.35-91.25	Neg	ND	ND
20	**Malic acid**	1.23	133.00	115.2-71.3	Neg	917.44±10.37	594.28±6.72
21	**Syringic acid**	9.02	196.95	182.2-167.3	Neg	ND	ND
22	**Hesperetin**	31.76	300.95	164.2-136.2	Neg	17.75±1.00	23.24±1.31
23	**Naringenin**	30.68	270.95	151.2-119.3	Neg	2532.19±131.93	15.36±0.80
24	**Rutin**	12.61	609.05	300.1-271.1	Neg	ND	ND
25	**Quercetin**	28.17	300.90	151.2-179.2	Neg	8.19±0.44	ND
26	**Quercitrin**	16.41	447.15	301.15-255.15	Neg	ND	ND
27	**Apigenin**	31.43	268.95	117.3-151.2	Neg	235.19±15.29	25.52±1.66
28	**Chrysin**	36.65	252.95	143.3-119.4	Neg	ND	ND
29	**Liquiritigenin**	25.62	254.95	119.25-135.15	Neg	ND	ND
30	**Isoquercitrin**	13.42	463.00	300.15-271.15	Neg	69.07±0.92	8.63±0.11
31	**Cosmosiin**	16.59	431.00	268.2-239.2	Neg	ND	ND
32	**Rhoifolin**	16.11	577.05	269.2-211.15	Neg	544.73±51.26	1067.04±100.41
33	**Nicotiflorin**	14.68	593.05	285.1-255.2	Neg	12878.31±355.44	47512.4±1311.34
34	**Fisetin**	19.30	284.95	135.2-121.25	Neg	18.2±0.27	ND
35	**Luteolin**	28.27	284.75	133.2-151.2	Neg	ND	ND
36	**Myricetin**	18.72	317.00	179.15-151.25	Neg	ND	ND
37	**Kaempferol**	31.88	284.75	255.1-117.3	Neg	ND	ND

[a]RT: Retention time, [b]Values in µg/g (w/w) of plant extracts.

Sarikurkcu et al. (2020a) carried out an LC–ESI–MS/MS analysis to investigate the phenolic constituents of *M. micromaria* aerial parts (from Muğla-Türkiye) water, methanol, and ethyl acetate extracts. Accordingly, the methanol and water extracts were particularly rich in rosmarinic, syringic, chlorogenic, caffeic, and protocatechuic acids, whereas rosmarinic acid and apigenin were the predominant compounds in the ethyl acetate extract. Küpeli Akkol et al. (2019) also conducted a study aiming to isolate the secondary metabolites of *M. myrtifolia* aerial parts collected from Antalya-Türkiye. Three extracts (methanol, ethyl acetate, and n-hexane) were prepared. Reportedly, rosmarinic acid, naringenin, apigenin, and myricetin were isolated from the methanol extract. In another study, *M. myrtifolia* methanol extract was shown to contain high amounts of rosmarinic acid, quercetin, and chlorogenic acid (Taskin et al., 2024). It can be said that the findings of the current study demonstrate a high degree of concordance with the results reported in previous studies.

Other *Micromaria* species were also investigated for their phenolic constituents. Speaking of which, gallic acid, caffeic acid, chlorogenic acid, rosmarinic acid, diosmin, and apigenin were identified in *M. graeca* aerial parts from Algeria (ethanol extract) by an HPLC analysis (Brahmi et al., 2017). In another study, different extracts (methanol, water, and ethyl acetate) of *M. nervosa* from Türkiye were shown to be rich in terms of rosmarinic acid. Besides, luteolin, apigenin, chlorogenic acid, protocatechuic acid, caffeic acid, and vanillic acid were determined in the extracts (Sarikurkcu et al., 2020b). In *M. inodora* extract, different phenolic acids and flavonoids, predominantly gallic acid, quercetin, rutin, vanillin, and naringenin were detected by an RP-HPLC-PDA analysis (Adjdir et al., 2021). In *M. fruticosa* (from Palestine) methanol extract, phenolic compounds including gallic acid, chlorogenic acid, catechin, protocatechuic acid, rosmarinic acid, apigenin, and quercetin were screened (Abu-Gharbieh & Ahmed, 2016). In a recent study by Yilmaz et al. (2024), an ethanol extract of *M. cymuligera* (from Türkiye, endemic) was found to contain notable amounts of rosmarinic acid, quinic acid, chlorogenic acid, and cynaroside. Incidentally, these species were thought to be extinct for almost 150 years until samples were rediscovered in 2011. In *M. frivaldszkyana* (from Bulgaria, endemic) aerial parts ethanol extract, rosmarinic acid was revealed as the major constituent. Furthermore, vanillic acid, caffeic acid, chlorogenic acid, protocatechuic acid, salicylic acid, hesperidin, (-)-epicatechin, and apigenin were reported to be present in the extract (Mladenova et al., 2021).

