



Antioxidant Capacity and Antibacterial Potential of Rosehip (*Rosa canina*) Fruits Grown

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Abstract: It was aimed to investigate the antioxidant and antibacterial capacity of the rosehip plant grown in Samsun and its surroundings. Rosehip fruits were collected from 10 different regions around Samsun. In order to investigate the antioxidant capacity of the fruits, ascorbic acid, total tannin, total flavonoid, total phenolic substance, total protein amount, total antioxidant level (TAS), total oxidant levels were determined. Kirby-Bauer Disk diffusion method was used to investigate the antibacterial capacity of rosehip samples. In line with the findings obtained in our study, tannin, flavonoids, phenolic substance, total protein, TAS and TOS levels were 1.42-4.65 g of tannic acid/100 g of rosehip, 29.5-36.3 mg of quercetin/100 g of rosehip, 4.700-8.347 g gallic acid equivalent/100 g rosehip, 0.54-0.89 g bovine serum albumin/100 g rosehip, 2.59-2.62 mmol trolox equivalent/L, 6.13-7.41 mmol/ H₂O₂ equivalent/L, respectively. The highest average antibacterial activity was observed against *Enterococcus faecalis*, while the lowest antibacterial activity was against *Enterococcus faecium*. In this research, it was determined that the rosehip fruit grown in Samsun and its surroundings has a high antioxidant capacity due to the amount of ascorbic acid it contains, and also shows antibacterial activity. It was concluded that the rosehip fruits contain significant amounts of valuable bioactive substances, and adding it to the feeds of livestock such as cattle, poultry and pigs might have positive effects.

Keywords: Antibacterial activity, antioxidant, rosehip, TAS, TOS.

Kuşburnu (*Rosa canina*) Meyvesinin Antioksidan Kapasitesi ve Antimikrobiyel Potansiyelinin Araştırılması

Öz: Yapılan bu çalışma ile Samsun ve çevresinde yetişen kuşburnu bitkisinin antioksidan ve antibakteriyel kapasitesinin araştırılması amaçlandı. Yapılan çalışmada Samsun ve çevresinde bulunan 10 farklı bölgeden kuşburnu meyveleri toplandı. Örneklerin antioksidan kapasitesinin araştırılması üzere askorbik asit miktarı gram (g) askorbik asit, toplam tanen miktarı gram tannik asit, toplam flavonoid miktarı mg kuersetin, toplam fenolik madde miktarı gram gallik asit eşdeğeri (GAE), toplam protein miktarı g bovin serum albumin (BSA)/100 g kuşburnu cinsinden; toplam antioksidan seviyesi (TAS) mmol troloks eşdeğeri/g, toplam oksidan miktarı mmol H₂O₂ eşdeğeri/g cinsinden hesaplandı. Kuşburnu örneklerinin antibakteriyel kapasitesinin ölçülmesi için disk difüzyon yöntemi kullanılmış, örneklerin oluşturdukları inhibisyon çapları milimetre (mm) cinsinden tespit edildi. Çalışmamızda elde edilen bulgular doğrultusunda kuşburnu örneklerinin askorbik asit miktarı 0.781-1.120 g askorbik asit/100 g kuşburnu, tanen miktarı 1.42-4.65 g tannik asit/100 g kuşburnu, flavonoid miktarı 29.5-36.3 mg kuersetin/100 g kuşburnu, fenolik madde miktarı 4.700-8.347 g GAE/100 g kuşburnu, toplam protein miktarı 0.54-0.89 g BSA/100 g kuşburnu, toplam antioksidan seviyesi 2.59-2.62 mmol troloks eşdeğeri/g, toplam oksidan seviyesi 6.13-7.41 mmol/ H₂O₂ eşdeğeri/g aralığında bulundu. En yüksek ortalama antibakteriyel aktivite *Enterococcus faecalis*'e karşı gözlenirken, en düşük antibakteriyel aktivite *Enterococcus faecium*'a karşı olmuştur. Bu araştırma Samsun ve çevresinde yetişen kuşburnu meyvesinin içerdiği askorbik asit miktarı sebebiyle antioksidan kapasitesinin yüksek olduğunu, ayrıca antibakteriyel aktivite gösterdiği belirlendi. Çalışma sonucunda, kuşburnu meyvesinin bileşimi ile sığır, kümes hayvanları ve domuz gibi çiftlik hayvanlarının yemlerine eklenmesinin olumlu etkilerinin olacağı kanaatine varıldı.

