



Total phenolic and flavonoid contents and antioxidant capacity of home-made Isabella grape (*Vitis labrusca* L.) vinegar

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ABSTRACT

Vitis vinifera is commonly consumed and utilized to produce juice, which has health benefits. The purpose of the current study is to determine antioxidant activity of the vinegar obtained from *V. vinifera*. While total phenolic content was found as $160.23 \pm 0.007 \mu\text{g GAE/ml}$, total flavonoid content was determined as $298 \pm 0.069 \mu\text{g CE/ml}$. Moreover, total antioxidant capacity was detected as $113.12 \pm 0.011 \mu\text{g AAE/ml}$. CUPRAC activity and DPPH radical scavenging activity of the vinegar was higher than standards (BHT for CUPRAC activity, BHT and Rutin for DPPH activity). The grape vinegar exhibited lower ABTS radical scavenging activity than standards (BHT and Rutin). Both ABTS and DPPH radical scavenging activity of the grape vinegar and standards (BHT and Rutin) increased with the increasing concentration. ABTS activity of the vinegar ranged between 27.47% and 63.72%. This study reveals that grape vinegar possess significant antioxidant properties and may be an alternative to synthetic antioxidant agents.

Keywords: Phenolic content, antioxidant activity, *Vitis labrusca*, vinegar.

Ev yapımı Isabella üzümü (*Vitis labrusca* L.) sirkesinin toplam fenolik ve flavonoid içerikleri ve antioksidan kapasitesi

ÖZ

Vitis vinifera sağlığa yararlı olan meyve suyu üretiminde kullanılmakta ve sıklıkla tüketilmektedir. Mevcut araştırmanın amacı *V. vinifera*'dan elde edilen sirkenin antioksidan aktivitesini belirlemektir. Total fenolik içeriği $160.23 \pm 0,007 \mu\text{g GAE/ml}$ olarak bulunurken; toplam flavonoid içeriği $298 \pm 0.069 \mu\text{g CE/ml}$ olarak saptanmıştır. Ayrıca, total antioksidan kapasitesi $113.12 \pm 0.011 \mu\text{g AAE/ml}$ olarak belirlendi. Sirkenin CUPRAC aktivitesi ve DPPH radikal süpürme aktivitesi standartlardan (CUPRAC aktivitesi için BHT, DPPH aktivitesi için BHT ve Rutin) daha yüksektir. Üzüm sirkesi standartlardan daha düşük bir ABTS radikal süpürme aktivitesi sergiledi. Üzüm sirkesinin ve standartların hem ABTS hemde DPPH süpürme aktiviteleri artan konsantrasyonlarla artmıştır. Sirkenin ABTS aktivitesi 27.47% ve 63.72% arasında değişmiştir. Bu çalışma üzüm sirkesinin önemli antioksidan özelliklere sahip olduğunu ve sentetik antioksidan ajanlara alternatif olabileceğini ortaya koymaktadır.

Anahtar Kelimeler: Fenolik içerik, Antioksidan aktivite, *Vitis labrusca*, sirke.

1. INTRODUCTION

Vinegar forms after fermentation process. Vinegar mainly includes carbohydrates-rich foods.¹ Ethyl alcohol provides partial oxidation during the conversion

of wine or fruit juice into vinegar, and then acetaldehyde is generated. Finally, the acetaldehyde is converted into acetic acid.²

For more than 2000 years, the vinegar has been utilized for flavoring and preserving foods, curing

wounds, struggling infections, cleaning surfaces, and controlling diabetes.³ Daily intake of the vinegar was recorded to protect against some illnesses, such as hypertension, obesity and hyperlipidemia. Besides this the vinegar has also some healthy features such as promoting recovery, adjusts blood glucose, blood pressure, helps digestion, induces the appetite, and helps calcium absorption.⁴

Acetic acid and other components which found in vinegar might be attributed to its therapeutic impact¹. Natural vinegar contains various medicinal components such as carbohydrate, organic acid (e.g. acetic, formic, gallic acid catechin, lactic and malic), alcohols and amino acid and peptides, vitamins and minerals salts.⁵ Friedman and co-workers have revealed that some properties of the vinegar such as acidity, alcohol content, and content of polyphenolic compounds like tannins and resveratrol might have antimicrobial effects against foodborne microorganisms.⁴

The ingestion of the vinegar is stated to be linked with satiety and to decrease intake of subsequent meals, which this situation can contribute to weight loss.⁶ Recently, vinegar has an importance in gastronomy and its quality is protected by title of origin in different vinegar-producing areas. Vinegar is generally utilized as an ingredient in the food, and it is a suitable solvent for the essential oils of herbs and spices in the salads or meals.⁴

An antioxidant can be explained as any substance that inhibits oxidative damage to a target molecule. An antioxidant have the ability to scavenge free radicals. Antioxidant compounds such as phenolic acids, polyphenols and flavonoids scavenge free radicals, so they inhibit the oxidative mechanisms that cause to degenerative illnesses.⁷ Recent studies has demonstrated that bioactive compounds found in the vinegar may reduce degenerative diseases through antioxidant effects.⁸

In this study, antioxidant activity of home-made vinegar which prepared from fragrant black grapes (isabella) grown in Black Sea, Giresun region was investigated.

