

Comparison of antioxidant, antiradical and antibacterial activities of mistletoe (*Viscum album* L.) fruit and leaves growing on different host tree genus

BEYZA ÇİFTÇİ (✉ beyzacftc.58@gmail.com)

Erciyes University: Erciyes Üniversitesi <https://orcid.org/0000-0002-3080-0880>

Kevser Karaman

Erciyes Üniversitesi: Erciyes Üniversitesi

Mahmut KAPLAN

Erciyes Üniversitesi: Erciyes Üniversitesi

Research Article

Keywords: Mistletoe, bioactivity, extraction, tree genus

Posted Date: August 14th, 2023

DOI: <https://doi.org/10.21203/rs.3.rs-3156420/v1>

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Version of Record: A version of this preprint was published at Waste and Biomass Valorization on November 6th, 2023. See the published version at <https://doi.org/10.1007/s12649-023-02307-0>.

Abstract

Mistletoe is a hemi-parasitic shrub and grows on different host trees and used as a remedy for some disease therapies. In this study, mistletoe fruits and leaves growing on different host tree genera were compared in terms bioactivity and antibacterial efficiency. For this purpose, mistletoe samples were collected from seven different tree genera (black locust, wild pear, pine, hawthorn, willow, apricot and almond) and removed their fruits and leaves. At the beginning, the most suitable solvent for the extraction was determined using optimization procedure of simplex lattice mixture design. The most effective solvent showing the highest total phenolic content (TPC) and antiradical activity (ARA) was determined as ethanol:water (92.5:7.5) for the fruit and ethanol:water (30:70) for the leaf extraction. TPC values of the leaf and fruit samples ranged between 7-21.2 and 3.2–6.9 mgGAE/g, respectively. The results showed significant differences based on the tree genus and the highest antioxidant and antiradical activities were monitored for the black locust tree while the lowest values were in willow tree. For antibacterial activity, the highest inhibition zone was obtained from wild pear leaf extract for *Salmonella typhimurium*. Mistletoe leave extracts showed a remarkable superiority bioactive performance than those of the mistletoe fruits for all tree genus.

1. Introduction

Mistletoe (*Viscum album*) is a semi-parasitic and an evergreen plant which is growing on some tree genera such as pine, poplar, apple and locust trees (Vicaş et al. 2011a). The members of the genus *Viscum* have a woody shrub having branches 15–80 cm long growing on the host trees and it has fruits which are berry, white and they show a sticky structure when they are matured (Vicaş et al. 2012). The mistletoe takes some nutrients from the host trees and they can biosynthesize their own compounds. So, there is a hypothesis that the biologically active compounds present in the host tree may pass from the tree to the parasitic mistletoe plants (Büssing and Schietzel. 1999). The mistletoe is used as a remedy for the traditional medicine and it shows important biofunctional properties namely anticancer, apoptosis-inducing, antimicrobial, antibacterial, antiviral, and immunomodulatory performance (Hajtó et al. 2005). Maier et al. (2002) reported that the mistletoe which is a medicinal material had a good pharmacological performance in the quite wide spectrum and it is widely used as a therapeutic agent due to its anti-neoplastic activity. Also, it was reported that the mistletoe had a functional role in the management of chronic metabolic disorders such as diabetes (Obatomi et al. 1994). Luczkiewicz et al. (2001) reported that the host tree genus had a significant effect on the phytochemical composition which affects the biological activities of the samples. Vicaş et al. (2011b) investigated the four different host tree genus and harvesting season on some biological activities of the mistletoe stems and leaves and reported that the both tree genus and harvesting season caused very significant differences in the antioxidant and antiradical performances of the samples. Also, they reported that the leaves of mistletoe showed better bioactivity compared to stems. In another research, the effect of the mistletoe leaves extracts on arterial blood pressure and heart rate in albino Wistar rats was analyzed and it was observed that the mistletoe lowered the blood pressure significantly (Ofem et al. 2007).

The main aim of the present research was to investigate the host tree genus effect on the total phenolic and flavonoid contents, antiradical activities, antioxidant activities and antibacterial performance of the mistletoe extracts. An also, the different parts of the mistletoe plant such as leaf and fruits were compared in terms of the studied bioactive parameters. Published reports on *Viscum album* L. are limited, and to our knowledge no study on the effect of such a different tree genus on bioactive properties of this plant has appeared in the literature.

2. Materials and Methods

2.1 Materials

The mistletoe leaves and fruits were collected in 2019 summer from the around of Kayseri, Turkey. To make a detailed comparison, different tree genus having mistletoe hosted on the branches were selected. The tree geniuses were Black

locust (*Robinia pseudoacacia*), wild pear (*Pyrus elaeagnifolia*), pine (*Pinus sylvestris*), hawthorn (*Crataegus monogyna*), willow (*Salix alba*), apricot (*Prunus armeniaca*), and almond (*Prunus dulcis*) and the images of the materials were illustrated in Fig. 1. The collected samples were packaged with a paper bag and immediately transferred to the laboratory. The leaves and fruits of the mistletoes were removed from the branched and exposed to drying at room conditions, then grounded and kept at -18 °C for further analyses.

2.2 Methods

2.2.1 Determination of suitable solvent for extraction using optimization procedure

Before starting to the extraction, the best suitable solvent mixture was determined using simplex lattice mixture design approach which is a statistical-mathematical optimization technique. For this purpose, three different solvents namely ethanol, methanol and water were used, and their mixture combinations were prepared according to Table 1. Fifteen experimental points were applied for the extraction of both fruit and leaves of mistletoe. For the extraction, 1 g of the sample was mixed with the extraction solvent (Table 1) and the sample was mixed well by vortex. Then the samples were exposed to extraction for 1 h in the shaking water bath at room temperature. At the end, the samples were centrifuged at 7500 g for 5 min at 4 °C. After that, the supernatant was filtered using 0.45 µm syringe filter and the filtrate was used for bioactive analysis (total phenolic content and antiradical analysis) to determine the best suitable solvent for the extraction of both fruit and leaves of mistletoe. To optimize the results and so to determine the best solvent mixture, Design Expert 7.0 which is statistical-mathematical optimization software was used. Following the optimization procedure of the software, the solvent mixture which results the maximum total phenolic content and antiradical activity of the extracts was determined and after validation for this mixtures, these solvents were used for the extraction of all mistletoe samples collected from the different tree genus.

