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Fruit Bioactive Profile of “Alişar” Pear Variety Grown in Şebinkarahisar, Giresun

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Abstract: With a history of at least three centuries, “Alişar” is a local pear (*Pyrus communis* L.) variety that is widely grown in the district of Şebinkarahisar in Giresun province. In recent years, researches on the bioactive compounds of fruits that are valuable for human health has gained importance. This research was performed to determine fruit bioactive profiles of 27 clones belonging to this variety. The total phenolic content, antioxidant activity and total flavonoid content in the fruits of the clones were investigated as bioactive properties. Total phenolic contents of the clones were found between 72.61-147.38 mg 100 g⁻¹. In the FRAP and DPPH assays, the antioxidant activity was determined between 2.61-4.27 mmol kg⁻¹ and 0.18-0.76 mmol kg⁻¹. The total flavonoid content was detected between 3.05-18.01 mg 100 g⁻¹. According to the FRAP assay, there was a high positive correlation ($r = 0.679^{***}$) between total phenolic and antioxidant activity, while total phenolics showed a moderate relationship with antioxidant activity ($R^2 = 0.461$). The findings revealed that Alişar pear clones showed variation in fruit bioactive components, and also some clones were rich in bioactive contents. A-19 in total phenolic content, A-23 in antioxidant activity and A-18 in total flavonoid content were evaluated more remarkably than other clones. These clones are thought to contribute as genetic material to pear breeding studies.

Şebinkarahisar (Giresun) Yöresinde Yetiştirilen “Alişar” Armut Çeşidinin Biyoaktif Profili

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Anahtar kelimeler

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Öz: “Alişar”, Giresun ili Şebinkarahisar ilçesinde en az üç asırlık bir geçmişe sahip olan ve yörede yaygın olarak yetiştirilen yerel bir armut (*Pyrus communis* L.) çeşididir. Son yıllarda, insan sağlığı için değerli olan meyvelerin biyoaktif bileşenleri üzerine araştırmalar önem kazanmıştır. Bu araştırma, bu çeşide ait 27 klonun biyoaktif özelliklerini belirlemek amacıyla yapılmıştır. Klonların meyvelerinde, toplam fenolik içeriği, antioksidan aktivite (FRAP ve DPPH deneylerine göre) ve toplam flavonoid içeriği biyoaktif özellikler olarak araştırılmıştır. Alişar armut klonlarında toplam fenolik içeriği 72.61-147.38 mg/100 g arasında bulunmuştur. FRAP ve DPPH deneylerine göre, antioksidan aktivite 2.61-4.27 mmol/kg ile 0.18-0.76 mmol/kg arasında belirlenirken, toplam flavonoid içeriği 3.05-18.01 mg 100 g⁻¹ arasında kaydedilmiştir. FRAP testine göre, toplam fenolik ve antioksidan aktivite arasında güçlü pozitif korelasyon ($r=0.679^{***}$) belirlenirken, toplam fenolikler antioksidan aktivite ile orta derecede bir ilişki göstermiştir ($R^2=0.461$). Araştırma bulguları, Alişar armut klonlarının biyoaktif özellikler yönünden varyasyon gösterdiğini, bazı klonların zengin

biyoaktif içeriklere sahip olduğunu ortaya çıkarmıştır. Toplam fenolik içerik bakımından A-19, antioksidan aktivite açısından A-23 ve toplam flavonoid içeriği yönünden A-18, diğer klonlardan daha dikkat çekici bulunmuştur. Biyoaktif özellikler açısından öne çıkan klonların armut ıslah çalışmalarına genetik materyal olarak katkı sağlayabileceği düşünülmektedir.

1. Introduction

Pear is one of the most common fruit species in temperate climate areas of the world (Özçağırın et al., 2014). Turkey has rich genetic resources of pear. Local and old pear cultivars that are well adapted to different ecological conditions are distributed in most regions of Turkey. They could contribute as valuable germplasm to cultivar breeding programs. To reveal this genetic richness of our country, clonal selection studies are of great importance and offer the opportunity to evaluate our current breeding potential (Bostan, 2009; Özrenk et al., 2010; Karadeniz and Çorumlu, 2012; Öztürk and Demirsoy, 2013; Bostan and Çelikel-Çubukçu, 2018; Özrenk et al., 2018).

With its different taste and aroma, pear is a popular fruit due to its low caloric level and high nutritive value (Qin et al., 2012; Versini et al., 2012). It contains many vitamins and minerals, free sugars, organic acids, and high in fiber (Tanrıöven and Ekşi, 2005; Chen et al., 2006).

