ATATURK UNIVERSITY PUBLICATIONS

Research Article

DOI: 10.5152/Pharmata.2024.23030

Evaluating the Antioxidant Capacity of Rheum Ribes via Cupric Reducing Antioxidant Capacity, Ferric Reducing Antioxidant Power and 2,2-Diphenyl-1-picrylhydrazyl Methods

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ABSTRACT

Objective: It was aimed at determining the antioxidant capacity of the above-ground parts of the *Rheum ribes* species, known as "Iskın," which grows in the high parts of Erzurum province, using different methods.

Methods: Methanol extracts have been prepared to determine the antioxidant capacity of the *Rheum ribes* plant. The samples were then analyzed with cupric reducing antioxidant capacity, ferric reducing antioxidant power, 2,2-diphenyl-1-picrylhydrazyl radicals. As a reference sample, gallic acid compounds were reacted with the same radicals in different concentrations. The reference sample was determined for equivalent antioxidant capacity methanol extracts.

Results: Gallic acid was used as a reference standard along with the in vitro capacity measurements. The working range was determined where the absorbance graph was linear, and the calibration curves were plotted. The "Iskın" sample was then measured using the same workflow, with 6 consecutive samples. The antioxidant capacity of rhubarb samples was calculated in terms of gallic acid equivalent µg/mL.

Conclusion: In the study, the antioxidant capacity of rhubarb plant was determined using different methods. The response of the rhubarb plant against triphenyltetrazolinechloride, neocuproin, diphenyl picryl hydrazine radicals was determined by spectrophotometric measurements, and the findings were compared with the reference gallic acid sample. According to the results, it was determined that this endemic plant exhibited a very high antioxidant capacity. For this reason, it is thought that crop planting for this wild plant may lead to a remarkable contribution to the industry of the local region and may be used in the cosmetic and drug industries.

Keywords: Antioxidant, CUPRAC, DPPH, FRAP, *Rheum ribes*, spectrophotometer

Received: 12.02.2023
Accepted: 12.19.2023
Publication Date: 01.17.2024

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Cite this article as: Özli S, Şenol O, Sezen Karaoğlan E. Evaluating the antioxidant capacity of rheum ribes via cupric reducing antioxidant capacity, ferric reducing antioxidant power, and 2,2,-diphenyl-1-picrylhydrazyl methods. *Pharmata* 2024;4(1):14-18.

INTRODUCTION

Inorganic molecules or atoms containing one or more non-associated electrons in their outer orbit are called free radicals.[1] Free radicals are compounds with high activity. Enzyme reactions, autooxidation reactions, and life activities can be caused by endogenous sources and various environmental factors such as air pollution, cigarette smoke, ionized rays, ultraviolet (UV) radiation, and xenobiotics.[2] Free radicals in life cause very simple chain reactions with biological molecules such as nucleic acids, carbohydrates, and lipids.[3] Free radicals occur in organisms as a result of metabolic reactions; in macrophages, they are involved in many metabolic responses, such as the destruction of bacteria, electron transfer, and biosignal production. The presence of these reactive and excessive amounts of radicals in life can lead to diseases such as premature aging, neurological disorders, cardiovascular diseases, and cancer.[4]

Antioxidants are molecules that minimize the effects of free radicals, destroy their effects, and prevent reactions that can cause premature aging and various diseases. Antioxidants bind unassociated electrons in free radicals to form a stable structure.[5,6]

Free radicals affect the organism when the oxidative balance is notined. The antioxidants, on the other hand, provide a balance between the oxidant and the antioxidant, ensuring that tissues and cells maintain their structural integrity and fulfill their functions.[7]

The protective effects of naturally produced antioxidants in the human body are limited, and oxidative stress occurs when the production of reactive oxygen derivatives (ROS) exceeds the antioxidant capacity in biological systems. Increased ROS formation in tissues and cells for a variety of reasons is called oxidative stress.[8] Free oxygen radicals are formed in life by 3 basic mechanisms: metabolic, reactive, and energetic. The most important mechanism in the formation of free oxygen radicals is the metabolic pathway. Free oxygen radicals cause harmful effects in cells because they are highly reactive. It is important for each cell and tissue to maintain the balance between antioxidants and intrinsic oxidation within physiological limits. Oxidative stress causes damage to deoxyribose nucleic acid (DNA), carbohydrates, enzymes, and proteins. Neurodegenerative and cardiovascular diseases, autoimmune disorders, diabetes, and cancer form the molecular basis for the development of the cell membrane due to the damage caused by random bonding and rupture in the DNA chain and damage to structural proteins and enzymes.[9]

