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**ARAŞTIRMA MAKALESİ** **RESEARCH ARTICLE**

# Phenolic Compounds and Bioactive Properties of cv. Piraziz Apple (Türkiye)

Piraziz Elma Çeşidinin (Türkiye) Fenolik Bileşikleri ve Biyoaktif Özellikleri

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## Abstract

There is a tendency towards natural products in many diseases (prevention and/or treatment). The aim was to determine the bioactive properties (antioxidant capacity, antimicrobial activity, enzyme inhibition) of a local apple variety from the Rosaceae family in Turkey *in vitro*. Peel and flesh of cv. Piraziz apples were extracted by ultrasonication using 99.8% ethanol, 99.7% methanol and 97.0% *n*-hexane solvents. Phenolic compounds of the extracts were determined quantitatively by LC-ESI-MS/MS using 53 standard compounds. Peel and water extracts contained the highest total phenolic substance and flavonoids. The highest number of phenolic compounds, 3 phenolic acids and 8 flavonoids, were detected in apple peel ethanol extracts. Among the phenolic compounds of the extracts, epicatechin, catechin, chlorogenic acid were dominant in the flesh, while epicatechin, catechin and isoquercitrin were dominant in the peels. Except for chlorogenic acid, other phenolic compounds were determined more in the peel extracts. Flavonols (isoquercitrin, astragalin, quercetin, and kaempferol) and flavanones (hesperidin and hesperetin) were identified only in apple peel extracts. While it was determined that peel extracts were better than fruit flesh extracts in inhibiting α-amylase and α-glucosidase, only peel extracts were effective in inhibiting acetylcholinesterase (AChE). Antibacterial activity values determined by disk diffusion and minimum inhibitory concentration of extracts varied according to microorganisms and apple part extracts and the best inhibition zones were obtained with water extracts. It was determined that peel extracts contained more bioactive components than fruit flesh extracts and accordingly showed better bioactive properties. In general, water extracts showed better biological activity. Bioactive components and antioxidant capacities of extracts, enzyme inhibition and antibacterial activities depend on apple parts and solvents. This study suggests that cv. Piraziz apple peel is an important bioactive source and may be valuable for pharmacological applications.

**Keywords:** Piraziz apple, Phenolics, Enzyme inhibition, Acetylcholinesterase inhibition, Antibacterial

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# Öz

Birçok hastalığın önlenmesi ve/veya tedavi edilmesi amacıyla doğal ürünlere yönelim vardır. Bu çalışmanın amacı, Türkiye'de Rosaceae familyasından yerel bir elma çeşidinin biyoaktif özelliklerini (antioksidan kapasite, antibakteriyel aktivite, enzim inhibisyonu) *in vitro* olarak belirlemektir. Piraziz elmasının kabuk ve eti %99.8 etanol, %99.7 metanol ve %97.0 *n*-hekzan çözücüleri kullanılarak ultrasonikasyon ile ekstrakte edilmiştir. Ekstrelerin fenolik bileşikleri, kantitatif olarak LC-ESI-MS/MS ile 53 standart fenolik bileşik kullanılarak belirlenmiştir. Elmanın kabuk ve su ekstreleri en yüksek toplam fenolik madde ve flavanoid içermektedir. Elma kabuk etanol ekstrelerinde 3 adet fenolik asit ve 8 adet flavonoid olmak üzere en fazla sayıda fenolik bileşik tespit edilmiştir. Ekstraktların fenolik bileşikleri arasında elma meyve etinde epikateşin, kateşin, klorojenik asit, elma kabuklarında ise epikateşin, kateşin ve izokuersetin baskındır. Klorojenik asit hariç diğer fenolik bileşikler daha fazla elma kabuk ekstrelerinde belirlenmiştir. Flavonoller (izokuersetin, astragalin, kuersetin, kaempferol) ve flavanonlar (hesperidin ve hesperetin) sadece elma kabuğu ekstraktlarında tanımlanmıştır. Kabuk ekstraktlarının α-amilaz ve α-glikozidaz inhibisyonunda meyve eti ekstraktlarından daha iyi olduğu belirlenirken, sadece kabuk ekstraktları asetilkolinesteraz (AChE) inhibisyonunda etkili olmuştur. Disk difüzyon ve ekstraktların minimum inhibitör konsantrasyonu ile belirlenen antibakteriyel aktivite değerleri mikroorganizmalara ve elma kısmının ekstraktlarına göre değişmiş ve en iyi inhibisyon zonları su ekstraktları ile elde edilmiştir. Kabuk ekstraktlarının meyve eti ekstraktlarından daha fazla biyoaktif bileşen içerdiği ve buna bağlı olarak daha iyi biyoaktif özellikler gösterdiği belirlenmiştir. Genel olarak su ekstraktları daha iyi biyolojik aktivite göstermiştir. Ekstraktların biyoaktif bileşenleri ve antioksidan kapasiteleri, enzim inhibisyonu ve antibakteriyel aktiviteleri elmanın kısmına ve çözücülere bağlıdır. Bu araştırma Piraziz elmasının özellikle kabuğunun önemli bir biyoaktif kaynak olduğunu ve farmakolojik çalışmalar için değerli olabileceğini düşündürmektedir.


