J. Fac. Pharm. Ankara / Ankara Ecz. Fak. Derg., 48(2): 406-411, 2024
Doi: 10.33483/jfpau.1372535

ORIGINAL ARTICLE / ÖZGÜN MAKALE

DETERMINATION OF BIOLOGICAL ACTIVITIES OF *MICROMERIA MYRTIFOLIA* BOISS. & HOHEN

MICROMERIA MYRTIFOLIA BOISS. & HOHEN'İN BİYOLOJİK AKTİVİTELERİNİN BELİRLENMESİ

Yasemin Yücel YÜCEL[1,2]*, Ebru ÖZDEMİR NATH[2,3]

[1]Altınbaş University, Faculty of Pharmacy, Department of Biochemistry, 34356, Istanbul, Türkiye
[2]Altınbaş University, Natural Products Research and Development Center, DÜAGEM, 34356, Istanbul, Türkiye
[3]Altınbaş University, Faculty of Pharmacy Department of Pharmaceutical Botany, 34356, Istanbul, Türkiye

ABSTRACT

Objective: *Lamiaceae family has a wide variety of well-known and lesser-known plants with strong medicinal qualities. The genus Micromeria Benth. is a member of this family consisting of herbaceous plants with a variety of significant biological, phytochemical, and ethnobotanical uses. In this study, the biological activities of methanol and ethanol extracts of Micromeria myrtifolia were evaluated.*
Material and Method: *To demonstrate the antioxidant activity DPPH radical scavenging activity and total phenolic content assays were done. The effects of the extracts on acetylcholinesterase (AChE) and monoamine oxidase-A were then assessed.*
Result and Discussion: *Methanol extract showed the highest DPPH scavenging activity, at the dose of 10 mg/ml with a value of 96.55%. For the highest concentration that can be applicable, AChE inhibitions for the methanol and ethanol extracts were 25% and 27%, respectively. On the other hand, the inhibitory effects of the ethanol and methanol extracts of the plant on MAO-A were determined; for the ethanol extract IC_{50} value was found as 32.5876 ± 0.89 µg/ml, and for the methanol extract it was found as 34.6544 ± 0.76 µg/ml. It can be told that M. myrtifolia can act as a potential antioxidant. With further research and investigation, it is thought that Micromeria myrtifolia could be used as a natural source for the treatment of various neurological diseases.*
Keywords: *Acetylcholinesterase, antioxidant activity, Micromeria myrtifolia, monoamine oxidase-A, neurodegenerative diseases*

ÖZ

Amaç: *Lamiaceae familyası, güçlü tıbbi niteliklere sahip bilinen veya az bilinen değişik bitkileri içeren bir bitki ailesidir. Micromeria Benth cinsi bu familyaya ait, çok çeşitli biyolojik, fitokimyasal ve etnobotanik kullanımı olan, çoğunlukla otsu bitkilerden oluşan cinslerden biridir. Bu çalışmada, Micromeria myrtifolia'nın metanol ve etanol ekstreleri, bitkinin biyolojik aktiviteleri gösterilmek üzere seçilmiştir*
Gereç ve Yöntem: *Antioksidan aktiviteyi göstermek için DPPH radikal süpürücü aktivitesi ve total fenolik içerik deneyleri yapılmıştır. Daha sonra ekstrelerin asetilkolinesteraz (AChE) ve monoamin oksidaz-A (MAO-A) üzerindeki etkileri değerlendirilmiştir.*
Sonuç ve Tartışma: *Çalışma sonunda metanol ekstraktı, 10 mg/ml dozunda % 96.55 değeriyle en yüksek DPPH radikal süpürücü aktivitesi göstermiştir. Uygulanabilecek en yüksek*

* **Corresponding Author / Sorumlu Yazar:** Yasemin Yücel Yücel
e-mail / e-posta: yasemin.yucel@altinbas.edu.tr **Phone / Tel.:** +902127094528

