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Antimicrobial, Antioxidant Activities and Total Phenolic Contents of the Traditional Turkish Beverages Produced by Using Grapes#

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A R T I C L E I N F O

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A B S T R A C T

In the present study, antimicrobial effects of traditional beverages produced from grapes such as traditional grape pickles, grape juice, home-made hardaliye, and commercially produced hardaliye were investigated using microdilution method against *Staphylococcus aureus* ATCC 6538P, *Escherichia coli* ATCC 25922, *Salmonella* Typhimurium NRRL B4420, *Bacillus cereus* ATCC 10876, *Saccharomyces cerevisiae* NRRL Y-12632, *Lactobacillus acidophilus* La-5 and *L. rhamnosus* LGG. In addition, total phenolic content was measured by the Folin-Ciocalteau method, and antioxidant activities of grape products were evaluated using DPPH assay. Results showed that grape containing beverages have antimicrobial effects on *S. aureus*, *E. coli*, *S. typhimurium* and *B. cereus* at various minimum inhibitory concentration (MIC) values in the range of 4.53-150 mg/mL. The lowest MIC value of 4.53 mg/mL was obtained against *E. coli* for home-made hardaliye. MIC values of the traditional grape pickles were determined as 4.69 mg/mL and 9.38 mg/mL against *S.aureus* and *E.coli*, respectively. On the other hand, traditional grape pickles showed weak inhibitory effects against *B.cereus* with MIC value of 150 mg/mL. The bactericidal effect of these grape products was not detected for any of the test microorganisms however traditional foods produced by using grapes were showed inhibitory effects at different concentrations against tested microorganisms except for probiotics and the yeast. The total phenolic contents of the grape products were within the range of 865.27-2193.08 mg gallic acid equivalent (GAE)/L. Free radical scavenging activities of grape samples ranged from 46% to 90% and the grape juice was found to have the highest antioxidant activity. In conclusion, grape beverages have the potential to act as a antimicrobial and antioxidant agents for use as a natural antimicrobial and antioxidant product in the food industry.

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Üzüm Kullanılarak Üretilen Geleneksel Türk İçeceklerinin Antimikrobiyal, Aktioksidan Etkileri ve Toplam Fenolik İçerikleri

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Ö Z

Bu çalışmada üzüm turşusu, üzüm suyu, ev yapımı ve ticari olarak üretilmiş hardaliye gibi üzüm ile üretilen geleneksel içeceklerin mikrodilüsyon yöntemi kullanılarak *Staphylococcus aureus* ATCC 6538P, *Escherichia coli* ATCC 25922, *Salmonella* Typhimurium NRRL B4420, *Bacillus cereus* ATCC 10876, *Saccharomyces cerevisiae* NRRL Y-12632, *Lactobacillus acidophilus* La-5 ve *L. rhamnosus* LGG'ye karşı antimikrobiyal etkisi araştırılmıştır. Ayrıca, üzüm ürünlerinin toplam fenolik içeriği Folin-Ciocalteau yöntemi ile ölçülmüş ve antioksidan aktiviteleri DPPH yöntemi ile değerlendirilmiştir. Sonuçlar, üzüm içeren bu içeceklerin *S. aureus*, *E. coli*, *S.* Typhimurium ve *B. cereus* üzerinde 4.53-150 mg/mL aralığında minimum inhibisyon konsantrasyonu (MİK) değerlerinde antimikrobiyal etkiye sahip olduğunu göstermiştir. Ev yapımı hardaliyenin en düşük MİK değerinin 4,53 mg/mL ile *E. coli*'ye karşı olduğu belirlenmiştir. Geleneksel üzüm turşunun *S.aureus* ve *E.coli* için MİK değerleri sırasıyla 4,69 mg/mL ve 9,38 mg/mL olarak belirlenmiştir. Diğer yandan, geleneksel üzüm turşusu, 150 mg/mL MİK değeri ile *B.cereus*'a karşı zayıf inhibisyon etkisi göstermiştir. Kullanılan bu üzüm ürünlerinin test edilen mikroorganizmaların hiçbirinde bakterisidal etkisi tespit edilememiştir, ancak üzüm kullanılarak üretilen geleneksel gıdaların, probiyotikler ve maya dışındaki test edilen mikroorganizmalara karşı farklı konsantrasyonlarda inhibe edici etki gösterdiği görülmüştür. Üzüm ürünlerinin toplam fenolik içerikleri 865,27-2193,08 mg gallik asit eşdeğeri (GAE)/L aralığındadır. Üzüm örneklerinin serbest radikal süpürme aktiviteleri %46 ile %90 arasında değişmiş olup, üzüm suyunun en yüksek antioksidan aktiviteye sahip olduğu bulunmuştur. Sonuç olarak, üzüm içeren geleneksel içeceklerin antimikrobiyal ve antioksidan ajan olarak kullanım potansiyelinin bulunduğu ve gıda sektöründe doğal bir antimikrobiyal ve antioksidan ürün olarak kullanılabileceği saptanmıştır.

