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Investigation of the effects of some plant varieties on tyrosinase activity and antioxidant properties

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Abstract

Free radicals and reactive oxygen derivatives that cause damage to cells are effectively reduced by antioxidants and converted into products with less toxicity. Antioxidants must be taken into the body continuously to maintain living health. The enzyme tyrosinase is an oxidase that catalyzes the oxidation of tyrosine in the synthesis of melanin, the pigment that gives color to the skin. Over or under-expression of the enzyme can cause various skin problems and neurodegenerative damage in living things. This study aimed to determine the antioxidant capacity of rosemary and green tea, two important plants that we consume in our daily life in meals and as herbal tea, by determining the tyrosinase enzyme inhibition effects, total phenolic substance content, and DPPH free radical scavenging activity. The natural extraction method is preferred. According to this method, green tea and rosemary plants were dried and extracted with pure water by brewing method. The rosemary plant inhibited the tyrosinase enzyme at 750 µg/ml concentrations by 75% and the green tea plant at 1000 µg/ml concentration by 77%. Total phenolic contents were determined by the Folin-Ciocalteu method. Green tea (186 mg/GAE) was found to have the highest phenolic content. Antioxidant capacities were determined by the DPPH(1,1-diphenyl-2-picryl-hydrazil) method, and their free radical scavenging effects were determined. Trolox and BHT were used as positive controls in the DPPH method. Green tea and rosemary showed the highest DPPH free radical scavenging activity at 1000 µg/ml concentration
Keywords: *Tyrosinase, Antioxidant, Phenolic Substance, Rosemary, Green Tea*

Bazı bitki çeşitlerinin tirozinaz aktivitesine etkileri ve antioksidan özelliklerinin incelenmesi

Öz

Hücrelerde hasar oluşturan serbest radikaller ve reaktif oksijen türevleri, antioksidanlar tarafından etkili şekilde indirgenerek daha az toksisitesi olan ürünlere dönüştürülür. Canlı sağlığının devamlılığı açısından antioksidanların sürekli olarak vücuda alınması gerekmektedir. Tirozinaz enzimi, deriye renk veren pigment olan melaninin sentezinde tirozinin oksidasyonunu katalizleyen bir oksidazdır. Enzimin çok ya da az sentezlenmesi canlılarda çeşitli cilt sorunlarına ve bazı nörodejeneratif hasarlara yol açabilmektedir. Bu çalışmada günlük hayatımızda yemeklerde ve bitki çayı olarak tükettiğimiz iki önemli bitkiden biberiye ve yeşil çayın tirozinaz enzimi inhibisyon etkilerini, içeriğindeki toplam fenolik madde miktarı ve DPPH serbest radikal giderimi aktivitesinin belirlenerek antioksidan kapasitesinin tespit edilmesi amaçlanmıştır. Doğal ekstraksiyon yöntemi tercih edilmiştir. Bu yönteme göre biberiye and yeşil çay bitkileri kurutularak demleme metoduyla saf su ile ekstrakte edilmiştir. Tirozinaz enzimini, biberiye bitkisi 750 µg/ml konsantrasyonda %75, yeşil çay bitkisi ise 1000 µg/ml konsantrasyonda % 77 oranında inhibe etmiştir. Toplam fenolik içerikleri Folin-Ciocalteu yöntemiyle belirlenmiştir. En yüksek fenolik içeriğe sahip olan yeşil çay (186 mg/GAE) bulunmuştur. Antioksidan kapasiteleri DPPH(1,1-difenil-2-pikril-hidrazil) metoduyla serbest radikal giderim etkileri belirlenmiştir. DPPH metodunda pozitif kontrol olarak Trolox ve BHT kullanıldı. En yüksek DPPH serbest radikal giderim aktivitesini yeşilçay ve biberiye 1000 µg/ml konsantrasyonda göstermiştir.
Anahtar kelimeler: Tirozinaz, Antioksidan, Fenolik Madde, Biberiye, Yeşil Çay

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1. Introductıon

Toxic substances known as prooxidants, which cause oxidative stress in the structure of oils, nucleic acids and proteins, can cause various diseases and pathological conditions. Free radicals can cause DNA and cell damage by causing the structure of proteins to break down [1]. Antioxidants are known as preservatives that prevent or delay the effects of these toxic substances, prooxidants. Free radicals and reactive oxygen derivatives that cause damage to cells are effectively reduced by antioxidants and converted into products with less toxicity. Antioxidants have a very important place in our lives for a healthy life and the smooth continuity of metabolic systems [2].

