Determination of Some Chemical Properties of Wild Pear (*Pyrus spinosa* Forsk.)

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Wild pear plant species *Pyrus spinosa* Forsk., naturally grown in woodlands in Turkey, are one of the most important wild fruits consumed by local people as food, as well as used for medical purposes. In this study, some chemical properties were determined such as fruit seed and fruit pulp antioxidant and antimicrobial activities, fruit seed fatty acid components of *Pyrus spinosa* determined, which grow naturally in Ödemiş, İzmir, in the south of Turkey. The qualitative and quantitative analyses of fatty acid components of fruit seed of *P. spinosa* were determined by GC/MS analysis. A total of 16 fatty acids in the fruit seed were found in the analysis results and 44.8% linoleic acid (C18:2n6c), 40.5% oleic acid (C18:1n9c), and 7.6% palmitic acid (C16:0) components were found in higher proportions. Free radical removal effects of fruit seed and fruit pulp extracts were determined using the DPPH method. The antimicrobial activity of fruit pulp extract of *P. spinosa* was determined by the disk diffusion method against *E. coli, S. aureus*, and *C. albicans*. This study revealed that the fruit pulp and fruit seed of *P. spinosa*, which had a significant level of antioxidant activity, antimicrobial activity, and fruit seed had high levels fatty acids components.

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INTRODUCTION

Turkey has three biogeographical regions called Euro-Siberian, Mediterranean, and Irano-Turanian, as well as their transition zones. Because its climatic and geographical features change within short intervals of space due to its position as a bridge between two continents, Turkey has acquired the character of a small continent from the point of biological diversity. Hence, there are more flora and endemic plant type richness in Turkey than all of Europe (Özhatay *et al.* 2009; Demir 2013; Şengül *et al.* 2017). There are more than 12,000 plant taxa that naturally grow in Turkey, and approximately 3,649 of those (one of three) consists of endemic taxa (Güner *et al.* 2012). As Turkey has diverse climate areas with various ecological conditions, it is on migration routes, and has been a host for many civilizations. Since the first ages, its plant diversity and richness has increased, as gene sources got richer. As a result, Anatolia has very rich fruit types and varieties (Özbek 1977; Özbek 1985; Demir 2013). The term wild fruit is used for plants that grow naturally in a certain region without planting (Chua-Barcelo 2014). Wild fruits can grow in unproductive soils and in extreme conditions; therefore, they have to develop adaptations that are more resistant to natural conditions. They generally are more fertile, resistant to drought, and are smaller. They have more seeds, and fruits that are sour, fibrous, and

astringent with more secondary metabolites (Smatana *et al.* 1988). Edible wild fruits have been used as a food source in the world for centuries and are also important locally for their economic return (Doğan *et al.* 2004). Wild fruits contain various types of antioxidant components such as flavonoids, phenolics, carotenoids, and vitamins; these are accepted as healthy constituents for human health as they prevent the oxidation of macromolecules and decrease the risk of degenerative diseases by reducing oxidative stress (Markham 1982; Prior *et al.* 2003; Heber 2004; Rangkadilok *et al*. 2007; Kubola *et al.* 2011).

One of the wild fruit species that are commonly grown in Anatolia is the wild pear. The wild pear that grows naturally in Turkey is included in the wild pear and almond leaf wild pear taxon Rosaceae family in systematics, within the Pomoideae sub-family inside *Pyrus* species (Davis 1972; Anşin and Özkan 1993). Although the *Pyrus* species has more than 80 types (Browicz 1993), new taxons are being detected as time goes by (Aydın Uğurlu and Dönmez 2014). The homeland of wild pear is stated as Anatolia, and its distribution area is Ukraine, South Eastern Europe, and Turkey. Different varieties and forms of this species in the appearance of *Carya* and brown are found in different regions of Anatolia. Wild pear has completely xerophyte plant characteristics, has small brown hairy branches, leaves in the shape of a small crow, thick thorny branches, and a pile root that goes deep (Dumanoğlu *et al.* 1999). Wild pear is a species resistant to air pollution and drought. It is common with its various thorn tree species (*Crataegus*) in the far forests, in arid places, moorlands, and especially in farms that are opened from the forest. It blooms in April, the fruit ripens fully in autumn, color turns brown, and is eaten at that time (Kartal 2013).