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Introduction

Grapes *(Vitis vinifera* L.*)* are one of the commercially important fruit worldwide. Grapes are one of the main natural sources of phenolic compounds, therefore it has been associated with substantial health benefits such as antioxidant, anti-carcinogenic, cardioprotective and anti-inflammatory properties (Toaldo et al., 2014). Harvest dates of grapes are closely connected to the timing of grape maturation and which is not available throughout the year (Cook and Wolkovich, 2016). Generally, grapes are consumed either raw or in processed form. The beverages produced by using grapes are a good opportunity for consumers who want to consume grapes all seasons (Coskun, 2017). Grape juice is a fresh and nutritious beverage containing minerals, vitamins and phenolics (Faria et al., 2016).

Grapes are rich in phenolic compounds, such as flavonoids (catechin, epicatechin, quercetin, anthocyanins and procyanidins), and resveratrol, which are mainly found in red grape products (Dani et al., 2007). These compounds have been identified and quantified in several fruits and vegetables and show a high correlation with antioxidant activity (Soares, 2002; Einbond et al., 2004; Pehlivan and Sevindik, 2018). Moreover, these polyphenol compounds have been proved to show various biological effects such as antioxidant or antimicrobial (Jayaprakasha et al., 2003; Baydar et al., 2006; Sevindik et al., 2017). Grape juice consumption in the world has increased significantly in recent years (Spinelli et al., 2016). Grape pickle is a traditional fermented beverage made by adding molasses, horseradish (*Armoracia rusticana* P. Gaertn., B. Mey. & Scherb.) roots and grapes in the Thrace region of Turkey. Hardaliye is a grapefruit based a non-alcoholic traditional beverage in the Thrace region of Turkey. Hardaliye is mostly manufactured homemade by the traditional method. Red grape (Papazkarasi, Alphonse or Cardinal) or grape juice, 0.2% of crushed raw mustard seeds, sour cherry leaves and 0.1% of benzoic acid are mixed in preferably in wooden (or plastic) barrel for the production of hardaliye and then the mix is fermented for 5–10 days. After fermentation, the ingredients are filtered and hardaliye is stored at 4℃ for 3–4 months (Arici and Coskun, 2001; Altay et al., 2013; Amoutzopoulos et al., 2013; Marsh et al., 2014; Panghal et al., 2018). Hardaliye is a valuable, traditional beverage due to it is highly delicious, non-alcoholic, non-dairy and low-fat that is used in the nutrition of a wide range of consumer groups including children, vegetarians, people with a dairy intolerance (Amoutzopoulos et al., 2013).