Focusing on the current study, the abundance of nicotiflorin in both extracts is remarkable, highlighting its contribution to the bioactivity of the extracts. Nicotiflorin (kaempferol-3-β-rutinoside) is a flavonoid glycoside with well-established anti-inflammatory, antioxidant, antibacterial, antiviral, analgesic, anti-hypertensive, anti-anaphylactic, and neuroprotective effects. Medicinal plants that are rich in nicotiflorin have been used for the treatment and prevention of cardiovascular diseases in Traditional Chinese Medicine (Li et al., 2006; Yu et al., 2021). Incidentally, to the authors' best knowledge, this is the first report indicating the significant presence of nicotiflorin in *Micromeria* species. Sarikurkcu et al. (2020a) did not report the determination of nicotiflorin in *M. myrtifolia* in their analysis. Yilmaz et al. (2024) did not detect any nicotiflorin in *M. cymuligera* extract although it was among the standard compounds.

The composition, content, and proportions of bioactive compounds within a single plant species may vary significantly due to the influence of diverse environmental factors in their habitats. Additionally, a notable correlation exists between the flavonoid profiles of plants and their ecological and morphological attributes (Mykhailenko et al., 2020). As a case in point, Ersoy et al. (2023) indicated significant differences in the phenolic compositions of *Teucrium multicaule* samples collected from two different locations in Türkiye. Nevertheless, while present phenolic compounds can vary due to numerous factors, the compositions of *Micromeria* species were observed to be notably similar to one another. Brahmi et al. (2017) also emphasized this situation. In their study, *Micromeria* species in general were reported to contain mostly phenolic acids (such as gallic, chlorogenic, vanillic, ferulic, *and p*-coumaric acids) and flavonoids (such as apigenin, luteolin, naringenin, hesperetin, and quercetin). As additional studies investigating the phenolic constituents of different *Micromeria* species emerge, a clearer understanding will be developed on this subject.

The results of the calculation of total phenolic and flavonoid contents of *M. myrtifolia* aerial parts and root extracts as well as the extract yields were given in Table 2. The ethanol extract of *M. myrtifolia* aerial parts exhibited a total phenolic content of 58.07±1.46 µg PEs/mg extract, while the total flavonoid content for the same extract was determined to be 16.17±0.83 µg QEs/mg extract. In contrast, the ethanol extract of *M. myrtifolia* roots showed a total phenolic content of 50.12±1.59 µg PEs/mg extract, with a total flavonoid content calculated at 25.10±1.02 µg QEs/mg extract. Both extracts were found to contain high amounts of total phenolics and flavonoids.

Table 2. Yields (%), Total Phenolic and Flavonoid Contents of the Studied Extracts

Extracts	Yield %	Phenolic content (*µ*g PEs/mg extract)[a]	Flavonoid content (*µ*g QEs/mg extract)[b]
M. myrtifolia aerial parts	3.69	58.07±1.46	16.17±0.83
M. myrtifolia roots	0.98	50.12±1.59	25.10±1.02

*Values expressed are means ± standard deviation of three parallel measurements (p<0.05)

[a] PEs, pyrocatechol equivalents (y = 0.0409x + 0.0495, R^2 = 0.9975).