Submitted / Gönderilme : 06.10.2023
Accepted / Kabul : 17.01.2024
Published / Yayınlanma : 20.05.2024

konsantrasyonlarda metanol ve etanol ekstreleri için AChE inhibisyonları sırasıyla %25 ve %27 olarak saptanmıştır. Ayrıca bitkinin MAO-A üzerine etkisi de tespit edilmiştir. Buna göre etanol ekstresi için IC_{50} değeri 32.5876 ± 0.89 µg/ml ve metanol ekstresi için ise 34.6544 ± 0.76 µg/ml olarak bulunmuştur. Elde edilen sonuçlara göre M. myrtifolia'nın potansiyel bir antioksidan etkisi gösterebileceği söylenebilir. Daha ileri çalışmalar ile Micromeria myrtifolia'nın çeşitli nörolojik hastalıkların tedavisi için doğal bir kaynak olarak kullanılabileceği düşünülmektedir.
Anahtar Kelimeler: *Antioksidan aktivite, asetilkolinesteraz, Micromeria myrtifolia, monoamin oksidaz-A, nörodejeneratif hastalıklar*

INTRODUCTION

The pathogenetic pathways of many illnesses, including neurological disorders are linked to oxidative stress [1,2]. Besides, acetylcholinesterase (AChE) and monoamine oxidases (MAOs) are very important enzymes for their significance in the treatment of these diseases. So, their activities and inhibitions play crucial roles in treating some neurologic conditions [3,4].

Since ancient times plants have been used for the therapy of different illnesses. Today, practically all pharmacopoeias in the world recommend plant-derived medicines with actual therapeutic benefits. There are also some regions with unique herbal pharmacopoeias [5].

Lamiaceae family has strong biological effects with secondary metabolites whose structure has been elucidated and secondary metabolites that have not yet been elucidated [6]. *Micromeria* Benth., a genus of this family, mostly consists of herbaceous plants with a variety of significant biological, phytochemical, and ethnobotanical uses. *Micromeria* genus is represented by eighty-nine species in the world and nine species in Türkiye. There are seventeen taxa in Türkiye and five of them are endemic [7,8]. *Micromeria myrtifolia* Boiss. & Hohen. (Ayaklı kekik, Boğumluçay) used for flu, gallstones, gastrointestinal disorders, pleasure, relaxing, stomachache, and throat disease [9-12].

Studies on the phytochemistry of *Micromeria* species have revealed the presence of several flavonoid chemicals, saponins, tannins, anthraquinones, and essential oils [13-15]. Numerous investigations have established that certain *Micromeria* species have biological properties that include antifungal, antimicrobial, antioxidant, anticholinesterase, anti-inflammatory, gastroprotective, hepatoprotective, and cytotoxic effects [6,16-19]. According to Formisano et al., the methanol extracts of *M. myrtifolia* were found to be substantially more active antioxidants than chloroform and hexane extracts, when tested using DPPH radical scavenging and ferric ion reduction (FRAP) assays [20].

Here in this study, some of the biological functions of *Micromeria myrtifolia* were examined. Our goal was to demonstrate the DPPH activity of the extracts of the plant and the relationship between that activity and the phenolic content of the plant. AChE and MAO-A are two crucial enzymes that have drawn a lot of interest from researchers due to their significance in the therapy of neurodegenerative illnesses. We also tried to find out how the extracts of *Micromeria myrtifolia* influenced these two enzymes.

MATERIAL AND METHOD

Plant Materials

In June 2021, *M. myrtifolia* was collected from Gemlik, Bursa, Türkiye. Assistant Professor Dr. Ebru Ozdemir Nath observed and identified the plant sample. Herbarium of Altınbaş University Faculty of Pharmacy (HERA) has received a specimen of the plant with the herbarium number HERA255.

Preparation of the Extract

The plant parts were ground into a fine powder using a mechanic's grinder, air dried at room temperature in a dark, shaded environment, and weighed with a digital balance. *M. myrtifolia* powder was macerated with a ratio of 1 part of plant (25 g) soaked in 10 parts of solvent (250 ml), by using 96% ethanol and methanol solvents, in a tightly closed container for 24 hours, and protected from light, while stirring frequently. Under a rotary evaporator (Heidolph Hei-VAP Advantage rotary evaporator), the solvent evaporated to dryness. *M. myrtifolia* plant extract was kept at +4ºC until biological activity

research.