Interest in the use of natural substances to prevent microbiological spoilage of food has significantly increased in the last years, due to the high demand of high nutritional value and safe foods (Soliva-Fortuny and Martín-Belloso 2003; Raybaudi-Massilia et al., 2009). The development of alternative food products for the preservation of microbiological quality continues and traditional natural products are considered as good alternatives. The objective of this study was to evaluate the antimicrobial, the antioxidant activities and total phenolic contents of different types of traditional Turkish beverages produced using grapes.

Materials and methods

Samples

In this study, traditional beverages produced from grapes such as traditional grape pickles, grape juice, supernatant from the grape juice, home-made hardaliye and commercially produced hardaliye were used. The home-made hardaliye was obtained by a producer who produces the traditional hardaliye for their own consumption. Commercially produced hardaliye was purchased from a local market in Kırklareli. Grape pickle samples were produced in our laboratory according to traditional procedures. Grape juice samples were provided from Samsun, Turkey and used in two different ways. One grape juice sample was used as it was and for the second sample, the grape juice was centrifuged at 4,000×g for 10 min for the experiments and the supernatant was used. All the test materials were filtered through a 0.45 μm cellulose acetate filter (Minisart, Sartorius) directly to an amber coloured bottle. Sterile test samples were stored at 4℃.

pH and °Brix Values of the Beverages

Total soluble solid content (°Brix) of samples was measured using a refractometer. The pH values were determined by a digital pH meter (NEL Mod 821).

Total Phenolic Content of The Beverages

The total phenolic contents of the beverages were determined spectrophotometrically at 760 nm according to the Folin-Ciocalteu colorimetric method (Singleton and Rossi, 1965). Estimations were carried out in triplicate and calculated from the standard curve was prepared with gallic acid. Total phenolics were expressed as gallic acid equivalents (mg GAE/mL).

Free Radical-Scavenging Ability (DPPH)

The free radical-scavenging ability of grape products was also studied through the evaluation of the free radical-scavenging effect on the 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical. The DPPH assay was determined using the modified method of Singh et al. (2002). The sample (0.1 mL) was pipetted into 5 mL of 0.1 mM DPPH methanolic solution to initiate the reaction. The mixture was thoroughly vortex-mixed and kept in the dark for 15 min. The absorbance was measured later, at 517 nm using the Agilent Technologies Cary 60 UV-Vis, against a blank of methanol without DPPH. Results were expressed as a percentage of inhibition of the DPPH radical. Percentage of inhibition of the DPPH radical was calculated according to the following equation:

$$\%\text{Inhibition of DPPH} = \left(\frac{\text{Abs control-Abs sample}}{\text{Abs control}}\right) \times 100$$

where Abs control is the absorbance of DPPH solution without samples.

Test Cultures and Culture Preparations

The antimicrobial activity of products was assessed against six bacteria: *Staphylococcus aureus* ATCC 6538P, *Escherichia coli* ATCC 25922, *Salmonella* Typhimurium NRRL B4420, *Bacillus cereus* ATCC 10876, *Lactobacillus acidophilus* La-5, *L. rhamnosus* LGG and the yeast *Saccharomyces cerevisiae* NRRL Y-12632. The test cultures were subcultured twice before the inoculation. Bacterial cultures were inoculated into Tryptone Soya Broth (TSB, pH 7.3±0.2, Oxoid) and incubated at 37°C for 24 h and for the studied yeast Malt Extract Broth (MEB, pH 4.6±0.2, Merck) was used and incubated at 30℃ for 24 h. Activated (18–24 h) microbial cells were collected by centrifugation (4000×g, 10 min, 4°C) and re-suspended in sterile peptone water (0.1%), and then microbial suspensions were adjusted to 0.5 McFarland scale from this suspension (Romulo et al., 2018). Total microbial count in the standard inoculum was determined using the spread plate technique. Appropriate dilutions were prepared and inoculated on TSA and MEA plates for bacteria and yeast, respectively. Inoculated plates were incubated at 37°C for 24−48 hours for bacteria and at 30°C for 3-5 days for yeast.

