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Determination of Some Antioxidant and Antiradical Properties of T. Polium Ethanol Extract

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ABSTRACT

T. polium, a member of the Lamiaceae family, is a traditional herb used in the treatment of various diseases. In this study, *in vitro* antioxidant and antiradical properties of *T. polium* ethanol extract collected in Ağrı-Patnos were investigated. For this purpose, total antioxidant activity according to ferric thiocyanate method, 2,2 Diphenyl 1 picrylhydrazyl (DPPH˙) radical scavenging activity, FRAP and CUPRAC reduction forces were used. BHA, Trolox and pirogallol study results, which are standard antioxidants, were compared with the results of the plant. According to the results of the study, it was determined that especially the total antioxidant capacity of *T. polium* ethanol extract was close to the standards, and the other antioxidant activities were lower than the standards.

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1. INTRODUCTION

Since ancient times, herbs have been used in the treatment of various diseases by extracting them in different ways. According to the World Health Organization, 70% of the population in developing countries consume these herbs as tea or spice for basic health care. Plants are considered to be natural sources with potential therapeutic effects [1].

The Lamiaceae family is a large family of plants that includes medicinal plant species. Teucrium genus, a member of this family, includes flowering, aromatic plant species with a height of 20-50 cm. *Teucrium* genus grows at an altitude of about 1000 meters and mostly in arid rocky regions of South West Asia, North Africa, the Mediterranean and Europe [2].

The *Teucrium* genus, which contains species with various therapeutic effects, has been reported to contain more than 300 species in previous studies. It is stated that this plant is antimutagenic, anticancer, hypoglycemic, antibacterial, anti-inflammatory, antipyretic and anti-ulcer, it is effective against hypolipidemic and diarrhea [2,3], it is also used for treatment against diabetes, rheumatism, inflammation and gastrointestinal disorders [4]. *T. polium*, which is from the Lamiaceae family, has been used for many years because it is good for cough and asthma ailments [2]. It has also been reported that *T. polium* is used in the treatment of different diseases such as gastrointestinal diseases (kidney and liver diseases, abdominal and intestinal pain), inflammation, eczema, urinary tract inflammation, diabetes and rheumatic diseases [1].

It has been reported that plants may differ both in size and content according to the geography and environmental conditions in which they grow, and thus the plants may have different therapeutic effects [5].

Because of their important biological activities, plants are one of the scientific research topics from past to present. In this study, it was aimed to elucidate some biological properties of *T. polium*. For this purpose, ethanol extracts were prepared from *T. polium* leaf parts and total antioxidant activity, DPPH˙ radical scavenging activity and finally iron and copper reduction activities were investigated.

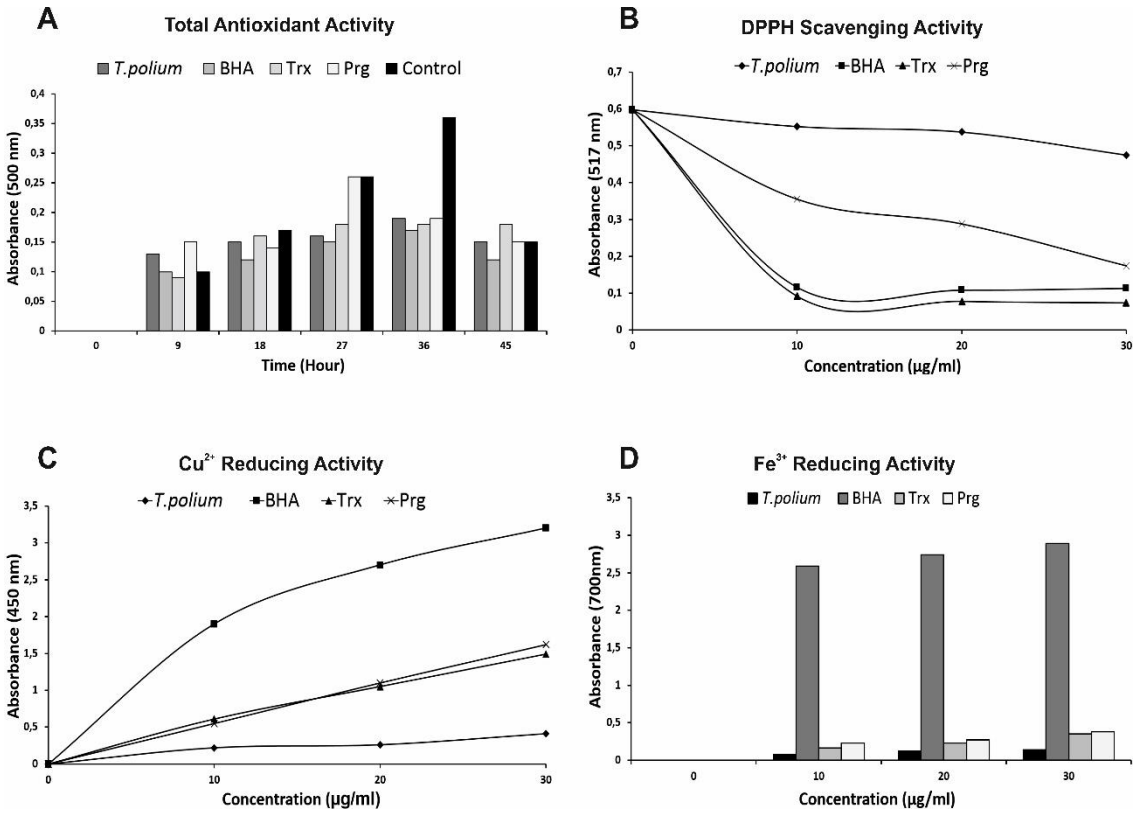

Figure 1. Antioxidant and antiradical activity results of *T.polium* and BHA, Trx, Prg at different concentrations.