DPPH Radical Scavenging Activity

Brand-Williams method was used to determine the DPPH activity of the extracts of *M. myrtifolia* [21]. 10 µl of ethanol/methanol extracts of the plant sample in different concentrations (0.5-10 mg/ml) were combined with 240 ml DPPH radical and incubated in the dark at room temperature for 10 minutes. By measuring the absorbance at 517 nm, the decrease of the DPPH radical was quantified. Quercetin was used as the standard in this activity test and the result is given in Table 1.

Table 1. DPPH radical scavenging activity of quercetin

Concentration (mg/ml)	DPPH %
0.250	86.853
0.125	75.148
0.062	49.864
0.031	30.740
0.015	21.124
0.008	9.850
0.004	4.736

The formula below was used to determine the radical scavenging activity (Inh %) as a percentage of DPPH discoloration.

$$\text{Inh \%} = [1- (Abs_{extract} / Abs_{DPPH})] \times 100$$

Total Phenolic Content

The total phenolic contents of the extracts of *M. myrtifolia* were found using a modified Folin Ciocalteu technique [22]. The reduction that resulted in a blue color was observed at 760 nm. Various concentrations (0.5-10 mg/ml) of the ethanol/methanol extracts of the plant samples were combined with distilled water and Folin Ciocalteu reagent, respectively. The combination underwent a room-temperature incubation. 15 µl of 2% Na_2CO_3 were added after 3 minutes. After 2 hours of incubation at room temperature in the dark, the absorbance at 760 nm was measured using a multimode microplate reader, the BioTek Synergy H1 (Agilent). Gallic acid was used as the reference solution.

Acetylcholinesterase Activity

The acetylcholinesterase activities of the extracts of *M. myrtifolia* were measured with the AChE activity of *Electrophorus electricus* (electric eels) spectrophotometrically using the Ellman Method [23]. The substrate used in the study was acetylthiocholine iodide (ATC). The reactions were carried out at 25°C, in 100 mM Tris HCl (pH 8.0) buffer. To start each enzymatic reaction approximately 0.05 U/ml AChE was added to the reaction mixture. The breakdown of acetylthiocholine was detected with a UV spectrophotometer (Carry 60 Single Beam spectrophotometer, Agilent Technologies, USA) over an elevation in absorbance at 412 nm.

Monoamine Oxidase-A (MAO-A) Activity

Spectrofluorometric analysis and the previously described protocols were used to determine the effects of the *M. myrtifolia* extracts on MAO-A activity [24,25]. Each reaction was carried out in a 100 mM potassium phosphate buffer at a pH of 7.4. Kynuramine was used as the substrate of MAO-A. The substrate, buffer, and extracts had a 10-minute preincubation at 37°C. MAO-A was added to the assay mixtures to start the reactions, and the processes were then maintained at 37°C for an additional 20 minutes. 1000 µl of distilled water was added after the reactions were stopped by NaOH (2N). A multimode microplate reader was used to measure the quantity of fluorescence at 310 nm for excitation and 400 nm for emission. The related IC_{50} values were used to express the extracts' inhibitory potencies.

To compare the IC_{50} values of the plant clorgyline was used.

RESULT AND DISCUSSION

The DPPH radical scavenging activity and the total phenolic content of different concentrations of these extracts were determined. The results are summarized in Table 2. The antioxidant activity of methanol extracts was higher than that of ethanol extract. Methanol extract showed the highest DPPH scavenging activity, at the dose of 10 mg/ml with a percentage value of 96.55%. In this concentration, quercetin equivalent was obtained as 0.4392 mg. As the amount of tested dose increases, the DPPH activity increases for both extracts. DPPH percentage of the 0.5 mg/ml of the methanol extract was found to be close to the IC_{50} value (46.0967%). Similar to antioxidant activity assays, the highest TPC was found in the methanol extracts at the dose of 10 mg/ml with gallic acid equivalent 0.8629 mg/ml, and the gallic acid the equivalent phenolic content of methanol extract was higher at all doses. Furthermore, it was found that, as the TPC was getting higher, the DPPH activity test was found to be higher. All these findings suggest that *Micromeria myrtifolia* methanol extracts have high antioxidant content, even in low doses, and have a high potential in terms of antioxidant product development.

