**ABSTRACT**

**Objective:** Antioxidants prevent and/or slow down many adverse effects on the body, especially oxidative stress caused by free radicals. Phenolic compounds in fruits and vegetables have beneficial effects by neutralizing free radicals. Since most phenolic compounds have antioxidant effects, they help to protect cells against oxidative damage caused by free radicals. In our study, we aimed to determine some enzyme activities thought to be present in black plum, carob and black mulberry plants.

**Material and Methods:** In this study, antioxidant enzyme activities were determined using the spectrophotometric method. The extracts of the fruits used in the study were measured manually by preparing reagent solutions. Statistical significance level was taken as 5%, and SPSS 26 statistical package program was used for calculations.

**Results:** Plum (Prunus domestica), black mulberry (Morus nigra) and carob (Ceratoniasiliqua) fruits were found to have higher Superoxide Dismutase (SOD) and Catalase (CAT) values. SOD and CAT values were much higher in black mulberry than in white mulberry. CAT activity was low in black plum and high in green plum. Similarly, SOD activity was low in black plum and high in green plum. Green plum was significantly higher than black plum in terms of both CAT and SOD activity. SOD activity measured in carob was almost twice as high as CAT activity.

**Conclusion:** Antioxidants, which are known to be effective against diabetes, the disease of our age, and many various diseases, protect the body against diseases. Due to the high amounts of antioxidants found in black mulberry, carob and plum, adding extracts to the treatment of chronic patients can stop and/or slow the progression of diseases.

**Keywords:** Plum, Black Mulberry, Carob

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**INTRODUCTION**

Every year, new diseases emerge in the world and the number of chronic diseases such as cardiovascular diseases, diabetes, Alzheimer’s, asthma and allergic diseases increases. Oxidative stress contributes to the formation of these diseases. Knowing the effects of antioxidants and their protection against diseases is a very popular topic of scientific research. Since cellular damage in the human body is directly related to the increase in the amount of free radicals, it is important to maintain their levels below a certain threshold. The most important and healthy way to inhibit oxidative stress and reduce free radicals is to have a balanced diet and a certain level of antioxidants in foods taken from outside (1).

Plum ‘Prunus domestica’ is green, yellow, red or purple in color. Plum trees, which bear sour or sweet fruit, belong to the genus Prunus of the Rosaceae family. According to the oldest written documents, it has been known for 2000 years. It is accepted to have spread around the Caucasus and the Caspian Sea (2). With its many species and varieties, it has found the opportunity to spread by adapting to different climatic regions. The large number of species and the fact that they originated from regions with different climates were also effective in spreading plum over such a wide area (3). Some plum varieties grown in Turkey are indigenous, while others are foreign.
In world plum production, Yugoslavia ranks first, followed by the USA and Turkey ranks eighth. Most of the plum production in Turkey is grown in the Black Sea region. Plum is a fruit rich in vitamin B, potassium and magnesium minerals. In addition to being consumed fresh, plums are used in the production of jam, paste, pestil, marmalade, and fruit juice, as well as in the development of the alcoholic beverage and canning industries. It is also marketed by drying (3).

Black mulberry (Morus nigra) is a fruit of the Morus species of the Moraceae family (4). India and China are the leading countries where black mulberry is grown. In these countries, mulberry leaves are generally used in silkworm breeding. Mulberry cultivation has been practiced in Turkey for about 400 years. In Turkey, 95% of the mulberry trees are white mulberry (Morus alba), 3% are red mulberry (Morus rubra), and 2% are black mulberry (Morus nigra) (5). The main components of black mulberry are sugar, organic acid (malic acid and malic acid citric) and phenolic compounds (6). The characteristic red-violet color of black mulberry is due to anthocyanins, a subgroup of polyphenols. Black mulberry contains a large proportion of polyphenols (5,7). Polyphenols are compounds found in all fruits and vegetables and play an important role in their color, taste, texture properties and antioxidant and antimicrobial effects (9). It has been determined that polyphenols have an important effect on plants' defence mechanism and texture due to their ability to cross-link biopolymers (10). Grape fruits contain many bioactive and phytochemical compounds that are important for human health. Nowadays, many studies have been conducted on the health effects of plant polyphenols, which are composed of flavonoids and non-flavonoid compounds, and it has been stated that these compounds are powerful antioxidants and that they delay cardiovascular diseases, cancer, inflammatory disorders, diabetes, and even aging by destroying free radicals formed in the body (11-14). Polyphenols are easily bound to DNA, Enzymes and Proteins due to their high chemical activity, and thus it has been determined that they show defense against free radicals that cause the inactivation of these molecules (11,15).

