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# Cytotoxic activity of the aqueous extract of *Micromeria fruticosa* (L.) Druce subsp. *serpyllifolia* on human U-87 MG cell lines

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**Abstract:** *Micromeria fruticosa* (L.) Druce subsp. *serpyllifolia*, which is widely used in folk medicine as a medicinal herbal tea, is grown in different areas of Turkey and the Mediterranean region. The present study was conducted to evaluate the aqueous extract of *Micromeria fruticosa* subsp. *serpyllifolia* for its antioxidant and antiproliferative activity on a human glioblastoma multiforme cell line (U-87 MG), which has not been reported before. Here, the extract was added to cultures at 8 different concentrations (0-200 μg/mL). Cell viability and cell membrane damage was determined using the MTT and LDH assays for 48 h, respectively. To examine the oxidative effects, total antioxidant capacity (TAC) and total oxidant status (TOS) levels were measured. The extract displayed considerable antiproliferative activities at the high concentrations of 175 and 200 μg/mL. Furthermore, the extract caused a significant increase in the release of the lactate dehydrogenase (LDH) enzyme in a concentration-dependent manner; 200 μg/mL of extract enhanced the release of LDH. Treatments with extract at higher doses increased TOS levels and decreased TAC levels in human U-87 MG cells. Our study suggests that the aqueous extract of *Micromeria fruticosa* ssp. *serpyllifolia* was capable of inducing growth inhibition of cancer cells. These results encourage further research to assess the value of the extract in modern phytotherapy.

**Key words:** *Micromeria fruticosa* ssp. *serpyllifolia*; aqueous extract; anticancer activity; U-87 MG cell line; oxidative stress

## INTRODUCTION

The genus *Micromeria*, a member of the Lamiaceae family, is perennial or dwarf shrubs that usually grow in warm rocky and in dry open habitats [1]. *Micromeria* spp. is distributed from the Himalayan region to the Macaronesian Archipelago and from the Mediterranean to South Africa and Madagascar, some of which are endemic [2,3]. Several species in this genus are widely used in traditional herbal medicine to treat a variety of diseases and ailments [4-13]. A number of *Micromeria* species are also used for their insecticidal, herbicidal and culinary properties [14-16].

*Micromeria fruticosa* (L.) Druce., also called as White Micromeria, is a perennial aromatic herb found in Turkey and other eastern Mediterranean countries [8,17]. In Turkey, *M. fruticosa* has four subspecies, one of which is *Micromeria fruticosa* (L.) Druce subsp. *serpyllifolia*, growing naturally in clefts of fissured rocks in northern Turkey, and traditionally used as medicinal herbal tea and spice with names such as "taş nanesi" by local citizens [18,19]. A few studies have been made into the biologic activity of *M. fruticosa* ssp. *serpyllifolia* [8,20-23]. The aim of the present work was therefore to evaluate the antioxidant and antiproliferative activities of extracts of the aerial parts of *M. fruticosa* ssp. *serpyllifolia* on the human glioblastoma multiforme cell line (U-87 MG), which is the most common and aggressive form of malignant brain tumor and generally refractory to treatment with current therapeutic modalities [24]. In the current study, the U-87 MG cell line was chosen to investigate the anticancer effects of the mentioned plant species since its usefulness in cancer research has been demonstrated, with about 1700 studies over four decades [25].

## MATERIALS AND METHODS

### Plant material and extraction

*Micromeria fruticosa* ssp. *serpyllifolia* was collected at the full flowering stage in Tortum in northeast Turkey in 2015. The plant was identified by Dr. Meryem Şengül, Ataturk University. One hundred g of the aerial parts of the plant were infused in 1 L of hot water for 15 min. The initial temperature of the added water was 98°C. Infusions were kept at room temperature without additional heating. After the infusion cooled, the liquate of aqueous extract was carefully decanted away from the residual solids and diluted at different concentrations (0, 25, 50, 75, 100, 125, 150, 175, and 200 μg/ml).