Table 2. DPPH radical scavenging activity and TFC of the methanol and ethanol extracts of *Micromeria myrtifolia*

Extract	Concentration (mg/ml)	DPPH Quercetin Equivalent (mg/ml)	DPPH, %	TFC, Gallic acid Equivalent (mg/ml)
Ethanol Extracts	10	0.2603±0.024	83.1417±1.795	0.5836±0.040
	5	0.1059±0.001	64.1762±0.010	0.3214±0.018
	1	0.0333±0.001	39.4157±0.707	0.0789±0.002
	0.5	0.0162±0.001	23.8984±1.764	0.0368±0.003
Methanol Extracts	10	0.4392±0.003	96.5517±2.441	0.8629±0.045
	5	0.2540±0.012	83.0459±1.005	0.6106±0.012
	1	0.0586±0.038	59.6024±1.221	0.1860±0.009
	0.5	0.0456±0.004	46.0967±2.083	0.0901±0.002

For the inhibitory effects of *Micromeria myrtifolia*'s ethanol and methanol extracts on AChE, a wide range of concentrations were screened. The highest (final) concentration that could be tested was 1.5 mg/ml for each extract. The inhibition percentages for the methanol and ethanol extracts were 25% and 27%, respectively. At higher concentrations, transparency and measurability in the cuvette were greatly reduced, preventing tests from being performed with higher concentrations.

On the other hand, the inhibitory effects of the ethanol and methanol extracts of the plant on MAO-A were determined and the IC_{50} values were calculated. For the ethanol extract the IC_{50} value was found as 32.5876 ± 0.89 μg/ml and for the methanol extract it was found as 34.6544 ± 0.76 μg/ml which is a very close result to the former. We have identified the IC_{50} value for the well-known inhibitor "clorgyline" as 3.128 nM $\pm$ 0.023. On the other hand, the IC_{50} values of another selective inhibitor of MAO-A "moclobemide" is reported as 6.1 μM [26]. According to these results, it can be assumed that the plant has a moderate inhibitory effect on MAO-A activity.

There are various studies on the antioxidants, anti-tyrosinase, anti-amylase, and antidepressant activities of *Micromeria* species, but very limited of them have been done with *M. myrtifolia* species [27,28]. In the study that have been conducted by *M. myrtifolia* collected from Lebanon the DPPH test revealed that the methanol extracts exhibited higher antioxidant activity compared to the extracts than that of the chloroform and hexane extracts. Finally, *Micromeria myrtifolia* collected from Antalya was studied for its antidepressant activity. It was determined that the methanol extract of the plant was shown to have antidepressant activity both in vivo and in vitro [29].

In this work, we have identified some of the biological activities of *M. myrtifolia*'s ethanol and

methanol extracts. In the study, we found that methanol extract showed high activity in all doses administered by DPPH. If we compare the findings we obtain with the ones in the literature, the results we found for the antioxidant tests were consistent with the results obtained from different species of the same genus. They were also parallel to that of the same species collected from different locations. So, it can be accepted that our plant *M. myrtifolia* can act as an antioxidant.

On the other hand, our research is the first to examine the inhibitory effects of the methanol and ethanol extracts of *M. myrtifolia* on AChE and the first to address the inhibitory effects of the ethanol extracts of *M. myrtifolia* on MAO-A. The results are beneficial and encouraging. With further research and investigation, it is thought that *M. myrtifolia* could be used as a natural source for the treatment of various neurological diseases.

ACKNOWLEDGEMENTS

This research project was supported by Altınbaş University Scientific Research Fund. Project Number: PB2020-ECZ-3.

AUTHOR CONTRIBUTIONS

Concept: Y.Y.Y., E.Ö.N.; Design: Y.Y.Y., E.Ö.N.; Control: Y.Y.Y., E.Ö.N.; Sources: Y.Y.Y., E.Ö.N.; Materials: Y.Y.Y., E.Ö.N.; Data Collection and/or Processing: Y.Y.Y., E.Ö.N.; Analysis and/or Interpretation: Y.Y.Y., E.Ö.N.; Literature Review: Y.Y.Y., E.Ö.N.; Manuscript Writing: Y.Y.Y., E.Ö.N.; Critical Review: Y.Y.Y., E.Ö.N.; Other: -

CONFLICT OF INTEREST

The authors stated that there are no conflicts of interest regarding the publication of this article.

ETHICS COMMITTEE APPROVAL

The authors declare that the ethics committee approval is not required for this study.

