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COMPARISON OF PHENOLIC CONTENT AND ANTIOXIDANT ACTIVITIES OF EXTRACTS OF *Phytolacca americana* L. PLANT BASED ON SOME DIFFERENT HABITATS FROM TÜRKIYE

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Abstract: In this study, leaf and fruit samples of *Phytolacca americana* collected in the early, mature and senescence period from localities with different habitat characteristics (non-polluted, polluted and wetland) were studied. The total phenolic content, 2,2-diphenyl-1-picrylhydrazyl (DPPH), radical scavenging activity and iron (III) reduction antioxidant power (FRAP) of the water and methanol extracts of the samples dried in oven and at room temperature were evaluated and compared as statistically. According to the results of the analyses, the highest phenolic content (263.25 mg GAE/g sample) was found in the leaf parts of the plant collected from the wetland during the senescence period, dried at room temperature and extracted with water. The lowest phenolic content (0.22 mg GAE/g sample) was determined in water extract of the fruit parts of the plant collected from the same locality in the same period. Among the antioxidant activity values determined, the highest and lowest findings belong to these samples. It can be concluded that each tested variable is statistically significant for both the leaf and fruit parts, and by bringing these variables under suitable conditions, biochemically more efficient use of plants can be achieved.

Keywords: Growth season, Wetland, Polluted area, Total phenolic, DPPH, FRAP

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1. Introduction

Although there was an increase in the use of synthetic drugs with the development of the chemical industry in the 19th century, with the understanding of the importance of natural and healthy nutrition from the middle of the 20th century, plants have become the focus of attention again, as in ancient times, to be used both for food and medicinal purposes. Today, plants are also used for a wide variety of purposes such as additives, beverages, paints, perfumes, cosmetics and decorations. Their antimicrobial, antioxidant and pharmaceutical properties are the basis of their widespread use (Kiralan et al., 2012; Kırıcı, 2015; Goktaş and Gidik, 2019).

Thanks to its rich flora, Türkiye makes home coastlines for many plants used for medicinal, food and aromatic purposes (Güner, 2012). Secondary substances such as alkaloids, phenolic compounds, essential oils, tannins, terpenoids, anthocyanins and saponins, which do not have a direct role in the important physiological and metabolic activities of the plant, resist environmental stress factors such as drought, salinity, UV, protect the plant against herbivores and microorganisms, as well as pollination and seed dispersal thanks to important ecological functions, they increase the defense power of plants and ensure their survival and adaptation to the environment (Taiz and Zeiger, 2002; Akçam Oluk, 2006; Faydaoğlu and Sürücüoğlu, 2011; Eray Vuran and Türker, 2021).

As an invasive weed, *Phytolacca americana*, which can cause problems in different places such as field crops, meadow, and pasture areas, is used for medicinal purposes by the local people of America. In our country, it is especially common in the eastern Black Sea region and is known as Şekerci boyası, Acımur, Miss World (Baytop, 1994; Ravikiran et al., 2011; Tubives, 2014).

Reactive oxygen species, which can inevitably occur even during normal metabolism, often cause irreparable damage to lipids, proteins, and nucleic acids. These damages, known as oxidative stress, are linked to many diseases such as cataracts, rheumatoid arthritis (Hadjigogos, 2003), cancer (Khanna et al., 2014), lung damage (Erol et al., 2019), neurodegenerative diseases (Rekatsina et al., 2020) and diabetes (Yaribeygi et al., 2020). Antioxidant substances, which prevent cells from being damaged by catching and neutralizing free radicals in living things, have the potential to minimize the negative effects of free radicals on health (Diplock, 1998; Elliot, 1999; Harman, 2009).

The aim of this study is to investigate the effect of this difference on biochemical activities, with the thought that *P. americana* collected from localities with different habitat characteristics will contain different secondary metabolites in terms of species and/or amount. In addition, by changing the extraction conditions, which are known to affect biochemical activities, the phenolic content and antioxidant activity of different parts of the plant were investigated comparatively. Thus, the results obtained will enable the plant to be used more effectively and in different ways upon need.

2. Materials and Methods

2.1. Plant Material and Preparation of Extracts

Phytolacca americana L. (Phytolaccaceae) also known as pokeweed and pokeberry is a poisonous, herbaceous perennial plant that dispersal 0-500 m altitudes and different habitat conditions such as slopes, forest edges, bushes, and wetlands. It has been reported that *P. americana* is used as treatment, food, and animal feed (Baytop, 1994; Nabavi et al., 2009). It is used in the treatment of mumps, arthritis, cancer, herpes, and AIDS.

P. americana leaf samples were collected from three localities with different habitat characteristics in early, mature and senescence period from Ordu province in Black Sea Region of Türkiye. The fruit samples were collected at mature period. While determining the habitat characteristics, the criteria for being widespread in all selected areas, being exposed to one or more environmental pollutants (polluted area), being away from pollutants (non-polluted area) and being close to the aquatic ecosystem (wetland) were taken as basis. According to these criteria, a cement factory edge was determined as a polluted area, a forest edge as a non-polluted area, and a river edge as a wetland. The identity of the plant specimen was clarified based on the book 'Flora of Türkiye and the East Aegean Island' (Davis, 1988).

