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Research Article

Antioxidant Capacity of *Micromeria fruticosa* L. Druce Methanol Extract

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ABSTRACT:
Micromeria species have various biological activities including antimicrobial, antibacterial, antifungal and antioxidant activities, and therefore it has been reported that they are used in traditional treatment in many areas. This study was carried out to investigate the antioxidant effects of *Micromeria fruticosa* L. Druce methanol extract obtained from the leaves of *Micromeria fruticosa* L. Druce plant. Extracts were prepared at the concentrations of 125, 250 and 500 µg/mL. Antioxidant activity of the samples were analyzed by FRAP (Iron ion reducing antioxidant power) and DPPH (1,1-diphenyl-2-picrylhydrazil) methods. Equivalent antioxidant capacity was determined by using different concentrations of 1-100 µg/mL of the reference samples. It was determined that the methanol extract of *Micromeria fruticosa* L. Druce plant exhibited antioxidant capacity according to FRAP and DPPH methods, thus further studies are needed to investigate therapeutic effects of this plant.

Keywords *:* Antioxidant, DPPH, FRAP, methanol extract, *Micromeria fruticosa* L. Druce.

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1. INTRODUCTION

Micromeria fruticosa L. Druce is a member of the Lamiaceae family. *Micromeria fruticosa* L. Druce is a Mediterranean herb with medicinal properties. *Micromeria fruticosa*, also called 'Taş nanesi' in Turkey, is a perennial herb that grows up to 20-60 cm high in the rocky regions of the southern and eastern Anatolian region of Turkey. *Micromeria* species are commonly used as herbal teas. Traditionally it is used in heart diseases, headaches, wounds and skin infections. *Micromeria* species have biological activities such as antimicrobial, antibacterial, antifungal and antioxidant, and are used as a sedative, anesthetic, antiseptic, abortifacient, antirheumatic, and in the treatment of colds [1-3]. The existence of protective mechanisms that are effective in reducing and treating oxidative stress may be crucial. Antioxidants are molecules that prevent the formation of radical groups in living metabolism and neutralize the formed radicals.

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Antioxidants are among the defense systems that resist the harmful effects of free radicals [4]. The antioxidant activity of methanol extract of *Micromeria fruticosa* L. Druce was determined using two antioxidant capacity assessment techniques (FRAP and DPPH).

2. MATERIALS & METHODS

2.1. Materials

Micromeria fruticosa (L.) Druce was collected from Uzundere district of Erzurum province in August 2022. Trolox was purchased from Fluka Chemica (Switzerland) and NH4Ac from Riedel De Haen (Germany). TPTZ (2,4,6-Tri(2-pyridyl)-s-triazine) and DPPH (1,1-diphenyl-2-picrylhydrazil) were obtained from Sigma Chemicals Co. (St. Louis, USA). $FeCl_3.6H_2O$ was purchased from Merck (Germany). Millipore (Direct-Q® 3UV, USA) was used to obtain ultrapure water. All of the other solvents and reagents were of the analytical grade.

2.2. Extraction

For the preparation of methanol extract, dried *Micromeria fruticosa* (L.) Druce leaf samples taken as representative of the leaves were ground into powder with the help of a laboratory blender. 250 mL of solvent was added to 50 g of dried herb and mixed in a horizontal shaker water bath for 72 hours (filtered every 24 hours) at 50 ^oC. The solvents were evaporated at 50°C with the aid of an evaporator and the extract was made dry. The extract was kept in dark and at 2-8 ^oC in an airtight bottle for further studies.