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Anahtar kelimeler: Antibakteriyel aktivite, antioksidan, kuşburnu, TAS, TOS.

INTRODUCTION

Rosehip, also known as dog nose or rosehip, is the false fruit of the rose plant (Blumenthal et al., 1998). Members of the Rosa family have been used both in the food industry and in the field of medicine from past to present. After it was discovered that rosehip strengthens immunity, it did not take long for it to reach a very common use among the people, with its effects in the treatment of infection proven. Rosehip fruit, leaves and even roots have been consumed for many years by boiling or leaving them in water (Yılmaz et al., 2011). The structure of the fruit is hairy and contains many seeds. The fruits ripen in autumn. Due to the vitamin C contains, its antioxidant capacity is quite high. The phenolic chemicals included in rosehips, such as tannins, flavonoids, phenolic acids, and anthocyanins, have been found to constitute an extremely significant category of biologically active components (Balta & Tekin, 2021; Oğah et al., 2014).

Rosehips are frequently used in food preparations such juice, wine, tea, jelly, and jam (Moerman, 2002). Turkey is quite diverse in terms of rosehip species. About 25% of rose species grow naturally in the country. Rosehip fruit has an important place both in the food sector and in alternative treatment methods (Ercişli, 2005). It contains lots of phenols. These substances have been shown to have anticarcinogenic, antimutagenic, and antioxidant properties. Potential antioxidants and inhibitors of the emergence of human disease include polyphenol molecules. Bacterial resistance to antibiotics used today has become a general health problem and threatens the whole world. With the developing resistance, the antibiotics available on the market are not sufficient to treat the bacterial infection, which encourages scientists to find new antimicrobial sources. These sources may be newly designed antibiotics as well as antimicrobial substances obtained from natural sources (Monroe et al., 2000; Yılmaz et al., 2011). Up to now, alternative compounds except for antibiotics are fields of interest to the researchers.

In this study, the antibacterial and antioxidant capacities of rosehip fruit were investigated in order to find new antimicrobial sources that can be used in both human and animal health.

MATERIAL AND METHOD

Material Determination: The samples used in the study were naturally grown rosehip fruits collected from center of Samsun and surrounding villages. Samples were collected from 10 different sites, washed with distilled water, dried and stored at +4 °C until homogenization.

Preparation of Rosehip Extract: Rosehip samples were washed and dried. Then, 1 g of rosehip sample was weighed. Nine mL of phosphate buffer (0.3 M Phosphate Buffer, pH 7.2) was added and fragmented with the help of

a homogenizer. The homogenized sample was centrifuged at 10 000 rpm for 10 min. At the end of the process, the supernatant was taken and stored at -20 °C to be used in the measurement of parameters. Homogenization steps were repeated for 10 groups of rosehip samples.

Ascorbic Acid Determination: The amount of the vitamin C (L-ascorbic acid) was determined with 2,6-dichloroindophenol by using the titrimetric methods (AOAC, 1995). For the determination of ascorbic acid, rosehip samples were crushed with 0.4 % oxalic acid and homogenized. Samples left for 2 h were filtered. One mL of the filtrate prepared with oxalic acid was taken and 9 mL of 2,6-dichlorophenol indophenol (12 mg/L) was added. Measurements were made at 520 nm with a UV spectrophotometer. 10, 20, 30, 40 and 50 mg/mL solutions of ascorbic acid were prepared and their absorbance was measured in a spectrophotometer at 520 nanometers and the standard curve was drawn. According to the standard curve, the amount of ascorbic acid in the samples were calculated in g of ascorbic acid/100 g of rosehip according to the equation ($y=0.0169x+0.5054$).

