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Antioxidant Properties and Alpha-Amylase Inhibition of Wild Blackberry Leaf

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Abstract: *In this study, the possible antioxidant properties of ethanolic extract of wild blackberry leaves were investigated by using antioxidant assays including DPPH˙ scavenging and β-carotene bleaching method. The leaf extract was showed DPPH radical scavenging activity at the 100 µg/mL and above concentrations and inhibited lipid peroxidation at the high concentrations. The natural compounds known as potential antioxidants such as phenolics, flavonoids were also determined. Total phenolic content was determined using Folin-Ciocalteu method and was found to be 186.05±1.46 µg of gallic acid equivalents. Total flavonoid content was studied using $AlCl_3$ reagent and was determined as 19.39±0.83 µg of quercetin equivalents. The different concentrations of leaf extract had α-amylase inhibitory activity in the range of 21 - 44 %. The observed inhibition effect against α-amylase could be due to phenolic contents of the plant. In conclusion, the leaves of wild blackberry may be used as a natural antioxidant source in both food and nutritional applications.*

Keywords: *α-Amylase inhibition, Wild blackberry, DPPH˙ scavenging, Phenolic.*
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I. Introduction

The role of free radicals and reactive oxygen species (ROS) in the pathogenesis of human diseases, including cancer, aging, atherosclerosis, neurological damage, has been recognized [1]. Antioxidants, exogenous and endogenous, are vital substances which possess the ability to protect the body from possible harms caused by the free radical induced oxidative stress [2]. Hence, the antioxidants are the important elements for human nutrition, also pharmaceutical and food industries. In recent years, there has been an increasing interest to natural antioxidants present in cultivar and wild plants. Diabetes mellitus is a metabolic disorder of multiple etiologies characterized by chronic hyperglycemia with disturbance of carbohydrate, fat, and protein metabolism resulting from defects in insulin secretion or insulin action. One of an important therapeutic approach for treating diabetes is to decrease the postprandial hyperglycemia by suppressing glucose absorption through the inhibition of the carbohydrate-hydrolyzing enzymes, α-amylase and α-glucosidase, in the digestive tract [3]. The use of natural α-amylase inhibitors from fruits and vegetables is proposed to control postprandial hyperglycemia. In addition to antioxidant capacities of dietary polyphenols which are a large and diverse group of molecules in plant kingdom, their inhibitory effects against α-amylase have attracted great interest in recently [3, 4]. Blackberry (*Rubus fruticosus*, Rosaceae) is a perennial shrub and its fruit called blackberry fruit. Berries contain a variety of phenolic compounds, vitamin C, dietary fibre, α-tocopherol, minerals and carotenoids [5, 6]. Not only the fruit, but also the blackberry leaves and roots are used in traditional folk medicine as medicinal agents. Infusion from the leaves are used for colds, sore throat, diarrhoea, colic pain and various respiratory problems. This present study was aimed to detailed investigation of the antioxidant properties of ethanolic extract from wild blackberry leaves using two different antioxidant models and determining total phenolic and total flavonoid contents. And also α-amylase inhibitory effect of blackberry leaf extract was evaluated *in vitro* in the study.

II. Experimental

2.1. Sample Preparation

Wild blackberry brambles were collected from Kırklareli province (Vize), Turkey, in May 2015. The fresh leaves of the plant were chopped using Warring blender and extracted with ethanol at room temperature for 6 h in a shaking water bath. After the filtration the solvent was removed using a rotary evaporator and obtained leaf extract was used in all assays.

2.2. Determination of Total Phenolic Content

The concentration of total phenolic compound in the extract was determined by using Folin-Ciocalteu reagent [6]. Gallic acid was used as standard, and plotted calibration curve. The results were expressed as µg gallic acid equivalent (GAE) per mg of extract.

2.3. Determination of Total Flavonoid Content

Total flavonoid content was determined with aluminum chloride ($AlCl_3$) [7], using quercetin as a standard. The method is based on the formation of a flavonoid–aluminum complex which has maximum absorbance at 430 nm. The content of flavonoid in extract was expressed in terms of µg quercetin equivalent (QE) using the calibration line equation.