Recently, researches on the bioactive compounds of fruits those are valuable for human health has gained importance. Fruits, which are considered as a natural antioxidant sources, and their positive effects on human health have been revealed in many studies (Szajdek and Borowska, 2008). Phenolic compounds are the most common group of substances in plants and defined as secondary metabolic products of plants (Kafkas et al., 2006). These are divided into two groups as phenolic acids and flavonoids. Some of these substances affect the formation of flavors such as the unique sourness-bitterness in fruits, and some of them the formation of color of the products (Cemeroğlu, 2004; Karataş and Şengül, 2018; Zor and Şengül, 2020). Phenolic compounds show antioxidative properties, especially effective in preventing oxidative effects of free radicals in cells (Li et al., 2012). In addition, it is well known that they have protective effects against many diseases such as cancer and cardiovascular diseases (Vauzour et al., 2010).

Pear is also rich in terms of phenolic compounds and antioxidant promoting human health (Li et al., 2016; Yim and Nam, 2016). Studies to determine the bioactive properties of pear genetic resources are limited (Duric et al., 2015; Abacı et al., 2016; Azzini et al., 2019). Pear genetic resources with high antioxidant capacity are important for breeding materials (Ieguchi et al., 2015). In Turkey, local pear varieties from ancient times should be evaluated in terms of bioactive properties. Therefore, pear germplasm resources should be investigated not only for fruit and plant characteristics but also for phytochemical components. It is also a known fact that local fruit varieties evoke old flavors and attract the attention of many consumers today.

'Alişar' is a popular local pear (*Pyrus communis* L.) variety that is widely grown in the district of Şebinkarahisar in Giresun province and has a history of at least three centuries. This study aims to reveal the fruit bioactive profiles of 27 clones belonging to this variety.

2. Materials and Methods

2.1. Plant material

The plant material of the study includes fruit samples collected from 27 different clones of Alişar local pear grafted on seedling rootstock grown in Şebinkarahisar district of Giresun province. All of them are naturally grown and are popular with local people.

2.2. Determination of biochemical properties

From the pear clones, 10 fruit that have not been mechanically and physically damaged, have been hand-harvested. The harvested fruit was washed with pure water and peeled. A slice of each fruit was taken and made homogeneous with hand blender. Then kept at -20°C in the falcon tubes. After that,

the total phenolics content, total antioxidant activity (according to FRAP and DPPH assays) and total flavonoids content were determined in these fruit samples.

2.3. Total phenolics content

Total phenolics content were determined using Folin-Ciocalteu's chemical. Initially, 4.2 mL of distilled water was added over 400 µL of fresh fruit extract. Then, 100 µL Folin-Ciocalteu and 2% sodium carbonate (Na₂CO₃) were added and left to incubate for 2 hours. After incubation, a bluish solution was obtained and then measured spectrophotometrically at 760 nm wavelength. The values obtained were expressed as mg 100 g⁻¹ (Beyhan et al., 2010).

2.4. Total antioxidant activity

FRAP assay: About 120 µL samples were supplemented initially with 0.2 M of PO₄³⁻ to get a volume of 1.25 mL and then with 1.25 mL 1% K₃Fe(CN)₆. Resultant mixture was vortexed and incubated at 50 °C for 1 hour. Incubated samples were supplemented with 1.25 mL 10% TCA and 0.25 mL 0.1% FeCl₃. Then, absorbance values at 700 nm were determined on the spectrophotometer. Obtained values were expressed as mmol kg⁻¹ (Benzie and Strain, 1996).

DPPH assay: 0.26 mM DPPH (1,1-diphenyl-2-picryl-hydrazil) solution was prepared for DPPH analysis. 2700 µL of ethyl alcohol and 1 ml of DPPH solution were added to 300 µL of fruit extract and vortexed. Then, 30 min kept in dark. After incubation of the samples, the absorbance values at 517 nm were determined on the spectrophotometer. Obtained values were expressed as mmol kg⁻¹ (Blois, 1958).

2.5. Total flavonoids content

Initially, 3.5 mL of methanol was added over 800 µL of fresh fruit extract. Then, 100 µL both %10 ammonium acetate and 1 M ammonium nitrate were added and left to incubate 40 min. After incubation of the samples, the absorbance values at 517 nm were determined on the spectrophotometer. Obtained values were expressed as mg 100 g⁻¹ (Zhishen et al., 1999).