Table 1
Simplex lattice mixture design used for the extraction of mistletoe fruits and leaves

Runs	Actual level of variables			Mistletoe Fruits		Mistletoe Leaves	
	Ethanol (X ₁)	Methanol (X ₂)	Water (X ₃)	TPC (mg GAE/g)	ARA (%)	TPC (mg GAE/g)	ARA (%)
1	50.0	0.0	50.0	5.51 ± 0.29	69.10 ± 3.61	19.7 ± 0.52	85.61 ± 3.52
2	66.7	16.7	16.7	3.61 ± 0.83	39.77 ± 2.63	15.6 ± 0.16	23.71 ± 1.61
3	16.7	66.7	16.7	3.86 ± 0.02	55.94 ± 5.18	14.8 ± 0.37	48.57 ± 1.78
4	0.0	0.0	100.0	5.46 ± 0.07	82.30 ± 4.41	17.6 ± 0.33	73.24 ± 2.89
5	100.0	0.0	0.0	1.78 ± 0.09	10.23 ± 0.49	5.20 ± 0.20	5.20 ± 0.54
6	0.0	50.0	50.0	5.03 ± 0.55	71.31 ± 1.80	19.2 ± 0.18	87.51 ± 0.49
7	33.3	33.3	33.3	4.51 ± 0.13	49.54 ± 1.45	17.7 ± 0.36	59.22 ± 0.94
8	16.7	16.7	66.7	4.95 ± 0.33	58.94 ± 0.70	18.7 ± 0.32	81.75 ± 4.29
9	50.0	50.0	0.0	1.36 ± 0.09	18.16 ± 1.03	6.60 ± 0.12	13.43 ± 0.91
10	0.0	0.0	100.0	5.61 ± 0.03	86.71 ± 0.36	19.4 ± 0.12	59.73 ± 0.67
11	100.0	0.0	0.0	1.64 ± 0.05	10.12 ± 0.41	5.10 ± 0.06	5.10 ± 0.68
12	0.0	100.0	0.0	2.38 ± 0.13	32.56 ± 1.74	9.10 ± 0.28	23.00 ± 0.46
13	50.0	50.0	0.0	1.14 ± 0.02	16.13 ± 0.93	7.00 ± 0.18	11.62 ± 0.24
14	0.0	100.0	0.0	2.30 ± 0.01	29.72 ± 2.00	9.70 ± 0.24	17.50 ± 0.55
15	50.0	0.0	50.0	5.06 ± 0.72	59.59 ± 1.83	20.4 ± 0.33	80.48 ± 3.01
TPC: Total phenolic content, ARA: Antiradical activity, The leaf extract was diluted as 1/5 for ARA test							

Table 2
Significance of the regression models (*F* values) and the effects of variables on TPC and ARA of both fruits and leaves of mistletoe

		Mistletoe Fruits		Mistletoe Leaves	
Source of variance	Df	TPC	ARA	TPC	ARA
Model		50.72 ^a	29.37 ^a	44.88 ^a	90.48 ^a
Linear mixture	1	102.89 ^a	68.92 ^a	77.06 ^a	194.1 ^a
X ₁ X ₂		3.28	0.17	0.02	0.001
X ₁ X ₃	1	31.16 ^a	6.14 ^b	53.48 ^a	97.17 ^a
X ₂ X ₃		11.29 ^a	2.41	15.57 ^a	58.29 ^a
Residual	1				
Lack of fit	3	9.75	9.80	9.42	7.25
Pure error	1				
Cor total	5				
<i>R</i> ²		0.966	0.942	0.961	0.986
Adj <i>R</i> ²		0.946	0.910	0.940	0.975
Pred <i>R</i> ²		0.935	0.889	0.925	0.923
X ₁ : Ethanol, X ₂ :Methanol, X ₃ :Water, TPC: Total phenolic content, ARA: Antiradical activity					
^a p < 0.01					
^b p < 0.05					

2.2.2 Preparation of the leave and fruit extracts using optimized solvent

Upon the determination of the best suitable solvent for the extraction of mistletoe leaf and fruit, main extraction process by the optimized solvent was started. For this purpose, 1 g of leaf or fruit powder sample was weighed and 20 mL of solvent (30% ethanol for leaf and 7.5% for fruit samples) was added into the falcon tubes. After that, all tubes were mixed by vortex for 1 min and then the tubes covered with parafilm were placed as horizontally in the shaking water bath and exposed to extraction for 1 h at 25°C. Finally the tubes were centrifuged at 7500 g for 5 min at 4 °C and the upper phase was filtrated using 0.45 µm syringe filter and the extracts were used for further analysis.

2.2.3 Bioactive analysis of leave and fruit extracts

2.2.3.1 Total phenolic content (TPC)

TPC of the samples was determined using the method suggested by Singleton and Rossi (1965). For this purpose, 200 µl of the extract was mixed with 1800 µl of distilled water. Then 1 mL of diluted (1/10) Folin Ciocalteu reagent and after 1 min later, 2 mL of sodium carbonate (2% w/v) was added into all tubes. The samples were incubated for 2 hours at room temperature and dark conditions. At the end of the incubation, the absorbance values of the samples were recorded at 765

nm using a UV-vis spectrophotometer (Shimadzu, Japan). Total phenolic content of the samples was calculated as mg GAE/g sample using a calibration curve.

2.2.3.2 Total flavonoid content (TFC)

TFC of the samples was measured according to method of Zhishen et al. (1999). For this aim, 0.5 ml of sample was mixed with 2 mL of distilled water and then 150 µl of sodium nitrite (5% w/v) was added into the tubes and the samples were waited for 5 min. Then 150 µl of AlCl₃ (5% w/v) was incorporated into samples and after 6 min waiting, 1 ml of NaOH (1 M) and 1.2 ml of distilled water was added and the final mixture was vortexed and the absorbance of these mixtures was recorded at 510 nm by a UV-vis spectrophotometer (Shimadzu, Japan) and the total flavonoid content of the samples was calculated as mg catechin equivalent (CE)/g sample.

2.2.3.3 DPPH radical scavenging activity

DPPH radical scavenging activity of the samples was determined as described by He et al. (2016). A 100 µL of extract sample was mixed 3900 µL of DPPH radical solution in methanol (2mM) and mixed well using vortex. After the incubation of the samples at room conditions in a dark place for 30 min, the absorbance values were recorded at 517 nm by a UV-VIS spectrophotometer (Shimadzu, Japan). DPPH radical scavenging capacity was calculated as % inhibition using the following equation):

$$\% \text{ Inh.}_{(\text{Remaining})} = 100 - [(\text{Abs}_{\text{control}} - \text{Abs}_{\text{sample}}) / \text{Abs}_{\text{control}}] \times 100 \quad (1)$$

2.2.3.4 ABTS.⁺ radical scavenging activity

Firstly, ABTS.⁺ radical was produced by preparation of ABTS.⁺ stock solution. For this purpose, 7mmol/L ABTS.⁺ stock solution was prepared and mixed with 2.45 mmol/L potassium persulfate. It was kept at dark conditions at room temperature for 16 h to complete the radical occurrence. At the end of the time, the stock radical solution was diluted with the buffer solution (pH 7.4) until the absorbance value of 0.7 ± 0.05 at 734 nm was obtained. After that, four different concentrations (15, 30, 45 and 60 µL) of diluted extracts (1:20) were placed into the spectrophotometer cuvettes and 2 mL of ABTS.⁺ solution was placed into the cuvettes having extracts and the samples were incubated for 6 min and the absorbance values of the samples were recorded at 734 nm using a spectrophotometer (Shimadzu, Japan). The radical scavenging activity of the samples as % inhibition was calculated using the following equation (Eq. 2).

$$\% \text{ Inhibition} = (A_{\text{control}} - A_{\text{sample}}) / A_{\text{control}} \times 100 \quad (2)$$

where A_{sample} is the absorbance of ABTS.⁺ with sample; A_{control} is the absorbance of ABTS.⁺ without sample. The % inhibition values were converted into the Trolox values and all results were expressed as Trolox equivalent antiradical capacity (µg TEAC/g sample) (Gong et al., 2012).