[b] QEs, quercetin equivalents (y = 0.0347x + 0.1174, R^2 = 0.9951)

Studies in this area have revealed that different plant organs possess varying levels of total phenolic and flavonoid contents. For instance, aerial parts of *Hypericum empetrifolium* were found to be richer than the roots of the plant in terms of total phenolic and flavonoid contents (Boga et al., 2021). Total phenolic and flavonoid contents of *Thymus praceox* subsp. *grossheimi* aerial parts and roots and *T. pubescens* aerial parts and roots were significantly different than each other (Eroglu Ozkan et al., 2022). Likewise, fruits and leaves of *H. androsaemum* were shown to contain different amounts of total phenolic and flavonoids (Bektaş et al., 2021).

Regarding total phenolic and flavonoid contents of different *Micromeria* species, in a study carried out with *M. croatica, M. juliana* and *M. thymifolia* (from Croatia), the dried plant samples were analyzed spectrophotometrically to determine their total contents of polyphenols (9.69–13.66%), phenolic acids (5.26–6.84%), flavonoids (0.01–0.09%), and tannins (3.07–6.48%) (Vladimir-Knežević et al., 2011). Total phenolic contents across different extracts of *M. graeca* were found to vary between 211 ± 11.9 mg GAE/g and 360 ± 22.1 mg GAE/g. The water extract exhibited the highest, whereas the ethyl acetate extract displayed the lowest total phenolic content. These results also indicate the influence of solvent on the total phenolic and flavonoid contents of the extracts (El Kamari et al., 2024).

Antioxidant activity of the aerial parts and root extracts of *M. myrtifolia* was determined using three different methodologies: DPPH free radical scavenging, ABTS cation radical scavenging, and CUPRAC activity assays.

The results of the DPPH free radical scavenging activity are presented in Fig. 6, pointing out the antioxidant potential of the ethanol extracts from both the aerial parts and roots of *M. myrtifolia*. These findings demonstrate the ability of extracts to scavenge DPPH free radicals, which is crucial for understanding their potential therapeutic applications regarding antioxidant activity. The graphical representations of the results, including comparisons to the standards BHT, BHA, and α-TOC, can be found in Fig. 6, illustrating the comparative efficacy of each extract in scavenging DPPH radicals.

As shown in Fig. 6, the ethanol extracts from *M. myrtifolia* aerial parts and roots demonstrated varying levels of inhibition activity across concentrations. At 10 µg/mL, *M. myrtifolia* aerial parts and roots exhibited 19.99% and 31.37% inhibition, respectively; at 25 µg/mL, they showed 40.96% and 51.72%; at 50 µg/mL, 76.11% and 73.09%; and at 100 µg/mL, 87.61% and 85.81%. At lower concentrations (10 and 25 µg/mL), both extracts had lower activity than all three standard compounds (BHT, BHA, and α-TOC). However, at 50 µg/mL, the activity of *M. myrtifolia* roots extract approached that of BHT, while aerial parts extract surpassed BHT. At the highest concentration, 100 µg/mL, both extracts exhibited DPPH radical scavenging activities similar to BHA and α-TOC and higher than BHT. Overall, these results indicate that both *M. myrtifolia* aerial parts and roots—extracts demonstrate good antioxidant activity, highlighting their potential as natural sources of antioxidants.

ABTS cation radical scavenging activity of ethanol extracts from the aerial parts and roots of *M. myrtifolia* are presented in Fig. 7, highlighting their antioxidant potential. Fig. 7 illustrates the scavenging efficiency of each extract, compared to standards BHT, BHA, and α-TOC, underscoring their promising antioxidant properties.

As shown in Fig. 7, the ethanol extracts from *M. myrtifolia* aerial parts and roots exhibited ABTS cation radical scavenging activities of 65.64% and 59.22% at 10 µg/mL, 86.63% and 85.12% at 25 µg/mL, 88.59% and 87.08% at 50 µg/mL, and 88.77% and 88.06% at 100 µg/mL, respectively. At concentrations of 10 and 25 µg/mL, the *M. myrtifolia* aerial parts extract demonstrated higher activity than the standards BHT and TOC, while at 50 and 100 µg/mL, it showed activity comparable to TOC and greater than BHT. The *M. myrtifolia* roots extract, on the other hand, exhibited higher activity than BHT and was close to TOC at 10 µg/mL, surpassed both standards at 25 µg/mL, and maintained levels near the other standards at 50 and 100 µg/mL.