Enzymes are protein-structured substances that act as a biological catalyst in a chemical reaction, accelerate the reaction, and remain unchanged after the reaction. There are various substances that increase or limit enzyme activity in a chemical reaction. Substances that increase the activity of the enzyme are called activators, and substances that have the opposite effect are called inhibitors. Inhibition of enzymes is used to treat many diseases or to control undesirable enzyme activity for various conditions in pesticides. The mechanism of action of many existing drugs is based on this. For example, most drugs used for Alzheimer's disease try to increase the amount of acetylcholine that decreases in this disease by inhibiting the acetylcholinesterase enzyme. In another example of inhibitor, It is aimed to treat diseases such as abnormal pigmentations on the skin, vitiligo and melasma, which are caused by suppression of the tyrosinase enzyme [3,4].

The tyrosinase enzyme is an oxidase that catalyzes the oxidation of tyrosine in the synthesis reaction of the substance that gives color to the skin, known as the melanin pigment in living things [5]. In the synthesis of melanin pigment, if this synthesis is more or less, various diseases and disorders may occur in living things. While skin aging, vitiligo and melasma are some of these disorders in humans, disorders in this enzyme can also cause quality problems by causing darkening in plants and foods [6]. This abnormal synthesis of melanin causes aesthetic problems for both humans and food in general [7]. In addition, the formation of neuromelanin can cause neurodegeneration, which paves the way for serious diseases such as Parkinson's. Considering all these situations, new natural compounds that will reduce melanin formation, act as a tyrosinase inhibitor and counteract the harmful effects of synthetic inhibitors are widely researched [8,9].

Rosemary *(Rosmarinus officinalis L.)* is a perennial herb that grows naturally in the Mediterranean climate and can stay green in summer and winter [10]. It is frequently used in folk medicine for therapeutic purposes and as a flavouring for meals. It has a high antioxidant effect because it contains flavonoids, phenolic acids and triterpenes.

Research on rosemary has proven its effects in treating microbial diseases and enhancing memory. In addition, the effective studies of this plant on anticancer have attracted great interest in recent years [11].

Green tea (*Camellia sinensis)* is produced from the green shoots of tea containing various polyphenolic compounds. Green tea has an important place among the popular herbal protection methods against many diseases. Due to its high antioxidant properties, regular consumption of green tea prevents many diseases. It is counted among the benefits of improving brain functions and protecting against various cancers. It has been determined that the amino acid L-theanine in green tea, which can pass the blood-brain barrier, plays a role in the prevention of serious neurodegenerative diseases such as Parkinson's and Alzheimer's [12].

Today, natural resources have begun to be preferred in cosmetic and pharmaceutical products. Therefore, the importance of herbal, medicinal and aromatic plants is gradually increasing. The use of these plants in the treatment of various diseases has been known since ancient times. The use of these medicinal plants as medicine inhibits enzymes that cause diseases. The use of synthetic inhibitors in the tyrosinase enzyme is very limited. Therefore, there is always a need for natural tyrosinase inhibitors [13,14].

This study aims to identify new natural inhibitors of tyrosinase enzyme. For this purpose, rosemary *(Rosmarinus officinalis L.)* and green tea *(Camella sinensis)* plants were used. The inhibitory effects of these plants on the tyrosinase enzyme were investigated and the total phenol content and antioxidant properties of these plants were also determined.