2.2.3.5 Ferric reducing antioxidant activity

Reducing power, which gives an idea about the antioxidant capacity of the samples, was determined based on the method applied by Malomo et al. (2011). One ml of the sample extracts of various concentrations and standard (ascorbic acid) were mixed with 2.5 ml of 0.2 M phosphate buffer solution (pH = 6.6) and then 2.5 ml of 1% w/v potassium ferricyanide [K₃Fe (CN)₆] was added. The samples were incubated for 20 min at 50 °C. After this step, 2.5 ml of trichloroacetic acid (10% w/v) was added to the reaction mixture and centrifuged at 1000 *g* for 10 minutes and 2.5 ml was taken from the top of the solution. 2.5 ml of distilled water and 0.5 ml of 0.1% FeCl₃ were added to the separated part of the solution (2.5 mL) and the samples were mixed by vortex. Then the absorbance values of the samples were measured by UV-vis spectrophotometer (Shimadzu, Japan). The results are given in mg ascorbic acid equivalent (mg AAE / kg).

2.2.3.6 Antioxidant capacity by phosphomolybdenum reduction

Antioxidant capacity of samples was also evaluated by phosphomolybdenum reduction assays according to Prieto et al. (1999). In this regard, 400 µL of extract was mixed with 4 mL of reagent solution (0.6 M sulphuric acid, 28 mM sodium phosphate and 4 mM ammonium molybdate). Then the tubes were mixed and placed in a water bath to incubate at 95 °C for 90 min. At the end of the incubation, the tubes were cooled in an ice bath and the absorbance of the samples was recorded at 695 nm using UV-vis spectrophotometer (Shimadzu, Japan). The results were expressed as mg ascorbic acid equivalent (mg AAE/g) using a calibration curve created by ascorbic acid standard.

2.2.3.7 Antibacterial activity

Antibacterial activity of the extract samples was evaluated according to the method as described by Karaman et al. (2015). For this purpose, *Salmonella typhimurium* ATCC 14028, *Staphylococcus aureus* ATCC 25923, *E. coli* O157:H7 ATCC 43888, *Klebsiella pneumoniae* ATCC 43816, and *Bacillus subtilis* ATCC 6633 were used as bacterial cultures. Firstly, all bacterial strains were activated in nutrient broth (Merck, Darmstadt, Germany) and fresh inocula was prepared by 22 h incubated cultures at 37 °C. To evaluate the antibacterial activity of the extracts (0.2 g freeze dried extract / 1 ml optimized solvent) the agar diffusion method was used. For this purpose, nutrient agar was prepared and sterilized and then the fresh culture for each bacteria was inoculated to be 10^5 - 10^6 cfu/mL into the agar at 42–45 °C and mixed well. The inoculated agar was poured into the petri dishes and waited to solidify the agar content. Then four holes were bored using a cork borer (5 mm) and 30 µL of the extracts (0.2 g freeze dried extract / 1 ml solvent) was placed in the holes and the petri dishes were incubated at 37 °C for 24h. At the end of the incubation, the diameter of the zones occurred around the holes was measured and the results were expressed as the mean of inhibition zone diameter (mm).

2.3 Statistical analysis

Statistical analysis of the data was evaluated using Windows based SAS 8.2 statistical analysis software (SAS Institute, Cary, North Carolina, USA). Duncan multiple comparison was performed with the significance level of 95%. All the analyses were carried out in duplicate with four repetitions. Principal Component Analysis was applied by XLSTAT software to illustrate the relationship among the tree genus in terms of studied bioactive parameters.

3. Results and Discussion

3.1 Solvent optimization for the mistletoe leaves and fruits

Before starting to the bioactive characterization of the mistletoe leaf and fruit samples, an optimization study based on the response surface methodology-simplex lattice mixture design approach was performed. For this aim, three popular solvents namely ethanol, methanol and water were selected and different mixtures of these solvents in addition to their sole usage were evaluated in terms of their phenolic extraction efficiency and the radical scavenging activity of the produced extracts. Table 1 shows the recorded values of total phenolic content (TPC) and antiradical activity (ARA) of both mistletoe leaves and fruits by different solvent mixtures. As is seen, the highest TPC was measured as 5.61 g/g GAE while the lowest value was 1.14 g/g GAE for the mistletoe fruits. The highest TPC value of mistletoe fruit was recorded for the 100% water while the lowest TPC was for ethanol:methanol mixture at 1:1. ARA values of the samples ranged between 16.13–86.71% while the highest ARA value was recorded for the sample having the highest TPC (Run 10) and the lowest one for the sample prepared with ethanol:methanol mixture (Run 13). For the mistletoe leaves, TPC values were in the range of 5.10–19.7 mg GAE/g sample. The highest TPC was observed for the sample extracted by ethanol:water mixture (Run 1, Table 1) while the lowest one was for the solvent of sole ethanol (Run 11, Table 1). But as is seen in Table 1, the TPC results recorded for the methanol:water mixtures were observed as quite close to the results of the ethanol:water mixture. ARA values of the mistletoe leaves extract ranged between 5.10-87.51%. As compared to leaf and fruit extracts, antiradical performance of the leaves was determined to be quite higher compared to fruit extracts due to the phenolic abundance in the structure of the mistletoe leaves. Pearson correlation analysis showed that there was a significant correlation between the TPC and ARA values of both mistletoe fruits ($r = 0.958$, $p < 0.05$) and mistletoe leaves (0.942 , $p < 0.05$). According to the ANOVA results, the

effect of linear mixture was determined to be significant ($p < 0.05$) which means both studied TPC and ARA values of the mistletoe leaf and fruit samples showed a significant change depending on the solvent type. In addition to that, the interaction effects between the ethanol and water and methanol and water were observed as significant ($p < 0.05$). According to the experimental results a polynomial predictive regression models were constructed for each processing variable and given in Eq. 1–4 as following:

$$Y_{\text{Fruit (TPC)}} = 1.75X_1 + 2.40X_2 + 5.39X_3 - 2.29X_1X_2 + 7.06X_1X_3 + 5.06X_2X_3 \quad (R^2 = 0.966) \quad \text{Eq. 1}$$

$$Y_{\text{Fruit (ARA)}} = 11.18X_1 + 33.23X_2 + 82.01X_3 - 10.32X_1X_2 + 62.6X_1X_3 + 46.6X_2X_3 \quad (R^2 = 0.942) \quad \text{Eq. 2}$$

$$Y_{\text{Leave (TPC)}} = 5.54X_1 + 9.42X_2 + 18.01X_3 - 0.70X_1X_2 + 34.3X_1X_3 + 22.01X_2X_3 \quad (R^2 = 0.961) \quad \text{Eq. 3}$$

$$Y_{\text{Leave (ARA)}} = 3.54X_1 + 21.33X_2 + 71.48X_3 - 1.77X_1X_2 + 175.6X_1X_3 - 399.5X_2X_3 \quad (R^2 = 0.986) \quad \text{Eq. 4}$$

The constructed predictive regression equations showed quite high determination coefficients as in the range of 0.942–0.986 which show the high fitting ability of experimental and predictive TPC and ARA results for both mistletoe fruit and leaf extract samples.