2.3. Determination of Antioxidant Capacity

2.3.1. FRAP method

When FRAP interacts with a potential antioxidant, it transforms a colorless Fe(III)-TPTZ complex into a bright blue Fe(II)-TPTZ complex. In this method proposed by Benzei and Strain, the total amount of antioxidants is evaluated by the reducing capacity of Fe(III) in acidic medium. The reduced Fe(II)-TPTZ complex has blue color and a maximum absorbance at 593 nm. Since the color change in the FRAP method indicates the presence of antioxidant substances, it is a method in which the antioxidant capacity is directly determined [5,6]. To obtain the FRAP solution, 10 mM solution of TPTZ (2,3,5-Triphenyltetrazolium chloride) in 40 mM HCl, 20 mM $FeCl_3$ solution and 300 mmol/L acetate buffer solution (pH 3.6) were prepared separately. A total of 30 mL of FRAP solution was obtained by taking 2.5 mL of TPTZ, 2.5 mL of $FeCl_3$ and 25 mL of acetate buffer from these prepared solutions. For the 96-well plate, 200 µL of FRAP solution and 10 µL of extract sample were added to each well. Absorbances were measured at 593 nm after 30 minutes of incubation.

2.3.2. DPPH method

The spectrophotometric method developed by Brand-Williams was used to determine the antioxidant capacity based on measuring the inhibition response of the samples against the DPPH radical [7] 39 mg of DPPH was dissolved in ethanol, and then the volume was made up to 100 mL to obtain DPPH solution. To the 96-well plate, 70 µL of DPPH solution

and 210 µL of the extract sample were added to each well. After shaking for about 1 minute, it was incubated in the dark for 30 minutes and then the absorbance at 517 nm was measured. Trolox was used as the standard antioxidant for the control sample. The sign of the presence of an antioxidant in the DPPH solution is the decrease in its color, the decrease in the color intensity facilitates the measurement in the spectrophotometer. This test has advantages such as ease of laboratory work, low cost, repeatability, automation options and applicability at room temperature [8].

3. RESULTS & DISCUSSION

3.1. Antioxidant Capacity Findings

3.1.1. Findings of iron ion reducing antioxidant power (FRAP)

The absorbance values corresponding to the iron (III) reducing/antioxidant power at 595 nm of the methanol extract prepared from the leaves of *Micromeria fruticosa* (L.) Druce plant and standard antioxidant compounds were measured spectrophotometrically. The analyzed concentration range (1-100 µg/mL) was determined as a result of studies on standard antioxidant compounds. Trolox was used as the reference compound and results are shown as Trolox equivalent. Trolox solutions were prepared in various concentrations (range 1-100 µg/mL), and by measuring their absorbance, the calibration line and the line equation were obtained.

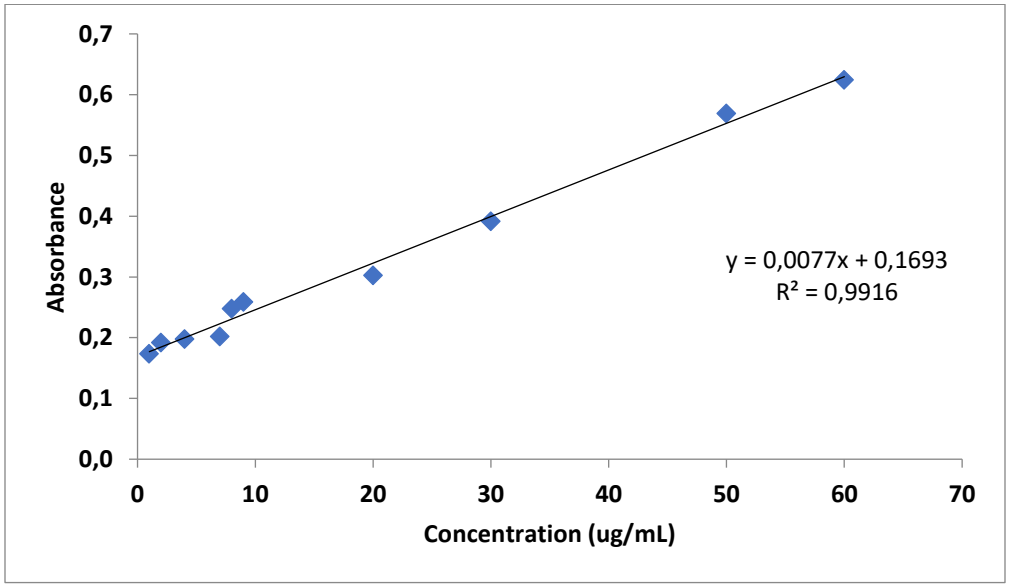

Figure 1. The calibration curve for the FRAP method.