The results demonstrate that both the ethanol extracts from the aerial parts and roots of *M. myrtifolia* possess significant ABTS cation radical scavenging activity, indicating their potential as effective natural antioxidants. The dose-dependent increase in scavenging activity suggests that higher concentration enhances their antioxidant effects. Notably, *M. myrtifolia* aerial parts extract exceeded the standards of BHT and TOC at lower concentrations and maintained comparable activity at higher concentrations. Meanwhile, *M. myrtifolia* roots extract also showed considerable antioxidant potential, particularly at 25 µg/mL. These findings highlight *M. myrtifolia* as a promising candidate for further research and development in antioxidant applications, reinforcing its value in both traditional and modern medicine.

Fig. 8 presents the CUPRAC (Cupric Ion Reducing Antioxidant Capacity) assay results, assessing the reducing power of ethanol extracts from the aerial parts and roots of *M. myrtifolia*. By measuring the ability of the extracts to reduce cupric ions (Cu^{2+}) to cuprous ions (Cu^{+}), it is possible to gain insights into their antioxidant activity. The results, shown in Fig. 8, illustrate the antioxidant capacities of both extracts at different concentrations, allowing comparisons with established standards.

At a concentration of 10 mg/mL, the aerial parts (0.55) and roots (0.63) of *M. myrtifolia* demonstrated notable reducing power, significantly lower than that of the standards BHA (1.25) and BHT (1.98), while α-TOC had a value of 0.48. As the concentration increased to 25 mg/mL, the reducing power of both extracts improved, with values of 1.11 for aerial parts and 1.31 for roots, indicating that they retained their antioxidant capacity but were still lower than BHA (2.35) and BHT (3.68), while surpassing α-TOC (1.06). At 50 mg/mL, both extracts showed enhanced activity, reaching values of 2.11 for aerial parts and 2.46 for roots, still below the standards BHA (3.63) and BHT (3.99). Notably, at 100 mg/mL, both extracts exhibited significant antioxidant activity with values of 3.37 for aerial parts and 3.99 for roots, aligning with the performance of the BHT and BHA standards, which also reached 3.99. These findings suggest that *M. myrtifolia* extracts possess promising antioxidant activity, particularly in the root extract, which demonstrates significant reducing power, especially at higher concentrations.