2.6. Vitamin C content

Vitamin C content was determined using reflectometer set (Merck RQflex plus 10, Germany). Obtained values were expressed as mg 100 g⁻¹.

2.7. Statistical analysis

The obtained data were analyzed with ANOVA using for SPSS 23.0 software and differences between pear clones were determined with LSD test at P<0.05. Principle component analysis (PCA) were performed using the JMP 10 software. Hierarchical clustering method was used for cluster analysis based on biochemical characteristics of pear clones.

3. Results and Discussion

The total phenolics content, antioxidant activity and total flavonoids content of Alişar pear clones are seen in Table 1.

With regard to the total phenolics content, Alişar pear clones differed statistically (p<0.05). The total phenolics content of the clones ranged from 72.61 mg 100 g⁻¹ (A-11) to 147.38 mg 100 g⁻¹ (A-19). In related studies, the total phenolics content were reported between the ranges 307.06-717.08 mg 100 g⁻¹ for pear genotypes grown in Bosnia and Herzegovina (Duric et al., 2015), 8-140 mg 100 g⁻¹ for pear genetic resources in Northern Japan (Ieguchi et al., 2015), 112.6-230.5 mg 100 g⁻¹ for local pear variety grown in Ardahan (Abacı et al., 2016), 126.1-215.2 mg 100 g⁻¹ for local pear variety grown in Posof (Erbil et al., 2018), 9.88-22.09 mg 100 g⁻¹ for pear genetic resources in Italy (Azzini et al., 2019), 15.17-190.46 mg 100 g⁻¹ for pear variety grown in Northeast Bosnia (Salkic et al., 2019). Findings regarding

the total phenolic content showed differences rather than partial similarities with those of pear genetic resources from different countries and growing ecologies.

Table 1. Total phenolics content, antioxidant activity, total flavonoids content and vitamin C content of Alişar pear clones

Clones	Total Phenolics Content (mg 100 g ⁻¹)	Antioxidant Activity (mmol kg ⁻¹)		Total Flavonoids Content (mg 100 g ⁻¹)	Vitamin C Content (mg 100 g ⁻¹)
		FRAP	DPPH		
A-01	85.71	2.63	0.44	3.38	13.0
A-02	95.53	2.72	0.20	5.18	13.8
A-03	116.81	2.93	0.37	4.85	19.2
A-04	106.17	3.88	0.76	5.02	13.4
A-05	85.71	2.71	0.55	3.49	17.5
A-06	75.88	2.84	0.18	3.65	18.9
A-07	93.08	2.83	0.32	3.05	18.5
A-08	88.98	2.71	0.23	3.38	12.3
A-09	84.62	2.63	0.38	3.60	14.7
A-11	72.61	3.28	0.47	3.57	13.6
A-12	91.71	3.03	0.40	3.57	14.5
A-13	109.72	3.62	0.46	3.95	14.7
A-14	97.71	2.81	0.52	4.20	15.1
A-15	120.36	4.01	0.62	8.48	20.4
A-16	97.44	2.90	0.57	3.98	25.6
A-17	98.81	2.83	0.38	4.06	15.0
A-18	96.08	3.78	0.59	18.01	14.9
A-19	147.38	3.87	0.41	7.77	15.1
A-20	114.63	3.58	0.37	7.50	22.5
A-21	113.27	3.21	0.58	13.04	29.0
A-22	90.07	2.76	0.45	3.57	14.9
A-23	138.10	4.27	0.76	7.97	16.5
A-25	111.36	3.26	0.37	4.12	13.1
A-26	98.81	3.49	0.50	3.68	14.6
A-27	105.90	3.13	0.57	3.82	12.8
A-28	133.19	3.27	0.54	4.64	23.9
A-29	119.27	3.53	0.63	5.70	15.8
Significant	***	***	***	***	***
LSD	5.15	0.21	0.05	0.63	0.28