Carob is known as one of the oldest plants on earth. It is thought to have first appeared in Egypt around 4000 BC. It is also called carob. The carob tree belongs to the legume family and is a tree that grows in large quantities in regions with Mediterranean climate characteristics, generally without being cultivated and without the need for any artificial additives during growth (16). In addition to Mediterranean countries such as Italy, Spain, Portugal, Turkey, Greece, Morocco, Tunisia, Algeria, Cyprus and Israel, carob is cultivated in the USA, Australia and South and North Africa. Carob adapts to the climatic conditions in all these countries, and its yield decreases (17). Carob grows naturally in the Mediterranean region of Turkey on a coastal strip of about 1750 km2 extending from Tarsus and Mersin to Marmaris. The varieties grown in Turkey are fleshy, sesame and wild species (18). Although our country is not located in the homeland of carob, it has large areas for carob cultivation, especially in the Mediterranean region, and carob cultivation is becoming increasingly important (19).

Nowadays, fried carob pieces produced from carob fruits in our country are generally used as a substitute for chocolate, especially in bakery products, pastry and confectionery products and low-calorie snack products. Carob can be ground into flour, which can be mixed with milk and used as a substitute for cocoa. In addition, new products are created by extracting molasses, special drinks or water-soluble parts of carob fruit from carob and drying the extract in a spray dryer. Since the cellulose content of the remaining pulp is high, it can be used as animal feed (19). Carob has been reported to have positive effects on gastritis, digestive system disorders, liver and especially lung problems, tooth and gum disorders, lowering cholesterol and muscle development (16,18).

Carob is in the class of foods with low glycemic index due to its high amount of water-insoluble dietary fiber (20). It has been reported that a diet rich in dietary fiber has preventive and curative effects on common diseases such as obesity, cardiovascular and gastrointestinal diseases (21). The primary purpose of our study is to determine the levels of antioxidants found in plum, black mulberry and carob and to contribute to the healing of chronic and systemic diseases by using alternative treatment methods to be developed in the future and/or in addition to drug treatments applied to diseases.

MATERIAL and METHODS

Determination of superoxide dismutase (SOD) activity

Superoxide dismutase (SOD) enzyme activity was determined spectrophotometrically according to the method described by Sun et al. (22).

A-) (Manual Method)

Preparation of Reagent Solution:

1. 0.3mM Xanthine: 4.56 mg xanthine (Sigma X7375) was first dissolved in a few drops of 1N NaOH and dissolved in 100 ml bidistilled water.
2. 0.6mM EDTA: 4.46 mg EDTA was dissolved in 20 ml of bidistilled water.
3. 150 mg/L NBT: 12.3 mg NBT (Sigma N6876) was dissolved in 100 ml of bidistilled water.
4. 400 mM Na₂CO₃: 2.544 g Na₂CO₃ was dissolved in 60 ml bidistilled water.
5. Bovine serum albumin (1g/L): 12 mg BSA (Sigma A2153) was dissolved in 12 ml bidistilled water.

Preparation of the reagent solution: 40 ml xanthine solution, 20 ml EDTA solution, 20 ml NBT solution, 12 ml Na₂CO₃ solution, 6 ml BSA were mixed. (Store in a dark bottle)

-16 μl of xanthinoxidase (167 u/L) (Sigma X1875) was taken and dissolved in 1 ml of 2 M (NH₄)2SO₄.

-2M (NH₄) 2- SO₄: 2.643 g (NH₄)2SO₄ was made up to 10 ml with distilled water (stored at +4 °C).

-0.8 mM CuCl₂ 2H₂O 13.6 mg CuCl₂ 2H₂O was prepared and made up to 100 ml with distilled water.