The present study used the *Pyrus spinosa* Forsk. plant, which is in the category of wild fruits from the Rosaceae family. *P. spinosa* is mainly distributed in the Mediterranean region and Iran (Jovanovic 1972, 1977; Zamani *et al.* 2012). *P. spinosa* was selected as the material of this study from its homeland in Anatolia. It has become an extremely attractive plant, as its impacts on health that were forgotten have recently come to light. Due to its almost ubiquitous presence, which is not affected by harsh climatic conditions, the plant is even more attractive. It is a plant that can grow in most regions in Turkey, but is not very appreciated. The following are some of the known properties of wild pear among people: it is good for heart diseases, diarrhea (however, it leads to constipation in case of overconsumption), makes the heart stronger, dilutes and cleans the blood, reduces fever, it is beneficial for the nerve system as a sedative, reduces fatigue, benefits people with asthma and gingival diseases, and is used to treat insect stings (Keçeci 2018).

A review of the literature showed a lack of research about the chemical composition of *P. spinosa*, including the fatty acid components, antimicrobial activity of fruit seed, as well as the antioxidant activity and other properties of fruit pulp. Therefore, the aim of this study was to evaluate the physicochemical properties, fruit seed fatty acid composition, antioxidant, and antimicrobial properties of *P. spinosa* that can be beneficial to the development of nutraceutical and pharmaceutical uses of agro by-products.

EXPERIMENTAL

Materials

Plant material

Fruit samples of *P. spinosa* were collected from Izmir, Ödemiş-Bozdag (1050 m to 1110 m), Turkey, in 2020 during the fruit ripening period (October). Plant and fruit

samples were dried by herbarium techniques, and their identification was conducted in Hacettepe University herbarium. The samples were stored in the same herbarium. The harvested samples were analyzed after maturation at room temperature for a week.

Methods

Method of determination of fatty acid methyl esters *content of fruit seed*

Wild pear fruits (*P. spinosa*) and their samples collected were placed in packages and were transferred to the laboratory within the same day without waiting and exposure to sunlight. The seeds were obtained by cutting the fruit meet off the wild pear fruit (*P. spinosa*) using a knife without damaging it, and the seeds were carefully removed from nests with hands. At this stage, the hollow and blackened seeds were separated from intact ones and were not included in the analysis. Fruit pulp and fruit seeds were dried for about 2 weeks in the shade without direct sunlight.

Determination of fatty acid methyl esters content of fruit seed extracts via (FAME) GC/MSD

The fruit seeds of wild pear that were dried and ground in a mechanical grinder to fine powder before the extraction process with methanol. Briefly, 100 g the fruit seeds samples weighed into 400 mL methanol were mixed using an orbital shaker for 1 hour. Then extraction was continued for 10 days at 25 °C. After the extraction, methanol was evaporated in a rotary evaporator to obtain raw extracts. The obtained methanol extracts were dissolved in water, and again they were extracted with various organic solvents according to their increased polarity, first n-hexane followed by ethyl acetate. Then, the solvents were removed, resulting in raw extracts belonging to each solvent. Approximately 100.0 mg of the resulting hexane extract was weighed into a 20 mL test tube and 10 mL of hexane was added to it. After vortexing for 5.0 min, KOH solution (100 µL solution in 2N methanol) was added. The mouth of the tube was closed and vortexed for a minute. After centrifuging at 4000 rpm for 10 min, the upper phase machinery-Nagel Chromaphil Xtra was filtered through a PTFE - 20/25 0.20 µm filter and then 2.0 µL solution was injected into the Agilent 7890A GC-5975C MSD device.