2. Materıals And Methods

2.1. Chemicals Used

Rosemary *(Rosmarinus officinalis L.)* and green tea *(Camella sinensis)* plants used in the study were purchased from a local market in Sakarya. Tyrosinase (T3824-25 KU), L-DOPA, DPPH and other chemicals were obtained from Sigma-Aldrich.

2.2. Preparation of Plant Extracts

Rosemary and green tea plants were obtained from a local market in Sakarya. 2 g of the plants were weighed and 100 ml of distilled water at 100 ° C was added to them and left to infuse for 10 minutes. After the infusion process was finished, it was filtered through filter paper and the supernatant part was stored at 4 °C for use in experimental studies.

2.3. Tyrosinase Enzyme Inhibition

The enzyme inhibition method was modified and applied to plant extracts as in Yang et al.(2012)'s [15]. First of all, 100 µl of 0.1M pH:6.8 phosphate buffer solution, 20 µl of 250 U/ml tyrosinase solution and 20 µl of plant extracts (0, 10, 50, 250, 500, 1000 µg/ml) were mixed and added to the 96-well plate at 25°C for 10 min. Then, 20 µL of 3 mM L-DOPA substrate was added to these mixtures and incubated at 25°C for 30 min. The reaction absorbance was measured at 492nm. All experiments were carried out in 3 repetitions. Ascorbic acid was chosen as a positive control. Percent Inhibition of plant extracts was calculated with the following formula.

(%) = [(Acontrol-Plant) / Acontrol] x 100

Then, % relative activity was calculated and Ic_{50} values were determined. Ic_{50} is defined as the inhibitor concentration that halves the enzyme activity.

2.4. Determination of Total Phenolic Substance

Total phenolic content of plant samples was determined by the Folin-Ciocateu method [11]. 125 µl of 10% Folin-Ciocalteu reagent was mixed with 300 µL of plant extract and 1825 µl of distilled water and left for 5 minutes. After adding 250 µl of 20% sodium carbonate, it was kept for 30 minutes. The absorbance of the samples was measured at 650 nm. Experiments were carried out in 3 repetitions. A standard curve was created with gallic acid prepared at concentrations of 0, 10 50, 250, 500, 750 and 1000 µg'ml prepared with ethanol. The total phenolic content of the plant extracts was determined as gallic acid equivalent per dry weight of the extract (mg GAE/g dry extract weight).

2.5. DPPH Free Radical Removal Activity Analysis

Free radical scavenging activity was performed by changing the Blois method with 1,1-diphenyl-2-picrylhydrazil (DPPH) radical [12]. 1 ml of plant extract with concentrations of 50, 250, 500, 750 and 1000 µg/µl was taken and 4 ml of 0.1 mM DPPH solution was added. 1 ml of pure water and 4 ml of DPPH solution were used as control solution. As a standard, BHT and Trolox were prepared at the concentrations determined for the plants and the same amount of DPPH solution was added. The reaction tubes were incubated for 30 min at 25^{o}C and in the dark. The absorbance was then measured with a spectrophotometer at 517 nm. The free radical scavenging activity was calculated from the equation below.

DPPH Removal Activity :

(% inhibition)= ($A_{Control} - A_{sample}$)/$A_{Control}$ x100

$A_{Control}$ = control absorbance at 517 nm

A_{Sample} = sample absorbance at 517 nm

3. Results

3.1. Tyrosinase Enzyme Inhibition

Tyrosinase is a crucial enzyme both in the formation of melanin pigment in humans and in the browning reaction of plants. However, it causes disorders and diseases in these systems with its excessive work [18,19]. Therefore, many synthetic and natural inhibitors of the enzyme are currently in development. In this study, the inhibitory effects of infusing extracts of rosemary and green tea, which are widely used among the public, on the tyrosinase enzyme were investigated.