After pipetting, as indicated in Table 1, the blind and sample tubes were read against bidistilled water at 560 nm.
**Table 1. SOD activity determination method.**

<table>
<thead>
<tr>
<th></th>
<th>Blind</th>
<th>Example</th>
</tr>
</thead>
<tbody>
<tr>
<td>Reactive</td>
<td>1.425 µl</td>
<td>1.425 µl</td>
</tr>
<tr>
<td>Example</td>
<td>–</td>
<td>50 µl</td>
</tr>
<tr>
<td>Bidistile</td>
<td>100 µl</td>
<td>–</td>
</tr>
<tr>
<td>Xanthinoxidase</td>
<td>25 µl</td>
<td>25 µl</td>
</tr>
</tbody>
</table>

kept at room temperature at 25°C for 20 minutes

| CuCl₂          | 50 µl         | 50 µl         |

**Activity Account:**

% inhibition: \[(\text{Blind OD} - \text{Sample OD}) / \text{Blind OD}\] x 100

1 unit of SOD: Enzyme activity that inhibits NBT reduction by 50%. Activity = (% inhibition) / (50 x 0.1)

The activity was calculated in U/ml.

In this study, hydrogen peroxide was used as a substrate, and catalase activity was determined using the Aeibi method. The activity was performed as follows: First, two tubes were taken. 1.4 ml of 30 mM H₂O₂ was added to the blind tube and 0.1 ml of phosphate buffer was added. Add 1.4 ml of 30 mM H₂O₂ to the sample tube. 0.1 ml of enzyme was added and mixed with vortex. Absorbances at 240 nm were read twice at 30-second intervals, and thus activity was determined (23).

**Solutions used:**

1. Preparation of 30 mM H₂O₂: In 10 ml of bidistilled water, 34 µl of 30% H₂O₂ was added (25.8 µl of 35% H₂O₂ was added).

2. Preparation of 50 mM Phosphate Buffer: 6.81 g KH₂PO₄ and 7.1 g Na₂HPO₄ were dissolved in bidistilled water, the pH of the buffer was adjusted to 7.4 with 1N NaOH, and the volume was made up to 1 liter.

**Activity Account:**

E.U. = \((2.3 / \Delta x) \times [(\log A₁ / \log A₂)]\) Activity was calculated in U/L.

Δx = 30 seconds

2, 3 = 1 µmol H₂O₂ in a 1 cm light path

**Statistical Analysis:**

Descriptive statistics for the features emphasized were expressed as Mean and standard Deviation. In paired group comparisons, T- Test was used when normal distribution conditions were met, and Mann Whitney U test statistics were used when normal distribution conditions were not met. Statistical significance level was taken as p<0.05, and SPSS statistical package program was used for calculations.

**RESULTS**

Descriptive statistics and comparison results for CAT and SOD are given in Table 2. When Table 2 is examined, the difference between the averages for CAT and SOD in black plum and green plum, black mulberry and white mulberry is statistically significant (p<0.001). CAT activity was 0.19 U/L in black plum and 0.42 U/L in green plum, SOD activity was 0.20 U/ml in black plum and 0.47 U/ml in green plum (p<0.001). Green plum was significantly higher than black plum in terms of both CAT and SOD activity. CAT activity was 1.38 U/L in black mulberry and 0.76 U/L in white mulberry (p<0.001), SOD activity was 2.39 U/ml in black mulberry and 1.05 U/ml in white mulberry (p<0.001). Black mulberry was significantly higher than white mulberry in terms of both CAT and SOD activity. CAT activity was found to be 0.21 U/L on average in locust bean, while SOD activity was found to be 0.44 U/ml on average in locust bean (p<0.001).

**Table 2: Descriptive statistics and Comparison results**

<table>
<thead>
<tr>
<th>Group</th>
<th>n</th>
<th>Mean±Std. Deviation</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>CAT (U/L)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>black plum</td>
<td>12</td>
<td>0.19±0.001138 U/L</td>
<td>p&lt;0.001</td>
</tr>
<tr>
<td>green plum</td>
<td>12</td>
<td>0.42±0.013325 U/L</td>
<td></td>
</tr>
<tr>
<td>SOD (U/L)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>black plum</td>
<td>12</td>
<td>0.20±0.006331 U/ml</td>
<td>p&lt;0.001</td>
</tr>
<tr>
<td>green plum</td>
<td>12</td>
<td>0.47±0.016189 U/ml</td>
<td></td>
</tr>
<tr>
<td>CAT (U/L)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Black mulberry</td>
<td>12</td>
<td>1.38±0.10526 U/L</td>
<td>p&lt;0.001</td>
</tr>
<tr>
<td>White mulberry</td>
<td>12</td>
<td>0.76±0.02340 U/L</td>
<td></td>
</tr>
<tr>
<td>SOD (U/L)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Black mulberry</td>
<td>12</td>
<td>2.39±0.08581 U/ml</td>
<td>p&lt;0.001</td>
</tr>
<tr>
<td>White mulberry</td>
<td>12</td>
<td>1.05±0.02425 U/ml</td>
<td></td>
</tr>
<tr>
<td>CAT (U/L)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Carob</td>
<td>12</td>
<td>0.2113±0.003596 U/L</td>
<td>p&lt;0.001</td>
</tr>
<tr>
<td>SOD (U/L)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Carob</td>
<td>12</td>
<td>0.4442±0.018809 U/ml</td>
<td>p&lt;0.001</td>
</tr>
</tbody>
</table>