The change of TPC and ARA values of both mistletoe fruit and leaves depending on the solvent type and mixture levels was illustrated as ternary plot in Fig. 2. As is seen, TPC of mistletoe fruit and leaves increased towards to the edge of water and the lowest TPC values were recorded toward to the edge of ethanol and methanol for both sample extracts. Similarly, ARA values of the samples also showed a significant increase toward to the edge of water and the lowest values were measured towards to the edge of the ethanol and methanol. Depending on the recorded values for each solvent, an optimization study was performed to reveal the best solvent mixture for the extraction of the highest yield of phenolics. For this aim, desirability function was used and multiple response optimization process was carried out. By this way, the best solvent mixture concluding the highest TPC and ARA values for both mistletoe leaf and fruit sample extracts was determined. According to the optimization results, 70:30 water:ethanol and 92.5:7.5 water:ethanol mixtures were determined for mistletoe leaf and fruit extraction process, respectively. By using these solvent mixtures, all mistletoe fruit and leaves samples were extracted, and the related bioactive properties were studied. To validate the determined solvent mixture performance, experimental and predicted TPC and ARA values based on the constructed regression equations (Eq. 1–4) were illustrated in Fig. 3. As is seen, experimental and predicted TPC values were recorded as 5.61 and 5.30 mg GAE/g and 20.6–21.5 mg GAE/g for mistletoe fruit and leaves, respectively. Similarly, experimental and predicted ARA values were determined as 85 and 85.5% and 87.9 and 88.1% for mistletoe fruit and leaves, respectively. Desirability function values for both optimized values were calculated as 1.000 showing the best fitting ability of the constructed predictive regression equation. Vicas et al. (2011a) who investigated the biological activities of mistletoe samples collected from different trees reported that the total phenolic content of the samples was determined to be higher in aqueous extracts compared to sole ethanolic ones. But for the antiradical activity of the samples, ethanolic extracts showed better antiradical and antioxidant performance. In the current study, for both TPC and ARA values, sole aqueous extracts showed better performance when compared to values recorded at run 4 and 5 or 10 and 11. Pietrzak et al. (2014) studied the effect of different solvents and extraction methods on the bioactivity of mistletoe samples and they informed that the extracts produced by using water–polar solvent mixtures showed the highest TPC and antioxidant activity compared to organic polar solvents which acted as the least efficient extractants.

3.2 Total phenolic and flavonoid contents of the mistletoe leave and fruit extracts

Total phenolic content (TPC) and total flavonoid content (TFC) of the mistletoe leave and fruit samples collected from different tree genus (black locust, wild pear, pine, hawthorn, willow, apricot and almond) were illustrated in Fig. 4. As is seen, there were significant differences between both in TPC and TFC content of the mistletoe leaves and fruits. And, tree genus

showed a significant effect on the TPC and TFC of the samples ($p < 0.01$). As is seen in Fig. 4, for the leaves, the highest TPC (21.2 mg GAE/g) was recorded for the sample collected from black locust tree while the lowest one was for the willow (7.0 mg GAE/g). Similarly, black locust and also hawthorn mistletoe fruits showed the highest TPC while the lowest values were monitored for the willow mistletoe fruit samples (3.2 mg GAE/g). Similar to the TPC of the samples, the highest TFC was measured as 21.1 mg QE/g in black locust mistletoe leaf while the lowest one was for the willow mistletoe leaf (6.1 mg QE/g). TFC of the fruit samples was determined to be quite lower compared to leaf samples and the TFC ranged between 0.86–2.66 mg QE/g samples. Table 4 shows the correlation analysis results among the analyzed bioactive parameters for both mistletoe leaf and fruit extracts. As is seen, the correlation coefficients between TPC and TFC of the mistletoe leaf was quite high and significant ($r = 0.881$) while there was not observed similar correlation for the fruit samples. In a similar research, different tree genus were compared in terms of the highest TPC for the mistletoe leaves and it was reported that the mistletoe leaves host on black locust showed the highest TPC and they stated that the tree genus affected the TPC of the mistletoe samples (Vicaş et al. 2011a). Oluwaseun and Ganiyu (2008) reported that the TPC of mistletoe from cacao and cashew tree showed significant differences. It was also reported that the main constituents of the total phenolics are the phenolic acids represent the major fraction of bioactive compounds in all *V. album* (Vicaş et al. 2011a). In a study in which the compared the bioactivity of the leaves and stems of five different tree genus of mistletoes, analysis of phenolic compositions revealed that the leaves of mistletoe hosted on black locust tree (*R. pseudocacia*) had the highest concentration of gallic acid (39.93 µg/g dry matter) compared to the leaves and also stems of the mistletoe hosted on different tree genus namely *Acer campestre*, *Fraxinus excelsior*, *Populus nigra* and *Malus domestica* which had no gallic acid compound. Also the highest levels of sinapic acid, ferulic acid, kaempferol and quercetin phenolic compounds were measured as the highest for the black locust tree. The main reason of the highest TPC and TFC of the mistletoe leaf on the black locust tree could be attributed to the richest phenolic compounds such as gallic acid showing the phenolic acid structure and very high bioactive performance (Vicaş et al. 2011a; Shahidi and Yeao 2018). It was reported that the main phenolic compound in the black locust tree was gallic acid derivatives and the majority of the phenolic acids was detected in the pods of the trees. Sing et al. (2009) and Ee et al. (2011) reported the gallic acid presence in the pods and seeds only, respectively and the levels of the gallic acids in the pods of the black locust tree were several times higher than that of the seeds of black locust. As is well known, the mistletoe is a hemiparasite on several species of trees, from which it draws water and nutrients. So, the phenolic concentrations and also the profile of the phenolics in the tree pods or seeds could be similar to the corresponding mistletoe which grown on the tree and it could be also said that the main active compound providing the richness in the TPC and TFC in the leaf and fruit extracts of the mistletoe collected from the black locust tree is gallic acid.

