ORIGINAL ARTICLE

Eastern Journal of Medicine

East J Med 26(3): 470-474, 2021
DOI: 10.5505/ejm.2021.89725

The Effect of Currant (Ribes) on Human Health and Determination Certain Antioxidant Activities

Mustafa Bilici[1,2]

[1]*Van Yuzuncu Yil University Faculty of Medicine, Department of Basic Medical Sciences, Van, Turkey*
[2]*Van Yuzuncu Yil University Faculty of Science, Department of Chemistry, Van, Turkey*

ABSTRACT

This study aimed to determine some antioxidant activities at ribes fruit. Ribes is a strong reservoir of antioxidants. It has many vitamins, proteins, and mineral matters in its composition. Many *Ribes* types, especially black ribes types with darker fruits contains polyphenolic compounds in high concentrations, especially due to their antioxidant activities they have increasing amounts of anthocyanins and flavonols that are increasingly sought after due to their antioxidant activities. The most significant anthocyanin found in black ribes are cyanidine-3-glucoside, cyanidine-3-rutinoside, delphinidine-3-glucoside, and delfinidine-3-rutinoside. Natural antioxidants have protecting the body against infections, preventing anemia by inhibiting decomposition of red blood cells, preventing synthesis of cancerous compounds, diluting the blood by increasing prostaglandin synthesis (antithrombotic effect), preventing arteriosclerosis, and preventing unwanted effects on metabolism of active forms with oxidation ability causing liver damage. Its protective effects such as healing effects on some cancer types are known. Level of Catalase (CAT) was found to be 0.00011 U/mI on average for fresh ribes. Level of Superoxide Dismutase (SOD) was found to be 11.6960 U/mI on average for fresh ribes. The level of Reduced Glutathione (GSH) was found to be 0.00011 mmol/dI on average for fresh ribes.
In this study ribes was found to display a very strong antioxidant activity. Thus, ribes was demonstrated to be a protective antioxidant against oxidative damage against many diseases.

Keywords: Currant, Oxidation, Antioxidant.

Introduction

Currant (ribes) belongs to *Rosales* order *Saxifragaceae* family, *Ribes* genus. *Ribes* genus is separated to four sub-genus groups. These are; *Berisia*, *Ribesia*, *Coreosma* and *Grossularia* sub-genus groups. Ribes fall under *Ribesia* and *Coreosma* sub-genus groups. Ribes that belong to *Ribesia* sub-genus groups include red and white ribes while *Coreosma* sub-genus include black ribes. In Turkey ribes are known to have five types and these types are black fruit ribes (*Ribes nigrum* L.), East Black Sea ribes (*Ribes orientalis* L.), Alpine ribes (*Ribes alpinum* L.), and Caucasus ribes (*Ribes biebersteinii* Berl. Ex. Dc.) together with *Ribes rubrum* used in landscape planning and planted as decoration plant (1).

Free radicals damage membranes of body cells, lipids, nucleic acids and DNA in cell structure. Thus, they cause various conditions such as coronary diseases, diabetes, cancer, liver damage, and cataract (2). Antioxidants that we take from natural food sources as a result of natural and balanced diet are molecules that have the ability to prevent oxidations caused by free radicals and to catch and stabilize free radicals (3). The main impact of antioxidants on human health is their active role in mechanisms neutralizing free radicals and breaking chains (4).

Many *Ribes* types, especially the black ribes types with fruits in darker colors have polyphenolic compounds in high concentrations, especially anthocyanins and flavonols that are in increasingly high demand due to their antioxidant activities. The most important anthocyanin found in black ribes are cyanidine-3-glycoside, cyanidin-3-rutinoside, delphinidine-3-glucoside, and delfinidine-3-rutinoside (5). Natural antioxidants participating in biological activities have properties such as protecting the body against infections, preventing anemia by inhibiting decomposition of red blood cells, preventing synthesis of cancerous compounds, diluting the blood by increasing prostaglandin synthesis (antithrombotic effect), preventing arteriosclerosis, and preventing unwanted effects on metabolism of active forms with oxidation ability causing liver damage. Its protective effects

***Corresponding Author:** Mustafa Bilici, Van Yuzuncu Yil University Faculty of Medicine, Department of Basic Medical Sciences, Van, Turkey
E-mail: mbilici65@hotmail.com, Telephone: 0 (533) 451 21 99
ORCID ID: Mustafa Bilici: 0000-0002-8689-6463
Received: 10.06.2021, **Accepted:** 21.06.2021

Table 1. Method of Determination of SOD Activity

	Blind	Sample
Reactive	1.425 µl	1.425 µ
Sample	-	50 µl
Bidistilled	100 µl	-
Xanthine oxidase	25 µl	25 µl
Kept at room temperature for 20 minutes		
CuCI2	50 µl	50 µl

Table 2. CAT, SOD, and GSH Levels of Fresh Currant

	Group	n*	Antioxidant Activities
CAT (U/mI)	Fresh Currant	3	0.2695
SOD (U/mI)	Fresh Currant	3	66.15
GSH (mmol/dI)	Fresh Currant	3	0.00011

n*: mean result of three repeated measurements

such as healing effects on some cancer types are known (6). This study aims to determine some antioxidant activities at Ribes fruit.