Determination of Antimicrobial Activity

The antibacterial activities of samples were determined by using a microdilution method and the minimum inhibitory concentrations of grape products were determined. Different culture media of Mueller Hinton Broth (MHB), de Man, Rogosa and Sharpe (MRS) Broth and Malt Extract Broth (MEB) were used for the bacteria, the two probiotics and the yeast, respectively. The minimum inhibitory concentration of products against microorganisms was carried out by broth microdilution method using sterile 96-well cell culture microplates. The 96-well plates were prepared by dispensing into each well 100 µL of broth medium except first wells and 200 µL from the sample was added into the first wells. Then, 100 µL from their serial dilutions was transferred into consecutive wells. For the determination of antimicrobial activity, cultures were adjusted to 0.5 McFarland turbidity standard. Microplates were inoculated with respective microbial culture suspension and then incubated at 37°C for 24 h for bacteria and 30°C for 48h for yeast (No et al., 2002; Jung et al., 2010). The microplates were tested for the absence or presence of visible growth in comparison with that in negative control wells. At the end of the incubation, the microbial growth was indicated by the presence of a white “pellet” on the well bottom and an effective growth indicator for use of dye reagents by 2,3,5-Triphenyltetrazolium chloride (TTC) or the resazurin (Balouiri et al., 2016). The minimum inhibitory concentration (MIC) was determined as the lowest concentration of an antimicrobial agent that prevents visible growth of a microorganism in broth dilution susceptibility test. The minimum bactericidal concentration was defined as the lowest concentration at which there was no microbial growth. The minimum bactericidal concentration (MBC) was determined by inoculation of a loopful from each clear well onto agar plates (Filocamo et al., 2015).

Results and Discussion

In the present study, the antimicrobial effects of traditional grape-containing products on Gram-positive and Gram-negative bacteria, two probiotics and yeast were investigated. The pH and °Brix values of the samples are also determined (Table 1). Total soluble solids (°Brix) of the different samples showed differences ranged from 14% to 60% and the pH values of samples ranged from 3.00 to 5.36. The lowest pH value (3.00) was observed for grape juice compared to the other samples.

Most studies have shown that grapes are a rich source of phenolic compounds and these compounds have been proved to show a variety of biological effects, including antioxidant, anticarcinogenic, anti-inflammatory and antimicrobial activities (Baydar et al., 2004; Baydar et al., 2006, Jayaprakasha et al., 2003; Monagas et al., 2003; Serra et al., 2008). For this reason, total phenolic compounds in the beverages have been determined to find out the relationship between antimicrobial activity and phenolic compound content. There are many reports about the antimicrobial activity of the phenolic compound of different food sources against microorganisms. Mohammed et al. (2018) reported that *Rhus coriaria* var. *zebaria* had high antimicrobial activity levels at different concentrations between 25-100 µg/mL. It was considered that antioxidant, antimicrobial, DNA protective and cytotoxic effects of *R. coriaria* var. *zebaria* were attributed to the phenolic contents. Similarly, Pehlivan and Sevindik (2018) were determined antioxidant and antimicrobial activities of *Salvia multicaulis* Vahl plant against the test microorganisms including *S.aureus*, *Enterococcus faecalis* and *E.coli*. However, there are no available reports on the inhibitory effects on microorganisms of traditional Turkish beverages produced by using grapes. Table 2 gives the total phenolic contents of the beverages evaluated by the Folin-Ciocalteu colorimetric method. As shown in Table 2, total phenolic compounds of commercially produced hardaliye were more than the others, while total phenolic compounds of grape juice and grape pickles samples were lower than hardaliye samples.