Table 3
Antibacterial performance of both mistletoe leaf and fruit part on some pathogenic microorganism (mm inhibition zone) “-” Not determined

Mistletoe leaf	<i>Salmonella typhimurium</i>	<i>B.subtilis</i>	<i>E.coli</i> O157:H7	<i>S.aureus</i>	<i>K. pneumoniae</i>
Black locust	6 ± 0.0	9.33 ± 1.5	-	6.5 ± 0.7	6.75 ± 1.7
Wild pear	14 ± 1.5	-	11 ± 1.71	-	-
Pine	-	10.3 ± 1.5	-	9.5 ± 0.7	7 ± 1
Hawthorn	10 ± 1.4	-	11 ± 1.15	8.3 ± 1.2	9.75 ± 1.0
Willow	-	9.33 ± 1.2	11 ± 1.41	10 ± 1.3	8.5 ± 2.9
Apricot	7.5 ± 0.7	-	8.3 ± 1.41	8.7 ± 1.5	10.7 ± 0.6
Almond	-	-	11 ± 2.12	8.5 ± 0.7	9.67 ± 0.6
Mistletoe fruit					
Black locust	6.5 ± 0.7	-	8.7 ± 2.07	-	-
Wild pear	-	-	-	-	-
Pine	6 ± 0.0	7 ± 0.0	7.4 ± 0.55	8 ± 1.0	-
Hawthorn	7.3 ± 0.6	7.5 ± 1.5	9.26 ± 1.26	6.4 ± 1.1	7.5 ± 0.7
Willow	-	-	10 ± 1.15	9 ± 0.0	-
Apricot	-	-	-	-	8 ± 1.4
Almond	6.5 ± 0.7	10 ± 1.4	-	-	-

“-” Not determined

Table 4
Pearson correlation matrix showing the relationship among the studied parameters

Variables	TPC-L	TPC-F	TFC-L	TFC-F	DPPH-L	DPPH-F	ABTS-L	ABTS-F	FRAA-L	FRAA-F	AA-L	AA-F
TPC-L	1											
TPC-F	0.505	1										
TFC-L	0.881	0.562	1									
TFC-F	0.490	0.656	0.263	1								
DPPH-L	0.631	0.683	0.891	0.296	1							
DPPH-F	0.386	0.831	0.565	0.528	0.731	1						
ABTS-L	0.887	0.257	0.841	0.292	0.595	0.345	1					
ABTS-F	0.610	0.823	0.639	0.745	0.696	0.930	0.551	1				
FRAA-L	0.972	0.553	0.950	0.394	0.746	0.491	0.917	0.657	1			
FRAA-F	0.400	0.940	0.497	0.707	0.683	0.943	0.233	0.916	0.456	1		
AA-L	0.926	0.388	0.695	0.471	0.380	0.197	0.850	0.476	0.881	0.235	1	
AA-F	0.171	0.801	0.204	0.298	0.266	0.593	-0.115	0.482	0.216	0.682	0.137	1
<i>Values in bold are different from 0 with a significance level alpha = 0.05</i>												
TPC: Total phenolic content, TFC: Total flavonoid content, DPPH: 2,2-diphenyl-1-picrylhydrazyl radical scavenging activity, ABTS: 2,2-Azinobis (3-ethylbenzthiazoline-6-sulphonic acid) radical scavenging activity FRAA: Ferric reducing antioxidant activity, AA: Antioxidant activity by phosphomolybdenum reducing, L: Leaf, F: Fruit												

3.3 Antiradical activity of the mistletoe leave and fruit extracts

Antiradical performance of the both mistletoe leaves and fruits were evaluated by using DPPH and ABTS.⁺ radicals and the radical scavenging activities of the samples were illustrated in Fig. 5. As is seen from the figure, the highest DPPH radical scavenging performance (58.8%) was recorded for the leaf extracts obtained from the black locust tree while the lowest performance for the leaves was measured for leaf extract of the willow tree (18.6%). For the fruit extracts, the highest scavenging activity for DPPH radical was monitored for the extract obtained from the black locust tree also (22.2%) while the lowest one was for the sample of willow tree as similar to the leaf extracts. It was determined that there were significant differences among the tree genus in terms of DPPH radical scavenging activity of both mistletoe fruits and leaves ($p < 0.05$). Figure 5 also shows the change in ABTS.⁺ radical scavenging activity of the samples. As is seen, the highest antiradical activity was determined for the extract of the leaf sample collected from the black locust tree (32.7 $\mu\text{g TE/g}$) while the lowest one was observed for the extract of the leaf samples collected from the hawthorn and willow trees (18.1 and 19.3 $\mu\text{g TE/g}$, respectively). Also the extracts of the leaf samples collected from the apricot and almond showed similar antiradical activity to the leaf extracts of the hawthorn and willow trees ($p > 0.05$). The differences among the ABTS.⁺ radical scavenging performance of the both mistletoe leaves and fruits were found to be significant ($p < 0.05$). DPPH radical scavenging activity of the leaves showed a significant correlation with TFC of the leaves of the mistletoe and also significant correlation was also monitored between the DPPH radical scavenging activity of the fruit showed a significant correlation with the TPC of the fruit extracts. Additionally, ABTS.⁺ radical scavenging activity of the leaf and fruit extracts showed a significant correlations with the TPC of the leaves and fruits, respectively ($r = 0.887$ and 0.823 , $p < 0.05$, Table 4). Vicaş et al. (2011a) reported that the DPPH radical scavenging activity of the stem and leaf of the mistletoe (*V. album*)

showed significant differences depending on the tree genus where the mistletoe hosted on. Also, in other research, antiradical activity of mistletoe extracts was reported as in the range of 66.2–88.2% and also a significant correlation ($r = 0.940$, $p < 0.05$) between ARA and TPC (Roman et al., 2009). Vicaş et al. (2011a) informed that the mistletoe leaves showed good scavenging activity compared to stems of the mistletoe samples and the DPPH radical scavenging activity ranged between 2.20–11.49% and variations are affected by the tree genus. Among the tested tree genus samples, the mistletoe samples which growing on the *R. pseudoacacia* showed the highest antiradical activity. Also, they tested the effects of the extraction solvents and reported that the ethanolic extracts showed higher antiradical performance compared to aqueous extracts of the samples. Onay Ucar et al. (2006) reported that the mistletoe extracts showed good DPPH radical scavenging activity compared to vitamin E and butylated hydroxyanisole (BHA), but the harvesting season showed a significant effect on the antiradical activities of the mistletoe samples. They also informed that the samples harvested on January showed better bioactivity than the samples harvested on July. In another study showing the effects of harvest season and tree genus on the bioactivity of mistletoe leaf and stem extracts revealed that the harvesting month showed very significant effect on the DPPH radical scavenging activity and black locust tree showed the highest radical scavenging performance compared to others. Additionally, the inhibition performance was effected the season during the year and the lowest scavenging activity was measured as 41.62% in May and 76.60% in December for the leaf extract while the activities of the stem extracts in these months were 31.91 and 69.8%, respectively (Vicaş et al. 2011b). It was also reported that the mistletoe leaf and also stem extracts performed the ability of scavenging cation-radicals ABTS.⁺ (Vicaş et al. 2011a). It was also informed by Vicaş et al. (2011a) that the ABTS.⁺ radical scavenging activities of the mistletoe leaves and stems showed significant differences depending on the tree genus and the black locust (*R. pseudoacacia*) extracts showed quite high ABTS.⁺ radical scavenging activities after the extracts of mistletoe leaves growing on *Acer campestre* (field maple tree). As stated above, ABTS.⁺ radical scavenging activity of the both mistletoe leaf and fruit extracts well correlated with the TPC of the same samples. Similarly, Vicaş et al. (2011a) reported that the main reason of the high ABTS.⁺ scavenging activity of the water extracts of the mistletoe was attributed to the high concentrations of the bioactive compounds with the ability of scavenging cation-radicals.

