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ANTIMUTAGENIC AND ANTIOXIDANT ACTIVITIES OF TEUCRIUM MULTICAULE AND ITS CYTOTOXIC EFFECT ON MURINE LR7 CELL

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ABSTRACT. In present study, antimutagenic, antioxidant and cytotoxic effects of *Teucrium multicaule* Montbret Et Aucher Ex Bentham methanol extract (TME) was investigated. Cytotoxic effects and antimutagenic and antioxidant activities of TME were determined by MTT, DNA protecting, DPPH scavenging methods, respectively. As a result, high dose of TME exhibited a cytotoxic effect on murine LR7 cells. TME also showed a strong antimutagenic activity in DNA protection test system, it exhibited modest activity in DPPH test system. Consequently, it may be used as natural agent for antioxidant and antimutagenic properties.

1. Introduction

Traditional medicine practices are common in many parts of the world [1-3]. Being used as medicinal plants family, the genus *Teucrium* contain about 300 species worldwide [4], 27 of which is spread in Turkey flora [5]. The main distribution area of the genus *Teucrium* is not only the Mediterranean, but also it has a high spread in other continents [6-8]. *Teucrium* species have many biological activities including antioxidant, antimicrobial [9], antitumor [10], and DNA protecting [11].

Teucrium multicaule Montbret & Aucher ex Benth. is perennial and suffruticose form. Stems 12-40 cm, many, erect or ascending, pubescent. Inflorescence laxly racemose. *T. multicaule* spreads between 500-1600 m altitude, and blooms between April and July [5].
The information on the biological activities of *T. multicaule* is very limited. According to our literature review, there is no study about the antioxidant and antimutagenic activities and cytotoxic effects of *T. multicaule*. Therefore, in this

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study, antioxidant activity in DPPH system, DNA protecting activity against to hydroxyl radical and cytotoxicity effect on murine LR7 cells of *T. multicaule* were determined. Besides, some of the phenolic compounds of *T. multicaule* used were identified by LC-MS-MS.

2. Materials and Methods

2.1. Collection and extraction of T. multicaule

Collection of T. multicaule and extraction were performed as previously described [12]. Briefly, leaf and flower materials were air-dried and standard Soxhlet isolation procedure was followed.

2.2. Determination of DPPH scavenging activity

DPPH scavenging activity of TME was determined according to the method applied by [13].

2.3. DNA protecting activity of TME against hydroxyl radical

DNA protection activities of TME were detected using the pBR322 supercoiled DNA. Standard solutions were prepared at 25, 50, 100, and 200 µg/mL ratios of the extract. First, 0.5 µg of plasmid pBR322 supercoiled DNA was put into the Eppendorf tubes, then, 10 µL of the standard extracts solutions were added into tubes. Also, 10 µL of Fenton's agent (30 mM H2O2, 50 µM ascorbic acid, and 80 µM FeCl3) was added into the prepared solution. The tubes incubated for 10 minutes at the room temperature. The final volume of the mixture was prepared to be 20mL and incubated for 30 minutes at 37 °C. Then, the DNA was analyzed by electrophoresis on 1% agarose gel containing ethidium bromide [14].

2.4. Cytotoxic effect of the TME on Murine LR7 cells

The viability of LR7 was determined by MTT (3- [4,5- dimethylthiazol- 2- yl]- 2,5- diphenyl- tetrazolium bromide) method. Briefly, different doses of TME (25, 50, 100, and 200 µg/mL doses) was applied on LR7 cells (1 × 106 mL) and incubated for 24 hours. After, cells were incubated with 1 mg/mL MTT for 45 minutes at 37 °C. Later, MTT was removed, and dimethyl sulfoxide (DMSO) was used to dissolve the dyes absorbed. Then, the absorbance for each well was measured at 570 nm in

an EZ Read 400 Microplate Reader (Biochrom, Cambridge, UK). The experiments were repeated three times.

2.5. Photochemical screening by LC-MS/MS

The sample was prepared for phytochemical analysis of TME by dissolving in methanol and filtering through a 0.22-μM filter. The separation of phenolic was performed with a LC-MS-MS apparatus of Nexera UHPLC (Shimadzu) with two LC-20AD pumps, DGU-20A3R degasser, CTO-10ASVP column furnace, and SIL-20AC auto sampler. Besides, C18 Intersil ODS-4 analytical colon (3.0mm x 100mm, 2μm) was used. The injection volume was 2 μL and flow rate 0.3 mL/min. Mobile phase A (water and 0.1% formic acid) and mobile phase B (methanol and 0.1% formic acid) were used in a linear gradient flow and the initial column temperature was set to 40 °C.