**Anahtar Kelimeler:** Piraziz elması, Fenolikler, Enzim inhibisyonu, Asetilkolinesteraz inhibisyonu, Antibakteriyel

## 1. Introduction

Alzheimer's disease and diabetes are among the leading diseases of global health problems. There are many treatment methods for such diseases, but the most accepted is enzyme inhibition (Uysal et al., 2017). Cholinesterase inhibitors that terminate cholinergic signal transfer are used in the treatment of Alzheimer's disease. Similarly in patients with type 2 diabetes, it is necessary to reduce the postprandial blood glucose levels by inhibiting enzymes involved in the catabolism of carbohydrates (α-amylase and α-glucosidase). There is a tendency towards natural inhibitors due to the side effects of the drugs used for inhibition of these enzymes (Chai et al., 2016; Kumari and Jain, 2012). Because; fruit, vegetables, and herbs that are natural inhibitors contain secondary metabolites that are pharmacologically effective (Ağarmirzaoğlu et al., 2024; Aoudeh et al., 2024). The quantity of bioactive compounds varies considerably depending on the extraction method, the type of solvent used, and the analytical techniques applied (Simmonds and Howes, 2016). Since antioxidant activity is closely associated with the concentration of phenolic compounds, comparative studies aimed at determining the most suitable solvent for their efficient extraction are of great importance (Babbar et al., 2013).

Apples (*Malus domectica*), which are the subject of many scientific studies, have positive effects on human health. The compounds contained in apples, especially phenolics, show enzyme inhibition, high antioxidant and antimicrobial activity (Boyer and Liu, 2004). In addition to being consumed as a fruit, apple is a raw material widely used in the food industry for jam, marmalade, tea and vinegar (Sülük et al., 2018). The identification and characterization of biologically valuable compounds, especially of domestic and/or wild apples, which still have limited information about their antioxidant potential, and their potential use as nutraceuticals and functional foods should be further investigated (Mihailović et al., 2018).

Türkiye is an important apple producer in the world and has many domestic apple varieties. For example, cv. Piraziz apple, which has been grown in Giresun/Piraziz district for many years, can be easily distinguished from other apple varieties with its yellow, red, green color and unique flavor (Karadeniz and Aydın, 2015). Although there are studies on apple varieties with high production, there are limited data on domestic apple varieties. In this study, the phenolic compounds, antioxidant capacity, antibacterial activity and inhibition potential of key enzymes playing a role in the disease were investigated in cv. Piraziz apple. It is also aimed to select the appropriate extraction solvent that will provide the highest phenolic compound content and bioactive properties of the flesh and peel of the domestic apple variety. The results obtained may provide information about the potential of Turkey's domestic apple varieties.

## 2. Materials and Methods

### *2.1. Materials*

Chemicals and enzymes were purchased from Sigma-Aldrich (St. Louis, MO, USA), Streptomycin (10 µg) and Chloramphenicol (30 µg) from Bioanalyse Ltd. (Ankara, Turkey). *Staphylococcus aureus* and *Escherichia coli* were obtained from Çukurova University Food Engineering Department Food Microbiology Laboratory Culture Collection. Piraziz apple was obtained at harvest time from the orchard in the Piraziz Apple Project carried out in Giresun, Turkey. After the apple was washed and dried, they were stored in the refrigerator. Apple was peeled with an apple peeler and dried in a lyophilizer. The resulting flesh and peel were stored at -18 °C.