All samples were dried both in the shade at room temperature and in an oven at 65 °C. Dried samples were powdered using herb grinder. For the preparation aqueous and methanol extracts, dried and ground plant samples were extracted in appropriate amount of water/methanol using shaking water bath at 25 °C. After, then the extract was filtrated and lyophilized in case of water. The extraction solvent was removed under vacuum using a rotary evaporator for methanol extract. At the end of this process, the dry matters were weighed for quantification of extractable compounds from plants and resolved in water and ethanol, respectively (Çol Ayvaz, 2015).

2.2. Biochemical Analysis

Determination of total phenolic content and antioxidant activity tests were carried out to reveal the biochemical potential of the extracts prepared using water and methanol after drying at room temperature and in the oven, of the leaves and fruit parts of the plant collected at different stages of the development process from locations with different properties. All tests were performed in 3 repetitions.

2.3. Determination of Total Phenolic Content

The Folin-Ciocalteu method developed by Singleton and Rossi (1965) was followed to reveal the expected change in the total phenolic content of the samples with these differences. The total phenolic content of the samples was expressed as gallic acid equivalent (mg GAE/g extract), a commonly used phenolic acid.

2.4. Determination of DPPH Free Radical Scavenging Activity

The free radical scavenging potential of the extracts was investigated using the 2,2-diphenyl-1-picrylhydrazil (DPPH) radical (Blois, 1958). It is known that the decrease in absorbance in the prepared reaction mixture is proportional to the free radical scavenging activity, thanks to the method based on the bleaching of the purple-colored DPPH solution by the offer of a proton or electron by the extracts. For this purpose, firstly, different concentrations of ascorbic acid, which is used as a standard antioxidant, were brought together with the DPPH solution prepared in methanol and vortexed, then left in the dark for 30 minutes at room conditions, and at the end of this period, the absorbance of the tube contents at 517 nm was read against methanol. Using the obtained absorbance values, the %DPPH radical scavenging activity (%) was calculated with the formula given below. In this equation, ABS_{blank} represents the absorbance of the mixture prepared to contain only solvent and DPPH solution, not containing the sample.

Scavenging activity (%) = $(ABS_{blank} - ABS_{sample})/ABS_{blank}$

With the help of the graph created by plotting the % scavenging activity values calculated for different concentrations of ascorbic acid against the concentration, the DPPH radical scavenging activities of the samples tested simultaneously were expressed as mg AAE/g extract.

2.5. Determination of Iron (III) Reducing Antioxidant Power (FRAP) Capacity

The second method chosen to evaluate antioxidant activity is the FRAP test, which is known as simple, fast and inexpensive. The reducing ability of the antioxidants whose probable presence is known in the tested samples is followed by the ferric tripyridyl triazine (TPTZ) complex leading to the formation of the ferrous TPTZ form (Benzie and Strain, 1996). The change in absorbance of the tested sample at 595 nm was compared with the standard antioxidant Trolox, and the FRAP value of the samples were expressed as Trolox equivalent (µmol TXE/g extract).

For this purpose, after adding an appropriate amount of freshly prepared FRAP reagent (300 mM acetate buffer (pH 3.6), 10 mM 2,4,6-tris(2-pyridyl)-s-triazine (TPTZ) solution, and 20 mM $FeCl_3 \bullet 6H_2O$ in a 10:1:1) on Trolox solution in ethanol at varying concentrations and sufficient amount of samples, developing absorbances were measured at 595 nm after incubation at 37 °C for 30 minutes.

2.6. Statistical Analysis

The findings of DPPH, FRAP and Total phenolic substance amounts calculated for the fruit part of the plant were evaluated with three-way analysis of variance (three-way ANOVA), and the values calculated for the leaf part were evaluated with four-way analysis of variance (four-way ANOVA). After analysis of variance, if necessary, different means were determined by Tukey's multiple comparison test. Before the analysis of variance, the assumptions were checked with the Levene's test and the Kolmogorov-Smirnov test. 5% significance level was considered in calculations and interpretations. All calculations were made with Minitab 19 (Minitab LLC., USA) statistical program (Genç and Soysal, 2018).

3. Results

In this study, which was planned with the hypothesis of "there may be changes in some phytochemical properties of the samples of the same species living in different ecological conditions", the total phenolic content (TPC), DPPH radical scavenging activity and Iron (III) reduction antioxidant power (FRAP) capacity determinations of the extracts prepared from the leaf parts of the early, mature and senescence periods of *P. americana* plant obtained from three habitats with different characteristics determined in Ordu province Ünye district , were made. Similar analysis was carried out on the fruit parts of the plant, which were collected only in the mature and senescence period. All analysis was carried out on the extracts prepared with water and methanol from the powder form obtained by drying the fruit and leaf parts separately, either at room temperature or in an oven. However, in the case of fruit, there is no raw period and there is no drying parameter in the oven.