2.4. DPPH Free Radical Scavenging Activity

Free radical scavenging activity of blackberry leaf extract was evaluated with 1,1-diphenyl-2-picryl-hydrazil (DPPH$^{\bullet}$) using the Blois method [8]. This assay is based on the principle that DPPH on accepting a hydrogen atom from the scavenger molecule i.e. antioxidant, resulting the purple colour of DPPH$^{\bullet}$ solution changes to yellow. Briefly, one milliliter of the sample solution was added to ethanol solution of DPPH$^{\bullet}$ (0,1 mM), and the mixture was kept at room temperature. After 30 min, the absorbance was measured at 517 nm with a spectrophotometer. BHA was used as standard compound. The antiradical activity was calculated using the formula

% Inhibition $= [(A_{control} - A_{sample}) / A_{control})]x100$

Where $A_{control}$ is the absorption of the DPPH$^{\bullet}$ solution and A_{sample} is the absorption of the DPPH$^{\bullet}$ solution after the addition of the sample.

2.5. Total Antioxidant Activity (TAA) Assay

The total antioxidant activity of extract was evaluated by the β-carotene bleaching assay [9]. The β-carotene bleaching method is based on the loss of the yellow colour of β-carotene due to its reaction with radicals which are formed by linoleic acid oxidation in β-carotene/linoleate emulsion. The rate of β-carotene bleaching can be slowed down in the presence of antioxidants. BHT was used as a standard compound. Total antioxidant activity (TAA) was expressed as percent of inhibition relative to the control after a 120 min incubation period [10] as below:

$TAA = 100(DR_C - DR_S)/DR_C$

Where DR_C = degradation rate of control = ln(a/b)/120; DR_S =degradation rate of sample = ln(a/b)/120; a and b = absorbance of samples and controls at 0 and 120 min.

2.6. α-Amylase Inhibition Assay

α-Amylase inhibition assay was performed according to Apostolidis et al [11]. After pre-incubation the extract and α-amylase (Porcine pancreatic α-amylase), starch solution was added and incubated. Dinitrosalicylic acid (DNS) color reagent was used to determine released reducing sugars. The absorbance was recorded at 550 nm. Acarbose was used as possitive control. The results were expressed as % inhibition of α-amylase and was calculated according to the following equation:

% Inhibition = [(Absorbance of control – Absorbance of extract) / Absorbance of control)]x100

2.7. Statistical Analysis

The assays were carried out in triplicate and the results expressed as mean values ± standard deviations.

III. Results and Discussion

Phenolic phytochemicals are secondary metabolites of plant origin and they have antioxidant activity and certain therapeutic properties. Table 1 presents the total phenol content (TPC) and total flavonoid content (TFC) expressed as µg GAE/mg and QE/mg in plant extract, respectively. Oszmiański et al have been reported that the total content of phenolic compounds extracted from leaves of wild blackberry was highly diverse and ranged from 83.02 to 334.24 mg/g dry matter in twenty-six wild *Rubus* L. species. And also the flavonoid content of these *Rubus* L. species was ranged from 8.68 to 61.27 mg/g dry matter in leaves [13]. The obtained results are in agreement with those of Oszmiański et al.

Table 1. The total phenolic and flavonoid contents of blackberry leaf ethanolic extract.

	Calibration curve	Correlation coefficient	Quantities of tested extract
Total Phenolic	y = 0.0005x + 0.0377	0,9983	186.05±1.46 µg GAE/mg extract
Total Flavonoid	y = 0.0049x + 0.0615	0,9834	19.39±0.83 µg QE/mg extract

Several concentrations ranging from 10-750 µg/mL of ethanolic extracts of blackberry leaf were tested for its antioxidant activity using DPPH free radical. As seen in Fig 1. the results showed close radical scavenging activities to standard compound. At 100 µg/mL concentration inhibition values were 75.46±0.68 % and 83.95±2.2 % for ethanolic leaf extract and BHA, respectively. Other studies have also shown high DPPH$^{\bullet}$ scavenging activities for blackberry extracts [14, 15].