2. MATERIALS AND METHODS

2.1. Chemicals

Butylated hydroxytoluen (BHT), 2,2'-azinobis (3-ethylbenzthiazoline-6-sulfonic acid) (ABTS) were purchased from Fluka Chemical Co. (Buchs, Switzerland). 2,2-diphenyl-1-picryl-hydrazyl (DPPH), gallic acid, catechin, neocuproine, CuCl_2 , ammonium acetate, sulfuric acid, sodium phosphate, ammonium

molybdate, ascorbic acid and rutin were obtained from Sigma Chemical Co. (St. Louis, MO, USA).

2.2. Preparation of the vinegar

Fragrant black grapes (isabella) used in the production of the vinegar, grown in Giresun region, were collected from Giresun-Piraziz-Gökçeali Village in November 2017. Grapes which used for production of vinegar were also collected and brought to the laboratory. The washed grape dices were taken into a large clean container and 3 times as much water was added. Clean and untreated village water was used. It was kept in the dark for 30 days and shaken for oxygenation. After 30 days, it was drained, and the mouth was closed with cloth for 60 days. Then it was kept in the dark for maturation. After 90 days, the formation of the vinegar was completed and analyzes were made.

2.3. Total phenolic content

Total phenolic content of the vinegar was examined by Folin-Ciocalteu assay. Total phenolic contents of grape vinegar were determined in accordance with the method of Slinkard and Singleton (1977) utilizing gallic acid standard. Shortly, 0.1 ml vinegar was diluted with 4.5 ml distilled water. Then, 0.1 ml Folin-Ciocalteu reagent (previously diluted 3- fold with distilled water) was put into the mixture. After 3 minutes, 0.3 ml Na_2CO_3 (2%) was added. The absorbance was measured at 760 nm after incubating the mixture for 90 min. Total phenolic content of the extracts was expressed as μg gallic acid equivalents (GAE)/ml by using the calibration curve.⁹ The tests were performed in triplicate.

2.4. Total flavonoid content

0.25 ml vinegar, 1.25 ml distilled water and 75 μl NaNO_2 (5%) were mixed and vortexed. After 6 min, 150 μl of $\text{AlCl}_3 \cdot 6\text{H}_2\text{O}$ (10%) was added and the mixture was kept at room temperature for 5 min. Then, 0.5 ml NaOH (1M) and 725 μl distilled water added the mixture. Absorbance was measured at 510 nm. Catechin was used as standard and the results were expressed as μg catechin equivalent (CE)/ml.¹⁰ The tests were performed in triplicate.

2.5. Antioxidant activity

2.5.1. ABTS radical scavenging activity

ABTS⁺ solution was prepared by mixing 7.4 mM ABTS and 2.6 mM potassium persulfate, and the mixture was kept at room temperature for 12 h in the dark to complete reaction. Then, ABTS⁺ solution diluted with

methanol to obtain an absorbance of 0.700 ± 0.02 units at 734 nm.²³ Vinegar (150 μ l) was allowed to react with 2850 μ l of the ABTS⁺ solution for 2 h in a dark, and the absorbance was measured at 734 nm.¹¹ The tests were performed in triplicate. The ABTS radical scavenging activity was calculated using the following equation.

ABTS radical scavenging activity (% inhibition):

$$[(A_0 - A_1) / A_0] \times 100 \quad (1)$$

A_0 = Absorbance of control, A_1 = Absorbance of sample.

2.5.2. DPPH radical scavenging activity

Appropriate dilution series (50-200 μ g ml⁻¹) were added 1.5 ml of a 6×10^{-5} M methanolic solution of DPPH. After vortexing the tubes, the tubes were incubated in the dark. After 30 minutes, the absorbance was read at 517 nm. Synthetic antioxidant reagents BHT and rutin were used as positive control.¹² The tests were performed in triplicate. The DPPH radical scavenging activity was calculated using the following equation:

DPPH radical scavenging activity (% inhibition):

$$[(A_0 - A_1) / A_0] \times 100 \quad (2)$$

A_0 = Absorbance of control, A_1 = Absorbance of sample.