As a result of the three-way analysis of variance of the total phenolic content calculated as gallic acid equivalent of the extracts prepared from the fruit parts, considering all the variables, it was found that the triple interaction of *locality x period x chemical* was statistically significant ($P<0.001$). The highest phenolic content (40.233 mg GAE/g extract) of the fruit parts of the *P. americana* plant was calculated in the case of water extracts of the samples collected in the senescence period from non-polluted area, while the lowest phenolic content (0.213 mg GAE/g extract) was calculated in the case of the extracts prepared under the same conditions from the samples obtained from the wetland in the same period. Furthermore, a statistical difference was observed between the phenolic content of methanol and water extracts of the fruit parts of the samples collected from the polluted area in the mature period, from the wetland area in the senescence period, and from the non-polluted area in both periods (Table 1).

On the other hand, there is a statistical difference between the amount of phenolic content obtained in the case of water extracts of the fruit samples collected from the polluted and wetland areas in the mature and senescence period, and in the case of both water and methanol extracts of the samples collected from the non-polluted area. In addition, the phenolic content of the methanol extract of the samples collected from the wetland in the mature period is different from those obtained from other areas. In the senescence period, the methanol extract of the fruit collected from the polluted area and the water extract of the fruit collected from the non-polluted area are statistically different from the others.

The total phenolic contents of the extracts prepared in water and methanol were calculated after the leaf parts of the plant samples collected from each area at different growth stages were dried both in the oven and at room temperature (RT). As a result of the four-way analysis of variance performed with the obtained results, it was found that the interaction of *Locality × Period × Chemical × Drying* was statistically significant ($P<0.001$). Tukey's multiple comparison test results are expressed as letters next to the averages, and in the case of leaf samples, the highest phenolic content (263.253 mg GAE/g extract) was calculated in the extract prepared with water after drying at room temperature of the samples collected from the wetland during senescence period. The lowest value (0.227 mg GAE/g extract) was calculated for the extract prepared with water after drying the samples collected in the mature period from the clean area in the oven (Table 2).

Table 1. Descriptive statistics and comparison results for TPC values (mg GAE/g extract) of fruit parts

Locality	Chemical	Period			
		Mature period (n=3)		Senescence period (n=3)	
		Average	Std. Deviation	Average	Std. Deviation
Polluted area	Methanol	2.313Ba^{B}	0.133	3.740Aa^{B}	0.304
	Water	18.870Aa^{A}	5.370	1.693Ab^{B}	0.309
Wetland	Methanol	16.130Aa^{A}	1.597	10.503Aa^{A}	0.401
	Water	13.447Aa^{A}	3.440	0.213Bb^{B}	0.015
Non-polluted area	Methanol	5.453Bb^{B}	0.657	13.460Ba^{A}	0.861
	Water	14.887Ab^{A}	1.845	40.233Aa^{A}	2.034

There is a difference between the chemical averages without a common capital letter at the same locality and same period ($P<0.05$), There is a difference between the period averages without a common lowercase letter in the same locality and same chemical ($P<0.05$), There is a difference between the locality averages without a common exponential capital letter at the same chemical and same period ($P<0.05$).

Table 2. Descriptive statistics and comparison results for total phenolic content values (mg GAE/g extract) of leaf parts

Locality	Chemical	Drying	Period					
			Young Period(n=3)		Mature Period (n=3)		Senescence period (n=3)	
			Average	Std. Deviation	Average	Std. Deviation	Average	Std. Deviation
Polluted area	Methanol	Oven	8.787Aa^{Aa}	0.593	6.237Aa^{Ab}	0.108	20.067Aa^{Aa}	7.404
		RT	12.183Aa^{Aa}	0.525	5.847Aa^{Aa}	0.713	6.140Aa^{Ba}	0.346
	Water	Oven	8.050Aa^{Aa}	0.939	8.957Aa^{Aab}	0.843	21.757Ba^{Ab}	4.815
		RT	18.870Ab^{Aa}	5.370	14.893Ab^{Aa}	1.036	43.353Aa^{Ab}	9.781
Wetland	Methanol	Oven	13.503Aa^{Aa}	0.252	26.183Aa^{Aa}	1.201	8.267Aa^{Aa}	0.206
		RT	10.620Aa^{Aa}	0.800	7.387Aa^{Aa}	0.227	5.153Aa^{Ba}	0.152
	Water	Oven	8.063Aa^{Aa}	0.774	26.183Aa^{Aa}	1.201	10.187Ba^{Ab}	1.173
		RT	9.547Ab^{Aa}	0.785	15.487Ab^{Aa}	1.497	263.253Aa^{Aa}	19.241
Non-polluted Area	Methanol	Oven	13.757Aa^{Aa}	0.685	7.327Aa^{Aab}	1.371	19.337Aa^{Ba}	11.936
		RT	10.003Aa^{Aa}	0.335	3.543Aa^{Aa}	0.097	18.127Aa^{Aa}	2.769
	Water	Oven	7.023Ab^{Aa}	0.803	0.227Ab^{Ab}	0.099	78.860Aa^{Aa}	24.200
		RT	5.603Aa^{Aa}	0.405	13.227Aa^{Aa}	1.544	17.177Ba^{Ac}	1.737

RT= room temperature, There is a difference between the drying averages without a common capital letter at the same locality, chemical and period ($P<0.05$), There is a difference between the period averages without a common lowercase letter at the same locality, chemical and drying ($P<0.05$), There is a difference between the chemical averages without a common exponential capital letter of the same locality, drying and period ($P<0.05$), There is a difference between the locality averages without a common exponential lowercase letter for the same chemical, drying and period ($P<0.05$).