2.6. Statistical Analysis

The obtained data were determined as the mean ± standard deviation. SPSS version 22.0 software was used for statistical analysis. Intergroup evaluations were performed by a one-way ANOVA and post-hoc Tukey test. In all the analyses value of $p<0.05$ was considered statistically significant.

3. Results

3.1. Determination of DPPH scavenging and DNA protecting activities

DPPH scavenging percentage of different concentration of TME is shown in Figure 1. DPPH scavenging activity was increased after extract application in a dose-dependent manner (Figure 1). The TME exhibited a moderate antioxidant activity. 2 mg/ml dose of TME showed the highest scavenging activity ($p<0.05$)(40.8%). Furthermore, DNA protection activity of TME against the hydroxyl radical on plasmid DNA pBR322 was shown figure 2. As can be seen figure 2, supercoiled pBR322 DNA was protected by the presence of the all extract concentrations when compared with the control group.

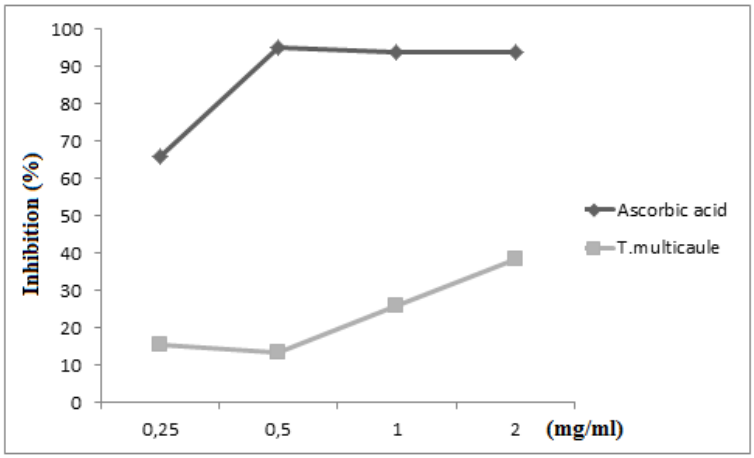

FIGURE 1. DPPH scavenging activity of TME.

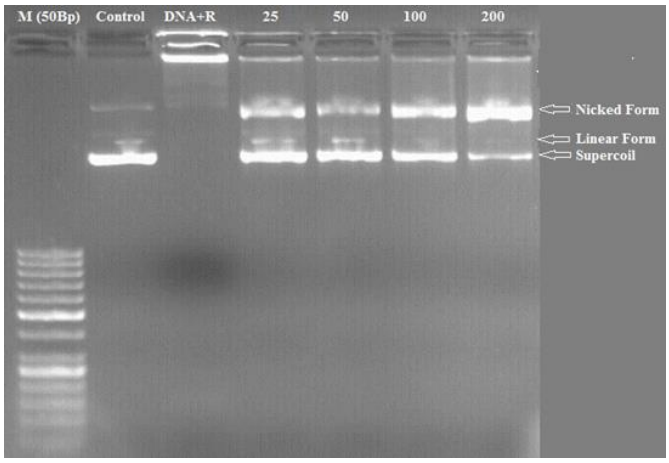

FIGURE 2. DNA protecting activity of TME. M: Marker, Negative control (DNA + R): pBR322 + OH radical, 25, 50, 100 and 200 µg / mL doses + OH radical.

3.2. Cytotoxic effects of TME on Murine LR7 cell

The murine LR7 cells were cultured in presence of the 25 to 200 µg/mL of TME at overnight (figure 3). It was observed that 25, 50, and 100 µg/mL of extracts did not

have an efficient cytotoxic effect on the cells, but the 200 µg/mL extract showed a significant cytotoxic effect ($p<0.05$).

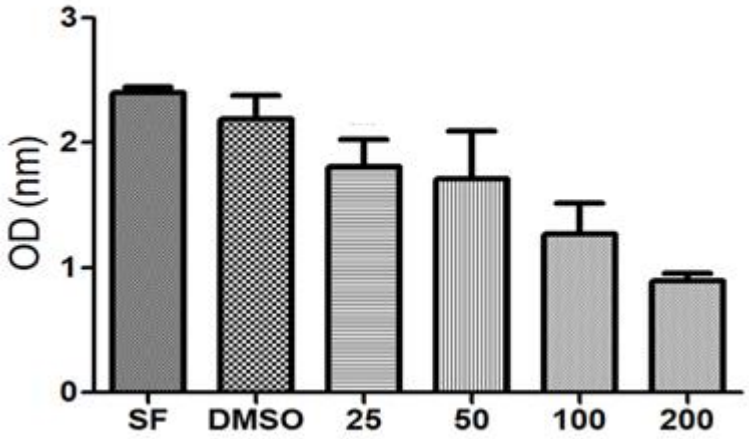

FIGURE 3. Effects of TME on cell viability of murine LR7 cells.