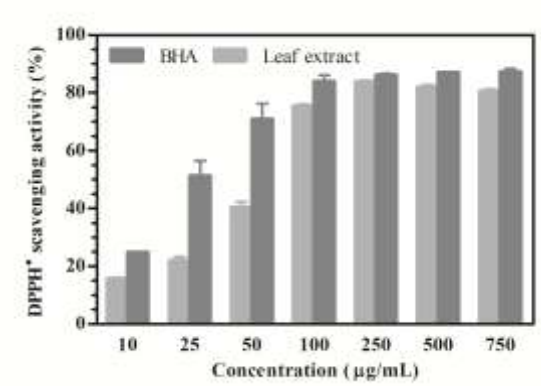

Figure 1. Free radical scavenging activities (%) of blackberry leaf extract and BHA on DPPH radical solution.

Antioxidant activity with β-carotene bleaching method of blackberry leaf was investigated in linoleic acid emulsion system. The results of researched activity were shown in the Fig 2. as a percent inhibition of lipid peroxidation. Total antioxidant activity of leaf extract was increased with increasing extract concentration. The extract showed close activity to standard compound only at high concentrations. Activity of leaf extract was about 82 % whereas that of BHT was about 98 % at 750 µg/mL concentration.

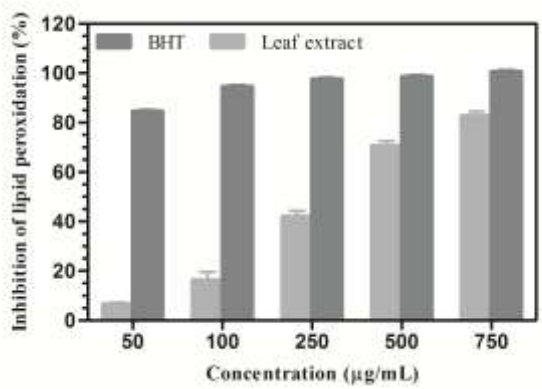

Figure 2. Total antioxidant activities (%) of blackberry leaf extract and BHT on β-carotene/linoleic acid model system.

Phenolic compounds are widely distributed in plant foods. It was reported that dietary polyphenols have the inhibitory effects against α-amylase. Recently they have been receiving much attention for their ability of managing hyperglycemia [3, 4]. α-Amylase inhibitory activity of blackberry leaf extract was changed in the range of 21 % - 44 % at the different concentrations (10-500 µg/mL), but the inhibition rates didn't depend the concentration (Fig 3). Antidiabetic activity of two blackberry cultivars were investigated by Saponjac et al [16] and the cultivars exhibited stronger α-glucosidase inhibitory activity even at the lowest concentration (0.02 mg/mL), while complete inhibition was achieved at 0.63–2.50 mg/mL.

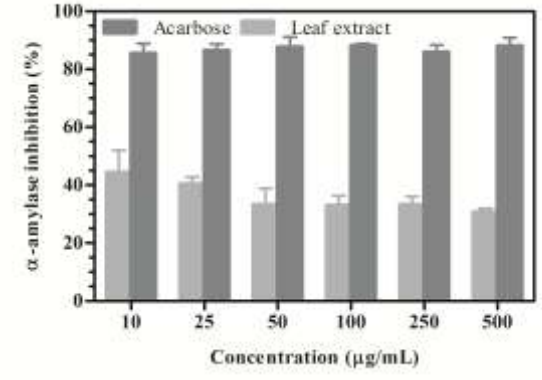

Figure 3. Inhibitor effect of blackberry leaf extract and acarbose on alpha-amylase activity.

IV. Conclusion

Blackberry leaf extract exhibited good free radical scavenging activities at 100 µg/mL and higher concentrations, while it inhibited lipid peroxidation at only high concentrations, which is comparable with standard compounds. The phenolic and flavonoid compounds determined in the extract may be responsible for the α-amylase inhibition as well as the antioxidant activities. The blackberry leaves can be considered a cheap raw material and safely natural potential of antioxidants. In conclusion it could be suggested blackberry leaf extract may be used as a promising source for pharmaceutical application, nutraceutical and functional food industries, however further trials are desirable for characterization of extract properties.

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