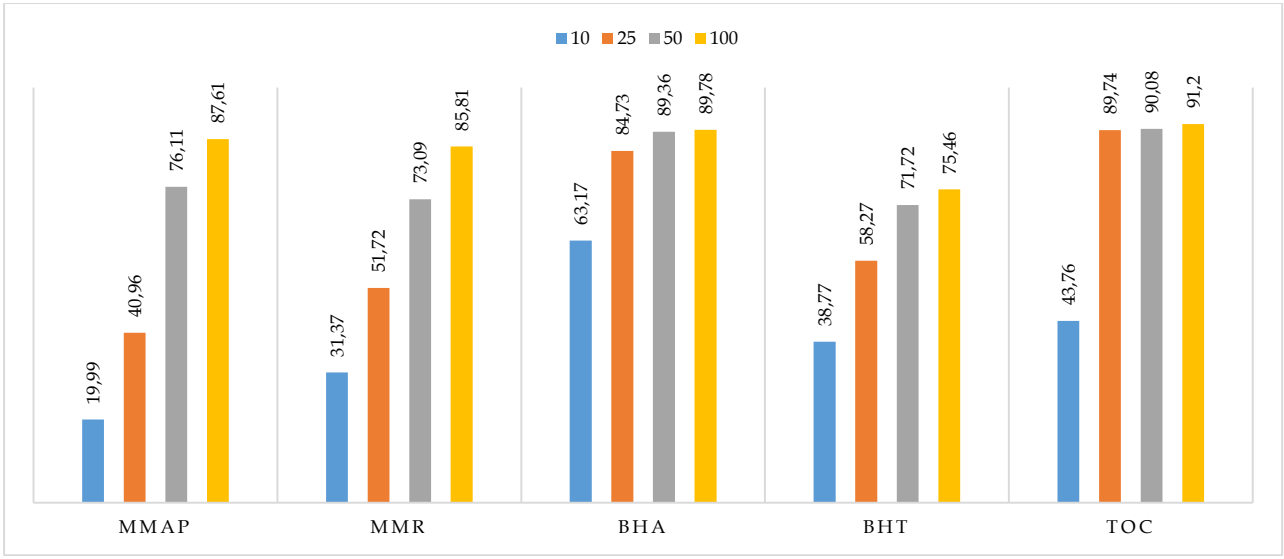

Figure 6. DPPH Free Radical Scavening Activity Results (Concentration-Inhibition%), MMAP: *M. myrtifolia* Aerial Parts, MMR: *M. myrtifolia* Root, BHA: Butylated Hydroxyanisole, BHT: Butylated Hydroxytoluene, TOC: α-Tocopherol

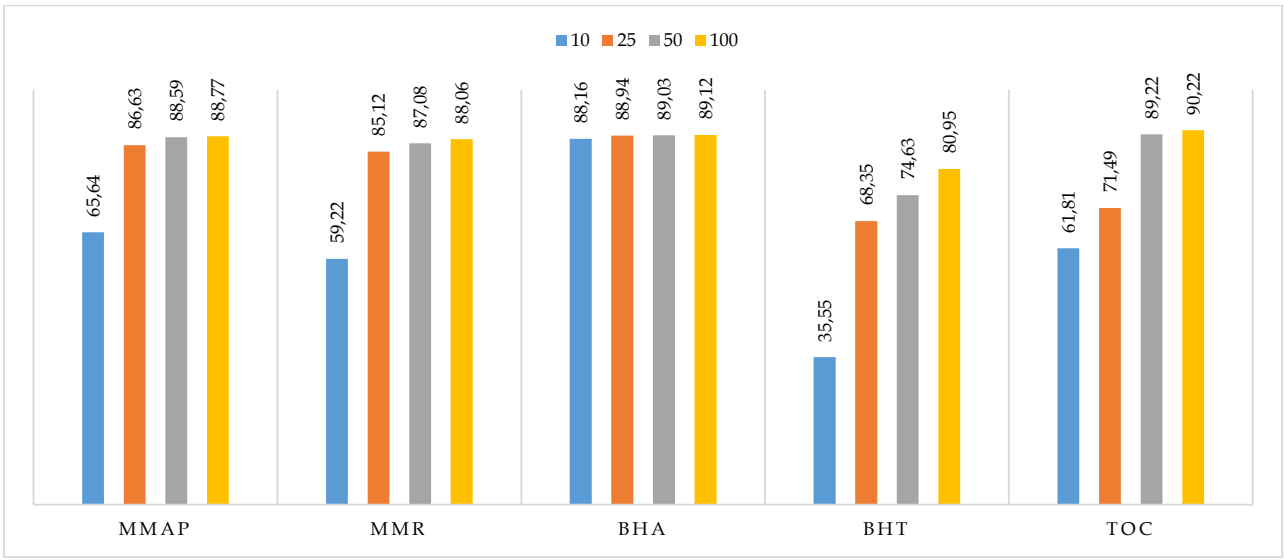

Figure 7. ABTS Cation Radical Scavening Activity Results (Concentration-Inhibition%), MMAP: *M. myrtifolia* Aerial Parts, MMR: *M. myrtifolia* Root, BHA: Butylated Hydroxyanisole, BHT: Butylated Hydroxytoluene, TOC: α-Tocopherol