Antioxidants can be synthesized in the body or can be obtained from the outside. Antioxidant systems in living organisms are divided into 2 groups: those externally supplied by diet (exogenous) and those formed in the body (endogenic). While the endogenous defense systems are made up of enzymes, the exogeneous antioxidant defenses are divided into natural and synthetic. Synthetic antioxidants are compounds such as hydroxysianol, propyl galate, hydroxytoluene, and trolox. Natural antioxidants are mostly found in bacon, seeds, green vegetables, and fruits.[4]

Studies on plants show that plants are a good source of antioxidants. The natural sources of antioxidants are generally phenolic compounds in plants. It is associated with the antioxidant activity of the hydroxyl group found in phenolic molecules.[10] Plant phenolic compounds can reduce reactive oxygen species that have toxic effects on a range of biological and pathological processes.[11]

Rheum ribes is a perennial alpine plant in the Polygonaceae family.[12] The *R. ribes* plant, which grows on rocks and cliffs, grows in stress-induced natural environments, and its excessive consumption in Eastern Anatolia has led to research on the plant. It has some healing properties, such as vomiting, preventing hemorrhoids, reducing stomach pain, reducing symptoms of inflammation, measles, and diabetes, and increasing appetite. The root has laxative and bleeding-stop effects. It regulates the digestive system.[13]

R. ribes contains chemicals called polyphenols that help prevent cancer. These substances prevent the development of many cancers, especially leukemia.[14] Polyphenols are one of the most common groups of plant metabolites and constitute the most important single group of flavonoids. Phenolic compounds destroy free radical chains, exhibit antioxidant activity, and form chelates with metal ions that catalyze lipid peroxidation.[15]

Considering the phenolic component profile of *R. ribes*, especially its flavonoids, stilbens, and antrakinons, it is believed that they provide a potential source of antioxidants.[16] Our study aims at determining the antioxidant capacity of the aboveground portions of the species *R. ribes*, of the family Polygonaceae, known in our country as the "Iskın," using different methods.

METHODS

The study collected approximately 2 kg of surface samples of the *R. ribes* species. An antioxidant activity study was carried out by extracting methanol extract from these samples. For methanol extraction, samples were dried and powdered. The dust samples were then left to be macerated for 3 days. Extracts were obtained by rotating steam when the samples were kept at a temperature of under 2 hours in the fractional distillation scheme.

Ferric Reducing Antioxidant Power Method

In order to produce the ferric reducing antioxidant power (FRAP) radical, 100 mL balloons were first taken into a bowl containing 40 mM HCl acid in a 10 mM TPTZ solution and completed with a volume of pure water to 100 mL. Another container produced a 20 mM $FeCl_3$ solution. In a third container, an acetate buffer containing 0.3 M acetates at pH 3.6 was prepared. From these 3 solutions in equal volumes, 30 mL of FRAP solution was obtained. Subsequently, 285 µL of this solution was transferred to pleytes, and 15 µL of samples were added to it. After a 30-minute incubation phase, the dissolutions were measured at a wavelength of 593 nm.

Cupric Reducing Antioxidant Capacity Method

0.4262 grams of $CuCl_2$ are used to prepare the reactive first. $2H_2O$ was diluted and dissolved in 250 mL of pure water (10 mM). Then 19.27 grams of NH4Ac were dissolved in 250 mL of water to prepare the acetate buffer. For the preparation of a 7.5 mM neocuproin solution, a 25 mL balloon jojed is obtained by preparing 0.039 grams of the neocuproid compound with ethanol at 96% purity. The solution was then mixed with 60 µL acetate buffer, 60 µl CuCI2, 60 µ L neocuproin solution, and 66 µL sample. It was incubated for 30 minutes, and then 450 nm wavelength measurements were taken. This method is based on the conversion of the Cu (II) Neocuproin complex to Cu (I) Neo-Cuproine through compounds that have an antioxidant effect in the environment and its absorption in the composite complex at 450 nm wavelengths.