### *2.2. Preparation of phenolic extracts from apples*

Powdered apple parts (15 g) were homogenized with extraction solvent (200 mL, 99.8% ethanol, 99.7% methanol or 97.0% n-hexane) for 2 min. The mixture was extracted by ultrasonication (30°C, 45 min) and then centrifuged (3500 g, 10 min). The same solvent was added to the pellet separated from the upper phase and the process was repeated. With the removal of solvents in the evaporator, the extracts were kept in the freezer (-18 °C) (Kam et al., 2013).

### *2.3. Determination of total phenolic content (TPC)*

The mixture (1 mL the various concentrations of extracts and 60 mL d$H_2O$) containing Folin Ciocalteu reagent (5 mL) was incubated for 8 min followed by $Na_2CO_3$ (20%, 15 mL) and d$H_2O$ volume was completed to 100 mL. The mixture was kept for 2 h in the dark and the absorbance (760 nm) was measured by UV-1700 spectrophotometer (Shimadzu, Kyoto, Japan). TPC values were expressed as Gallic acid equivalents $g^{-1}$ Dry Weight (Singleton et al., 1999).

### *2.4. Determination of total flavonoid content (TFC)*

The various concentrations of extracts (1 mL), $NaNO_2$ (5% 0.3 mL) and $dH_2O$ (4 mL) were mixed in a 10 mL volumetric flask and after 5 min, $AlCl_3$ (100% 0.6 mL) was added to the mixture. After 6 min, NaOH (2 mL 1M) was added and after the mixture volume was completed with $dH_2O$, absorbances (510 nm) were measured. Results were expressed as Rutin equivalents $g^{-1}$ Dry Weight (Aidi-Wannes et al., 2010).

### *2.5. Quantitative analysis of apple extract by LC-MS/MS*

The analysis of 53 phenolic compounds was quantitatively determined in UHPLC (Shimadzu-Nexera) with a tandem mass spectrometer calibrated by Yılmaz (2020).

### *2.6. Antioxidant capacity*

It was carried out according to 1,1-diphenyl-2-pic-rylhydrazyl radical (DPPH) and ferric-reducing antioxidant power (FRAP) methods as antioxidant capacity methods. DPPH (2850 µL) dissolved in methanol and various concentrations of extracts (150 µL) were mixed and absorbances were measured at 515 nm after 24 h. The FRAP solution prepared using acetate buffer, TPTZ, and $FeCl_3.6H_2O$ (10:1:1, v/v/v) was kept at 37 °C. After waiting for 30 minutes for FRAP solution (2850 µL) and various concentrations of extracts (150 µL), absorbances (593 nm) were measured. Antioxidant analyses were expressed as trolox equivalents (µmol TE $g^{-1}$ DW) (Thaipong et al., 2006).

### *2.7. Enzyme inhibition*

Inhibitory effects of extracts on diabetes enzymes were determined in a previous method (Liu et al., 2013). AChE inhibition experiments were performed according to Yabo-Dambagi et al. (2020). In all inhibition experiments, control and blank were prepared using buffer instead of extract and enzyme. Inhibition was calculated as shown İN Equation 1 below:

$$\text{Inhibition (\%)}: \frac{A-Ae}{A} \times 100 \qquad \text{(Eq. 1)}$$

where A and Ae are absorbances without and with inhibitor, respectively. The half-maximal inhibitory concentrations ($IC_{50}$) were calculated from the regression equation of the standard curve of the various concentrations extract and acarbose or galanthamine.

α-glucosidase inhibitory activity; the reaction mixture contained phosphate buffer (1.15 mL, pH6.8, 0.1 M), α-glucosidase (50 µL, 2U $mL^{-1}$), p-nitrophenyl-α-D-glucopyranoside (50 µL, 5 mM) as substrate and 50 µL various concentrations of extracts or acarbose solution. After preincubation of the reaction mixture without substrate for 10 min at 37 °C, substrate (50 µL) was added, and after 30 min, 2.0 mL NaCO (200 mM) and 4.7 mL $dH_2O$ were added. The absorbance was measured at 405 nm.