Antimicrobial activity

Escherichia coli ATCC 25922, *Staphylococcus aureus* ATCC 6538P, and *Candida albicans* ATCC 14053 were used in this study. The test microorganisms were obtained from the culture collection of the microbiology laboratory (Department of Biology of the Faculty of Science and Literature, University of Muğla Sıtki Koçman). The disk diffusion method was used to determine the inhibition impacts of fruit pulp and fruit seed extract of *P. spinosa* on *E. coli* ATCC 25922, *S. aureus* ATCC 6538P and *C. albicans* ATCC 14053 (Collins *et al.* 1995; Murray *et al.* 1995). From the liquid cultures that reached 0.5 McFarland standard turbidity, about 1000 µL were transferred to sterile petris and approximately 20 mL of Mueller Hinton Agar (Merck) for bacteria, Sabouraud Dextrose Agar (Merck) for *C. albicans* were transferred, and planting was made by the pour plate technique. The 20 µL extracts were then conveniently placed on the agar. Seed extract was directly dissolved in the fruit extract with dimethyl sulfoxide (DMSO) and used in 200 mg/mL concentration (Fig. 1). The Petri dishes in which planting was made were incubated for 24 h at 37±0.1 °C for *S. aureus* and *E. coli*, and 24 to 48 h at 30±0.1°C for *C. albicans*. At the end of incubation, the diameters of the inhibition zones formed around the discs were measured in mm. As a negative control, the fruit pulp and fruit seed extract of *P.*

spinosa was dissolved in DMSO, and for positive control, amoxicillin + clavulanic acid disc (Oxoid) for bacteria, and Nystatin (Oxoid) for *C. albicans* were used. All of the studies were carried out in triplicates in parallel.

Determination of DPPH free radical removal activity (*Radical scavenging activity)*

Radical scavenging activities of extracts were determined using the DPPH method (Burits and Bucar 2000). According to this method, 5 mL of 0.004% DPPH (2,2-diphenyl-1-picryl-hydrazyl-hydrate, Sigma) solution was added to a sample of 50 µL solution taken from concentrations of 100, 50, and 25 mg/mL of DMSO extracts. Samples were incubated for 30 min at room temperature and absorbance values were measured at a wavelength of 517 nm. DMSO was used as control.

RESULTS AND DISCUSSION

Fruit Seed Fatty Acid Compositions

The fruit seed fatty acid methyl esters components of *P. spinosa* were determined both qualitatively and quantitatively using (FAME)GC/MSD system. The fatty acid components were identified based on certified reference standard substances in Wiley 2008 and NIST 2008 libraries, and their amounts were calculated. Results of the analysis obtained were given in Table 1. When the fatty acid content in the fruit seed of *P. spinosa* was examined, 44.837%, of linoleic acid (C18:2n6c), 40.524% of oleic acid (C18:1n9c), and 7.625% of palmitic acid (C16:0) were obtained as major components (Table 1).

Table 1. Fatty Acid methyl esters Contents in the Fruit Seed of *P. spinosa* via (FAME)GC/MSD Analysis

Compounds	Percentage (%)	Retention Time
Myristic acid	0.024 ±0.002	15.836
Palmitoleic acid	0.117±0.013	21.515
Palmitic acid	7.625 ±0.57	22.472
Cis 10 Heptadecenoic acid	0.058±0.01	24.630
Margaric acid	0.071 ±0.004	25.581
Linoleic acid	44.837 ±0.001	27.636
Oleic acid	40.524 ±0.207	27.997
Elaidic acid	0.521±0.172	28.034
Stearic acid	2.544 ±0.060	28.724
*Cis*11 Eicosenoic acid	0.848 ±0.058	33.589
Arachidic acid	2.004 ±0.012	34.530
Heneicosanoic acid	0.042±0.116	37.276
Behenic acid	0.438±0.007	39.940
Tricosanoic acid	0.077±0.022	42.506
Tetracosanoic acid	0.242±0.11	44.986
Pentacosanoic acid	0.030±0.02	47.377