2,2-Diphenyl-1-picrylhydrazyl Method

This method is used to measure the antioxidant capacity of a radical called diphenyl-1 picryl hydrazyl (DPPH). This substance is a stable and affordable nitrogen radical that absorbs at 515 nm wavelengths. During scanning, the DPPH solution loses its color and can be easily measured on a spectroscopic photometer. When the DPPH method is studied closely, a 390 µL and 25 mg/L DPPH solution is prepared in methanol and mixed with a sample solution of 10 µL volume according to the original DPPH procedure. The final solution, prepared until the absorbance value is stable, is measured in a 515 nm wavelength spectroscopic photometer. This measurement can last up to 30 minutes in some samples. The percentage of non-reacting DPPH is determined by the following formula:

$$\text{DPPH\%} = (\text{DPPH remaining}/\text{DPPH first added}) \times 100$$

There is a correlation between DPPH inhibition and the concentration of antioxidant substance.

RESULTS

Ferric Reducing Antioxidant Power Method

Gallic acid was used as a control sample in the study. The average absorption values for each aliquot are shown in Table 1. Calibration curves were derived from the 1-50 µg/mL working range, where the absorbance graph was linear (Figure 1) and statistical calculations were made (Table 2). Subsequently, samples of *R. ribes* were measured by the same method to be 6 square samples, and the antioxidant capacity of the gallic acid equivalent was calculated. Average equivalent levels of gallic acid are shown

Table 1. Absorbance Values Vs. Concentration for the Ferric Reducing Antioxidant Power Method

Concentration µg/mL	1	2.50	5.00	7.50	10.00	15.00	20.00	25.00	30	35	40	45.00	50.00
ABSORBANCE	0.052	0.121	0.158	0.244	0.329	0.442	0.621	0.741	0.892	1.027	1.145	1.325	1.481
	0.053	0.124	0.154	0.240	0.334	0.451	0.625	0.744	0.878	1.036	1.154	1.331	1.480
	0.048	0.125	0.161	0.243	0.338	0.448	0.632	0.742	0.881	1.031	1.146	1.340	1.460
	0.048	0.122	0.160	0.251	0.330	0.453	0.630	0.750	0.895	1.035	1.157	1.323	1.492
	0.051	0.116	0.152	0.253	0.339	0.452	0.636	0.752	0.886	1.041	1.159	1.317	1.484
	0.049	0.113	0.159	0.252	0.327	0.450	0.631	0.741	0.891	1.032	1.137	1.330	1.472
Average	0.050	0.122	0.158	0.245	0.335	0.449	0.629	0.747	0.885	1.033	1.150	1.328	1.476

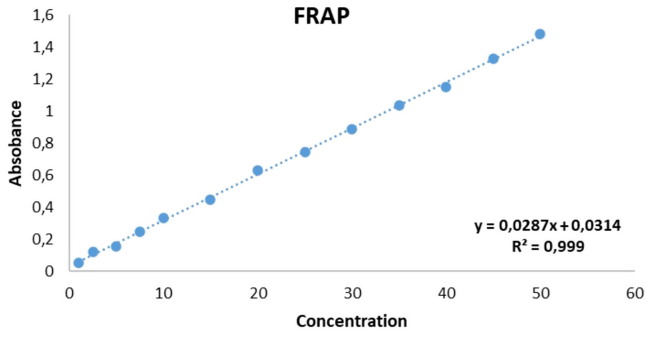

Figure 1. Concentration versus Absorbance Plot for Ferric Reducing Antioxidant Power Method.

Table 2. Statistical Values of the Calibration Curve for the Ferric Reducing Antioxidant Power Method

Method	WR (mg/mL)	LR[a]	Sa	Sb	R^2
FRAP	1-50	$y = 0.0287 \times 0.0314$	4×10^{-4}	7×10^{-5}	0.999

LR, linear regression; R2, correlation coefficient; Sa, standard deviation of the shift in the regression curve; Sb, standard deviation of the slope of the regression curve; WR, working range.[a]6 calibration curves.

Table 3. Average Equivalent Amount of Gallic Acid for Ferric Reducing Antioxidant Power Method

	Gallic Acid equivalent Antioxidant Capacity (µg/mL)
Rheum Ribes	48.0 ± 0.8

in Table 1. Measurements showed that 30 µg/mL of extrene was approximately equivalent to 48 µg/mL of gallic acid (Table 3).

Cupric Reducing Antioxidant Capacity Method Method

Gallik acid was used as a control sample for the CUPRAC method. Six different gallic acid samples were taken from each concentration, and the absorption of these samples was measured. The average absorbance values for each concentration are shown in Table 4. Calibration curves were derived from the 1-50 µg/mL working range, where the absorbance graph was linear (Figure 2) and statistical calculations were made (Table 5). Then the Iskın sample was measured using the same method, making it 6 square samples. The gallic acid equivalent antioxidant capacity was calculated. The average equivalent levels of gallic acid are shown in Table 6.