### Cell line

The U-87 MG (glioblastoma multiforme) cell line used in this study was obtained from the American Type Culture Collection (ATCC, Rockville MD). U-87 MG cells were grown in DMEM supplemented with 100 IU/mL penicillin, 100 μg/mL streptomycin, 2 mM L-glutamine, 1 mM sodium pyruvate, 0.1 mM non-essential amino acids and 10% heat-inactivated fetal bovine serum.

### Cell viability assay

Viability of the cells was studied using an MTT Cell Proliferation Assay kit (Sigma Chemical Company, St. Louis, USA). The MTT assay, a simple and reliable technique that measures cell viability, can be used for the screening of antiproliferative agents. The aqueous extract from *M. fruticosa* ssp. *serpyllifolia* was applied in a range of concentrations from 0 to 200 μg/mL. Cells were plated in a 96-well plate ($5x10^3$ cells/well) and incubated for 48 h. Following treatment, the MTT stock solution (5 mg/mL in phosphate buffered saline, PBS) was added to the medium, and incubated with cells at 37°C for 3 h to allow cell-mediated reduction of MTT. The absorbance was measured at 570 nm in a microtiter plate reader. The viability was determined as the percentage of absorbance of *M. fruticosa* ssp. *serpyllifolia* extract-treated cultures compared with those of untreated control cultures. The experiment used as a positive control cells treated with the DNA crosslinker mitomycin C (MMC: $10^{-7}$ M, Sigma-Aldrich®), which is used as a chemotherapeutic agent because of its antitumor activity. Cells without extracts of *M. fruticosa* ssp. *serpyllifolia* served as negative controls

### Lactate dehydrogenase (LDH) release assay

Cytotoxicity was assessed by measuring the amount of LDH released from the cells following treatment with the aqueous extract of *M. fruticosa* ssp. *serpyllifolia*. U-87 MG cells were treated with extracts for 48 h and the LDH released from damaged cells in culture medium was quantified by using a kit (Cayman Chemical Company, USA). The rate of NAD reduction, which is directly proportional to LDH activity, was measured as an increase in absorbance at 490 nm.

### TAC and TOS analysis

Automated colorimetric assays are frequently used to determine oxidative alteration, and the major advantage of the assays is their ability to measure the antioxidant capacity of all antioxidants in a biological sample and not just the antioxidant capacity of a single compound [26]. The automated Trolox equivalent antioxidant capacity (TAC) and total oxidant status (TOS) assays were carried out in cellular media using commercially available kits (Rel Assay Diagnostics®, Gaziantep, Turkey).

### Statistical analysis

Statistical analysis was performed using SPSS software (version 18.0, SPSS, Chicago, IL, USA). Duncan's test was used to determine whether any treatment significantly differed from controls or each other. Statistical decisions were made with a significance level of 0.05.

## RESULTS AND DISCUSSION

### Effects of the aqueous extract of aerial parts of *M. fruticosa* ssp. *serpyllifolia* on U-87MG cells

In the present study, the potential *in vitro* cytotoxicity of *M. fruticosa* ssp. *serpyllifolia* aqueous extracts was screened against the U-87 MG cell line for 48 h by MTT bioassay. Fig. 1 shows the effects of increas-

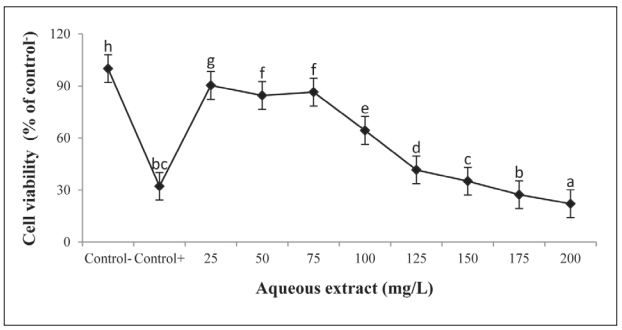


**Fig. 1.** Viability of U-87 MG cells exposed to *Micromeria fruticosa* ssp. *serpyllifolia* aqueous extract for 48 h. Cell viabilities are shown as percentages; the untreated cells were considered as 100% viable. Control$^-$ – negative control, cultured with no treatment. Control$^+$ – positive control, incubated with mitomycin C ($10^{-7}$ M). Different letters in the figure indicate statistically significant differences (P<0.05). Means±SD values followed by the same letter are not significantly different based on 95% confidence limits (P>0.05).