The comparison of iron (III) reducing/antioxidant powers at 593 nm with spectrophotometric method at 125, 250 and 500 µg/mL concentrations in terms of µg Trolox equivalent Antioxidant Capacity (TEAC) is shown in Table 1.

Table 1. Antioxidant capacities of *Micromeria fruticosa* (L.) Druce methanol extract as measured by FRAP method.

Concentration (ug/mL)	Trolox (Eq µg/mL)
125	3,038
250	11,264
500	17,519

3.2. DPPH radical scavenging activity

DPPH radical scavenging activity determinations of methanol extract prepared from leaves of *Micromeria fruticosa* (L.) Druce plant and standard antioxidant compounds were performed. Trolox was used as the reference compound and results are shown as Trolox equivalent. Trolox solutions were prepared in the concentration range of 1-100 µg/mL and by measuring their absorbance, the calibration line and the line equation were obtained.

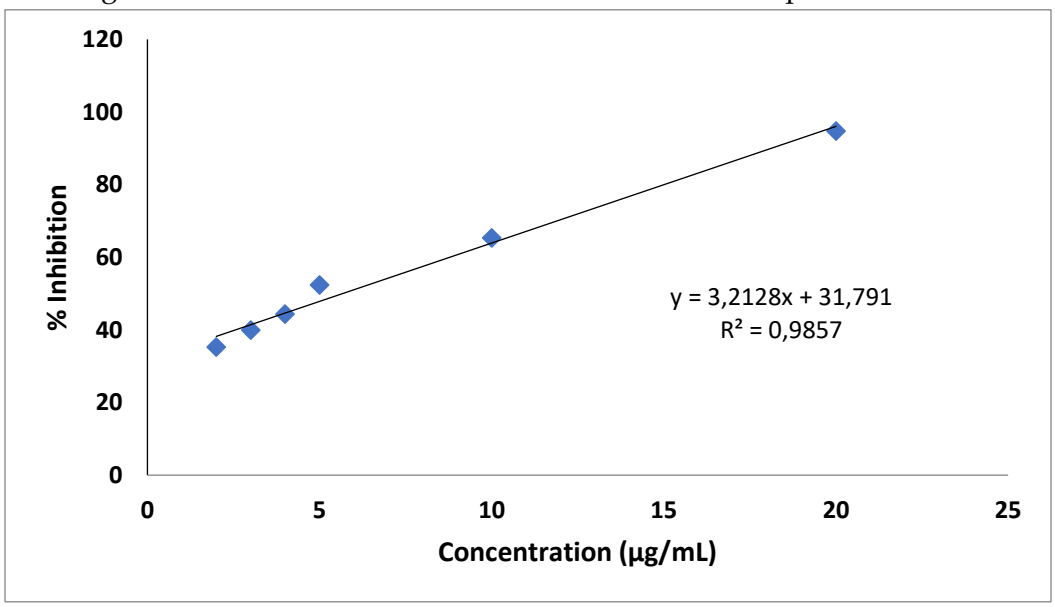

Figure 2. The obtained calibration curve for the DPPH method.

The DPPH radical scavenging capacities of the methanol extract prepared from the leaves of *Micromeria fruticosa* (L.) Druce plant at 125, 250 and 500 µg/mL concentrations are shown as % inhibition (Table 2). It was determined that the concentration with the highest DPPH free radical scavenging capacity from the methanol extract prepared from the leaves of *Micromeria fruticosa* (L.) Druce plant was 500 µg/mL.

Table 2. DPPH % Inhibition Values

Concentration (ug/mL)	% Inhibition
125	3,977
250	9,670
500	12,834

4. CONCLUSIONS

Micromeria fruticosa (L.) Druce extract has high antioxidant capacity, thus, the extracts can used as antioxidant in pharmaceutical preparations. It is thought that this study will contribute to the studies on *Micromeria fruticosa* (L.) Druce.

Conflict of Interest

The authors declare that there is no conflict of interest.

Author Contributions

The authors declare that they have contributed equally to the article.

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