2,2-Diphenyl-1-picrylhydrazyl Method

Gallic acid samples, which were different from the DPPH method, were left to wait for 30 minutes, and after this incubation phase, measurements were made of each sample at a wavelength of 515 nm. Gallic acid was used as a control sample. The percentage of inhibition values for each concentration are shown in Table 7. Calibration curves were found (Figure 3) with a linear absorbance chart for the 1-20 µg/mL working range and calculated statistically. (Table 8). The measurements were done using the same method, with 6 samples from the *R. ribes* samples. Gallic acid-equivalent antioxidant capacities have been measured. The average equivalent inhibition levels of gallic acid are shown in Table 9.

DISCUSSION

Antioxidants are chemicals that protect cells by either blocking the formation of free radicals or cleaning out existing free radical.[17] Its structures usually contain phenolic components. Antioxidant activity refers to the process that occurs when antioxidant molecules and free radicals interact. Antioxidant capacity is defined as the reaction of an antioxidant chemical mixture with a radical.[18]

Oxidative stress is caused by oxygen-using metabolic pathways and is the result of a disturbance of the prooxidant and antioxidant balance in the body.[19]

Since free radicals interact with biological macromolecules in conditions of oxidative stress, causing a variety of diseases, the total definition of antioxidants that destroy these free radicals in biological samples is important. Many analytical methods have been developed to measure the antioxidant capacity.[11]

In this study, the antioxidant activity of the methanol extraction of the *R. ribes* plant, which grows in high places in the Erzurum region, were determined by various methods of in vitro activity.

The antioxidant levels of Iskın extracts have been determined with FRAP, an in vitro anti-oxidant method commonly used in the literature. In order to produce the FRAP sample, 100 mL of the balloon was initially taken into a bowl containing 40 mM HCl acid in a 10 mM TPTZ solution and completed with a volume of 100 mL pure water. Another container produced a 20 mM $FeCl_3$ solution. In a third container, an acetate buffer containing 0.3 M acetates at pH 3.6 was prepared. From these 3 solutions in equal volumes, 30 mL of FRAP solution was obtained. Subsequently, 285 µL of this solution was transferred to pleytes, and 15 µL of samples were added to it. After a 30-minute incubation phase, the dissolutions were measured at a wavelength of 593 nm. Gallic acid was used as a control sample in the study. Calibration curves were derived for the absorbance of reference solutions prepared in the

Table 4. Average Absorbance Values Corresponding to Concentration for the Cupric Reducing Antioxidant Capacity Method

Concentration µg/mL	1	2.50	5.00	7.50	10.00	15.00	20.00	25.00	30	40	50
ABSORBANCE	0.1327	0.1864	0.2746	0.3907	0.5560	0.6620	0.8677	1.0373	1.3033	1.7832	1.9921
	0.1325	0.1860	0.2740	0.3901	0.5564	0.6622	0.8675	1.0371	1.3030	1.7830	1.9925
	0.1330	0.1865	0.2743	0.3905	0.5559	0.6624	0.8679	1.0374	1.3035	1.7828	1.9927
	0.1320	0.1862	0.2745	0.3906	0.5557	0.6618	0.8680	1.0370	1.3034	1.7836	1.9920
	0.1322	0.1869	0.2749	0.3910	0.5563	0.6615	0.8671	1.0376	1.3036	1.7835	1.9918
	0.1333	0.1866	0.2741	0.3912	0.5561	0.6623	0.8672	1.0377	1.3037	1.7831	1.9923
Average	0.1326	0.1864	0.2744	0.3907	0.5561	0.6620	0.8676	1.0374	1.3034	1.7832	1.9922

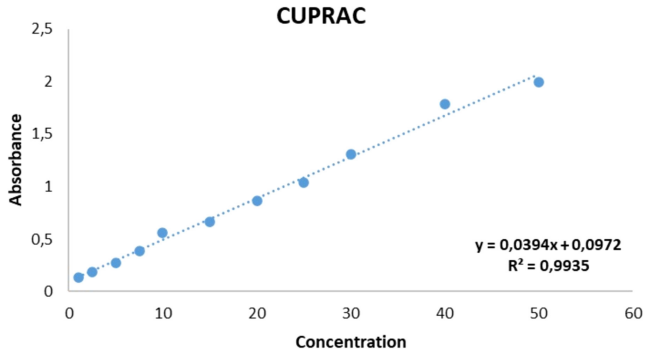

Figure 2. Concentration Versus Absorbance Pot for the Cupric Reducing Antioxidant Capacity Method.