It has been reported that antioxidants have positive effects on human health in the prevention of many diseases, especially cancer and cardiovascular diseases (Vauzour et al., 2010; Li et al., 2012). These effects increased the interest in fruits that contain polyphenolic compounds and are a natural source of antioxidants (Öğüt, 2014). Antioxidant capacity of the investigated clones were determined in the FRAP and DPPH assays. As seen in Table 1, antioxidant activity of Alişar pear clones showed significant variations among themselves ($p < 0.05$). The antioxidant activity was determined from 2.63 mmol kg⁻¹ (A-01 and A-09) to 4.27 mmol kg⁻¹ (A-23) in the FRAP assay. In the FRAP assay, the antioxidant activity was determined between the ranges 0.80-3.18 mmol kg⁻¹ for pear varieties grown in Tokat region (Küçükler et al., 2015) and 1.20-1.61 mmol kg⁻¹ for local pear varieties from Posof region (Erbil et al., 2018). Azzini et al. (2019) reported the antioxidant activity from 1.04 mmol kg⁻¹ to 2.91 mmol kg⁻¹ for Italian pear genetic resources. In present study, antioxidant activity in the DPPH assay was detected from 0.18 mmol kg⁻¹ (A-06) to 0.76 mmol kg⁻¹ (A-04 and A-23). In this assay, the total antioxidant activity was determined between the values 0.24-0.48 mmol kg⁻¹ in pear varieties grown in the southern area of Chile (Galvis Sánchez et al., 2003), 0.81-1.98 mmol kg⁻¹ in pear genetic resources in Northern Japan (Ieguchi et al., 2015) and 25.6-55.4% in local pear varieties grown in Ardahan (Abacı et al., 2016). When compared to the related works, some clones of this study had higher antioxidant values than those of some previous studies. In particular, the clones A-23, A-15 and A-04 with higher antioxidant activities were evaluated remarkable.

Flavonoids exhibit a wide range of biological effects, including antibacterial, antiviral, anti-inflammatory, antiallergic, antithrombotic, and vasodilatory actions (Cook and Sammon, 1996). Differing statistically ($p < 0.05$) among the clones, the content of total flavonoids was determined between $3.05 \text{ mg } 100 \text{ g}^{-1}$ (A-07) and $18.01 \text{ mg } 100 \text{ g}^{-1}$ (A-18) as seen in Table 1. Galvis Sánchez et al. (2003) recorded the total flavonoids content ranging from 162.0 to 559.0 $\text{mg } 100 \text{ g}^{-1}$ in pear varieties grown in the southern area of Chile. The total flavonoids content was determined between 32.1-38.1 $\text{mg } 100 \text{ g}^{-1}$ for pear cultivars grown in Ankara (Karadeniz et al., 2005). Duric et al. (2015) reported total flavonoids content between 43.73-120.20 $\text{mg } 100 \text{ g}^{-1}$ for pear genotypes from Bosnia and Herzegovina. Abacı et al. (2016) stated that red-colored pears have higher flavonoids content than yellow and green-colored pears. In present research, values of total flavonoids in the clones were lower than those of other researches.

The content of phenolics and flavonoids and antioxidant activity in the pear fruit might be affected by many factors such as genetic structure (variety or genotype), growing ecology, soil characteristics, harvest season and maturity stage of fruit.

On the other hand, there were significant differences ($p < 0.05$) with respect to vitamin C content among Alişar pear clones (Table 1). The vitamin C content of clones ranged between $12.3 \text{ mg } 100 \text{ g}^{-1}$ (A-08) and $29.0 \text{ mg } 100 \text{ g}^{-1}$ (A-21). The vitamin C content of pear were determined between the range $9.1\text{-}29.7 \text{ mg } 100 \text{ g}^{-1}$ in local pear varieties grown in Sinop province (Ozturk et al., 2015), $0.23\text{-}1.61 \text{ mg } 100 \text{ g}^{-1}$ in pear genotypes grown in Bosnia and Herzegovina (Duric et al., 2015), **$4.4\text{-}10.2 \text{ mg } 100 \text{ g}^{-1}$** in local pear varieties grown in Ardahan (Abacı et al., 2016), $9.03\text{-}16.02 \text{ mg } 100 \text{ g}^{-1}$ in local pear varieties from Posof (Erbil et al., 2018), $2.25\text{-}8.45 \text{ mg } 100 \text{ g}^{-1}$ in pear varieties grown in Northeast Bosnia (Salkic et al., 2019), and $8.19\text{-}23.29 \text{ mg } 100 \text{ g}^{-1}$ in pear genetic resources in Italy (Azzini et al., 2019). In the present study, Vitamin C contents of some pear clones were higher than those of many related studies. Especially, A-21, A-16 and A-19 clones might be also a good source of ascorbic acid. Ascorbic acid content in pear is influenced by genetic factors, ecological conditions and maturity stage of fruit (Lee and Kader, 2000; Colaric et al., 2006).