3.4 Antioxidant activity of the mistletoe leave and fruits

Antioxidant activities of the extracts produced from mistletoe leaves and fruits collected different tree genus were evaluated two different approaches namely ferric reducing and phosphomolybdenum reducing activity. The results of the antioxidant assays for the samples were showed in Fig. 6. As is seen, the highest ferric reducing antioxidant activity (35.9 mg AAE/kg) was determined for the leaf extracts of mistletoe collected from black locust tree while the lowest one was for the sample of willow tree genus (15 mg AAE/kg). For the extracts of the fruits, the highest antioxidant performances were observed for the samples of black locust (10.09 mg AAE/kg) and hawthorn trees (10.65 mg AAE/kg). Willow tree fruit extracts also showed the lowest FRAA values among the all tested samples (4.22 mg AAE/kg). It was observed that the differences among the samples were significant for both mistletoe leaf and fruit extracts ($p < 0.05$). Figure 6 also shows the change in antioxidant activity of the samples tested by phosphomolybdenum reagent. As is seen, the highest AA values were determined for the leaf extract collected from black locust tree (52.2 mg AAE/g) and pine tree (51.7 mg AAE/g) while the lowest AA values were was also for the extracts of the willow and apricot tree leaves. For the fruit samples, the highest AA was determined for the fruit of the almond tree (32.2 mg AAE/g) and the lowest value was for the sample of the willow tree fruits (15.8 mg GAE/g). The correlation analysis results among the analyzed bioactive parameters for both mistletoe leaf and fruit extracts were tabulated in Table 4. As is seen, a significant correlation was determined between the FRAA and TPC of the leaves ($r = 0.972$, $p < 0.05$) and FRAA and TPC of the fruits ($r = 0.940$, $p < 0.05$). Similarly, significant correlations were observed between the AA values of both mistletoe leaves and fruits and TPC of both mistletoe leaves and fruits ($p < 0.05$, Table 4). Lee et al. (2010) reported that the mistletoe extracts (50 mL/g) showed a very high electron donating ability compared to 1% of L-ascorbate solution and informed that the mistletoe is a potential source of the antioxidant compounds. It was also informed that the phenolic acids presents in the mistletoe plants are the digallic and o-coumaric acids in the free or glycosilated forms and they are also considered to be compounds with antioxidant activity (Luczkiewicz et al. 2001).

3.5 Antibacterial activity of mistletoe fruit and leaf extracts

Table 3 shows the antibacterial activity of mistletoe fruit and leaf extracts against some well-known pathogenic bacteria such as *Salmonella typhimurium*, *B.subtilis*, *E.coli* O157:H7, *S.aureus* and *K. pneumoniae*. As is seen, the antibacterial activity of leaf extracts was higher compared to fruits extracts significantly for most of the tested bacteria ($p < 0.05$). The most resistant bacteria were *S. typhimurium* and *B.subtilis* while the others were inhibited easily by the leaf extract. The highest inhibition zone was measured for the black locust extract (14 mm) on *S. typhimurium* while the weakest extract was wild pear (6 mm). Hawthorn leaf extract also showed high inhibition zone for *S. typhimurium* while it did not perform any inhibition activity on only *B. subtilis* among the tested bacteria. *E.coli* O157:H7 was the most sensitive microorganism against the extract but no inhibition was seen for black locust and pine leaf extract. The only wild pear leaf extract did not show any antibacterial activity for *S. aureus* and *K. pneumonia* among the samples and the differences were found as statistically significant ($p < 0.05$). Antibacterial activity of mistletoe fruits was lower than that of the mistletoe leaves as is seen in Table 3. Hussain et al. (2011) performed a detailed research on the antibacterial activities of different organic solvents on some pathogenic bacteria and reported that the ethyl acetate, chloroform, ethanol, and methanol crude extracts of selected plant parts had significant antibacterial activities on both Gram (+) and Gram (-) bacteria. When compared to results, inhibition zones of the mistletoe leaves were reported to be higher than those of the mistletoe twigs. Also, a significant difference among the solvent type was reported by the authors. They informed that the inhibition zone of ethanolic, methanolic and water extracts on *S. aureus* and *E.coli* was 15.9, 15.2 and 9.66 and 16.6, 16.9 and 9.16 mm, respectively. In the same study, inhibition zones of the mistletoe twigs were reported to be 11.7, 19.8 and 0 mm by ethanol, methanolic and aqueous extracts on *S. aureus* and 14.8, 24.8 and 0 mm by same solvents on *E.coli* (Hussain et al. 2011).

3.6. Principal component analysis and dissimilarity for the tree genus

Figure 7 illustrates the biplot and dissimilarity graphs showing the relationships among the samples and tested parameters. Two basic principal components having an eigenvalue higher than the unity were determined and 83.78% of the total variance was explained by these two principal components. As is seen from the biplot illustration, the tree geniuses are located at different zones of the graph showing the significant differences for the samples in terms of the tested biological activities. The mostly differed mistletoe samples was determined as the samples growing on the willow tree it showed the highest factor scores among the tree genera. As is seen from the factor loadings of the biplot (Fig. 7), the mistletoe fruits and leaves samples also differentiated well due to the significant differences between two parts of the mistletoe samples in terms of phenolic abundance, antiradical performance and antioxidant capacities. It was also observed that the mistletoe samples growing on the black locust tree were characterized well by the used bioactive parameters. In Fig. 7, the dendrogram of dissimilarity revealed that four different classes were created and the mistletoe samples growing on the willow is very different compared to other samples and also black locust showed the lower dissimilarity that that of the willow. The samples hosted on hawthorn, apricot and almond trees also showed the maximum distance to centroid while the lowest value were observed for the samples growing on the willow and black locust trees.

4. Conclusion

The effects of the host tree genus on the bioactive properties of the mistletoe leaf and fruits were investigated and it was revealed that the tree genus showed significant effect of the biological activities of the mistletoe extracts. At the beginning, a solvent optimization was performed to determine the best and suitable solvent mixture showing the highest bioactivity. As a conclusion, it was determined that there was a big difference between the leaf and fruit of the mistletoe in terms of the characterized phenolic and flavonoid concentrations, antiradical performance and antioxidant capacities. Among the tree genera, the richest bioactive compounds present and the, so the highest bioactive mistletoe was the one host on the black locust tree (*R. pseudoacacia*) while the weakest mistletoe samples were the ones growing on the willow tree (*S. alba*). The mistletoe leaves and fruits also showed a significant antibacterial activity on some well-known pathogenic bacteria and the tree genus showed a significant effect on the antibacterial performances of the samples.