α-amylase inhibitory activity; the various concentrations of extracts (100 µL), 560 µL 0.1 M phosphate buffer (containing 6 mM NaCl, pH6.9), and α-amylase prepared with phosphate buffer (40 µL, 6U $mL^{-1}$) were incubated after 20 min at 37 °C. Starch containing phosphate buffer containing 6 mM NaCl (1%, 300 µL) was added to the mixture. After 20 min, 750 µL of dinitro salicylic acid was added and a 100 °C water bath for 5 min was followed by 6 mL of $dH_2O$. The absorbance was measured at 540 nm.

AChE inhibitory activity; 1 M pH8.0 Tris-HCl buffer (400 µL), various concentrations of apple extract (10 µL), 10 µL AChE enzyme (0.05 mg $mL^{-1}$ Tris-HCl buffer), and $dH_2O$ (1880 µL) were mixed and incubated for 5 min. 100 µL of acetylcholine iodide (4 mg $mL^{-1}$) was added to the mixture. Finally, after 15 min, 100 µL of 0.5 mM 5,5′-dithiobis (2-nitrobenzoic acid) was added to the mixture and the absorbance was measured at 412 nm.

### *2.8. Antibacterial activity*

Disc diffusion was performed according to the method recommended by the European Committee for testing for antimicrobial susceptibility (Anonim, 2019). A single colony of a pure culture which was streaked on agar was re-suspended on Mueller-Hinton agar which was then incubated at 37 °C for 24 h. 100 µL specimen (McFarland standard turbidity was 0.5) taken separately from each test microorganism was spread on agar with a sterile swab. Three sterile blank disks (10 mm) were treated with 50 µL of extract, then placed in a petri dish and incubated for a day (37 °C). As controls, commercially available Streptomycin and Chloramphenicol were also tested against all test microorganisms under similar conditions (Béjaoui et al., 2017). A constant volume of serially diluted apple extract (100 µL) was added to the Mueller Hinton broth. Each test tube was inoculated with a 100 µL aliquot of the test organism adjusted to a 0.5 McFarland scale. After vortexing, the test tubes were incubated for a day (37 °C). The concentration without turbidity was determined as MIC (Anonim, 2019). MIC values are given in $\mu g\ mL^{-1}$.

### *2.9. Statistical analysis*

Statistical comparisons were estimated by ANOVA followed by Duncan's test was carried out to compare samples and values of $p<0.05$ were considered significantly different, using SPSS software package (SPSS Inc., Chicago, IL, USA). The correlations within variables were calculated by the Pearson correlation coefficient.

## 3. Results and Discussion

### *3.1. Extraction yields, total phenolics, total flavonoid, and antioxidant capacity*

The extraction yields based on dry matter were determined as 0.20-82.56% in apple flesh and 0.30-65.54% in apple peel. Hexane gave the lowest extraction yield, while other solvents gave close extraction yields. It has been reported by many researchers that extraction efficiency varies depending on the parts of the apple and the solvent (Wang et al., 2019). The peel of Piraziz apple has more TPC and TFC content than the flesh (*Table 1*). In other researchers, the total phenolic substance amount of apple extracts obtained by ultrasonication extraction is similar to the current study (Stojiljković et al., 2016). Solovchenko and Schmitz-Eiberger (2003), explained that the higher presence of phenolics in apple peel, which have an effect on protecting plants against pests, is due to the fact that these compounds protect the apple from damage such as ultraviolet rays and pathogens. Additionally, Jakopic et al. (2009), reported that the outer part of the apple, which is more exposed to sunlight, has more intense color content.

Apple extracts with high phenol content also have better antioxidant capacity. This can be explained by the fact that the hydroxyls of phenolics show better antioxidant activity (Singleton et al., 1999). Except for the DPPH reducing capacity of FM; the highest DPPH antioxidant capacity in flesh and peel extracts was obtained in water extraction. Similar to the study, Reis et al. (2012), reported the best antioxidant capacity of apple pulp in water extracts.