Based on DPPH and FRAP assays, significant positive correlations ($p < 0.05$) were computed between total phenolics and antioxidant activity ($r = 0.492^{***}$ and $r = 0.679^{***}$), respectively. Also, significant positive relationship ($r = 0.358^{**}$) was found between total phenolics and total flavonoids (Table 2). Total phenolics showed a moderate relation ($R^2 = 0.461$) with FRAP (Fig 1). Abacı et al. (2016) reported a positive correlation ($r = 0.758^*$) between total phenolic and DPPH. Azzini et al. (2019) computed a high positive correlation between total phenolics and FRAP ($r = 0.874^{**}$), and between total phenolics and total flavonoids ($r = 0.918^{**}$). In addition, there was positive correlation between DPPH and FRAP ($r = 0.623^{***}$), total flavonoids and antioxidant activity ($r = 0.370^{**}$ and $r = 0.531^{***}$) according to DPPH and FRAP assays, respectively ($p < 0.05$) (Table 2). A lower positive relation ($R^2 = 0.388$) was found between DPPH and FRAP. Total flavonoids also showed a weak relation with FRAP ($R^2 = 0.282$) (Fig 1). Azzini et al. (2019) reported a moderate positive correlation ($r = 0.436$) between FRAP and TEAC, and a high positive correlation ($r = 0.919^{**}$) between total flavonoid and FRAP (Table 2).

Table 2 Correlation matrix for fruit bioactive compounds of Alişar pear clones

	Total phenolics	DPPH	FRAP
DPPH	0.429***	-	
FRAP	0.679***	0.623***	-
Total flavonoids	0.358**	0.370**	0.531***

Pearson r values indicate significant correlations (^{ns} non-significant, * $P < 0.05$, ** $P < 0.005$, *** $P < 0.001$).

The five biochemical variables were used for principle component analysis (Table 3). Two of five components explained 71.87% of total variation. PC 1 was explained 51.86% of total variation, and related to total phenolics content, total flavonoids content and antioxidant activity in the FRAP and DPPH assays. PC 2 was related to vitamin C, and it explained 20.01% of total variation (Table 3 and Fig 2).

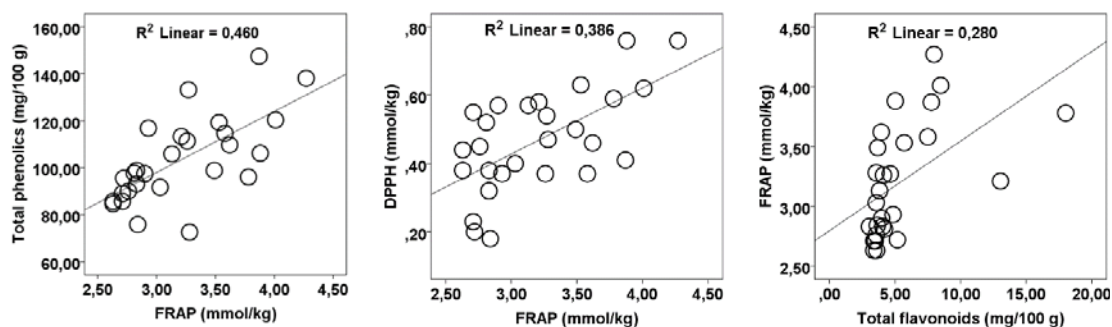


Figure 1. Relationship of total phenolics to FRAP, DPPH to FRAP and total flavonoids to FRAP in the clones.

Table 3. Principle component analysis of bioactive characteristics of Alişar pear clones

Variable	Component	
	1	2
Total phenolics	0.746	0.253
FRAP	0.939	0.007
DPPH	0.782	0.049
Total flavonoids	0.572	0.472
Vitamin C	0.043	0.962
Eigen value	2.59	1.00
% of variance	51.86	20.01
Cumulative %	51.86	71.87

Factor loading > |0.56| are marked in bold.