Declarations

Acknowledgement

This study was supported as financially by Scientific Research Project Unit of Erciyes University (Project Code: FYL-2020-10193)

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Figures

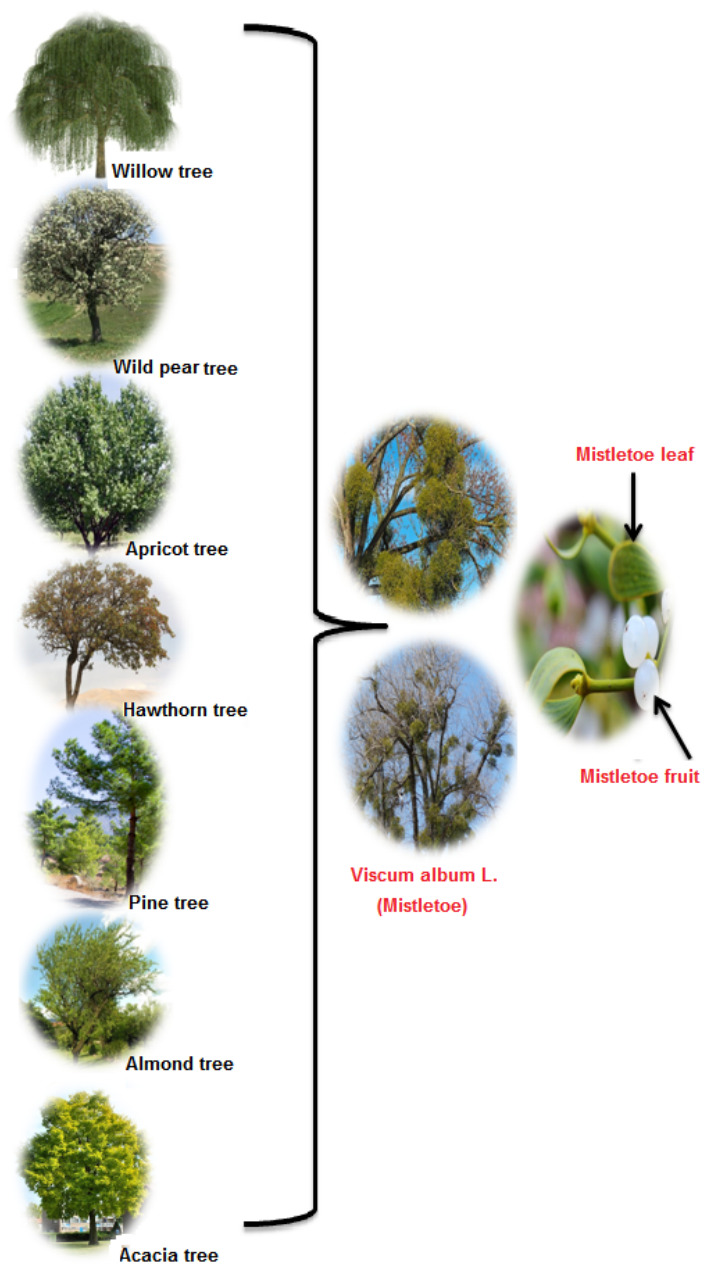


Figure 1

The images of the host tree genera and mistletoe plant parts used in the study

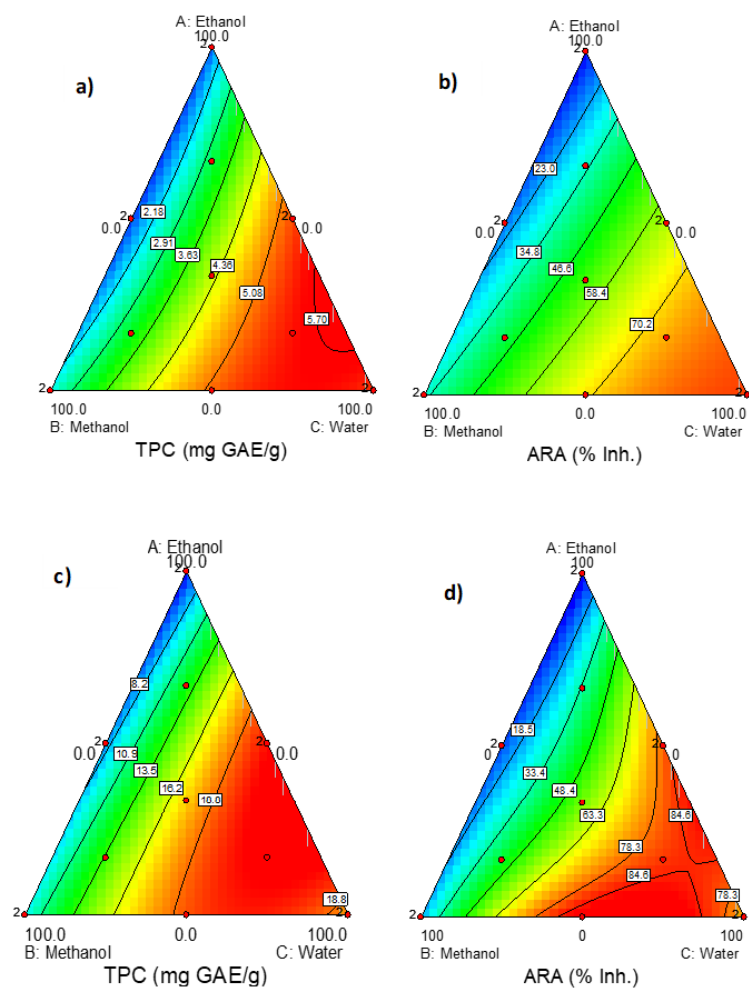


Figure 2

Ternary plots showing the effect of solvent type on total phenolic content (TPC) (a) and antiradical activity (ARA) (b) of mistletoe fruits and TPC (c) and ARA (d) of mistletoe leaves

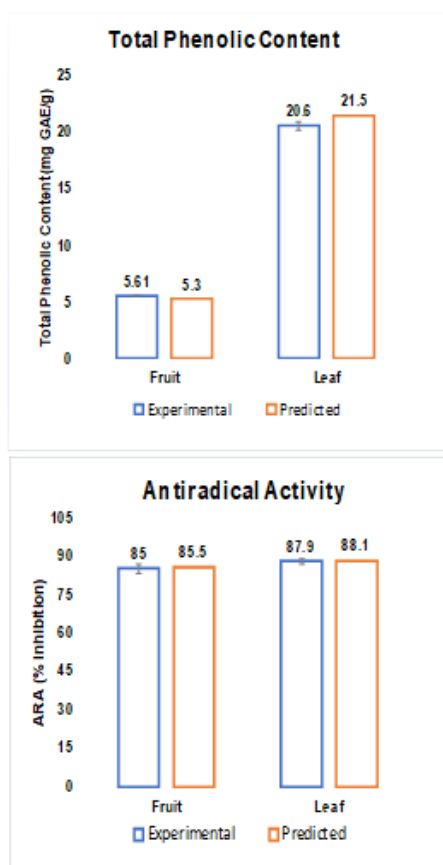


Figure 3

Experimental and predicted TPC and ARA values of mistletoe extract produced using optimized solvent mixture (The leaf extract was diluted 1/5 for ARA test)