Tannin Determination: The condensed tannins were determined according to the method as described by Bajaj and Devsharm (1977). One-g of rosehip was kept in 50 mL of hot distilled water for 1 h. One mL was taken from the filtered samples and placed in a 100 mL balloon jug. 90 mL of distilled water, 1 mL of Folin Denis reagent, and 5 mL of Na₂CO₃ were placed on it and the volume was completed to 100 mL with distilled water. It was kept in the dark for 30 min. Absorbance was measured at 760 nm with a UV spectrophotometer. To prepare the standard curve, 0.1 mg/mL tannic acid was taken from the stock solution and solutions were prepared at the concentrations of 10, 20, 30, 40 and 50 mg/mL and placed in 100-gauge flasks with 75 mL of distilled water. One mL of Folin Denis reagent and 5 mL of Na₂CO₃ were added and the volume was made up to 100 mL with distilled water. After waiting 30 min in a dark, absorbance's were measured in a spectrophotometer at 760 nm, and the standard curve was drawn. According to the standard curve, the amount of tannin in the samples were calculated in g of tannic acid/100 g of rosehip according to the equation ($y=0,0148x+0,8224$).

Determination of Total Flavonoids: The total flavonoids content was measured by the AlCl₃ colorimetric assay (Zhishen et al., 1999). 1 mL of homogenized fruit sample was taken and 4 mL of distilled water was added to it. 0.30 mL of 5 % NaNO₂ solution was added and left for 5 min. 0.30 mL of 10 % AlCl₃ was added to the mixture and left for 5 min. 2 mL of NaOH (1 mol/L) solution was added to it. The volume of the solution was made up to 10 mL with distilled water and mixed well. Measurements were made with the help of UV spectrophotometer at 510 nm against the blind. To draw the standard curve, 20, 40 and 60 µg/ml

quercetin solutions were prepared. The first 4 steps were applied to the quercetin solutions, the reading was taken at 510 nm and the standard curve was drawn. With the drawn standard curve, the total amount of flavonoids in the samples was calculated in mg quercetin/100 g rosehip according to the equation ($y=0.127x-0.0653$).

Determination of Total Phenolic Substance: The total phenolics were determined by using the Folin Ciocalteu method (Singleton et al., 1999). Four mL distilled water was added to 50 μ L fruit extract for each group from homogenized rosehip samples. 250 μ L of 1:1 diluted Folin-Ciocalteu was added to each tube and waited for 1 min. 750 μ L of sodium carbonate solution (2 g/10 mL) was added. It was left to stand at room temperature for 2 h in the dark. At the end of the waiting period, the absorbance value of 765 nm was measured in a spectrophotometer. Gallic acid solutions were prepared at 5 different concentrations, in the range of 10-50 mg/mL to form a standard curve. The first 5 steps were applied on the gallic acid samples and measurements were made with a spectrophotometer. A standard curve was drawn according to the results. With the drawn standard curve, the total amount of phenol in the samples was calculated in g of gallic acid/100 g of rosehip according to the equation ($y=0.0197x+0.7187$).

Determination of Total Protein: 100 μ L of rosehip extract was taken and 5 mL of Protein Reagent Blue G-250 dye was added to it. After waiting for 10 min, measurements were made with a spectrophotometer at a wavelength of 595 nm. The amounts of total protein in the samples were calculated according to the standard curve prepared with BSA (Bradford, 1976).

Determination of Total Antioxidant Level (TAS): The total antioxidant level (TAS) of rosehip samples was determined according to the Total Antioxidant Status kit of Rel Assay Diagnostics (Erel, 2004).

Determination of Total Oxidant Level (TOS): The Total Oxidant Level (TOS) of rosehip samples was determined according to the Total Oxidant Status kit of Rel Assay Diagnostics (Erel, 2005).