From Table 2 it is seen that, total phenolic contents of the extracts prepared with water after drying in oven and at room temperature from the leaf parts of the samples collected from all three areas in the senescence period were statistically different ($P<0.05$). There is a statistical difference between the total phenolic contents of the extracts prepared in methanol and water of the leaf samples obtained from polluted and wetland areas and dried at room temperature during the senescence period ($P<0.05$). The same difference emerged between the methanol and water extracts prepared because of oven drying in the case of samples collected from the non-polluted area. The phenolic contents of the leaf parts, which were dried at room temperature and extracted with water after being collected from polluted and wetland areas in the senescence period, are statistically different from the extracts prepared by the same processes but collected in other periods. The phenolic value of the extract prepared with water from the leaf part of the sample obtained from the clean area, which was dried in the oven, is different from the samples of the plant collected in other periods ($P<0.05$).

The total phenolic content values of the methanol extracts dried in the oven of the leaf parts of the samples obtained from the polluted and wetland areas in the mature period showed statistical differences ($P<0.05$). The phenolic contents of the water extracts of the samples collected from the wetland and non-polluted areas in the same period and dried in the oven were found to be also statistically different. The phenolic values of all three samples collected from all three areas in the senescence period and extracted with water after drying at room temperature differ statistically from each other ($P<0.05$). In addition, the phenolic content of the extract obtained from the non-polluted area in the senescence period and prepared with water after drying in the oven was found to be different from the samples obtained from the other two localities at the same time and extracted under the same conditions ($P<0.05$).

Changes on DPPH free radical scavenging activity of extracts according to different parameters were evaluated thanks to the three-way analysis of variance. For the values obtained because of the calculation of the DPPH radical scavenging activities of the fruit extracts prepared in both methanol and water, the triple interaction of *Locality × Period × Chemical* was found to be statistically significant ($P<0.001$).

The highest DPPH radical scavenging activity value (28.303 mg AAE/g extract) calculated for the fruit part was calculated in the case of the methanol extract prepared after drying the sample obtained from the wetland in the mature period at room temperature, and the lowest value (0.067 mg AAE/g extract) was recorded for the extract prepared with water after being collected from the wetland in the senescence period (Table 3).

As a result of the evaluation of all the average values in the Table 3 according to the Tukey multiple comparison test, a statistically significant difference was found between the DPPH radical scavenging activities of the extracts prepared with methanol and water after the fruit parts of the plants collected from the wetland were dried at room temperature in both mature and senescence periods ($P<0.05$). A statistically significant difference was also found between the calculated values of DPPH radical scavenging activities of both methanol and water extracts of fruit samples collected from the non-polluted area in the mature and senescence period ($P<0.05$). The same difference was also observed in the total phenolic content.

Table 3. Descriptive statistics and comparison results for DPPH radical scavenging activities (mg AAE/g extract) of fruit parts

Locality	Chemical	Mature Period (n=3)		Senescence period (n=3)	
		Average	Std. Deviation	Average	Std. Deviation
Polluted area	Methanol	3.347Aa^{B}	0.752	8.157Aa^{B}	1.082
	Water	10.750Aa^{A}	2.676	0.657Aa^{B}	0.075
Wetland	Methanol	28.303Aa^{A}	8.006	21.767Aa^{A}	4.591
	Water	7.547Ba^{A}	0.917	0.067Ba^{B}	0.015
Non-polluted area	Methanol	9.617Ab^{B}	1.717	26.927Aa^{A}	7.606
	Water	6.607Ab^{A}	0.827	24.180Aa^{A}	2.902

There is a difference between the chemical averages without a common capital letter in the same locality and same period (P<0.05), There is a difference between the period averages without a common lowercase letter in the same locality and same chemical (P<0.05), There is a difference between the locality averages without common exponential capital letters at the same period and same chemical (P<0.05).

Table 4. Descriptive statistics and comparison results for DPPH radical scavenging activities (mg AAE/g extract) of leaf parts

Locality	Chemical	Drying	Young Period (n=3)		Mature Period (n=3)		Senescence Period (n=3)	
			Average	Std. Deviation	Average	Std. Deviation	Average	Std. Deviation
Polluted area	Methanol	Oven	7.417Ab^{Aa}	1.117	4.107Ab^{Aa}	0.647	30.420Aa^{Aa}	6.211
		RT	9.730Aa^{Aa}	1.656	5.943Aa^{Aa}	1.107	6.113Ba^{Bb}	0.821
	Water	Oven	7.917Aa^{Aa}	1.792	6.230Aa^{Aab}	1.586	18.780Ba^{Ab}	1.919
		RT	11.683Ab^{Aa}	0.903	7.683Ab^{Aa}	1.736	37.740Aa^{Ab}	0.963
Wetland	Methanol	Oven	7.950Aa^{Aa}	1.277	14.530Aa^{Aa}	1.629	7.457Aa^{Ab}	1.320
		RT	8.517Aa^{Aa}	1.914	4.543Aa^{Aa}	0.231	2.210Aa^{Bb}	0.182
	Water	Oven	5.057Aa^{Aa}	0.776	14.183Aa^{Aa}	2.009	1.967Ba^{Ac}	0.850
		RT	8.990Ab^{Aa}	0.513	4.967Ab^{Aa}	1.842	99.627Aa^{Aa}	17.036
Non polluted Area	Methanol	Oven	8.153Ab^{Aa}	0.331	4.80Ab^{Aa}	0.868	35.837Aa^{Ba}	1.616
		RT	7.360Ab^{Aa}	1.000	1.963Ab^{Aa}	0.287	23.107Aa^{Aa}	4.519
	Water	Oven	6.067Ab^{Aa}	1.006	0.073Ab^{Ab}	0.049	62.807Aa^{Aa}	13.597
		RT	3.350Ab^{Aa}	0.221	5.247Aab^{Aa}	0.917	17.097Ba^{Ac}	0.592