Table 1 Total soluble content (°Brix) and pH values of beverages

Test materials	°Brix	pH
Grape pickles	60	5.36
Supernatant from the grape juice	14	3.08
Grape juice	15	3.00
Home-made hardaliye	14.5	3.82
Commercially produced hardaliye	19	3.66

Table 2 Total phenolic content of beverages

Test materials	Total phenolic content (mg/L)*
Grape pickles	865.27 (17.63)
Supernatant from the grape juice	1701.97 (6.63)
Grape juice	1515.27 (2.84)
Home-made hardaliye	2029.30 (4.53)
Commercially produced hardaliye	2193.08 (2.25)

*Values are shown as means (n=5) and standard deviations are given in parenthesis.

Total phenolic contents of hardaliye samples are between 2029.30 and 2193.08 mg/L. According to da Silva Padilha (2017), the total phenolic content varied from 2135 to 2647 mg/L for the grape juices. However, the total phenolic content obtained in this study is not in line with those mentioned in grape juices. Samples of grape juice presented values of phenolic contents smaller than those reported in the literature for several samples of grape juice, whose average values ranged from 1515.27 to 1701.97

mg/L (Burin et al., 2010; Margraf et al., 2016). In similar studies on total phenolic content in grape juices using the Folin-Ciocalteu method carried out by Frankel et al. (1998), the results for Concord grape juice ranged between 1654-1971 mg/L and for juices produced with a greater variety of grapes, the total phenolic content range was 1407-1541 mg/L. According to Frankel et al. (1998) mention that the phenolic contents of grape juice may be influenced by the geographical origin of grapes, the procedures employed in the juice production and reactions occurring during storage (Granato et al., 2016).

The minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) values of grape products for microorganisms were determined for the evaluation of the antimicrobial activity (Table 3). Results indicated that the grape pickles showed lowest antibacterial activity against *B. cereus* (MIC=150 mg/mL), on the other hand, the highest antibacterial activity was obtained against *S. aureus* (MIC=4.69 mg/mL). Göral (2019) investigated the survival of *Escherichia coli* and *Bacillus cereus* in grape pickles. For this purpose, *E.coli* and *B.cereus* inoculated into grape pickles at two levels of 10^3 CFU/mL and 10^6 CFU/mL. *E. coli* cells were not determined after one week. On the other hand, the population of *B.cereus* were found in the range of 1.56-1.72 CFU/g at the end of the storage period of 5 months.

In many studies, the inhibitory effects of many plant extracts were attributed to their acidity (Gutierrez et al., 2009; Mohammed et al., 2018). In the present study, there is no difference between the samples of grape juice and supernatant from the grape juice in their pH values. MICs of supernatant from the grape juice against the *S.* Typhimurium and *E. coli* were 8.75 and 17.5 mg/mL, respectively. According to the MIC values for supernatant of the grape juice, higher antimicrobial activities were obtained at lower concentrations against test microorganisms, compared to the grape juice. The difference of antimicrobial activity between grape juice and supernatant of the grape juice was considered due to the variation in the phenolic profile of samples. Also, this result suggests there might be a positive antimicrobial effect within the constituents of the supernatant of grape juice that reinforce the inhibition response and/or the natural compound contains other components in the matrices with antimicrobial activity (Serra et al., 2008).

Table 3 The minimum inhibitory concentration (MIC) and the minimum bactericidal concentration (MBC) values of test materials against the microorganisms tested in microdilution assay

Microorganisms	Test materials (mg/mL)									
	Grape pickles		Supernatant from the grape juice		Grape juice		Home-made hardaliye		Commercially produced hardaliye	
	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC
S. aureus	4.69	-	35	-	37.5	-	36.25	-	47.5	-
E. coli	9.38	-	17.5	-	37.5	-	4.53	-	11.88	-
S. Typhimurium	37.5	-	8.75	-	18.75	-	18.13	-	23.75	-
B. cereus	150	-	17.5	-	18.75	-	18.13	-	23.75	-
S. cerevisiae	-[a]	-	-[b]	-	-[c]	-	-[d]	-	-[e]	-
L. acidophilus	-[a]	-	-[b]	-	-[c]	-	-[d]	-	-[e]	-
L. rhamnosus	-[a]	-	-[b]	-	-[c]	-	-[d]	-	-[e]	-