Antibacterial Capacity: Kirby Bauer Disk Diffusion Method was used to determine the antibacterial capacity of rosehip samples (Wayne, 2020). For this aim, rosehip extracts (30 μ L) were absorbed into 6 mm diameter blank discs and left to dry. Tested bacteria (*Escherichia coli*, *Staphylococcus aureus*, *Enterococcus faecium*, *Enterococcus faecalis*, *Pseudomonas aeruginosa*, and *Staphylococcus epidermidis*) were inoculated on Mueller

Hinton agar and rosehip extract impregnated discs were applied on agar surfaces. After incubation at 37°C for 18 h, the diameters of the inhibition zones were measured.

RESULTS

Tannin, flavonoids, phenolic substance, total protein, TAS and TOS levels were found as in the range of 1.42-4.65 g of tannic acid/100 g of rosehip, 29.5-36.3 mg of quercetin/100 g of rosehip, 4.700 -8.347 g gallic acid equivalent /100 g rosehip, 0.54-0.89 g bovine serum albumin/100 g rosehip, 2.59-2.62 mmol trolox equivalent/L, 6.13-7.41 mmol/H₂O₂ equivalent/L, respectively. TAS level of the rosehip samples was found in the range of 2.59-2.62 mmol/L, and the TOS level in the range of 6.13-7.41 mmol/L (Figure 1 and 2).

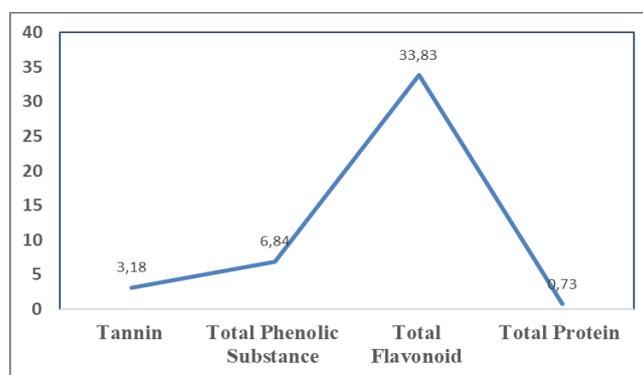


Figure 1. Total protein, phenolic, tannin, and flavonoid compounds derived from rosehip fruit extract and concentrations.

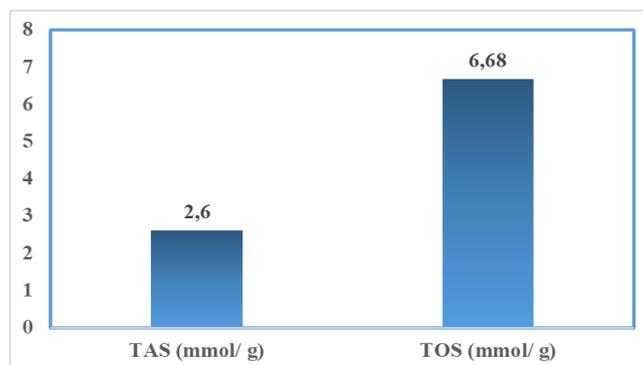


Figure 2. Rosehip fruit extract and concentrations were used to produce total antioxidant and total oxidant compounds.

The antibacterial capacity of rosehip samples was examined according to the inhibition zones, and the highest average inhibition diameter was 15.84 mm for *Enterococcus faecalis*. The inhibition zones of the rosehip for the tested bacteria were presented in Table 1 and Figure 3.

Table 1. Inhibition zones of rosehip samples against tested bacteriae (mm-diameter, AY: no activity)

	1	2	3	4	5	6	7	8	9	10	Average
<i>Enterococcus faecalis</i>	18	17	17,6	18,5	18,3	17	16	18	17,5	18,5	15,84
<i>Escherichia coli</i>	5	4,4	5,7	AY	4	8	8,5	AY	10	7	4,76
<i>Staphylococcus aureus</i>	15,5	17,2	18	15,2	16	17	18	18,4	19	18,5	15,73
<i>Enterococcus faecium</i>	AY	AY	AY	AY	6	8,2	7	AY	8,6	AY	2,98
<i>Staphylococcus epidermidis</i>	17	18,4	16,6	18,5	19	17,5	16,5	7	5	12,3	13,08
<i>Pseudomonas aeruginosa</i>	15,5	17,5	18,3	15	15,2	18	18,5	18	16	17,4	15,39