RT= room temperature.

The DPPH radical scavenging activity of the methanol fruit extract of the plant collected from the wetland in the mature period is statistically different from the activity of the extract collected from the other two areas and prepared under the same conditions (P<0.05). In the senescence period, fruit parts extracted with methanol collected from the polluted area have different DPPH radical scavenging efficiency compared to the methanol extracts of fruit parts collected from other areas. In addition, the DPPH activity of the sample collected from the non-polluted area and extracted with water during the senescence period was found to be statistically different from the extracts collected from the other two areas and analyzed under the same conditions (P<0.05).

The DPPH radical scavenging activities of the water and methanol extracts prepared from the powders dried both in the oven and at room temperature of the leaf parts of the plant samples collected in three different periods, different from the fruit parts, were examined and a four-way analysis of variance was performed according to these variables for the results obtained. The four-way interaction of *Locality × Period × Chemical × Drying* was found to be statistically significant. (P<0.001).

While the scavenging activity of DPPH radical is higher in the leaf part than fruit part, it is also higher in the leaf part, especially in the extracts prepared by collecting in the senescence period. (Table 4). In addition, statistical differences usually stand out between different parameters in this period.

The highest ~~calculated~~ value (99.627 mg AAE/g extract) was calculated in the extract prepared with water after drying at room temperature of the samples collected from the wetland during senescence period. The lowest value (0.073 mg GAE/g extract) was also calculated for the extract prepared with water after drying the samples collected in the mature period from the non-polluted area in the oven (Table 4). These values show a one-to-one correlation with the phenolic content values.

The DPPH radical scavenging activity of the leaf samples collected from the wetland during the senescence period and extracted using methanol after drying in the oven was found to be different from the samples collected from the other two areas in the same period and prepared for analysis in a similar way. Similarly, the antioxidant activity of methanol extracts of leaf parts collected from the clean area in the same period and dried at room temperature differ according to the value calculated for the extracts collected from other areas and prepared under the same conditions. Interestingly, the DPPH radical scavenging activity of the water extracts

from leaf parts of the plant samples dried both in an oven and at room temperature collected from all three areas in the senescence period were found to be statistically different from each other. In the mature period, the DPPH radical scavenging activities of the samples collected from wetland and non-polluted areas and prepared by extracting with water after drying in the oven are also statistically different from each other.

The antioxidant activities based on DPPH radical scavenging abilities of the extracts prepared with methanol of the leaf parts collected from the polluted area in the senescence period and dried in oven and at room temperature were statistically different. It was determined that the same difference was found in the extracts prepared with water from the leaf parts collected from all three areas.

The calculated DPPH radical scavenging activity values of the samples that were collected from the polluted area in the senescence period, dried in an oven and extracted with methanol, and extracted with water after drying at room temperature were found to be different from the samples prepared for extraction under the same conditions but collected at different times. A similar difference was observed compared to the samples collected in the other periods of the sample collected from the wetland in the senescence period and extracted with water after drying at room temperature. In addition, it was concluded that the extracts collected in the senescence period from the non-polluted area, dried both in the oven and at room temperature and prepared with both methanol and water, had a different degree of DPPH scavenging activity compared to the samples prepared under the same conditions but collected in the young and mature period ($P<0.05$).

The antioxidant activity values calculated according to the DPPH radical scavenging efficiency of the extracts prepared in methanol and water of the leaf samples collected from the non-polluted area during the senescence period and dried in the oven are statistically different. A similar difference arising from the solvent difference is also in question for the samples collected in the same period from the polluted and wetland areas and dried at room temperature ($P<0.05$).

The FRAP values of the water and methanol extracts prepared after drying at room temperature, *Phytolacca americana* fruit part collected from 3 different localities, which differ in two different periods, as mature and senescence, were calculated as Trolox equivalents and according to the analysis of variance using these values, it was concluded that the triple interaction of *Locality × Period × Chemical* was statistically significant ($P<0.01$).