REFERENCES

1. Ďuračková, Z. (2010). Some current insights into oxidative stress. Physiological Research, 59(4), 459-469. [CrossRef]
2. Reuter, S., Gupta, S.C., Chaturvedi, M.M., Aggarwal, B.B. (2010). Oxidative stress, inflammation, and cancer: How are they linked? Free Radical Biology and Medicine, 49(11), 1603-1616. [CrossRef]
3. Kim, D., Baik, S.H., Kang, S., Cho, S.W., Bae, J., Cha, M.Y., Sailor, M.J., Mook-Jung, I., Ahn, K.H. (2016). Close correlation of monoamine oxidase activity with progress of Alzheimer's disease in mice, observed by in vivo two-photon imaging. ACS Centeral Science 2(12), 967-975. [CrossRef]
4. Kaduszkiewicz, H., van den Bussche, H. (2009). Acetylcholinesterase inhibitors and Alzheimer's disease, Encyclopedia of Neuroscience, 9-13. [CrossRef]
5. Petrovska, B.B. (2012). Historical review of medicinal plants' usage. Pharmacognosy Reviews, 6(11), 1-5. [CrossRef]
6. Vladimir-Knežević, S., Blažeković, B., Štefan, M.B., Alegro, A., Kőszegi, T., Petrik, J. (2011). Antioxidant activities and polyphenolic contents of three selected *Micromeria* species from Croatia. Molecules, 16(2), 1454-1470. [CrossRef]
7. Dirmenci, T. (2012). *Micromeria*. Bizimbitkiler. Published October 28, 2013. http://www.bizimbitkiler.org .tr. Accessed date: June 5, 2023.
8. Bentham, (2023). World Flora Online. Retrieved October 28, 2023. From http://www.worldfloraonline.org. Accessed date: June 5, 2023.
9. Bentham, (1982). *Micromeria*. In: Davis PH, ed. Flora of Turkey and the East Aegean Islands. Vol 7, 353-367.
10. Korkmaz, M., Karakurt, E. (2015). An ethnobotanical ınvestigation to determine plants used as folk medicine in Kelkit Gümüşhane/Turkey district. Biyolojik Çeşitlilik ve Koruma, 8(3), 290-303.
11. Bulut, G., Tuzlaci, E. (2008). Folk Medicinal Plants of Bayramiç (Çanakale-Turkey). Journal of Pharmacy of Istanbul University, 40, 87-99.