The results were presented in Table 1. According to the results, it was observed that rosemary plant extract inhibited the tyrosinase enzyme over 50% at 500 µg/ml and 750 µg/ml concentrations. Green tea extract inhibited the enzyme more than 50% at concentrations of 500 µg/ml, 750 µg/ml and 1000 µg/ml. Ascorbic acid used as a positive control inhibited the tyrosinase enzyme by 75% at 750 µg/ml. The inhibition rate was 77% at 1000 µg/ml, where green tea showed the highest inhibition. This rate was even higher than the highest inhibition of ascorbic acid used as a positive control. Rosemary and ascorbic acid showed 75% inhibition at 750 µg/ml concentration.

Table 1. % inhibition values of samples to tyrosinase enzyme activites

Concentration (µg/ml)	**Rosemary % inhibition**	**Green tea % inhibition**	**Ascorbic acid % inhibition**

10	25.68	10	17
50	32	15	22
100	38	22	30
250	44	35	42
500	63	52	60.7
750	75	65	75
1000	-	77	-

IC_{50} value, their % relative activities were calculated first. The concentration versus % relative graph was plotted and the inhibitor concentration that halved the enzyme activity was calculated from there. The results are given in Table 2.

Table 2. IC_{50} values of inhibitors

	rosemary	**green tea**	**ascorbic acid**
IC_{50} ((µg/ml)	334.4	538.1	391.64

3.2. Determination of Total Phenolic Substance

In this study, the total phenolic content of rosemary and green tea extracts was determined. Therefore, a standard graph of gallic acid was drawn as seen in Figure 1.

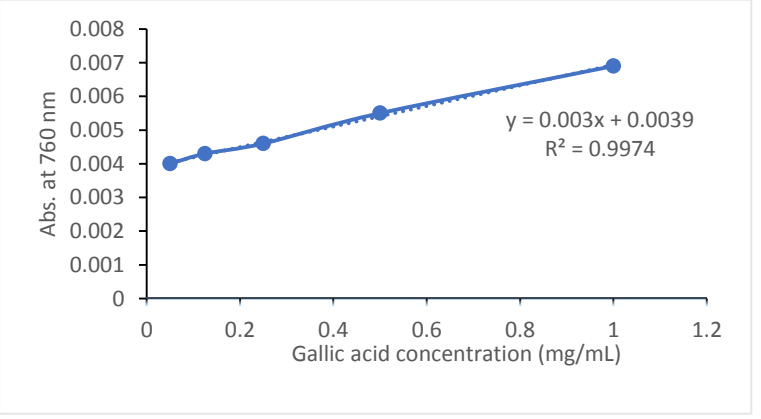

Figure 1. Gallic Acid Standard Curve

According to the results obtained from the plant extracts we used and the Figure 1 equation above, green tea with the highest total phenolic content (185 mg GAE/g) was determined. The total amount of phenol in our rosemary extract was calculated as 176 mg GAE/g. Total phenolic amounts of our extracts are shown in Figure 2.