Table 5. Descriptive statistics and comparison results for FRAP values (µmol TXE/g extract) of fruit parts

Locality	Chemical	Period			
		Mature Period (n=3)		Senescence period (n=3)	
		Average	Std. Deviation	Average	Std. Deviation
Polluted area	Methanol	20.653Ba^{B}	2.438	41.470Aa^{B}	6.670
	Water	84.590Aa^{A}	1.882	9.483Ab^{B}	0.821
Wetland	Methanol	234.800Aa^{A}	44.547	132.520Ab^{A}	16.353
	Water	79.480Ba^{A}	18.003	0.977Bb^{B}	0.138
Non-polluted area	Methanol	56.360Ab^{B}	7.511	184.450Aa^{A}	25.800
	Water	73.090Ab^{A}	5.121	211.540Aa^{A}	45.313

There is a difference between the chemical averages without a common capital letter at the same locality and same period ($P<0.05$), There is a difference between the period averages without a common lowercase letter in the same locality and same chemical ($P<0.05$), There is a difference between the locality averages without common exponential capital letters at the same period and same chemical ($P<0.05$).

Table 6. Descriptive statistics and comparison results for FRAP value (µmol TXE/g sample) of leaf parts

Locality	Chemical	Drying	Period					
			Young Period (n=3)		Mature Period (n=3)		Senescence Period (n=3)	
			Average	Std. Dev.	Average	Std. Dev.	Average	Std. Dev.
Polluted area	Methanol	Oven	53.900Ab^{Aa}	7.352	31.720Aa^{Aa}	8.410	193.000Aa^{Aa}	38.305
		RT	61.367Aa^{Aa}	7.392	32.710Aa^{Aa}	3.948	41.190Ba^{Ba}	8.281
	Water	Oven	64.713Aa^{Aa}	7.163	66.740Aa^{Aab}	9.257	157.240Ba^{Ab}	43.256
		RT	77.630Ab^{Aa}	6.744	82.683Aa^{Aa}	18.201	331.927Aa^{Ab}	68.912
Wetland	Methanol	Oven	61.567Aa^{Aa}	4.579	113.560Aa^{A}	14.091	56.447Aa^{Ab}	8.982
		RT	57.310Aa^{Aa}	10.277	37.213Aa^{Aa}	6.585	35.683Aa^{Ba}	11.180
	Water	Oven	42.177Aa^{Aa}	3.115	151.373Aa^{Aa}	17.916	54.037Ba^{Ab}	11.093
		RT	69.027Ab^{Aa}	11.896	80.797Aa^{Aa}	14.385	1193.637Aa^{Aa}	43.963
Non-polluted Area	Methanol	Oven	90.517Aa^{Aa}	22.757	32.527Aa^{Aa}	7.653	209.553Aa^{Ba}	36.790
		RT	56.020Aa^{Aa}	10.850	20.363Aa^{Aa}	4.682	142.943Aa^{Aa}	25.417
	Water	Oven	43.843Ab^{Aa}	5.633	0.697Aa^{Ab}	0.116	605.460Aa^{Aa}	208.071
		RT	28.747Aa^{Aa}	1.894	53.197Aa^{Aa}	1.292	141.477Ba^{Ac}	34.433

The highest FRAP value (234.800 µmol TXE/g extract) was obtained in the case of the plant sample collected from the wetland in the mature period and prepared with methanol after drying at room temperature. The second sample, which follows it with a very close value (211.540 µmol TXE/g extract), is the extract of the fruit parts of the plant collected in the senescence period from the non-polluted area, dried at room temperature and prepared with water. The lowest value (0.977 µmol TXE/g extract) was recorded in the case of the water extract of the sample obtained from the wetland in the senescence period (Table 5).

A statistical difference was found between the calculated FRAP values of the extracts prepared with water and methanol from the fruit parts of the samples collected from the polluted area in the mature period and from the wetland in both mature and senescence periods ($P<0.05$). The FRAP value of the fruit parts obtained from the wetland in the mature period and extracted with methanol was found to be statistically different from the extracts obtained from the polluted and non-polluted areas and exposed to the same processes ($P<0.05$). Likewise, the sample obtained from the polluted area in the senescence period and extracted with methanol gave statistically different results compared to the sample from other localities. Plus this, the water extract prepared from fruit sample grown at non-polluted area had statistically different FRAP value compared to similar extracts prepared with the fruit collected from other areas ($P<0.05$).

The statistical difference between the harvesting time of the plant and the activity recorded in the FRAP values of the extracts collected from three different areas in both periods and prepared with methanol and water stand out in all cases, except for the extract obtained from the polluted area and prepared with methanol.

According to the results of the 4-way analysis of variance for the values obtained because of the FRAP test for the leaf extracts prepared at different conditions in the 4 parameters, it was concluded that the *locality × period × chemical × drying* quadruple interaction was statistically significant ($P<0.001$).

The detailed examination of the average values from Table 6 shows that the first 3 values significantly higher were obtained in the case of samples collected in the senescence period. Although the values obtained for the samples collected in the mature and young period are similar, the lowest FRAP value in the case of leaf samples was obtained when the plant collected in the mature period was extracted with water after drying in the oven.

The FRAP values of the extracts prepared with water after drying the leaf parts of the samples collected from wetland and non-polluted areas in the senescence period, after drying in an oven and room temperature, show statistical differences. In the case of polluted area, the difference due to the change in drying conditions arises in the case of extracts prepared with both water and methanol ($P<0.05$).