Table 5. Statistical Values of the Calibration Curve for the Cupric Reducing Antioxidant Capacity Method

Method	WR (mg/mL)	LR[a]	Sa	Sb	R^2
CUPRAC	1-50	$y = 0.0394 \times 0.0972$	4×10^{-4}	7×10^{-5}	0.9935

CUPRAC, cupric reducing antioxidant capacity; LR, linear regression; R2, correlation coefficient; Sa, standard deviation of the shift in the regression curve; Sb, standard deviation of the slope of the regression curve; WR, working range.[a]6 calibration curves.

Table 6. Average equivalent amounts of gallic acid for the Cupric Reducing Antioxidant Capacity Method

	Gallic Acid equivalent Antioxidant Capacity (µg/mL)
Rheum ribes	46.7 ± 0.45

range of 1-50 µg/mL. The correct equation of the calibration curve is $y = 0.0287 \times 0.0314$. The Iskın extract was then measured at the same wavelength, and the gallic acid equivalent antioxidant activity was calculated at 48 µg/mL. It is one of the topics in the literature where emodine, aloe emodin, and flavonoids like parietine, antrachinons, and curcumin can show very high antioxidant activity.

Another method used is the CUPRAC method, which is widely used in literature. In order to produce the CUPRAC sample, 100 mL of the balloon was initially taken into a bowl containing 40 mM HCl acid in a 10 mM TPTZ solution and completed with a volume of 100 mL pure water. Another container produced a 20 mM $FeCl_3$ solution. In a third container, an acetate buffer containing 0.3 M acetates at pH 3.6 was prepared. From these 3 solutions, 30 mL of CUPRAC solution was obtained in equal volumes. Subsequently, 285 µL of this solution was transferred to pleytes, and 15 µL of samples were added to it. After a 30-minute incubation phase, the dissolutions were measured at a wavelength of 593 nm. Gallic acid was used as a control sample in the study. Calibration curves were derived for the absorbance of reference solutions prepared in the range of 1-50 µg/mL. The correct equation of the calibration curve was calculated as $y = 0.0394 \times 0,0972$. The extraction of Iskın was then measured at 450 nm wavelengths, and the gallic acid equivalent antioxidant activity was detected at 46.7 µg/mL.

Another method used is DPPH, one of the antioxidant capacity determination methods commonly used in literature. This method is used to measure the capacity of a radical antioxidant called diphenyl-1 picryl hydrazyl. When the DPPH method was studied, according to the original DPPH procedure, a 390 µL and 25 mg/L DPPH solution was prepared in methanol and mixed with a sample solution of 10 µL. The absorbance value was measured at 515 nm wavelengths in the final solution spectroscopic photometer, prepared until stable, and the gallic acid equivalent antioxidant activity was detected at 16.7 µg/mL.

Iskın contains many anthraquinons and flavonoids. These are the anthraquinones and flavonoids emodin, aloe emodin, and chersetin, along with parietine. There are many opinions in the literature that these substances may have very high antioxidant activity due to their presence.[20,21] The study revealed similar findings to previous studies in the literature on the antioxidant properties of methanol extracts from *R. Ribes*.[22] It exhibited significant antioxidant activity, consistent with findings reported in the literature.

As expected, a high antioxidant capacity has been detected for Iskın samples grown in Erzurum in the study. According to this study, a sample of 30 µg/mL of the methanol extraction of the Iskın plant showed antioxidant activity equivalent to approximately 48 µg/mL of gallic acid by FRAP, which is about 1.5 times the reference effect on the neuproin radical. The CUPRAC method showed antioxidant activity equivalent to approximately 47 µg/mL of gallic acid, which is about 1.5 times the reference, for example. The DPPH method showed antioxidant activity equivalent to approximately 47 µg/mL of gallic acid, about half of the reference sample. This study suggests that the samples of Iskın collected in Erzurum have a very high content of flavonoids and anthraquinones. It is thought to be an important starting point for the future culture, standardization, and industrial use of the Iskın plant.