-[a]: No inhibition activity in the tested maximum concentration of 300 mg/mL, -[b]: No inhibition activity in the tested maximum concentration of 70 mg/mL, -[c]: No inhibition activity in the tested maximum concentration of 75 mg/mL, -[d]: No inhibition activity in the tested maximum concentration of 72.5 mg/mL, -[e]: No inhibition activity in the tested maximum concentration of 95 mg/mL, -: No bactericidal activity in the tested concentrations.

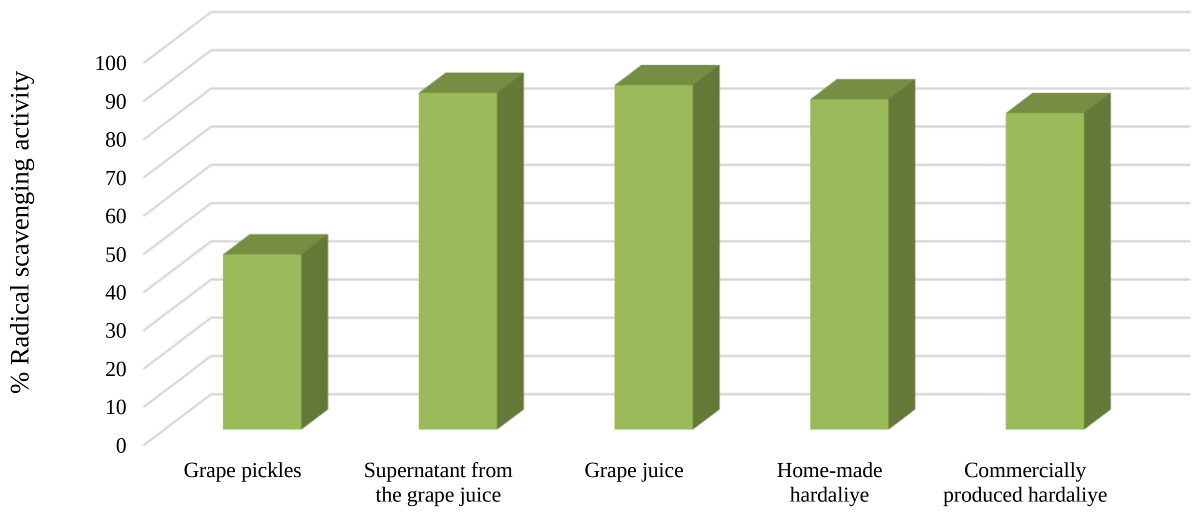

Figure 1 Radical scavenging activity of samples by DPPH method

Hardaliye is a grape beverage considered to have antioxidative effects as a potential source of bioactive components (Amoutzopoulos, 2013). The pH values of commercially produced and home-made hardaliye samples used in this study were 3.66 and 3.82, respectively. Similar pH values were obtained in the study conducted by Arici and Coskun (2001). Total soluble contents are higher in the commercially produced hardaliye compared with home-made hardaliye (Table 1). In this study, the total phenolic content in the commercially produced hardaliye was also higher than those found in the home made hardaliye. Similarly, the data reported by Coşkun et al. (2018) total polyphenol content from 368.8 to 2647.5 mg/ L was found in the hardaliye samples. MIC value of the home-made hardaliye sample were lower than that of commercially produced hardaliye samples. The home-made hardaliye has the strongest antimicrobial activity, with a MIC of 4.53 mg/mL against *E. coli*. Home-made hardaliye exhibited significant antibacterial activity against *E. coli* compared to commercially produced hardaliye, and those results contrast to higher polyphenolic content existent in the commercially produced hardaliye. Commercially produced hardaliye had been shown to be antibacterial against *E. coli*, *S.* Typhimurium, *B. cereus* and *S. aureus* with MICs of 11.88, 23.75, 23.75 and 47.5 mg/mL, respectively. On the other hand, no inhibitory effect on the growth of probiotic bacteria and the yeast was detected. Pehlivanoglu et al. (2015) reported that the antimicrobial effect of hardaliye against *E. coli* and *Listeria monocytogenes*. Similar to those obtained in this study, hardaliye samples has no antimicrobial effects on *S. cerevisiae*. Also, the bactericidal effect of these grape products was not detected at the tested concentrations for any of the test microorganisms (Table 3).