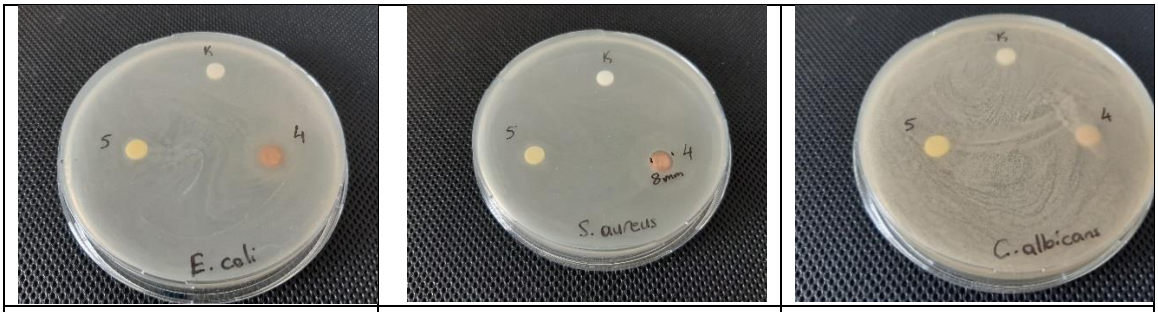
Hazrati *et al.* (2019) examined the fatty acid components of the fruit seed of *P. syriaca* and *P. glabra* and they determined the linoleic acid (46.99 ± 0.37%) and oleic acid (41.43 ± 0.23%) in the *P. syriaca* samples as main components, while they obtained linoleic acid (C18:2) 46.99 ± 0.37% and oleic acid (C18:1) 41.43 ± 0.23% in fruit seeds of *P. glabra* at high rates. Other fatty acid components found in higher amounts in *P. glabra* samples were palmitoleic acid, stearic acid, linoleic acid, alpha-linolenic acid, arachidic acid, and

heneicosanoic acid methyl ester. The fruit seed fatty acid components of *P. syriaca* were palmitic acid, palmitoleic acid, stearic acid, alpha-linolenic acid, arachidic acid, and gondoic acid.

In other studies, in the samples of *Pyrus* species, it was stated that 11 fatty acid components were found, and the main components were fatty acids and oleic acid (56.80 g/100 g oil), stearic acid (20.28 g/100 g oil), and palmitic acid (6.39 g/100 g oil) (Yukui *et al.* 2009). In *P. glabra,* Hashemi *et al.* (2018) determined that the linoleic and oleic acid components as main fatty acids. The study by Gornas *et al.* (2016) determined that in the cultivars of eight pear (*Pyrus communis* L.) species, the fatty acid components were palmitic acid (6.1 to 8.5%), oleic acid (27.4 to 38.2%), and linoleic acid (50.7 to 63.8%). When the fatty acid content in the fruit seed of *P. spinosa* was evaluated 44.8% of linoleic acid (C18:2n6c), 40.5% of oleic acid (C18:1n9c), and 7.6% of palmitic acid (C16:0) were obtained as major components. The fatty acid main components of *P. spinosa* determined in the present study show similarity to the other studies on other *Pyrus* species.

Antimicrobial Activity Results of *Pyrus spinosa* Fruit Extracts

The antimicrobial activity of the fruit seed and pulp extracts of *P. spinosa* samples was examined. The inhibition zone value in fruit pulp extract was 8 mm for *S. aureus*. In fruit seed extract, no activity on test strains was determined. The zone of inhibition measurements are given in Table 2, and inhibition zone images are shown in Fig. 1.