In the study, an endemic species, and ethnobotanical studies conducted in Erzurum, the antioxidant capacity of the Iskın plant used in the treatment of many diseases such as cancer and diabetes has been determined using different methods. The reaction of the Iskın plant to the radicals of triphenyl tetrazoline chloride, neokuproin, and Difenyl Pikril Hydrazine was detected by spectroscopic measurements, and the results were compared with the reference gallic acid sample. The results show that this endemic plant has very high antioxidant activity. The study is in line with similar studies in literature. According to these studies, it is believed that the Iskın plant found in our area has a very intense content of anthraquinones and flavonoids. Therefore, it is believed that the cultivation of this wildly grown plant, the standardization of its contents, and the introduction to the economically relatively underdeveloped East Anatolia and Southeast Anatolia economies can play a role in the development of both our region's industry and agriculture, as well as the extension to the industry of the compounds contained in its Iskın contents

Table 7. Average absorbance values corresponding to concentration for the DPPH method

Concentration µg/mL	1	2.50	5.00	7.50	10.00	12.50	15.00	17.5	20
ABSORBANCE	5.187	7.854	23.639	37.462	47.611	61.014	72.296	85.640	97.444
	5.213	7.850	323.608	37.412	47.650	61.040	72.282	85.624	97.440
	5.145	7.842	223.600	37.468	47.582	61.020	72.296	85.642	97.424
	5.223	7.874	223.650	37.476	47.610	61.032	72.264	85.618	97.480
	5.172	7.877	223.661	37.482	47.620	60.982	72.314	85.660	97.490
	5.201	7.891	223.642	37.470	47.620	61.024	72.318	85.640	97.512
Average	5,190	7,865	23,633	37,462	47,616	61,019	72,295	85,637	97,465

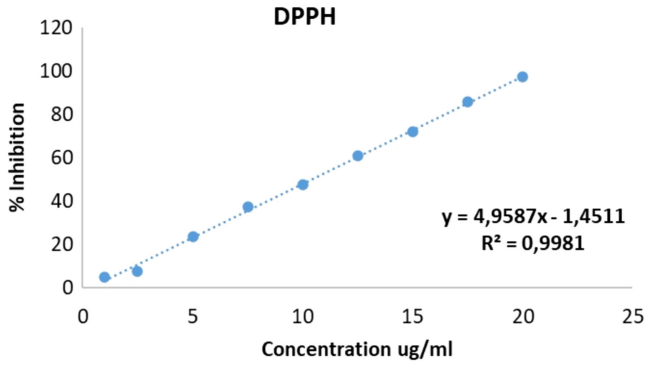

Figure 3. Absorbance Graph Corresponding to Concentration for the 2,2-Diphenyl-1-picrylhydrazyl Method.

Table 8. Statistical Values of the Calibration Curve for the 2,2-Diphenyl-1-picrylhydrazyl Method

Method	WR (mg/mL)	LR[a]	Sa	Sb	R^2
DPPH	1-20	$y = 4.9587 \times -1.4511$	4×10^{-4}	7×10^{-5}	0.9981

DPPH, 2,2-Diphenyl-1-picrylhydrazyl; LR, linear regression; R2, correlation coefficient; Sa, standard deviation of the shift in the regression curve; Sb, standard deviation of the slope of the regression curve; WR, working range. [a]6 calibration curves.

Table 9. Average Equivalent Amounts of Gallic Acid for the 2,2-Diphenyl-1-picrylhydrazyl Method

	Gallic Acid equivalent Antioxidant Capacity (µg/mL)
Rheum Ribes	16.7 ± 0.38

used in the pharmaceutical and cosmetic industries. This work establishes a foundation for identifying, separating, and investigating the effectiveness of chemicals believed to be accountable for the antioxidant properties of the plant.

Ethics Committee Approval: Ethical approval and informed consent are not required in our study as no research was conducted on human or animal specimens.

Peer-review: Externally peer-reviewed.

Author Contributions: Concept – O.Ş., E.S.Z.; Design – O.Ş., E.S.Z.; Supervision – O.Ş.; Resources – O.Ş.; Materials – S.Ö., E.S.Z.; Data Collection and/or Processing – S.Ö., O.Ş., E.S.Z.; Analysis and/or Interpretation – S.Ö.; Literature Review – S.Ö.; Writing – S.Ö., O.Ş.; Critical Review – S.Ö.

Declaration of Interests: The authors declare that they have no competing interest.

Funding: The authors declared that they received no financial support for this study.

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