The p value < 0.001 was considered statistically significant for Superoxide Dismutase (SOD) and Catalase (CAT) values between the groups.
Figure 1: SOD and CAT concentrations in locust bean (p value < 0.05).

Figure 2: SOD and CAT concentrations in black and green plum (p value < 0.05).

Figure 3: SOD and CAT concentrations in black mulberry and white mulberry (p value < 0.05).
DISCUSSION

With the increase in the world population, the increase in chronic diseases has become an important health problem. The high cost of treatment of diseases and the loss of workforce due to diseases shake the economies of the states. For such reasons, consuming fruits that are easy to access and provide protection against diseases is important. Anthocyanins, found in large amounts in mulberry, have positive health effects such as antithrombotic, antioxidant, antimicrobial, antimitogenic, anticarcinogenic, anti-inflammatory and protective of the nervous system. This effect is due to the phenolic compounds that give mulberry color. The interest in identifying appropriate dietary sources of antioxidant phenolic compounds has gradually increased. There are studies in which total phenolic matter, vitamin C content, total fat content and fatty acid profile of black mulberry fruit were determined (24).

Carob fruit has been proven to positively affect cardiovascular and gastrointestinal diseases. Carob is a natural raw material used for the production of D-pinitol, which is used for disease protection. Plums, which contain various phenolic compounds such as hydroxycinnamic acids, flavonols and anthocyanins, have been proven to have practical antioxidant effects due to their high polyphenolic content. The amount of phenolic compounds in black mulberry has been quite high (25).

Due to their antimicrobial and antioxidant activities, polyphenols have become natural alternatives to synthetic food additives, which are used in food preservation but also raise health concerns.

Korniyev et al. In another study conducted with a mutant cotton plant, it was determined that SOD activity increased approximately 3 times compared to the control as a result of low temperature application (26). In another study by Beak and Skinner, it was determined that the expression of MnSOD genes increased in winter wheat leaves during 4 weeks of cold adaptation (27). In another study by Vanacker et al., there are many studies on the factors affecting catalase activity in plants. After 24 hours of treatment of three different oat varieties with a fungus, it was determined that CAT activity increased in one sample, while the activity decreased in the second sample. It was observed that an antioxidant defense was formed in orange crops treated with different NaCl concentrations, and significant changes were observed in the activities of antioxidant enzymes such as SOD, GR, APX (28).

CONCLUSION

Some antioxidant enzyme activities such as SOD and CAT were found to be high in plum (Prunus domestica), black mulberry (Morus nigra) and carob (Ceratonia siliqua) fruits. Consumption of plum, black mulberry and carob and/or widespread consumption of these fruit extracts may strengthen the immune system and may be used as an alternative method in the treatment of chronic diseases. More comprehensive studies on this subject are needed. We think that this study will contribute to the literature.

Acknowledgements: None

Conflict of interest: The authors declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

Ethical approval: The present study was conducted in strict accordance with the principles outlined in the Declaration of Helsinki. Informed consent was obtained from the participant of this study.

Authors’ Contribution
Conceptualization: CG
Data curation: CG, HD, NA, CD, OB
Formal analysis: CG, HD, NA, CD, OB
Funding acquisition: -
Investigation: CG, HD, NA, CD, OB
Methodology: HD, CD
Project administration: CG
Resources: WOS, Scholar Google, Scopus, Index Copernicus Software: SPSS
Supervision: HD
Validation: CG, HD
Writing–original draft: CG, HD
Writing–review & editing: CG, HD

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