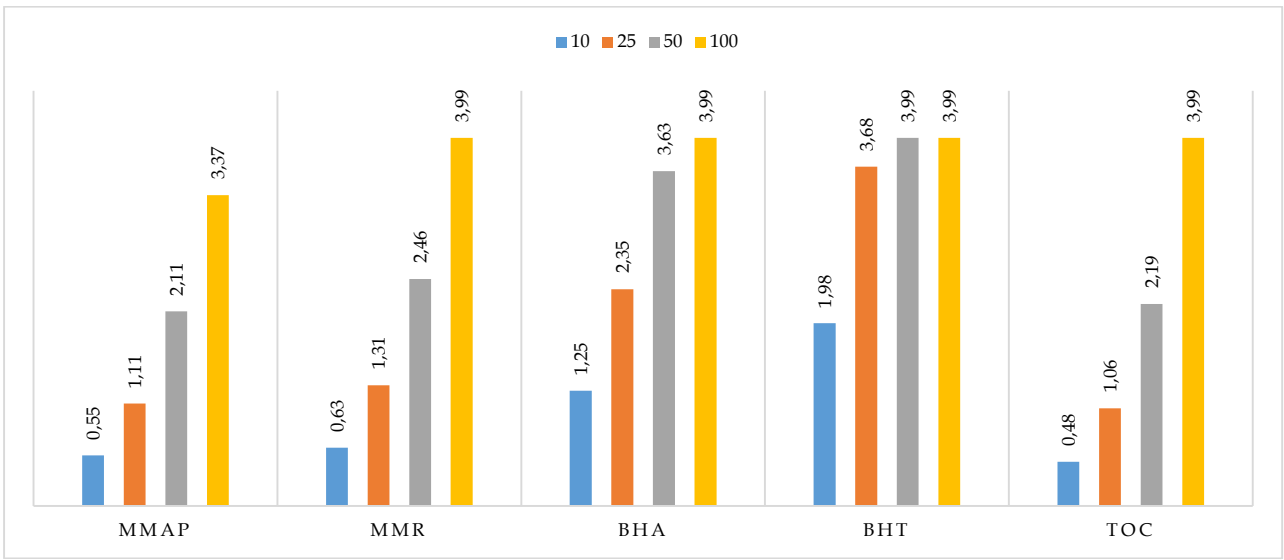

Figure 8. CUPRAC Activity Results (Concentration-Absorbance), MMAP: *M. myrtifolia* Aerial Parts, MMR: *M. myrtifolia Root*, BHA: Butylated Hydroxyanisole, BHT: Butylated Hydroxytoluene, TOC: α-Tocopherol

Micromeria species are renowned for being good sources of phenolic compounds, predominantly phenolic acids and flavonoids. As a consequence of this, their antioxidant properties are also well-established. The antioxidant activity of these extracts exhibits a dose-dependent relationship, indicating that higher concentrations generally lead to increased efficacy. Additionally, this activity is significantly influenced by the choice of solvents used for extraction, suggesting that different solvents can selectively extract various phytochemicals with antioxidant properties. This highlights the importance of solvent selection in optimizing the antioxidant potential of the extracts (Öztürk et al., 2011; Vladimir-Knežević et al., 2011; Formisano et al., 2014; Šamec et al. 2015; Abu-Gharbieh et al., 2016; Salameh et al., 2020; Sarikurkcu et al., 2020b; Adjdir et al., 2021; Mladenova et al., 2021; Sadeq et al., 2021; El Kamari et al., 2024). In the current study, the strong antioxidant activity of the extracts can be attributed to its rich composition of bioactive compounds. Nicotiflorin, the most abundant constituent, is a known radical scavenger with significant antioxidant properties (Patel, 2022). Rosmarinic acid, a well-established antioxidant, likely contributes through free radical scavenging and metal chelation. Other compounds such as quinic acid, caffeic acid, and naringenin further enhance the antioxidant activity due to their phenolic structures (Ersoy et al., 2022). The combined presence of these potent antioxidants highlights the efficacy of the studied extracts against oxidative stress.

The enzyme inhibitory activities of *M. myrtifolia* aerial parts and root extracts were assessed through acetylcholinesterase, butyrylcholinesterase, tyrosinase, urease, and α-glucosidase inhibition activity assays. The results are presented in Table 3.