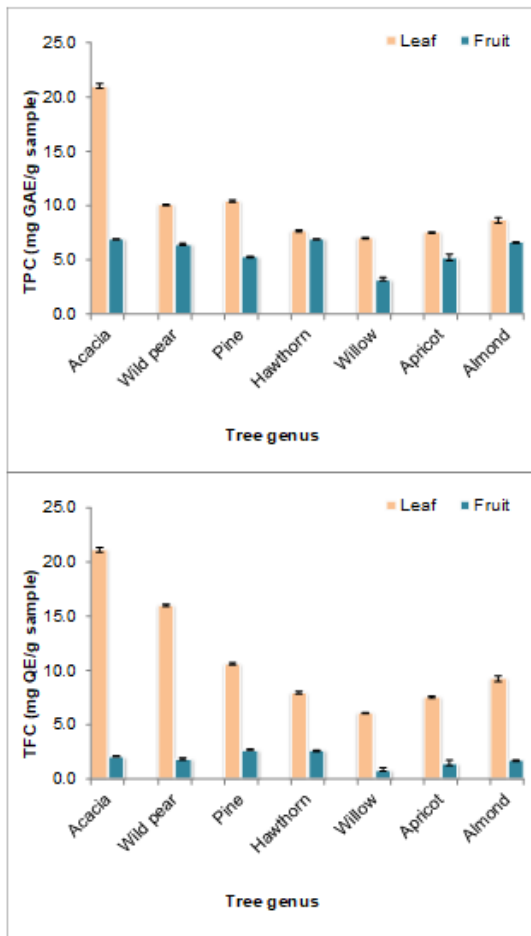


Figure 4

Change in total phenolic (TPC) and total flavonoid content (TFC) of mistletoe fruit and leaves depending on tree genus

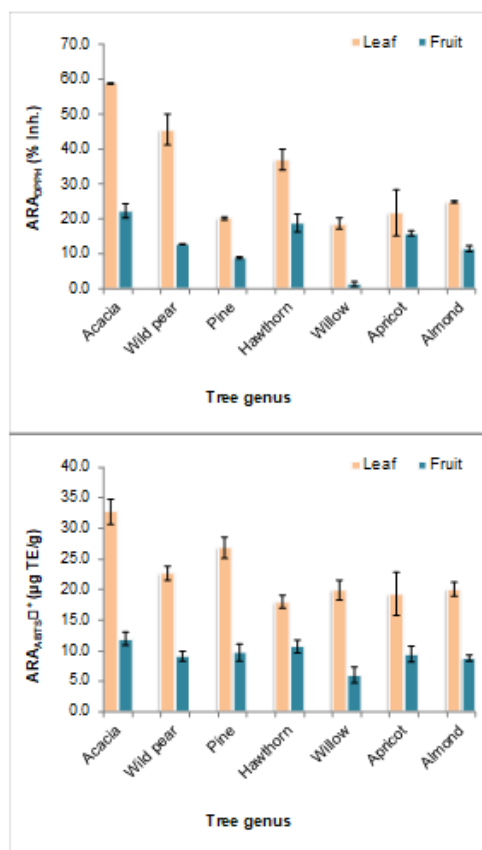


Figure 5

Change in DPPH and ABTS radical scavenging activity of mistletoe fruit and leaves depending on tree genus

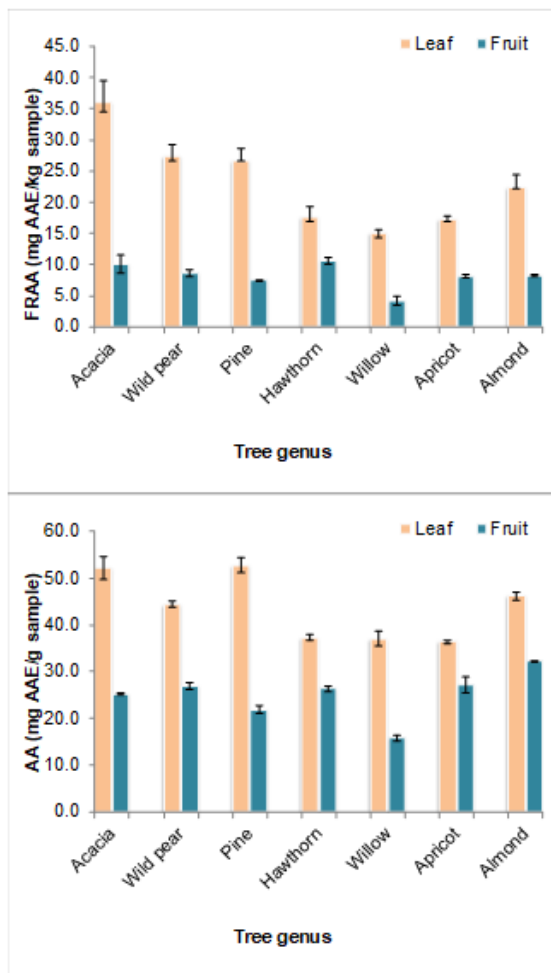


Figure 6

Change in ferric reducing antioxidant activity (FRAA) and iron chelating activity (ICA) of mistletoe fruit and leaves depending on tree genus

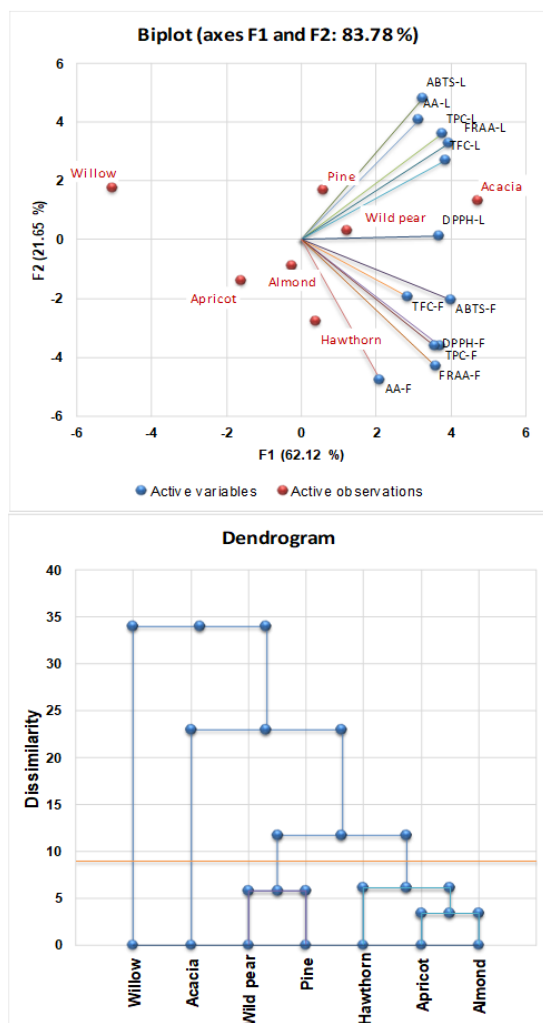


Figure 7

Biplot and dissimilarity graph showing the relations between active variables and observations