The antimicrobial effect of various fruit and vegetable juices were studied many times and the primary reasons for inhibition of microorganisms are concluded as organic acids and phenolic compounds in these materials (Öncül and Karabıyıklı, 2016). The juice of grapes was found to be highly inhibitory against *Listeria* species. The grape juice reduced the number of initial cells (10^6–10^7 CFU/mL) of *L. monocytogenes* to an undetectable level after 10 min of application (Rhodes et al., 2006). Additionally, Serra et al. (2008), reported that the grape aqueous extract had high phenolic compounds and had a good antimicrobial potential against *Bacillus cereus*, *S. cerevisiae* and *C. albicans*. Sanhueza et al. (2014), have reported an antibacterial effect of grape pomace extracts mainly against *S. aureus* and *E. coli* due to the polar phenolic content of grapes. Filocamo et al. (2015) have demonstrated that the MIC values of white grape juice extract ranged from 3.9 to >2000 µg/mL. Among the Gram-positive bacteria, *S.aureus* showed the highest sensitivity, with MIC and MBC values equal to 3.9 and 500 µg/mL, respectively (Filocamo et al., 2015). Similarly, a good growth inhibitory effect was found against *S.aureus* with a MIC value of 4.69 mg/mL in the present study.

Commercially produced hardaliye was contained the highest phenolic compound (Table 2). In many studies, it was reported that the inhibitory effects of phenolic compounds from natural extracts are more potent to Gram-positive bacteria than Gram-negative (Jayaprakasha et al., 2003, Furiga et al., 2009, Kao et al., 2010; Adámez et al., 2012). On the other hand, grape pickle has strong antimicrobial activity, with a MIC of 9.38 mg/mL against *E. coli*.

Free radical scavenging potentials of samples were tested by the DPPH method, and the results are shown in Figure 1. The greater the DPPH radical consumption associated with the higher the antioxidant activity of the sample (Miliauskas et al., 2004). The DPPH antioxidant activity of grape products were high for all samples, except in grape pickles, in this study. Grape pickles were considerably less effective radical scavengers compared to other beverages produced by using grapes. Samples of grape product exhibit free radical scavenging activities in the range of 46-90% (Figure 1). The results indicated that beverages produced by using grapes were free radical inhibitors and the most active radical scavengers were obtained for the grape juice.

The antioxidant capacity of samples generally associated with the presence of a mixture of polyphenolic compounds with good antioxidant properties (Adámez et al., 2012). In the case of this study, there may be a slight correlation between phenolic content and antioxidant activity. Differences in the antioxidant and antimicrobial activities among grape products were attributed to their different phenolic contents and compositions and to other non-phenolic antioxidants present in the samples (Dávalos et al., 2005). Recently, scientific investigations around the world have focused on traditional fermented food products. Turkey has a wide range of traditional fermented beverages. The results of the present study demonstrated that traditional grape products have an inhibitory effect against *S. aureus, E. coli, S*. Typhimurium and *B. cereus.* Data showed that all samples inhibited the growth of all tested microorganisms except probiotics and yeast. Grape products may have a potential role as a natural preservative for pathogenic microorganisms and also could be used as an additive for the probiotic beverages. Further studies need to be performed to better assess the antimicrobial effects of grape-based beverages produced by traditional procedures.

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