2.5.3. Total antioxidant capacity

Phosphomolybdenum method was used to determine total antioxidant capacity of the vinegar.¹³ 0.3 ml vinegar

and 3000 μ l reagent (contains 0.6 M sulfuric acid, 28 mM sodium phosphate and 28 M ammonium molybdate) was mixed and incubated at 95 °C for 90 min. Then, Absorbance was read at 695 nm. Ascorbic acid was used as the standard. The total antioxidant capacity was expressed as μ g ascorbic acid equivalent (AAE)/ml. The tests were performed in triplicate.

2.5.4. Cupric reducing antioxidant capacity (CUPRAC)

0.5 ml vinegar (prepared in 250-1000 μ g ml⁻¹), 1.0 ml CuCl₂ solution, 1 ml neocuproine solution and 1.0 ml ammonium acetate buffer were mixed in a test tube. Tubes were vortexed and stored in a dark place for 30 min. Absorbance was measured at 450 nm. BHT was utilized as standard antioxidant substance.¹⁴

3. RESULTS AND DISCUSSION

Phenols improve the status of various oxidative stress biomarkers. Phenols have many antioxidative mechanisms such as scavenge free radicals, break radical chain reactions, reducing peroxides, and stimulate the antioxidative defense enzyme activities.¹⁵

Flavonoids are secondary plant metabolites which are responsible for producing yellow and other pigments in the plants. Moreover, flavonoids exhibit significant activities in humans.¹⁶

Total phenolic and flavonoid contents, total antioxidant capacity are given in Table 1. Total phenolic and flavonoid contents, total antioxidant capacity of the grape vinegar were determined as 160.23 ± 0.007 μ g GAE/ml, 298 ± 0.0069 μ g CE/ml and 113.12 ± 0.011 μ g AAE/ml, respectively. DPPH, ABTS scavenging activi-

Table 1. Total phenolic, total flavonoid contents and total antioxidant capacity of the grape vinegar

	Total Phenolic Content (μ g GAE/ml)	Total Flavonoid Content (μ g CE/ml)	Total Antioxidant Capacity (μ g AAE/ml)
Grape Vinegar	160.23 ± 0.007	298 ± 0.0069	113.12 ± 0.011

ties and CUPRAC activity of the grape vinegar are demonstrated in Table 2. Grape vinegar exhibited strong DPPH radical scavenging activity even better than the standards such as BHT and Rutin. DPPH activity of the vinegar is illustrated in Figure 1. DPPH activity of the vinegar ranges between 83.66% to 95.81%. DPPH activity of the vinegar increases with the increasing concentrations. The activity was increased in the following order: Grape vinegar > Rutin > BHT.

Grape vinegar exhibited the weak ABTS radical scavenging activity. ABTS activity of the vinegar was summarized in Figure 2. Contrary to DPPH scavenging activity, standards have higher activity than vinegar in

ABTS assay. ABTS radical scavenging activity of the grape vinegar and standards increase with the increasing concentrations. ABTS activity of the vinegar ranges from 27.47% to 63.72%.

Moreover, vinegar demonstrated high CUPRAC activity when compared with DPPH and ABTS scavenging activities. Vinegar possess CUPRAC activity better than BHT which used as standard. There are some studies about antioxidant ability of vinegars from Isabella grapes (*Vitis labrusca*) in literatures. For example, Machado and co-workers¹⁷ found total phenolic contents of organic grape (*V. labrusca*) vinegar, Grape (*V. labrusca*) vinegar organic 5% enriched with grape

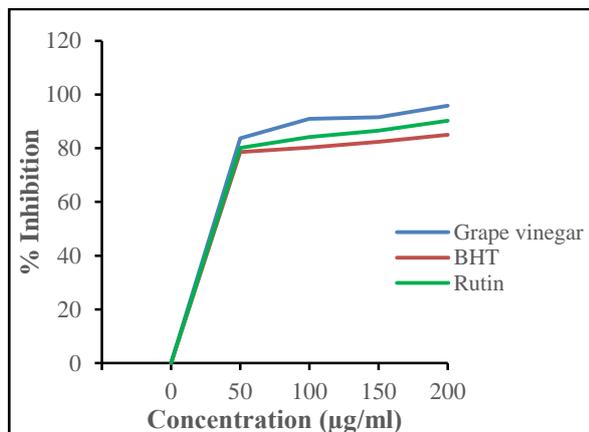


Figure 1. DPPH radical scavenging activity of the vinegar and standards.