***Table 1. Phenolic and antioxidant results of extracts[1]***

| | TPC (mg GAE $g^{-1}$ DW) | TFC (mg RE $g^{-1}$ DW) | DPPH (µmol TE $g^{-1}$ DW) | FRAP (µmol TE $g^{-1}$ DW) |
|---|---|---|---|---|
| PE | 20.24±0.07$^{b}$ | 4.67±0.11$^{a}$ | 221.46±0.14$^{b}$ | 184.20±0.86$^{b}$ |
| PM | 18.30±0.12$^{c}$ | 3.31±0.08$^{b}$ | 219.87±0.02$^{b}$ | 166.44±2.36$^{c}$ |
| PW | 20.89±0.08$^{a}$ | 4.80±0.18$^{a}$ | 229.10±0.48$^{a}$ | 218.48±0.26$^{a}$ |
| FE | 9.59±0.05$^{e}$ | 1.12±0.05$^{d}$ | 52.86±0.13$^{d}$ | 66.55±0.77$^{e}$ |
| FM | 7.12±0.01$^{f}$ | 1.09±0.01$^{d}$ | 57.83±2.23$^{c}$ | 62.92±0.37$^{f}$ |
| FW | 10.39±0.01$^{d}$ | 2.05±0.02$^{c}$ | 53.27±0.46$^{d}$ | 69.40±0.06$^{d}$ |

[1]Data are means ± SD (n = 3). a–f Values within column with the same letters are not significantly different at $p \leq 0.05$. PE: Ethanol Extract of Peel, PM: Methanol Extract of Peel, PW: Water Extract of Peel, FE: Ethanol Extract of Flesh, FM: Methanol Extract of Flesh, FW: Water Extract of Flesh.

### *3.2. LC-MS/MS analysis of polyphenols*

It was determined that fraction and solvent affected the individual phenolic compounds (*Table 2*). Phenolic compounds could not be identified in hexane extracts of cv. Piraziz apple with low extraction yield. Total phenolic compounds were determined more in the peels than in the flesh. As in the total amount of substance determined spectrophotometrically, total phenolic compounds were mostly determined in water extracts in peel and flesh. Since Folin Ciocalteu reagent has the ability to reduce components that are not specific to phenolic compounds (Savatović et al., 2008), there are differences in the total amount of phenolic compounds. In addition, 11 phenolic compounds were identified in peel ethanol extracts, including one hydroxybenzoic acid (protocatechuic aldehyde), two each flavanols (catechin, epicatechin), flavanone (hesperidin, hesperetin) and hydroxycinnamic acid (chlorogenic acid, caffeic acid) and four flavonols (isoquercitrin, astragalin, quercetin, kaempferol).

***Table 2. Quantitative determination of phenolic compounds in apple extracts by LC–MS/MS*** [1]

| Compound | * RT | Molecular ions (m/z) | Fragment ions (m/z) | Ion. mod | PE | PM | PW | FE | FM | FW |
|---|---|---|---|---|---|---|---|---|---|---|
| Catechin | 7.4 | 288.8 | 203.1 | Neg | 216.60±4.80b | 209.30±4.60c | 263.90±5.80a | 89.60±2.00e | 86.70±1.90f | 124.30±2.70d |
| Chlorogenic acid | 8.4 | 353.0 | 85.0 | Neg | 73.60±1.60e | 86.90±1.90c | 95.80±2.00b | 80.30±1.70d | 95.60±2.00b | 145.00±3.10a |
| Protocatechuic aldehyde | 8.5 | 137.2 | 92.0 | Neg | 15.20±0.60b | 10.20±0.40c | 22.90±0.90a | 2.90±0.10e | 3.80±0.20d | 3.80±0.00d |
| Epicatechin | 11.6 | 289.0 | 203.0 | Neg | 579.00±12.80c | 612.20±13.50b | 1015.00±22.40a | 168.20±3.70e | 154.60±3.40f | 227.00±5.00d |
| Caffeic acid | 12.1 | 179.0 | 134.0 | Neg | 3.50±0.0.00c | 3.80±0.00b | 6.30±0.10a | 1.00±0.00d | 0.80±0.00e | nd |
| Isoquercitrin | 25.6 | 463.0 | 271.0 | Neg | 185.30±4.10a | 176.70±3.90c | 182.80±4.00b | nd | nd | nd |
| Hesperidin | 25.8 | 611.2 | 449.0 | Poz | 17.70±0.60c | 58.70±2.00b | 65.60±2.20a | nd | nd | nd |
| Astragalin | 30.4 | 447.0 | 255.0 | Neg | 18.40±0.10b | 28.60±0.20a | 16.60±0.20c | nd | nd | nd |
| Quercetin | 35.7 | 301.0 | 272.9 | Neg | 100.50±1.20a | 81.60±1.10b | 20.40±0.30c | nd | nd | nd |
| Hesperetin | 36.7 | 301.0 | 136.0/286.0 | Neg | 9.10±0.20a | 6.90±0.20b | nd | nd | nd | nd |
| Kaempferol | 37.9 | 285.0 | 239.0 | Neg | 2.90±0.00 | nd | nd | nd | nd | nd |
| Total phenolic compounds | | | | | 1221.80 | 1274.80 | 1689.30 | 341.90 | 341.50 | 502.20 |