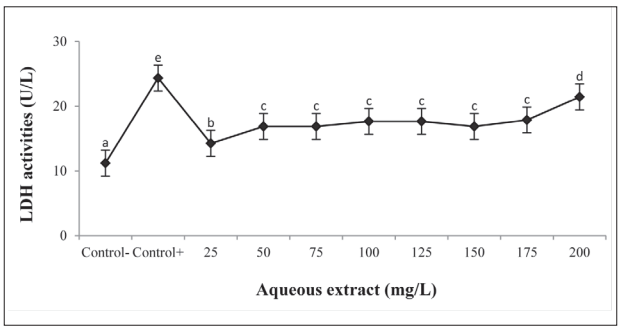


**Fig. 2.** Level of lactate dehydrogenase (LDH) in human U-87 MG cells maintained for 48 h in the presence of different concentrations of aqueous extracts from *Micromeria fruticosa* ssp. *serpyllifolia*. Control$^-$ – negative control, cultured with no treatment. Control$^+$ – positive control, incubated with mitomycin C ($10^{-7}$ M). Different letters in the figure indicate statistically significant differences (P<0.05). Means±SD values followed by the same letter are not significantly different based on 95% confidence limits (P>0.05).

ing amounts of *M. fruticosa* ssp. *serpyllifolia* aqueous extract on the proliferation of U-87 MG cells. The extracts inhibited U-87 MG cell growth in a concentration-dependent manner, although there were no significant differences between the three lowest concentrations (25, 50 and 75 μg/mL). It was found that with high concentrations of 175 and 200 μg/mL, considerable antiproliferative activities were observed when compared to the positive control.

The potential cytotoxic effect of aqueous extracts was also investigated using the LDH release assay. As shown in Fig. 2, the release of LDH was significantly enhanced in all the aqueous extract-treated groups in a concentration-dependent manner. Moreover, the highest concentration (200 μg/ml) showed a remarkable cytotoxic effect on U-87 MG cells.

## Level of oxidative stress

The effect of *M. fruticosa* ssp. *serpyllifolia* aqueous extracts on antioxidant/oxidant status of the human U-87 MG cell line determined by TAC and TOS analysis is presented in Figs. 3 and 4. As presented in Fig. 3, the TAC levels significantly decreased in all samples tested with the aqueous extract in a concentration-dependent manner. Similarly to the dose-response in TAC levels, a significant increase in TOS levels was observed in cells treated with high concentrations of aqueous extracts. Fig. 4 indicates that the last four

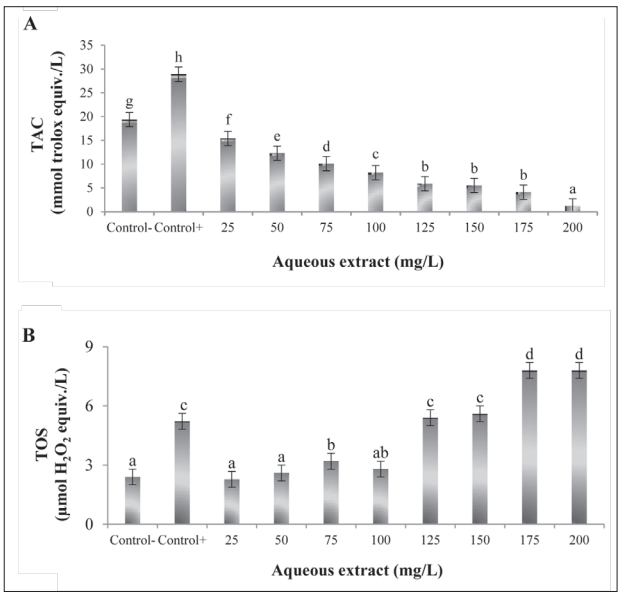


**Fig. 3.** The effects of aqueous extracts from *Micromeria fruticosa* ssp. *serpyllifolia* on the redox state of human U-87 MG cells. **A** – The effects of aqueous extracts from *Micromeria fruticosa* ssp. *serpyllifolia* on TAC levels in human U-87 MG cells. Positive control – incubated with ascorbic acid (10 μM) for TAC analysis. **B** – The effects of aqueous extracts from *Micromeria fruticosa* ssp. *serpyllifolia* on TOS levels in human U-87 MG cells. Positive control – hydrogen peroxide ($H_2O_2$, 25 μM) for TOS analysis. Different letters in the figure indicate statistically significant differences (P<0.05). Means±SD values followed by the same letter are not significantly different based on 95% confidence limits (P>0.05).