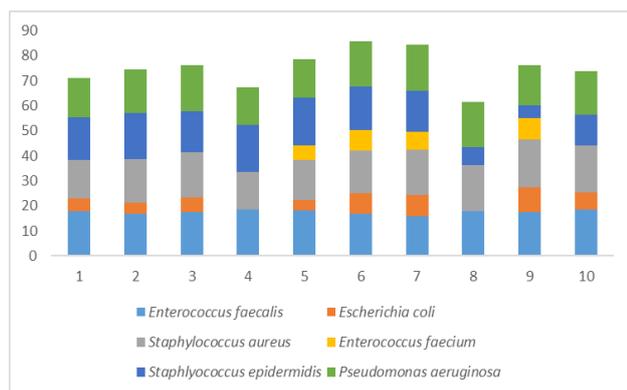


Figure 3. Inhibition zones of rosehip samples against tested bacteria.

DISCUSSION

Rosa is widely used in conventional medicine. Due to its biological properties, including immunosuppressive, antioxidant, anti-inflammatory, anti-arthritis, analgesic, anti-diabetic, cardioprotective, antibacterial, gastroprotective, and skin ameliorative actions, rosehip has long been used to treat a variety of diseases (Orhan et al., 2007; Barros et al., 2010; Willich et al., 2010; Marmol et al., 2017). It was reported that *R. cinnamomea* had the highest ascorbic acid level (5300 mg/100 g rosehip), and the lowest *R. tomentosa* (118 mg/100 g rosehip) species (Halasova and Jicinska, 1988). In a study examining the effect of ripening on the vitamin C level of rosehip fruit, it was observed that the amount of vitamin C in the fruits that reached full maturity was higher, and at the same time, light-colored rosehip fruits contained more vitamin C than dark-colored fruits (Razungles et al., 1989). It has been stated that the amount of ascorbic acid contained in the rosehip samples varies according to the height, temperature, climate, soil type, type of rosehip, and the maturity level of the fruit (Kurucu and Keskinoglu, 1990). When the amounts of ascorbic acid contained in different plant species were compared, it was stated that the highest level of ascorbic acid was in the rosehip fruit (Ileina and Bogdan, 1992). In another study conducted in the same year, it was shown that the amount of ascorbic acid in rosehip fruits ranged from 400 to 2330 mg/100 g rosehip (Kühn, 1992). The vitamin C content of frozen fruits of *R. canina* collected from Transylvania was measured in the range of 112.20-360.22 mg/100 g rosehip (Roman et al., 2013). The amount of ascorbic acid in rosehip fruits collected in Bulgaria was determined as 110 mg/100 g rosehip in the fruit, 230 mg/100 g rosehip in the fruit peel, and 40 mg/100 g rosehip in the seed (Georgieva et al., 2014). The amount of ascorbic acid contained in the rosehip samples was measured as 426 mg/100 g rosehip (Fan et al., 2014). In our study, ascorbic acid levels of rosehip samples collected from Samsun region were found to be between 781-1120 mg/100 g rosehip. There are a limited number of studies investigating the level of flavor

in rosehip fruit. According to the study in which the tannin content of different foods was determined in percent by the titration method, the tannin content of the rosehip plant was calculated as 3.41 % (Atanassova and Christova-Bagdassarian, 2009). The total amount of tannin in rosehip samples was determined as 3.86 g tannic acid/100 g rosehip (Taneva et al., 2016). In our study, the total tannin content of rosehip samples was measured by spectrophotometric method. Total tannin content of the samples was found to be maximum 4.65 g tannic acid/100 g rosehip, and minimum 1.42 g tannic acid/100 g rosehip.