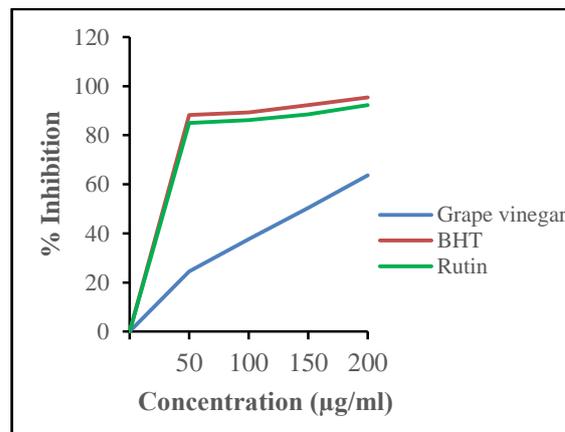


Figure 2. ABTS scavenging activity of the vinegar and standards.

Table 2. DPPH and ABTS scavenging activities of the grape vinegar (% inhibition)

	Concentration (µg ml ⁻¹)	DPPH radical scavenging activity (% inhibition)	ABTS radical scavenging activity (% inhibition)	Concentration (µg ml ⁻¹)	CUPRAC Activity
Grape vinegar	50	83.66±0.006	27.47±0.014	250	1.1273±0.003
	100	91.00±0.014	37.65±0.028	500	1.9936±0.078
	150	91.52±0.005	50.40±0.018	750	2.5125±0.039
	200	95.81±0.005	63.72±0.006	1000	2.5328±0.02
BHT	50	78.62±0.005	88.28±0.04	250	0.7026±0.003
	100	80.29±0.002	89.32±0.006	500	0.7151±0.026
	150	82.35±0.001	92.33±0.004	750	0.8822±0.010
	200	85.05±0.003	95.45±0.008	1000	0.9810±0.004
Rutin	50	80.17±0.003	84.94±0.048	-	-
	100	84.22±0.004	86.14±0.019	-	-
	150	86.54±0.003	88.46±0.010	-	-
	200	90.25±0.005	92.23±0.006	-	-

seeds and grape (*V. labrusca*) vinegar organic 10% enriched with grape seeds which prepared from grapes in Brazil as 23.62 ± 0.4 mg gallic acid/ml, 39.78 ± 0.29 mg gallic acid/ml and 24.80 ± 2.05 mg gallic acid/ml, respectively. Moreover, it was found that total flavonoid contents of organic grape vinegar, grape vinegar organic 5% enriched with grape seeds and grape vinegar organic 10% enriched with grape seeds as 0.110 ± 0.007 mg quercetin/ml, 0.118 ± 0.007 mg quercetin/ml and 0.124 mg quercetin/ml, respectively.¹⁷ In our study, it was determined total phenolic and flavonoid contents of grape vinegar as 160.23 ± 0.007 μ g GAE/ml and 298 ± 0.0069 μ g CE/ml. These different results might be attributed to use grapes from different geological areas. Machado and co-workers¹⁷ also stated that IC₅₀ values of DPPH activity of the grape vinegar organic grape vinegar, grape vinegar organic 5% enriched with grape seeds and grape vinegar organic 10% enriched with grape seeds as 6.70 μ g ml⁻¹, 9.01 μ g ml⁻¹ and 7.07 μ g ml⁻¹, respectively.¹⁴

Cottica and co-workers recorded that total phenolic content of grape (*V. labrusca*) vinegar as 162.91 ± 5.98 mg GAE/100 g⁻¹ and EC₅₀ value of DPPH activity of grape vinegar as 136.23 ± 0.82 μ g ml⁻¹.¹⁸

Kelebek and co-workers stated that antioxidant activity of grape vinegars changed between 5.39% and 14.43% according to DPPH assay while the values ranged from 7.72% to 17.96% with respect to ABTS assay, respectively.¹⁹ In our study, DPPH activity of the vinegar ranges between 83.66% to 95.81% and ABTS activity of the vinegar ranged from 27.47% to 63.72%. This difference could be explained by using vinegars obtained from grapes collected from different geographical locations and using different grape species when preparing vinegar.

Different vinegar types also have antioxidative properties. For example, Hafzan and co-workers investigated homemade and commercial dated vinegars. As a result of the study, it was concluded that homemade dated vinegar showed generally higher antioxidant activity than commercial dated vinegar.²⁰ Budak searched the antioxidative ability of pomegranate vinegar.²¹ Tagliazucchi and co-workers revealed the antioxidant potential of balsamic vinegar.²² Poiana and co-workers also studied the antioxidative effect of apple vinegars.²³

4. CONCLUSIONS

According to the data obtained, it can be concluded that grape vinegar has strong antioxidant activity. The findings of the current study confirm and improve the information on health-promoting and food safety-related potentials of grape vinegar. These results might associate with the chemical composition of the grape and repre-

sent a mechanism underlying the traditional use of *Vitis labrusca* vinegar.

Conflict of interest

Authors declare that there is no a conflict of interest with any person, institute, company, etc.

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