The FRAP value of the water extract of the leaf parts dried at room temperature after being collected from both the polluted and wetland areas in the young period is statistically different from the equivalent samples that differ only in the collection period. A similar difference, in which the period parameter is effective, is also in question in the case of samples that are dried in an oven and extracted with methanol after being collected from the polluted area and dried in an oven after being collected from the non-polluted area and extracted with water.

The effect of the solvent used in the preparation of the extract on the FRAP value of the sample, which was subjected to the same conditions in all other respects, is noticeable in the case of water and methanol extracts of leaf parts collected from polluted and wetland areas during the senescence period and dried at room temperature. In addition, the FRAP values of the extracts prepared in both solvents from the leaf sample collected from the non-polluted area and dried in the oven in the same period are statistically different.

When the FRAP values of the samples collected in the mature period, dried in the oven and extracted with water were examined statistically, it was observed that the results of the extracts prepared from the samples collected from the wetland and non-polluted areas were statistically different. The FRAP value of the methanol extract, which is collected from the wetland in the mature period and dried in an oven, is statistically different from the sample collected from other two areas in the same period and prepared for analysis in a similar way. The same difference emerged in the senescence period. The FRAP value of the sample, which was collected from the non-polluted area in the senescence period and extracted with water after drying in the oven, is statistically different from the FRAP value of the extracts collected from the other two areas in the same period and analyzed in a similar way. The FRAP values of the extracts prepared by drying the leaf samples obtained from all three areas in the senescence period at room temperature and extracting with water are statistically different from each other.

4. Discussion

Although oxygen is an essential element for life, it can also exacerbate the damage within the cell by oxidative events (Shinde et al., 2012). The formation of free radicals is associated with the normal natural metabolism of aerobic cells. Free radicals attack the body's healthy cells, causing them to lose their structure and function. Fortunately, free radical formation is naturally controlled by various beneficial compounds known as antioxidants. When the availability of antioxidants is limited, this damage can become cumulative and debilitating. The discovery of natural antioxidants is important instead of synthetic antioxidants whose use is restricted due to their harmful effects. Since plants have been used in traditional

medicine to treat various diseases for years, the potential of plant products to be antioxidants against various diseases caused by free radicals has been investigated for a long time (Zheleva-Dimitrova, 2013). The antioxidant effects of herbal products are mainly attributed to phenolic compounds such as flavonoids, phenolic acids, tannins and phenolic diterpenes (Nabavi et al., 2009). These active substances are the result of the interaction between plants and the environment during the long evolutionary period, and their production and changes have a strong correlation and relationship with the environment. In other words, environmental factors can affect the types and contents of active substances. In this way, active substances in the same plant species may differ in types, content, and proportions due to environmental differences in growing places. Certain substances are synthesized only in certain environments, while the contents of certain substances increase markedly in certain environments. Under different environmental conditions perceived as stress, an increase in the concentration of reactive oxygen species occurs in plants and oxidative stress develops. In this case, plants activate their enzymatic and non-enzymatic antioxidant systems to respond to abiotic stress (Bautista et al., 2016). In this way, environmental differences such as altitude, temperature, lighting, precipitation, humidity, soil in different production areas contribute to the differences in the active substance content of the plants and therefore the antioxidant activity. Thanks to this rich diversity in chemical compositions and antioxidant activity, different sources are provided in terms of drugs, functional foods and nutritional supplements (Liu et al., 2016). However, an appropriate extraction method and solvent selection is also very important to ensure efficient extraction of targeted nutraceuticals from plant material (Goli et al., 2005).

It is known that the extraction solvent can affect the phytochemical profile and antioxidant activity of prepared extracts (Sepahpour et al., 2018). Because the type, number, and position of the functional groups of phenolic compounds cause different properties, and thus the solubility of these compounds in different solutions may vary. Therefore, choosing the best solvent is an important factor affecting the quality and quantity of extracted phenolic compounds. Despite all this, a general extraction technique cannot be recommended to ensure the recovery of all phenolic compounds from all plant sources. A study was conducted in which solvents were compared for the extraction of phytochemicals from the leaves of *Datura metel* L. (Solanaceae) plant (Dhawan and Gupta, 2016). Among the 6 solvents (acetone, chloroform, distilled water, ethyl acetate, hexane and methanol) tested, the highest extraction yield was obtained in the case of methanol (85.36%) and water (78.00%). But this was not directly reflected in the phenolic content and antioxidant capacity. Namely, the highest phenolic content was obtained in the case of ethyl acetate extract, while the highest DPPH radical scavenging activity was obtained in the case of methanol. In the case of water extract, the DPPH radical scavenging activity is moderate, although the phenolic content is calculated to be quite low (Dhawan and Gupta, 2016).

There are some conditions in which the phenolic contents of the fruit sample collected in different periods are found to be statistically different. It is an expected situation that different types and amounts of phenolic compounds may accumulate in different soils at different periods and the amounts that can be extracted with water and alcohol in these accumulated phenolics will be different.

In the literature, there are biological studies on the same type of plant collected from different areas. For example, the antibacterial, antioxidant and anticancer properties of the tuber parts of the *Hydnophytum formicarum* Jack. (Rubiaceae) plant obtained from Setiu Wetland (Malaysia) and Muara Rupit (Indonesia) were investigated, with the view that different environment and habitat may cause possible differences in the medicinal properties of the metabolites. Although they showed that the antioxidant and antibacterial activities of the samples obtained from both locations were strong, their anti-cancer activities differed, and the authors concluded that the different geographical area, environment and habitat where the plant grows may influence the metabolite and activity produced (Andriani et al., 2017).