The total amount of phenolic substances in foods has been the subject of many studies. The highest level of phenolic substance in *Rosa* species was determined as 96 mg GAE/g rosehip in *Rosa canina* species. In the study examining the antibacterial and antioxidant activities of rosehip samples grown in Turkey, the total amount of phenolic substance was calculated in the range of 78-102 mg GAE/g rosehip in gallic acid equivalent (Yılmaz and Ercişli, 2011). In a study investigating the radical scavenging activity of the rosehip plant (*Rosa canina*), the total amount of phenolic substances was found to be 475 mg chlorogenic acid/g rosehip (Tumbas et al., 2012). In the study examining the chemical and physical properties of rosehip fruit and marmalade, the total amount of phenol in rosehip fruit was 9982 mg GAE/100 g rosehip, while the total amount of phenol in rosehip marmalade was 912 mg GAE/100 g rosehip in traditionally produced marmalade, and 761.2 mg GAE/100 g in commercially produced marmalade. It was calculated as 100 g rosehip (Yıldız and Alpaslan, 2012). The study, which examined the biochemical components of rosehip samples grown in northern Iran, showed parallelism with previous studies and the total phenolic content of the samples was found to be in the range of 57-152 mg GAE/g rosehip (Aptin et al., 2013). Total phenol, flavonoid and antioxidant activities of rosehip samples grown in Northern Romania were calculated, and the total amount of phenolic substances was calculated as 35.43-48.07 mg GAE/g rosehip (Soare et al., 2015). The total phenolic content of rosehip samples grown in Bulgaria was calculated as 6.9 g GAE/100 g rosehip (Taneva et al., 2016). In our study, the total phenolic content of rosehip samples grown in the Samsun region was measured in the range of 4.7-8.01 g GAE/100 g rosehip by colorimetric method. The total phenolic content of rosehip fruits differs in previous studies. The type of solvent used in the preparation of the rosehip extract, the room temperature, the time from the extraction of the extract to the determination of the phenolic substance, and the type of rosehip fruit may cause differences in the total amount of phenolic substances.

The flavonoid and organic acid contents of rosehip samples were measured. The total flavonoid

amount of rosehip samples used in the study was determined as 41 mg quercetin/100 g rosehip (Adamczak et al., 2012). In another study in which the total flavonoid content of rosehip fruit was measured, the total flavonoid amount was found to be 196.26 mg rutin/g rosehip (Tumbas et al., 2012). In the study conducted in Transylvania, the total flavonoid amount of the samples was stated as 163.3 mg quercetin/100 g rosehip (Roman et al., 2013). In a study conducted in Romania, the total amount of flavonoids contained in rosehip samples was stated in the range of 211.8-672.67 mg quercetin/100 g rosehip (Soare et al., 2015). In our study, the total flavonoid content of rosehip fruit samples was measured in the range of 29.5-36.3 mg quercetin/100 g rosehip. It is thought that the difference in the flavonoid content of the rosehip samples may be due to the differences used in the extraction of the fruit, as well as the freezing process between the homogenization and the experiment.

The total protein content of rosehip fruit is less than other fruits and the total protein amount in *Rosa corymbifera* and *Rosa nitidula* species from fresh rosehip samples was found to be 0.9-1.10 g/100 g rosehip. It was observed that freezing of fruits reduced the protein content by 15.24% in *Rosa corymbifera* species and 31.90% in *Rosa nitidula* species. In addition, the protein level decreased by 21.33% and 46.89% in dried *Rosa corymbifera* and *Rosa subcanina* species (Roşu et al., 2011). In the study, the protein content of rosehip fruit (*Rosa canina*) was determined as 2.68%. In the study we conducted, the total protein amount was calculated by the Bradford method and the results were determined as 0.89-0.62 g BSA/100 g rosehip. It was thought that the difference in the amount of protein found could be due to the differences in homogenization and ultrafiltration methods, temperature conditions, and the variety of fruit species.