concentrations of the aqueous extract (125, 150, 175 and 200 μg/mL) caused significant increases in TOS level when compared with the positive control value.

*Micromeria fruticosa* is described as a diaphoretic, laxative, sedative, analgesic and antiseptic that is generally used to treat a variety of respiratory and digestive diseases in folk herbal medicine of the eastern Mediterranean region [12,13]. The plant is usually consumed in the form of infusions or decoctions of the aerial parts [27-29]. In laboratory investigations, it has been also shown that the essential oil and extracts of *M. fruticosa* possess pharmacological properties with antiinflammatory, antimicrobial and antifungal activities [20,21,30,31]. Recent research has shown that these properties are partly due to the pulegone; *M. fruticosa* is a rich source of essential oil contents comprised mainly of (+)-pulegone, but also isomenthol, isomenthone, limonene, menthol, α-pinene, β-pinene, piperitone, piperitenone oxide and the sesquiterpenes b-caryophyllene and germacrene D [8,17,32]. Indeed, (+)-Pulegone is known to be a hepatotoxic, neurotoxic and analgesic agent [33-35].

Despite research on the bioactivity of *M. fruticosa* ssp. *serpyllifolia*, the antitumor activity of its aqueous extract has only been investigated in one study so far [23]. Therefore, in the present study we aimed to determine whether the aqueous extract of its aerial parts exhibits *in vitro* cytotoxic activity on cultured human glioblastoma multiforme cells. Our results clearly showed that the aqueous extract of *M. fruticosa* ssp. *serpyllifolia* could induce growth inhibition and oxidative stress in brain cancer cells. As presented in Fig. 1, the loss of cell viability that occurred with the high concentrations of aqueous extract were much more pronounced than the effects observed in smaller concentrations on U-87 MG cells for 48 h. Similarly, concentration-dependent changes in LDH release and TOS levels were observed in *M. fruticosa* ssp. *serpyllifolia* extract-treated U-87 MG cells (Figs. 2 and 4). A linear correlation between these two events was also found. It is suggested that the aqueous extract of *M. fruticosa* leads to cell inhibition through oxidative stress. Among the concentrations of aqueous extract analyzed, the most active was that of 200 μg/mL for all assays used in the present study. Small doses of aqueous extract showed weaker cytotoxicity to the tested cells.

Recently, it was found that the aqueous extract of *M. fruticosa* ssp. *serpyllifolia* possesses antiinflammatory and gastroprotective activities [22]. In addition, both the oil and the aqueous extract of the *M. fruticosa* ssp. *serpyllifolia* have been indicated to exhibit antitumor activities against human colon tumor (HCT) and mammary carcinoma F7 (MCF7) cells [23]. Hence, our findings are in accordance with previous reports on antiproliferative features, since our analyses suggest that treatment with the aqueous extract effectively suppressed the generation potential of brain cancer cells.

The cytotoxic and antiproliferative activities of the aqueous extracts toward the human U-87 MG cell line could also be related to some synergistic or antagonistic interactions of the many compounds present in the whole extract. Nonetheless, further investigations are required to determine the specific components within *M. fruticosa* ssp. *serpyllifolia* that may have cytotoxic activity. There is an upsurge in the use of natural products in drug discovery. The healing properties of *M. fruticosa* ssp. *serpyllifolia* which have been accepted by some cultures have been justified through laboratory investigations. Thus, the present study allows for new perspectives on the use of *M. fruticosa* ssp. *serpyllifolia* in situations involving oxidative stress and cell proliferation.

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**Conflict of interest disclosure:** The authors declare that there are no conflicts of interest.

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