Antioxidant: Chemicals that are responsible for antioxidant capacity at plant tissues are known as phenols, anthocyanins, and other flavonoids (7). Fruits that are in black, dark green, and blue have much higher antioxidant values (8). Especially when fruits of *R. nigrum* are compared with that of *R. rubrum* they contain 6 times more anthocyanins, thus have much higher antioxidant capacity (9, 10). In a similar study, black ribes was mentioned to have a much higher antioxidant capacity compared to red ribes and this rate was announced to be the highest at organically grown black ribes (10). Antioxidant tests conducted on leaf, bud, fruit extracts of black ribes revealed that leaf extract had the highest antioxidant activity connected to its high total phenol content (11). Ribes is a fruit with high nutritious value containing Vitamins A, B, B2, and C. In addition, it stimulates appetite and helps digestion. It is diuretic and it relaxes the body. It helps to pass kidney stones. It helps reduce edema at belly. It removes swelling of liver and hepatitis. It is also useful against rheumatism and osteoarthritis. It removes inflammation on digestive tract. Its syrup is very nutritious (12). Studies announced that red ribes leaves have a higher capacity of cleaning the free radicals compared to black ribes leaves. This property is based on phenolic substances such as rutin and quercetin it contains thus, red ribes is known as an important source of antioxidant (13). At the same time, antioxidants neutralize effects of free radicals, preventing diseases such as cancer, health diseases, Parkinson's and Alzheimer's disease together with breaking chains causing early aging (14).

Anthocyanins and other flavonoids together with phenolic acids were proven to have the effect of inhibiting free radicals and inhibit lipid peroxidation. Red and black ribes fruits were also determined to have similar antiradical effects. Extracts of all these fruits show high activity against superoxide radicals released by chemical means. In addition, these extracts show inhibitor effect against xanthine oxidase (EC 1.1.3.22) enzyme that start free radical formation in the cell (15,16).

It is announced that cyanidine-3-glucoside has highest antioxidant capacity among anthocyanins and it was followed by cyanidine-3-ramnoglucoside, cyanidine, cyanidine-3-galaktoside and malvidine in this order (17). Delfinidine was proven to have the highest antioxidant activity in inhibition of LDL (low density cholesterol) oxidation among anthocyanidins and it was followed by cyanidine, malvidine, and pelargonidin (18).

Materials and Methods

For this study red Ribes of Ribasia sub-genus was used from among the five ribes types grown in Turkey including black fruit ribes (*Ribes nigrum* L.), East Black Sea ribes (*Ribes orientalis* L.), Alpine ribes (*Ribes alpinum* L.), and Caucasus ribes (*Ribes biebersteinii* Berl. Ex. Dc.) together with *Ribes rubrum* used in landscape planning and planted as decoration plant. The fresh Ribes used in the study was not taken as test rat model was not used

for ethical approval of the study. Thus, ethics approval was not needed. Because some enzyme activities were described directly from fresh ribes seeds.

Biochemical Measurements

Determination of Superoxide Dismutase (SOD) Activity: Activity measurement of superoxide dismutase (SOD) enzyme was made according to the method studied by Sun et al. (9). Preparation of Reactive Solution: 1. 0.3 mM Xanthine: 4.56 mg xanthine (Sigma X7375) was solved in some drops of 1N NaOH and solved in 100 ml bidistilled water. 2. 0.6 mM EDTA: 4.46 mg EDTA was solved in 20 ml bidistilled water. 3. 150 mg/L NBT: 12.3 mg NBT (Sigma N6876) was solved in 100 ml bidistilled water. 4. 400 mM Na_2CO_3: 2.544 g Na_2CO_3 was solved in 60 ml bidistilled water. 5. Bovine serum albumin (1g/L): 12 mg BSA (Sigma A2153) was solved in 12 ml bidistilled water. Preparation of reactive solution: 40 ml xanthine solution, 20 ml EDTA solution, 20 ml NBT solution, 12 ml Na_2CO_3 solution, and 6 ml BSA were stirred. (Keep in a dark colored bottle) – 16 µl of Xanthine oxidase (167 u/L) (Sigma X1875) enzyme was taken and solved in 1 ml 2 M $(NH_4)2SO_4$. - 2M $(NH_4)2SO_4$: 2.643 g $(NH_4)2SO_4$ was completed to 10 ml with pure water (kept in +4 ˚C). - 0,8 mM $CuCl_2.2H_2O$ 13.6 mg $CuCl_2.2H_2O$ was prepared, completed to 100 ml with pure water. After pipetting was completed as shown on Table 1, blind and sample tubes were read against bidistilled water at 560 nm. Activity Measurement: inhibition %: [(Blind OD – Sample OD) / Blind OD] x 100 1 Unit SOD: enzyme activity that inhibits 50 % of NBT reduction. Activity = (inhibition %) / (50 x 0.1) Activity was calculated in terms of U/ml.