Antioxidant activities of rosehip samples were measured by ethanol extraction. The iron ion reduction antioxidant capacity method (FRAP) and trolox equivalent antioxidant capacity (TEAC) of the samples were found to be 983.4-2187.1 µmol FRAP/g rosehip and 457.2-626.2 µmol TEAC/g rosehip (Gao et al., 2007). Antioxidant and antiradical activities of *Rosa canina* and *Rosa pimpinellifolia* fruit samples collected from West Azerbaijan region were measured. The radical scavenging percentages of the samples were 22.41 and 58.10 for hydrogen peroxide in percent for *Rosa canina* and *Rosa pimpinellifolia*, respectively; 79.16 and 87.78 for DPPH; 236.76 for nitric oxide (Fattahi et al., 2012). Antioxidant capacities of rosehip samples collected from Transylvania region were measured by DPPH method. Results were found in the range of 63.35-127.8 µM trolox/100 g rosehip (Roman et al., 2013). Four distinct methods (DPPH,

ABTS, FRAP, and CUPRAC) were used to test the antioxidant activity of rosehip samples. The DPPH method produced a result of 295.0. The ABTS technique yields a value of 368.5. The FRAP approach produced a value of 390.1. The CUPRAC technique yielded a value of 1358.2 mM Trolox Equivalent/g rosehip (Taneva et al., 2016). In our study, TOS was measured in the range of 6.13-7.42 mmol/L, while TAS was found in the range of 2.58-2.62 mmol/L. The difference in the maturity level of rosehip fruits, the preferred homogenization method, and the storage conditions and duration of the extractions were thought to be effective in the lower measured total antioxidant level compared to other studies.

Antibacterial and antioxidant capacities of *Rosa damascena* flower extract were measured in one study, and according to the results of the study the most effective antibacterial activity was seen against *S. enteritidis* with an inhibition diameter of 21 mm, while the lowest activity was seen against *E. aerogenes*, *A. hydrophila*, *S. aureus* with an inhibition diameter of 12 mm (Özkan et al., 2004). The antibacterial effect of the naturally grown thyme plant (*Thymus vulgaris*) in Turkey was investigated. Two different methods were used with 14 different microorganisms. Among 8 different thyme extracts, only antibacterial effect was observed on *Bacillus subtilis* (Benli & Yiğit, 2005). The antibacterial and antifungal capacities of the samples of the *Rosa canina* plant, which grows naturally around Tokat, were investigated. The obtained fruit extracts were studied with a total of 15 microorganisms, 5 Gram-positive, 5 Gram-negative bacteria, and 5 fungi. Disk diffusion method was used in the study. At the end of the study, it was seen that the antibacterial effect of rosehip samples on *E. coli* and *K. pneumoniae* was negligible. Also, 10 mm inhibition diameter of *Salmonella enteritidis* was seen as a significant effect (Arik, 2011). Antibacterial properties of rosehip samples grown in Turkey were investigated. The most effective results were seen on *Bacillus cereus* (Montazeri et al., 2011). The phytochemical content and biological activity of *Rosa canina* fruit grown in Iran were investigated. In the study, it was stated that the antibacterial activity of rosehip extracts was not as effective as drugs, but it could be preferred due to the acquired antibiotic resistance (Yılmaz & Ercişli, 2011). Studies on the chemical composition and biological activity of the rosehip plant were compiled. In the study, it was stated that the extraction of rosehip seeds with methanol showed high antibacterial activity against *E. coli* 8110 (Deliorman & Hartevioğlu, 2013). In our study, the antibacterial activity of rosehip fruit on *Enterococcus faecalis*, *Escherichia coli*, *Staphylococcus aureus*, *Enterococcus faecium*, *Staphylococcus epidermidis* and *Pseudomonas aeruginosa* bacteria was investigated. The data obtained at the end of

the study were found to be in parallel with the literature. The highest antibacterial activity was observed against *Enterococcus faecalis*, while the lowest antibacterial activity was against *Enterococcus faecium*.

In conclusion, it was determined that the rosehip fruit grown in Samsun and its surroundings has a high antioxidant capacity due to the amount of ascorbic acid it contains, and also shows antibacterial activity. It was concluded that the rosehip fruits contain significant amounts of valuable bioactive substances, and adding it to the feeds of livestock such as cattle, poultry and pigs might have positive effects.

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