Wojdyło and Oszmiański (2020) investigated the antioxidant activity of apples and their leaves, which is modulated by their polyphenol content, during fruit development and ripening. They revealed that while the concentration of apple phenolics was high at the beginning of the season, it decreased during fruit development, while the leaf phenolics had a more constant level throughout the entire collection period compared to fruits. Together with these results, they stated that the chemical complexity and variations of the phenolic profile of apples are caused by the growing period, growing season, geographical location and most importantly genetic variation.

Here is a literature to support our findings: The antioxidant activity investigated by DPPH and ABTS radical removal techniques is higher when the apple is not yet ripe, and the leaves are young (Wojdyło and Oszmiański, 2020).

Our findings indicate that the environment in which the plant is grown has a significant effect on antioxidant activity. Another result, which is compatible with the literature, is that the leaf part contains more phenolic and antioxidant activities than the young fruit (Wojdyło and Oszmiański, 2020).

When all the results are evaluated in general, it is seen that the extracts of the samples prepared from both parts of the plant samples collected in the senescence period show higher phenolic content and antioxidant activity. As it is known, senescence is the death of some cells, tissues and organs of plants to complete their development.

Some researchers have reported that senescence can occur genetically in healthy plants grown under the most ideal growing conditions, as it can occur when exposed to unfavorable environmental conditions such as drought, heat, nitrogen deficiency, insufficient light, and disease and pathogen attacks. It is known that there is an increase in plant hormones such as ethylene hormone and some enzyme activities such as hydrolases during senescence. As it is understood, the senescence process can vary according to environmental stress factors. Stress can trigger early senescence and reduce yields. Various factors, which can be expressed as external factors, including length of day, temperature changes, light flux, drought, ozone, shade, injury, UV-B and pathogen infection, can be effective on the onset of senescence. Plants have also developed various defense mechanisms in response to this stress. These include early senescence, pigment synthesis, and accumulation of signaling molecules such as salicylic acid and jasmonic acid. Plant responses to stress result in transcriptional activation of many genes, such as proteins involved in photosynthesis, pigments, antioxidants, and genes encoding proteins associated with pathogens. For example, there are findings related to increased H_2O_2 level and enzymatic antioxidants such as superoxide dismutase (SOD), dehydroascorbate reductase (DHAR) and manganese-superoxide dismutase (Mn-SOD) activities in the peroxisomes of senescence pea leaves (Sağlam, 2015). On the other hand, there are reports showing that the activities of various antioxidant enzymes can both increase and decrease during aging (Prochazkova et al., 2001). For example, in *Arabidopsis thaliana* (L.) Heynh (Brassicaceae), a five-fold decrease in ascorbate peroxidase activity during the developmental transition to flowering causes an increase in lipid peroxidation (Ye et al., 2000). An increase in mitochondrial and peroxisomal oxidants and antioxidant activity during aging has also been reported (Jimenez et al., 1998; Del Rio et al., 1998).

Within the scope of the current study, the leaf and fruit parts of the plant were tested separately for each parameter, and it is impossible to make a comment that any of them make a significant difference compared to the other. It has not been subjected to statistical analysis.

There are also reports in the literature that support our findings. In the study examining the antioxidant activity of the fruit and leaf parts of the *Cudrania tricuspidata* (Carr.) Bur. ex *Lavallee* (Moraceae) plant, the total phenolic content was found to be higher in the leaf part, and the DPPH radical scavenging activity was found to be higher in the fruit part (Kim and Chin, 2020).

As a result of the study in which the polyphenolic content and antioxidant activity of *Pistacia lentiscus* L. (Anacardiaceae) plant were evaluated by optimizing the microwave extraction of the leaf and fruit parts, it was revealed that the fruit parts required a more intense extraction process than the leaf. The differences in these optimal extraction conditions observed between leaves and fruits have been attributed to plant characteristics, particularly differences in morphology and structure between fruit and leaves (Elez Garofulić et al., 2020).

5. Conclusion

It can be concluded that each variable examined in this study is significant for both leaf and fruit part and that more benefits can be obtained from plants in terms of antioxidant additives by bringing these variables under suitable conditions.

Author Contributions

The percentage of the author(s) contributions is presented below. All authors reviewed and approved the final version of the manuscript.

	T.Ö.	A.S.	M.Ç.A.
C	100		
D	50		50
S	50		50
DCP	10	40	50
DAI	30	10	60
L	40	20	40
W	15	15	70
CR	20	10	70
SR	50		50
PM	70	30	
FA	100		

C=Concept, D= design, S= supervision, DCP= data collection and/or processing, DAI= data analysis and/or interpretation, L= literature search, W= writing, CR= critical review, SR= submission and revision, PM= project management, FA= funding acquisition.

Conflict of Interest

The authors declared that there is no conflict of interest.

Ethical Consideration

Ethics committee approval was not required for this study because there was no study on animals or humans.

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