Determination of Catalase (CAT) Activity: In this study where hydrogen peroxide was used as substrate, catalase activity was determined according to Aeibi (10) method. For the activity, initially two tubes were taken and 1.4 ml of 30 mM H_2O_2 was put on blind tube and 0.1 ml phosphate buffer was added on it. 1.4 ml 30 H_2O_2 was put in sample tube. 0.1 ml enzyme was added on it and stirred with vortex. Absorbance were read twice in 30 second intervals at 240 nm, thus activity was determined. Solutions used: 1. Preparation of 30 mM H_2O_2: 34 µl of 30% H_2O_2 was added in 10 ml bidistilled water (25,8 µl of 35% H_2O_2 was added). 2. Preparation of 50 mM Phosphate Buffer: 6.81 g KH_2PO_4 and 7.1 g Na2HPO4 were solved in bidistilled water and pH pf buffer was set to 7.4 with 1N NaOH and volume was completed to 1 liter. Activity Measurement: E.Ü.= (2,3 / Δx) x [(log A1 / log A2)] Activity was calculated in terms of U/L. Δx= 30 seconds 2,3= optic density 1 µmol H_2O_2 gives on 1 cm optical path.

Designation of Reduced Glutathione (GSH): Reduced glutathione (GSH) was measured with formation of yellow color as a result of reaction of sulfhydryl groups in erythrocyte with DTNB (5',5'-(2-dithiobis nitrobenzoic acid). Reduced glutathione level in EDTA bloods was measured in 24 hours at spectrophotometer at 412 nm (19).

Calculation: Glutathione concentration was calculated in terms of mmol/g protein unit.

$C / 1000 = (OD_2-OD_1) / 13600 \times E_1 \times 5/2 \times ½$

13600: Molar extinction coefficient of yellow color created during interaction of GSH with DTNB.

E_1: In case a band with width greater than 6 nm is used, a derivative extinction coefficient is used to correct optical path and bandwidth differences. The band we use has a width of 2 nm. In calculations E1=1 was accepted.

1000: conversion factor to mmol.

C: mmol / glutathione (mg/dl)

OD_1: Optical density measured at 412 nm wavelength before DTNB was added.

OD_2: Optical density measured at 412 nm wavelength after DTNB was added.

Findings: Findings for CAT, SOD, and GSH were presented in Table 2.

Discussion

Antioxidant defense systems block free oxygen radicals. Free oxygen radicals (SOR) that are also contained in hydroxyl radicals (HO), peroxy radicals, superoxide anions (O_2-), and hydrogen peroxide and hydrogen peroxide (H_2O_2) created by free radicals are frequently produced in living organisms. Even a very low level of SOR would cause damages by affecting the metabolism. As a result of damage created by such SOR levels, oxidative stress occurs in cell (20).

Antioxidants are collected in two groups being enzymatic and non-enzymatic. Enzymatic antioxidants are superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx), glutathione reductase (GR), glutathione S-transferase (GST), and glucose 6-phosphate dehydrogenase (G6PD); while non-enzymatic antioxidants are vitamin E (tocopherols), vitamin C (ascorbic acid), vitamin A (β-carotene), selenium, transferrin, lactoferrin, uric acid, glucose, ascorbate, albumin, bilirubin, and ceruloplasmins. Antioxidants can frequently be intracellular while they can sometimes be extracellular (21-22).

Studies announced that consumption of fruits and vegetables rich with high amounts of phenolic

substances play a major role in reduction of primarily coronary heart diseases and cancer. Red and dark colored fruits contain high amounts of phenolic substances especially, anthocyanins. Natural pigments of such fruits were determined to have different biological effects such as antioxidant, anti-inflammatory, and anticarcinogenic effects.

CAT level was found to be 0.00011 U/mI on average for fresh ribes. SOD level was established to be 11.6960 U/mI on average for fresh ribes. GSH level was found to be 0.00011 mmol/dI on average for fresh ribes. The said ribes is significant for extension of shelf live without use of any chemicals and protection of human life in addition to easier sale of export products. Results of this study demonstrated that ribes is an important reservoir of antioxidants. Thus, as a result of this study it could be argued that ribes is a strong antioxidant both as food and in terms of human health against oxidative stress. Considering the product variety in our country, the authors believe that this study would have economic returns and pioneer future research.

Ribes fruit is an important source of antioxidants due to its rich anthocyanin and other phenolic substance contents. Ribes fruit has high antioxidant capacity. Thus, consumption of grape-like fruits including ribes would be useful in protecting the body against various antioxidative stresses (23).

Consequently, ribes was found to have a strong antioxidant activity. Thus, ribes was demonstrated to be a protective antioxidant against various diseases.

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