Table 3. Enzyme Inhibitory Activity Results of the Studied Extracts

	Inhibition (%)				
Samples	AChE[a]	BChE[a]	Tyrosinase[a]	Urease[a]	α-glucosidase[b]
M. myrtifolia aerial parts	NA	NA	NA	NA	73.03±0.16
M. myrtifolia roots	NA	44.39±1.40	NA	NA	47.06±0.41
Galanthamine*	72.50±0.28	76.51±0.81	-	-	-
Kojic acid*	-	-	95.26±0.23	-	-
Tiyourea*	-	-		88,61±1,16	-
Acarbose*	-	-			81.53±0.50

a: The results are presented as the mean and standard deviation of three parallel measurements at a concentration of 200 µg/mL.

b: The study was conducted at a concentration of 2 mg/mL

*: Standard compounds

NA: Not Active

AChE: Acetylcholinesterase, BChE: Butyrylcholinesterase

In terms of anticholinesterase activity, only root extract of *M. myrtifolia* exhibited moderate inhibitory activity against butyrylcholinesterase with 44.39±1.40% at 200µg/mL concentration. Aerial parts extract was not active against acetylcholinesterase or butyrylcholinesterase. Regarding previous studies on the subject, in Öztürk et al. (2011)'s study, anticholinesterase activity was assessed in the extracts of *M. cilicica* in comparison to that of galantamine. Among the studied extracts prepared with different solvents, only the acetone extract demonstrated moderate inhibitory activity against butyrylcholinesterase, whereas others were not active at all. In Vladimir-Knežević et al. (2014)'s study, a total of 26 wild-growing species from the Lamiaceae family were tested for their anti-acetylcholinesterase potential. Four *Micromeria* species, namely *M. croatica*, *M. graeca*, *M. juliana*, and *M. thymifolia* were included in the study. Accordingly, only *M. graeca* exerted moderate-to-weak acetylcholinesterase inhibitory activity, while the other studied *Micromeria* species did not achieve acetylcholinesterase inhibition. Regarding current study, the lack of acetylcholinesterase inhibitory activity may be due to the lack of specific compounds that are shown to possess acetylcholinesterase inhibitory activity potential. Speaking of which, flavonoids such as quercetin, quercitrin, hyperoside, and rutin were found to be much more effective compared to phenolic acids in terms of anti-acetylcholinesterase activity (Orhan et al., 2006; Ersoy et al., 2020). The aerial parts of *M. myrtifolia* were found to contain only quercetin with 8.19±0.44 µg/g extract among the effective compounds. Rutin and quercitrin were not detected in the extracts.

Both extracts were found to be effective against the α-glucosidase enzyme. Compared to the standard molecule acarbose (81.53±0.50%), the aerial parts extract showed strong inhibitory activity with 73.03±0.16% and the roots extract demonstrated moderate inhibitory activity with 47.06±0.41% at 2 mg/mL concentration. In managing diabetes, controlling blood glucose levels after meals is crucial. One effective approach is through the inhibition of α-glucosidase enzymes, which play an essential role in carbohydrate digestion. Located on the brush-border membrane of intestinal cells, these enzymes catalyze the breakdown of oligosaccharides by hydrolyzing α-glycosidic bonds, releasing glucose molecules for absorption into the bloodstream. By slowing this process, α-glucosidase inhibitors help reduce the rapid rise in blood glucose that typically follows a meal (Lawag et al., 2012). Aleixandre et al. (2022) aimed to understand the role of phenolic compounds in alpha-glucosidase inhibition. Accordingly, the most potent molecules were phenolic acids, caffeic and protocatechuic acids in particular. Since the studied extracts were found to contain

these compounds, the activity is considered to be due to these molecules. They also noted that phenolic acids containing more than one hydroxyl group demonstrated the lowest IC_{50} values against α-glucosidase.

Neither the aerial parts nor the root extracts were found to inhibit tyrosinase and urease enzymes.

4. Conclusions

Micromeria species are recognized as valuable sources of biologically active compounds and highly regarded as medicinal plants within Türkiye. This study provided compelling evidence that extracts from the aerial parts and roots of *M. myrtifolia* are particularly rich in phenolic compounds, with exceptional levels of nicotiflorin. Furthermore, these extracts demonstrated notable antioxidant activity and anti-glucosidase inhibitory effects that are worth mentioning, highlighting their potential as therapeutic agents. The findings underscore the importance of *M. myrtifolia* not only as a traditional remedy but also as a candidate for developing functional foods or nutraceuticals aimed at managing oxidative stress and diabetes. Further research in this area will enhance our understanding of the medicinal properties of *M. myrtifolia*, paving the way for its application in diabetes management and other health-related interventions.

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