[1](mg 100 $g^{-1}$ DW). *RT: Retention time. nd: Not detected. Different lower-case letters represent statistical differences ($p<0.05$) PE: Ethanol Extract of Peel, PM: Methanol Extract of Peel, PW: Water Extract of Peel, FE: Ethanol Extract of Flesh, FM: Methanol Extract of Flesh, FW: Water Extract of Flesh.

Similar to the literature, chlorogenic acid was determined more in the flesh extracts (Mihailović et al., 2018). The most dominant phenolic in the extracts was epicatechin. This result is consistent with the result in the study of Chinnici et al. (2004) on Golden Delicious apples. Quercetin, which was determined only in peels, was mostly determined in ethanol, methanol and water extracts, respectively. This result may be due to the fact that quercetin is less soluble in water. Similarly, kaempferol, which has low water solubility, was determined only in peel ethanol extracts. In the literature, gallic acid could not be detected in ultrasonic extracts of some apple varieties (Nkuimi-Wandjou et al., 2020). Skoko et al. (2022) reported the presence of astragalin in traditional Croatian apple varieties, marking the first documented occurrence of this phenolic compound in apples. In the present study, astragalin was likewise detected in the analyzed apple samples.

### *3.3. Enzyme inhibitory effects of apple extracts*

The $IC_{50}$ values required for enzyme inhibition of Piraziz apple are given in *Table 3*. The degree to which extracts obtained with different solvents inhibit enzymes varies. All extracts except hexane have α-amylase and α-glucosidase inhibition effect, while only peel extracts have AChE inhibition effect. The degree of inhibition of water extracts from both flesh and peel against α-amylase and α-glycosidase was found to be higher than other solvents. It has been stated that this effect may be due to the solvent polarity and effective compounds and amount, and it has been stated that water extracts also show good α-glycosidase enzyme as in the study (Khan et al., 2016) and α-amylase inhibition (Wickramaratne et al., 2016). The amount of Acarbose that inhibits α-amylase and α-glucosidase by 50% is 13.34±0.83 mg $L^{-1}$ and 5.31±0.97 mg $L^{-1}$, respectively. Compared to acarbose, extracts, especially peel extracts, have potential against diabetes enzymes. Oboh et al. (2015) stated that rutin and quercetin found in apples are potent inhibitors of these enzymes. The $IC_{50}$ value of the extracts for AChE was found to be much higher than the $IC_{50}$ value of galantamine (2.80±0.96 mg $mL^{-1}$). Similarly, the literature also reported that apple pieces had no effect on acetylcholinesterase inhibition (Lima et al., 2018).

***Table 3. The results of the extracts for enzyme inhibition ($IC_{50}$)*** [1]

| Extract | α-amylase (mg $L^{-1}$) | α-glucosidase (mg $L^{-1}$) | AChE (mg $mL^{-1}$) |
|---|---|---|---|
| PE | 12.78±0.17$^{d}$ | 6.32±0.03$^{d}$ | 374.53±1.50$^{a}$ |
| PM | 11.26±0.26$^{e}$ | 5.78±0.01$^{d}$ | 356.86±2.25$^{b}$ |
| PW | 9.62±1.03$^{f}$ | 4.95±0.02$^{e}$ | 193.67±2.23$^{c}$ |
| FE | 29.29±0.26$^{b}$ | 81.97±0.04$^{a}$ | - |
| FM | 34.23±1.25$^{a}$ | 75.94±0.59$^{b}$ | - |
| FW | 26.70±210$^{c}$ | 35.02±0.05$^{c}$ | - |

[1]Data are means ± SD (n = 3). a–f Values within column with the same letters are not significantly different at $p \leq 0.05$. PE: Ethanol Extract of Peel, PM: Methanol Extract of Peel, PW: Water Extract of Peel, FE: Ethanol Extract of Flesh, FM: Methanol Extract of Flesh, FW: Water Extract of Flesh.