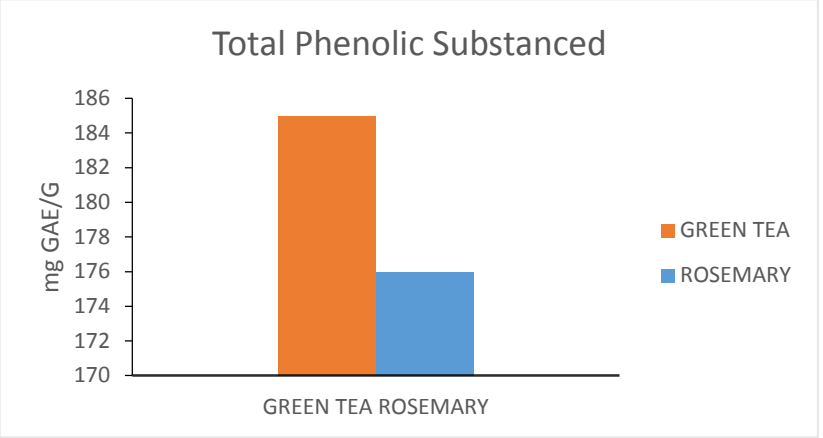

Figure 2. The average amount of phenolic compound content of extracts

3.3. DPPH Free Radical Removal Activity

One of the most widely used methods to determine the antioxidant properties of antioxidant compounds is the determination of DPPH free radical scavenging activity. In this study, DPPH free radical scavenging activities of water extracts of rosemary and green tea plants were determined. For DPPH free radical scavenging activity, samples were prepared at concentrations of 50, 250, 500, 750 and 1000 µg/µl and absorbance was measured at 517 nm. the results are given in graphic form in Figure 3 and Table 3.

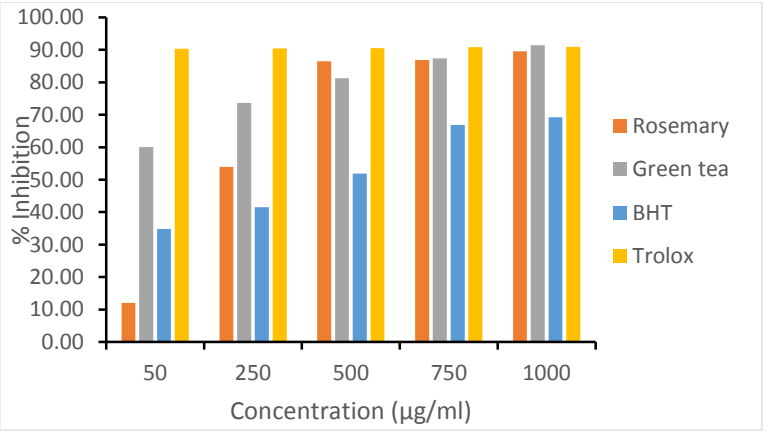

Figure 3. DPPH free radical removal % inhibition results

BHT and trolox were used as standard. Trolox showed greater than 90% inhibition at all concentrations. At a concentration of 50 µg/ml, green tea showed 60.08% inhibition, an effect above standard BHT. Rosemary at a concentration of 250 µg/ml had an effect of 53.95% and green tea had an effect of 73.63% on BHT. The other

standard, Trolox, showed more inhibition of all with 90.4%. At 500 µg/ml concentration, rosemary showed more inhibition than green tea with 86.45%. rosemary and green tea showed greater inhibition of BHT. Trolox showed greater inhibition in this set as well.

Table 3. % inhibition values of DPPH free radical scavenging activity according to concentrations

Concentration (µg/ml)	Rosemary % inhibition	Green tea % inhibition	BHT % inhibition	Trolox % inhibition
50	12.09	60.08	34.8	90.3
250	53.95	73.63	41.5	90.4
500	86.45	81.30	51.9	90.5
750	86.85	87.39	66.9	90.8
1000	89.58	91.41	69.2	90.9

Green tea showed more effect than rosemary and BHT with 87.39% effect at 750 µg/ml concentration. Trolox showed the highest inhibition effect at this concentration with 90.8%. Rosemary showed 89.58% and green tea 91.41% inhibition effects at a concentration of 1000 µg /ml. In these two values, it showed more inhibition than BHT used as standard. Green tea, on the other hand, was the sample with the highest DPPH free radical scavenging activity by showing more effect than all the substances used in this study.

4. Conclusıon

In this study, the inhibition effect of rosemary and green tea plants on tyrosinase enzyme was investigated and their antioxidant properties were determined. According to the findings, it was determined that the two selected plants, especially green tea, had higher DPPH radical scavenging activity than the synthetic standard antioxidants used. In addition, it has been determined that both plant extracts can be used as natural tyrosinase enzyme inhibitors by inhibiting the tyrosinase enzyme. Green tea and rosemary are often used in alternative medicine for their therapeutic properties. The results of our study also support this theory. It is thought that green tea and rosemary extracts, which are natural tyrosinase enzyme inhibitors, can be a positive support in the treatment of various diseases.

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