E. coli | *S.aureus* | *C. albicans*

Fig. 1. The results of the antimicrobial activities of *P. spinosa* (Inhibition zone images in the test) (4: fruit extract 5: fruit seed extract, K: control)

Table 2. Inhibition Zone Diameters of *P. spinosa* Fruit Seed and Fruit Pulp Extracts

Microorganisms	Fruit Pulp Extract (mm)	Seed Extract	Amoxicillin+Clavulanic Acid (mm)	Nystatin (mm)
E. coli	-	-	16	Not tried
S.aureus	8	-	20	Not tried
C. albicans	-	-	Not tried	19

In a study conducted in 2012, the antimicrobial activity assay showed 12 bacteria (*Aeromonas hydrophila*, *Bacillus cereus*, *Bacillus subtilis*, *Enterobacter cloacae*, *Escherichia coli*, *E. coli* O157:H7, *Klebsiella pneumoniae*, *Listeria monocytogenes*, *Proteus vulgaris*, *Pseudomonas aeruginosa*, *Salmonella typhimurium*, *Staphylococcus aureus*), and 2 yields (*Saccharomyces cerevisiae*, *C. albicans*). The prepared extract showed inhibition effect only on *K. pneumoniae* (10.06 mm inhibition zone at 10% concentration) and *A. hydrophila* (9.56 mm inhibition zone at 10% concentration) (Polat 2012). It was found that the aqueous extract of şakok pear (*P. elaeagnifolia* Pallas) has

antimicrobial activity at different levels against *Streptococcus agalactiae* ATCC 13813, *Bacillus megaterium* DSM 32, *S. aureus* ATCC 6538, and *K. pneumoniae* (Murathan *et al.* 2019). Similar to the results of the present study, Güdücü (2014) found that the 4 different concentrations (50, 100, 200, 500 mg/mL) of methanol and acetone extracts of fruits of *P. elaeagnifolia* did not form an inhibition zone on 7 bacteria (*S. aureus* ATCC29213, *K. pneumoniae* ATCC33495, *E. faecalis* ATCC29212, *P. aeruginosa* ATCC27853, *E. cloacae* ATCC13047, *Serratia marcescens*, *E. coli* ATCC25923) and did not exhibit any antimicrobial activity. Erbil *et al.* (2018) conducted tests on different pear types; it was stated that the highest microbial activity was in banda pear with a 20.14 mm inhibition diameter against *P. aeruginosa* ATCC 9027, while the lowest activity was in Güğüm pear with a 12.34 mm inhibition diameter against *E. coli*. In another study, the antimicrobial activity of the wild pear (*P. elaeagnifolia*) extracts' antimicrobial activities on *S. aureus*, *B. subtilis*, *B. cereus*, *E. coli*, *L. monocytogenes*, and *P. aeruginosa* microorganisms were followed by disk diffusion method. Prepared wild pear leaf extracts were found to show effect at different levels on 6 microorganisms, and they showed high antimicrobial effects against *S. aureus* and *E. coli* (18±1.6mm) (Keçeci 2018). Various results can be reported in antimicrobial activity and antioxidant studies. These observed differences can be caused by factors such as changes in plant content depending on the climatic conditions and geographical conditions in which the sample grows, different types and/or strains of microorganisms used, and different extraction methods and/or solvent selection methods used.

Antioxidant Activities of Fruit Pulp and Fruit Seed Extracts from *Pyrus spinosa* Forssk.

Free radical removal activities of *P. spinosa* extracts were determined using the DPPH method (Burits and Bucar 2000). Table 3 shows the antioxidant activities of the samples. DPPH radical removal activity was observed to vary depending on concentration. High rates of activity were achieved in fruit pulp, while low rates of activity were determined in fruit seeds. The highest activity was found to be 91.2% in fruit pulp extract. An inhibition of 20% was determined in the fruit seed at a concentration of 200 mg/mL.