2. MATERIAL AND METHODS

2.1. Preparation of extracts

T.polium was collected in Ağrı-Patnos (Altitude: 1800 m) and converted into herbarium material and stored in the Central Research Laboratory of Muş Alparslan University. It was mixed with ethanol (1:10) and stirred on a magnetic stirrer at room temperature for 24 hours. After incubation, ethanol was removed using the evaporator at 75ºC and the plant extract was obtained. *T.polium* was dissolved in ethanol at 1mg / ml and stock samples were prepared for antioxidant methods. Likewise, the antioxidant standard substances butylated hydroxyanisole (BHA), Trolox (Trx) and Pyrogallol (Prg) were dissolved in ethanol at 1mg / ml and prepared as stock for comparison.

2.2. Antioxidant Assays

2.2.1. Determination of total antioxidant

Total antioxidant studies were done according to the ferric thiocyanate method [6]. 10 µl, 20 µl and 30 µl of the stock solutions were taken into eppendorf tubes and the total volume was completed to 500 µl with phosphate buffer (pH: 7.4). 500 µl linoleic acid was added on it and left for incubation. Every 9 hours, 20 µl of the samples in the incubation was transferred to containers with 940 µl ethyl alcohol and 20 µl of $FeCl_2$ and NH_4SCN solutions were added on it. The absorbance was measured at 500 nm wavelength using a microvolume spectrophotometer. The experiment was terminated when the control reached the maximum absorbance.

2.2.2. DPPH˙ radical scavenging assay

Blois's DPPH˙ radical scavenging method [7] was used for antiradical studies. After transferring from the samples in different concentrations (10, 20, 30µl) to the Ependorf tubes, the total volume was completed to 600µl with ethanol. Then 200µl DPPH˙ radical solution was added on each. After 30 minutes of incubation, the absorbances of the samples were measured at 517 nm wavelength using a Microvolume Spectrophotometer. For control purposes 600µl ethanol and 200µl DPPH˙ radical solution were used.

2.2.3. Iron reduction power according to FRAP method

Total reduction power was determined according to Oyaizu method [8]. 10, 20 and 30µl of the stock solutions were taken into test tubes and the volume was completed to 200 µl with distilled water. Then, 500µl phosphate buffer (pH: 6.6) and 1% $[K_3Fe(CN)_6]$ were added to the test tubes. After 20 minutes of incubation at 50ºC, 500µl trichloracetic acid (TCA) was added to the mixture. 500µl was taken from the upper phase of the solution and 500µl of pure water and 100µl of $FeCl_3$ were added to the absorbance of the samples at 700 nm wavelength.

2.2.4. Copper reduction power according to CUPRAC method

Cu^{+2} reduction capacities of the samples were made according to the Cuprac method [9]. 250µl $CuCl_2$ was put into the test tubes. 250µl neocuprine solution and acetate buffer (pH: 6.5) were added on it. Then, different concentrations (10, 20, 30µl) of plant and standard samples were added and incubated for 30 minutes. After the incubation, the absorbances of the samples were measured at 450 nm wavelength using a Microvolume Spectrophotometer.

3. RESULTS AND DISCUSSİON

In this study, antioxidant and antiradical properties of *T.polium* ethanol extract were studied with different *in vitro* methods. According to the results of the total antioxidant study, it was determined that the lipid peroxides removal percentage of *T.polium* was very close to the standard antioxidants (Figure 1.A). In addition, it was observed that the total antioxidant activities of the samples generally increased due to the increase in concentration. Percentages of lipid peroxides removal of 30 mg / ml extract and standards at 36th hour were respectively as follows: BHA (52.70%) > Trx (50%) > *T.polium* = Prg (47.20%). When the DPPH˙ radical scavenging activities of the samples were examined, it was found that the activity increased as the concentration increased. While BHA showed the highest activity, it was determined that the radical scavenging activity of *T.polium* activity was lower than other standards (Figure 1.B). DPPH˙ radical scavenging activities of plants and standards at a concentration of 30mg/ml were in the following order: Trx (87.60%) > BHA (81.10%) > Prg (70.90%) > *T.polium* (20.70%). According to the results of FRAP and CUPRAC methods, while BHA showed the highest activity, *T.polium* extract did not show a significant activity. All samples showed better activity depending on the concentration increase (Figures 1.C and 1.D).

In a study conducted with extracts of *T.polium* prepared with different solvents, DPPH˙ radical scavenging activity of methanol extract was determined similar to BHT antioxidant. However, the activities of petroleum ether, chloroform and water extracts were recorded as very low compared to the standard (BHT). Similar measurements to DPPH˙ results were obtained in lipid peroxidation removal activities [10]. It has been reported that different extracts of *T.polium* species (methanol, ethyl acetate, petroleum ether, water) have important *in vitro* antioxidant Properties [11]. *T.polium* methanol extract has been reported to inhibit lipid peroxides [12]. In this study, the percentage of inhibition of lipid peroxides of the plant was close to the standards, while the radical scavenging activity was low compared to the standards. This may be because the solvent used is different from the solvents used in previous studies.In this study, while ethanol extract DPPH˙ radical scavenging activity was lower than the standards, the total antioxidant activity was very close to the standard antioxidants.

4. CONCLUSION

In this study, some antioxidant properties of *T.polium* ethanol extract were determined using different methods. The results were compared with the results of BHA, Trx and Prg antioxidants. In general, it was determined that the extract showed less activity than other antioxidants, but it had an activity close to standards in removing lipid peroxidation. The solvents used affect the antioxidant results. For this reason, it is planned to prepare different extracts of this plant and carry out other studies. It is thought that the results obtained from this study will make important contributions to the literature.

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