3.3. Determination of phenolic compounds of TME by LC-MS-MS

TME was screened to determine the phenolic compounds by LC-MS-MS. In total, 19 compounds were screened and only 13 were quantified. (Table 2). Among the identified phenolic compounds, it was found that TME contained the highest amount vanillic acid (50229.082 mg/kg) and Myrcetin (1005.257mg/kg). On the other hand, fumaric acid, gallic acid, ellagic acid, protocatechuic acid, kaempherol and thymokinone could not be detected in TME methanol extract.

TABLE 2. Compounds in TME by LC-MS-MS

	Compounds	**Amonts (mg/kg)**
1	Quercetine	12.78
2	Vanillic acid	50229.082
3	Resveratrol	212.608
4	Fumaric acid	0
5	Gallic acid	0
6	Caffeic acid	11.82
7	Phloridzindyhdrate	323.993
8	Oleuropein	77.631
9	Hidroxy cinnamic acid	418.114
10	Ellagic acid	0
11	Myrcetin	1005.257
12	Ptotocathecuic acid	0
13	Naringenin	0.857
14	Luteolin	11.858
15	Kaemperol	0
16	Thymoqunione	0
17	Alizarin	575.782
18	Hydroxybenzoic acid	5.486
19	Salicilic acid	3.972

4. DISCUSSION

In the present study, antimutagenic and antioxidant activities were determined by DNA protection and DPPH scavenging test systems, respectively. The DPPH method is one of the most practical methods used to determine the antioxidant activity of compounds. In the present study, different doses of TME showed a moderate antioxidant activity in a dose-dependent manner. The highest DPPH radical scavenging activity was observed at a dose of 2 mg/mL of extract. In previous studies, antioxidant activities of different species of *Teucrium* genus have been reported. *T. polium* exhibited strong antioxidant activity at low doses, while its petroleum ether and chloroform extracts exhibit low activity [15]. [16], reported that the methanol extracts of *T.* orientale collected during vegetative period showed strong DPPH scavenging activity.

DNA protecting activity was used as antimutagenic method. In this test, the hydroxyl radical formed as a result of the Fenton reactions cause fractures on DNA by

targeting the sugar-phosphate backbone [17]. If DNA is protected by an antioxidant molecule, the hydroxyl radical does not damage DNA. It was observed that in the DNA protecting activity test, the pBR322 was fragmented by the hydroxyl radical in the absence of TME, however, the harmful effect of hydroxyl radical on pBR322 was minimized at different concentrations of the TME. It can be said that our data is the first report to demonstrate in vitro DPPH scavenging and DNA protection activity of TME. In an *in vivo* study of ischemia-reperfusion conducted by [18], it was determined that *T. multicaule* reduce MDA level, as an indicator of tissue damage caused by radicals, and increased the level of GSH as an antioxidant compound.

Previous studies have also reported the effects of extracts of *Teucrium* species on different cells. For instance, [19] reported that *T. polium* water and ethanol extracts showed cytotoxic effects on human HepG2, A549, and HeLa cells. In addition to that, it reported that *T. polium* ethanol extract has cytotoxic effects on human A549, MCF7, BT20, and PC12 cells [20]. It was determined that neo-clerodane diterpenoid compounds obtained from *T. fruticans* did not show cytotoxic effects on U-2OS (human osteosarcoma cell line), NCI-H460 (human lung cancer cell line), and MCF-7 (human breast tumor cell line) cells. In our study, the cytotoxic effects of TME on murine LR7 cells were observed at a dose of 200 µg/mL.

Studies on the phytochemicals of TME are very limited. In a previous study, [21], identified germacrene D (13.2%), caryophyllene oxide, spathulenol, β-caryophyllene, and (6Z, 10Z)-pseudo phytol as major components among the 56 compounds in *T. multicaule* volatile oil. However, no studies on the phenolic compounds of TME have been found in the literature. In our study, TME was screened by LC-MS-MS for phenolic compounds. As shown in Table 2, 19 compounds were screened. It was found that vanillic acid and hydroxycinnamic acid among the phenolic acids, and myricetin, alizarin and resveratrol among the flavonoids, have the highest amounts among the determined compounds. On the other hand, fumaric, gallic, ellagic, and protocatechuic acid among phenolic acids and kaempferol and thymoquinone among flavonoids could not be detected.

In the light of the obtained data, it can be considered as an natural antioxidant and antimutagenic source due to it's the DNA protecting and DPPH radical scavenging activities and the determined phenolic compounds. Moreover, cytotoxic effect of TME on LR7 cells was found unimportantly.

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