Table 3. Antioxidant Activity of *P. spinosa* Fruit Pulp and Fruit Seed Extracts

Pyrus spinosa Extract	Concentration (mg/mL)	Radical Removal (%)
Fruit	100	91.2
	50	90.6
	25	90.1
Seed	200	20
	100	1
	50	0.0
	25	0.0
Control (Ascorbic acid)	5	93.2

Wild fruits contain high amounts of phytochemicals (Hu 2003; Ikram *et al.* 2009). Phytochemical studies show that *Pyrus* species contain such phenolic components as β-arbutin (Aydin *et al.* 2015), chlorogenic acid derivatives (Nishimura *et al.* 2003; Yerliturk *et al.* 2008), catechin derivatives (Nishimura *et al.* 2003; Ziyan *et al.* 2004; Yerliturk *et al.* 2008, Kim *et al.* 2013), as well as flavonoids (Siddiq *et al.* 2000; Dincer *et al.* 2002), hydroxycinnamoyl acids, and methyl esters (Kostic *et al.* 2015), caffeoyl triterpenes (Sahu *et al.* 2013), and sterol glucosides. Wild Pear (*Pyrus* spp.) is among the widely consumed

fruits with different species. Various studies indicated that pears contain vitamins and minerals which result in antipyretic, cough-relieving, and diarrhea-relieving properties. It is also reported to be good for urinary system disorders because it affects the liver and menstrual cycle. The leaves of *Pyrus communis* are urea disinfectant. Wild Pear flowers are used as an analgesic and spasmolytic medication among people (Gudej and Rychlınska 1999). Wild pear leaves with diuretic properties are known to be good for bladder inflammations, bacteriuria, high blood pressure, and the treatment of kidney stones (Zargari 1996). What makes *Pyrus* spp. important as a diet source is dietary fiber, mineral, vitamin C, and organic acids in it (Chen *et al.* 2007). In another study, pear is cited as the best source of polyphenols and triterpenes, while also noting the fruit as functional due to its antioxidant and anti-inflammatory effects (Li *et al.* 2014).

Güven *et al.* (2006) examined the impact of various pear types (*Pyrus* spp.) on microorganisms and found that Serik pear (*P. serikensis*) showed antibacterial effect at certain levels on all bacteria that were examined; however, it was effective on only some of the yeast and mold samples. Pear (*P. communis*) extract, examined within the context of the same study, was found to prevent the development of all other bacteria and yield except from *Salmonella typhimurium*, *Brucella spp.*, *Streptococcus faecalis*, *Pseudomonas fluorescens*, *P. tobacco*, *P. lachrymans*, *P. syringae* pv. *phaseolicola*, *P. gingeri*, and *Rhodotorula rubra*. However, it was found that it did not have any inhibition impact on molds. Antioxidants can be defined as compounds that, at low concentrations, react with free radicals, thus preventing their oxidation. Many epidemiological studies show that foods rich in antioxidants have a protective effect against diseases, and their consumption reduces the risk of cancer, heart disease, hypertension, and stroke (Polat 2012).

The use of antioxidants to strengthen the body's antioxidant defense system, and prevent or treat diseases. Because of the side effects of synthetic antioxidants, the tendency to use natural antioxidants has increased (Kurutaş 2016). Natural antioxidants are found in different parts of plants, such as roots, stems, leaves, flowers, and fruits. In the present study, the highest antioxidant activity in the samples of *P. spinosa* was found to be 91.2% in the fruit pulp extract. An inhibition of 20% was determined in the fruit seed at a concentration of 200 mg/mL.

CONCLUSIONS

1. This study determined that *P. spinosa* contains phytochemicals important for health. According to the results obtained, it was found that the fruit seed of *P. spinosa* contains high amounts of fatty acid components such as linoleic acid and oleic acid while the fruit pulp of *P. spinosa* contains undetermined fatty acid components. The fruit pulp shows antioxidant activity at high rates and antimicrobial activity in low rates. It was also revealed that the extracts obtained from wild pear contained chemical compounds and as a result of the bioactivity owned by these compounds it can be utilized in medicine and health, and functional food/additives production.

2. In the study, the linoleic acid component was determined at high rates (44%) in fruit seeds of *P. spinosa*. The double bond structure of the linoleic acid component provides a high rate (91.2%) of antioxidant properties, and when compared with the GS-MS analysis results, it has been determined that the results obtained support each other.

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