12. Everest, A., Ozturk, E. (2005). Focusing on the ethnobotanical uses of plants in Mersin and Adana provinces (Turkey). Journal of Ethnobiology and Ethnomedicine, 1, 1-6. [CrossRef]
13. Sargin, S.A. (2015). Ethnobotanical survey of medicinal plants in Bozyazı district of Mersin, Turkey. Journal of Ethnopharmacology, 173, 105-126. [CrossRef]
14. Nath, E.Ö., Kültür, Ş. (2022). An ethnobotanical study of medicinal plants In Savaştepe (Balıkesir-Turkey). Clinical and Experimental Health Sciences, 12(4), 954-980. [CrossRef]
15. Marin, P.D., Grayer, R.J., Veitch, N.C., Kite, G.C., Harborne, J.B. (2001). Acacetin glycosides as taxonomic markers in *Calamintha* and *Micromeria*. Phytochemistry, 58(6), 943-947. [CrossRef]
16. Tomas-Barberan, F.A., Gil, M.I., Marin, P.D., Tomas-Lorente, F. (1991). Flavonoids from some Yugoslavian *Micromeria* species: Chemotaxonomical aspects. Biochemical Systematics and Ecology, 19(8), 697-698. [CrossRef]
17. Küpeli Akkol, E., Gürağaç Dereli, F.T., Ilhan, M. (2019). Assessment of antidepressant effect of the aerial parts of *Micromeria myrtifolia* Boiss. & Hohen on mice. Molecules, 24(10), 1869. [CrossRef]
18. Özek, T., Kirimer, N., Baser, K.H.C. (1992). Composition of the essential oil of *Micromeria myrtifolia* Boiss. et Hohen. Journal of Essential Oil Research, 4(1), 79-80. [CrossRef]
19. Formisano, C., Oliviero, F., Rigano, D., Saab, A.M., Senatore, F. (2014). Chemical composition of essential oils and *in vitro* antioxidant properties of extracts and essential oils of *Calamintha origanifolia* and *Micromeria myrtifolia*, two Lamiaceae from the Lebanon flora. Industrial Crops and Products, 62, 405-411. [CrossRef]
20. El-Seedi, H.R., Khattab, A., Gaara, A.H., Mohamed, T.K., Hassan, N.A., El-kattan, A.E. (2008). Essential oil analysis of *Micromeria nubigena* HBK and its antimicrobial activity. Journal of Essential Oil Research, 20(5), 452-456. [CrossRef]
21. Duru, M.E., Öztürk, M., Uğur, A., Ceylan, Ö. (2004). The constituents of essential oil and *in vitro* antimicrobial activity of *Micromeria cilicica* from Turkey. Journal of Ethnopharmacology, 94(1), 43-48. [CrossRef]
22. Abu-Gharbieh, E., Ahmed, N.G. (2016). Bioactive content, hepatoprotective and antioxidant activities of whole plant extract of *Micromeria fruticosa* (L) Druce ssp *serpyllifolia* F Lamiaceae against carbon tetrachloride-induced hepatotoxicity in mice. Tropical Journal of Pharmaceutical Research, 15(10), 2099-2106. [CrossRef]
23. Koc, K., Ozdemir, O., Kizilkaya, F.O., Sengul, M., Turkez, H. (2017). Cytotoxic activity of the aqueous extract of *Micromeria fruticosa* (L.) Druce subsp. *serpyllifolia* on human U-87 MG cell lines. Archives of Biological Sciences, 69(3), 449-453. [CrossRef]
24. Formisano, C., Oliviero, F., Rigano, D., Saab, A.M., Senatore, F. (2014). Chemical composition of essential oils and *in vitro* antioxidant properties of extracts and essential oils of *Calamintha origanifolia* and *Micromeria myrtifolia*, two Lamiaceae from the Lebanon flora. Industrial Crops and Products, 62, 405-411. [CrossRef]
25. Brand-Williams, W., Cuvelier, M.E., Berset, C.L.W.T. (1995). Use of a free radical method to evaluate antioxidant activity. LWT-Food Science and Technology, 28(1), 25-30. [CrossRef]
26. Slinkard, K., Singleton, V.L. (1977). Total phenol analysis: Automation and comparison with manual methods. American Journal of Enology and Viticulture, 28(1), 49-55. [CrossRef]
27. Ellman, G.L., Courtney, K.D., Andres Jr, V., Featherstone, R.M. (1961). A new and rapid colorimetric determination of acetylcholinesterase activity. Biochemical Pharmacology, 7(2), 88-95. [CrossRef]
28. Krajl, M. (1965). A rapid microfluorimetric determination of monoamine oxidase. Biochemical Pharmacology, 14(11), 1684-1685. [CrossRef]
29. Urban, P., Andersen, J.K., Hsu, H.P., Pompon, D. (1991). Comparative membrane locations and activities of human monoamine oxidases expressed in yeast. FEBS Letters, 286(1-2), 142-146. [CrossRef]
30. Sarikurkcu, C., Hanine, H., Sarikurkcu, R.B., Sarikurkcu, R.T., Amarowicz, R. (2020). *Micromeria myrtifolia*: The influence of the extracting solvents on phenolic composition and biological activity. Industrial Crops and Products, 145, 111923. [CrossRef]
31. El Hamaoui, B.A.S.S.E.M., Bakkour, Y., El-Omar, F. (2015). Chemical composition, antimicrobial and antioxidant activities of the ethanolic extract of *Micromeria fruticosa* growing in Lebanon. International Journal of Chemical Sciences, 13(1), 325-335.
32. Küpeli Akkol, E., Gürağaç Dereli, F.T., Ilhan, M. (2019). Assessment of antidepressant effect of the aerial parts of *Micromeria myrtifolia* Boiss. & Hohen on mice. Molecules, 24(10), 1869. [CrossRef]
33. Selleck Chemicals web site (2023). MAO-A Selective Inhibitors from https://www.selleckchem.com/sub units/MAO-A_MAO_selpan.html. Accessed date: 29.10.2023.