### *3.4. Antibacterial effects*

Antibacterial activity varied depending on the apple part and the solvent (*Table 4*). Chloramphenicol and Streptomycin, used as positive control, showed higher inhibition zones in both microorganisms than the extracts. While methanol extracts of flesh had no inhibitory effect against *S. aureus*, they showed the higest inhibitory effect against *E. coli*. Similar to the study, it affects the antimicrobial activity of the solvent (Agourram et al., 2013). When classified according to inhibition zone diameter (Shahbazi, 2017), the antibacterial effects of the extracts can generally be considered to be of moderate level.

***Table 4. Antibacterial activities of cv. Piraziz apple extracts[1]***

| Extract | *S. aureus* | | *E. coli* | |
|---|---|---|---|---|
| | Zone diameter (mm) | MIC (µg $mL^{-1}$) | Zone diameter (mm) | MIC (µg $mL^{-1}$) |
| PE | 14.92±0.95$^{b}$ | 18.75 | 15.00±1.00$^{abc}$ | 18.75 |
| PM | 15.50±0.50$^{ab}$ | 37.5 | 14.34±0.60$^{bc}$ | 18.75 |
| PW | 16.17±0.76$^{a}$ | 18.75 | 15.50±0.60$^{ab}$ | 18.75 |
| FE | 14.67±0.60$^{b}$ | 75.00 | 14.00±0.50$^{c}$ | 75.00 |
| FM | nd | - | 15.67±0.60$^{a}$ | 75.00 |
| FW | 16.33±0.60$^{a}$ | 18.75 | 15.83±0.65$^{a}$ | 37.50 |
| Chloramphenicol | 22.00±0.63 | | 23.34±0.58 | |
| Streptomycin | 24.50±0.55 | | 25.69±0.82 | |

[1]Data are means ± SD (n = 3). nd: not detected. a–c Values within column with the same letters are not significantly different at $p \leq 0.05$. PE: Ethanol Extract of Peel, PM: Methanol Extract of Peel, PW: Water Extract of Peel, FE: Ethanol Extract of Flesh, FM: Methanol Extract of Flesh, FW: Water Extract of Flesh

## 4. Conclusions

The phenolic profile of extracts of the cv. Piraziz apple variety was determined and enzyme inhibition and antibacterial properties were investigated. The extraction of apple peel and flesh was carried out by ultrasonication method using solvents with different polarity. The extraction yield was similar in other extracts except hexane extracts. Phenolic components could not be determined in hexane extracts and no biological activity was detected. Phenolic compounds were detected in higher amounts in water extracts and in the peel of the apple by both spectrophotometric and LC-MS/MS. Antioxidant capacity (FRAP and DPPH) is parallel to the total amount of phenolics. The extracts exhibited inhibitory activity on diabetes enzymes. The peel extracts inhibited AChE at high concentration, while the flesh extracts did not have any inhibitory effects. The peel extracts showed moderate antibacterial activity. A total of 11 different phenolics were characterized. The most common phenolic compound was epicatechin, followed by catechin. These compounds identified in cv. Piraziz apple are thought to be responsible for the bioactive properties. The amount of phenolics is of critical importance in the evaluation of apple parts. In addition, especially water extracts of apple showed the best bioactive properties and/or activity.

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**Ethical Statement**

There is no need to obtain permission from the ethics committee for this study.

**Conflicts of Interest**

We declare that there is no conflict of interest between us as the article authors.

**Authorship Contribution Statement**

Concept: Unal, M. U.; Design: Kilic, T., Unal, M. U.; Data Collection or Processing Kilic, T., Unal, M. U.; Statistical Analyses: Kilic, T., Unal, M. U.; Literature Search: Kilic, T., Unal, M. U.; Writing. Review and Editing: Kilic, T., Unal, M. U

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