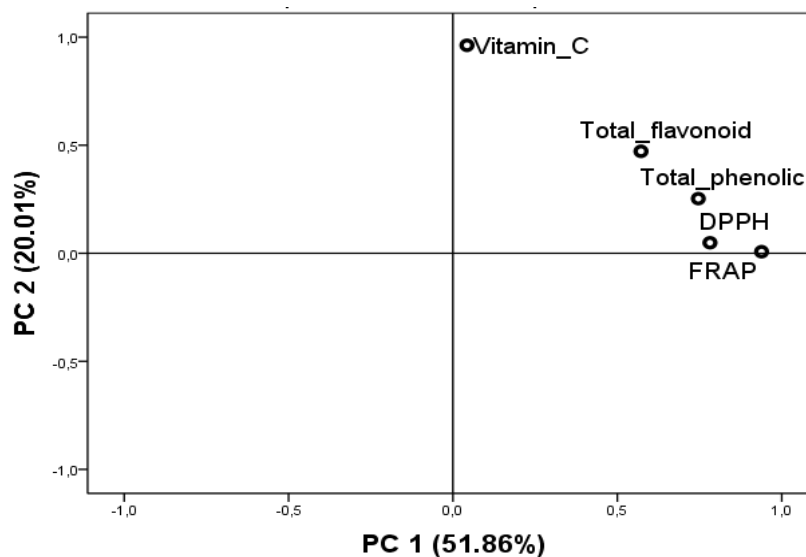


Figure 2. Component plot of the first two principle components based on bioactive profiles for Alişar pear clones.

The dendrogram formed according to PCA results, Alişar pear clones were divided into two main group. The first main group consisted of 16 clones. The second group included in 11 clones. Also, the second main group was divided into two sub-group. The first sub-group consisted of 5 clones. The second sub-group included in 6 clones. The means of biochemical profiles of the clones located in the second main group were higher than those of first main group. Similarly, the means of biochemical compounds of the clones included in the second sub-group was higher than those of first sub-group (Fig 3).

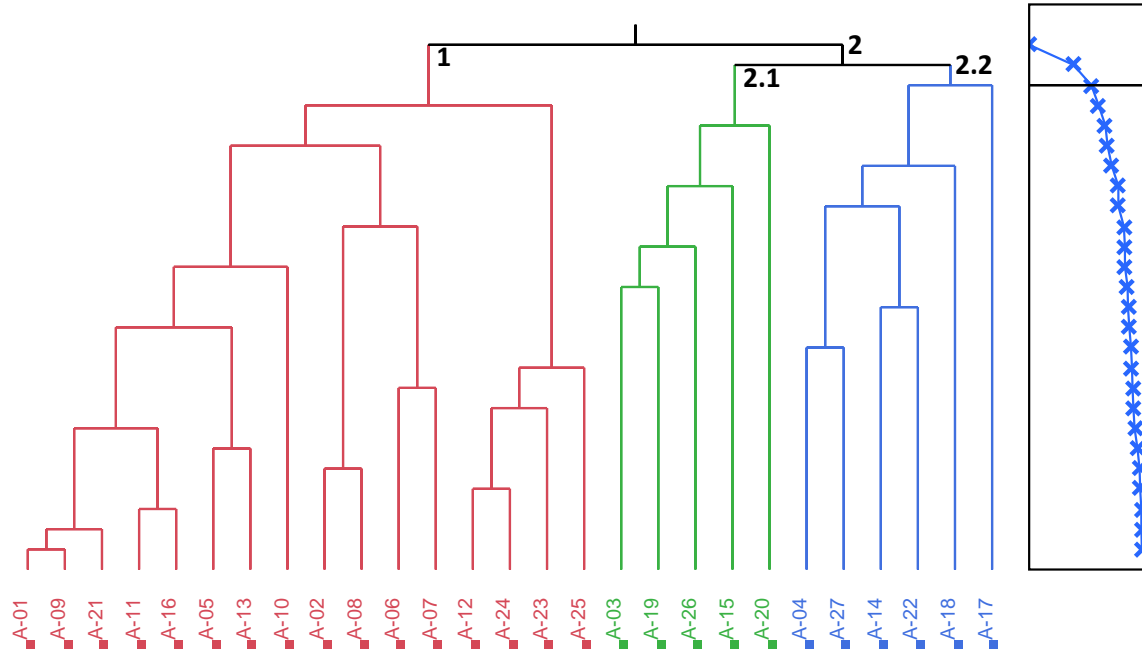


Figure 3. Dendrogram grouping based on bioactive profiles for Alişar pear clones.

4. Conclusion

With a history of at least three centuries, 'Alişar' is a local pear (*Pyrus communis* L.) variety that is widely grown in the district of Şebinkarahisar in Giresun province. In recent years, researches on the bioactive compounds of fruits that are valuable for human health has gained importance. Phenolic compounds are significant antioxidant source. They have protective effects against some diseases such as cancer, asthma and heart diseases (Cano and Arnao, 2005). Alişar pear clones investigated in this study showed variation in fruit bioactive components. Some clones were rich in bioactive compounds. The highest total phenolic content was determined as 147.38 mg 100 g⁻¹ in A-19. In the FRAP and DPPH assays, the highest antioxidant activity was detected in A-23 as 4.27 mmol kg⁻¹ and 0.76 mmol kg⁻¹, respectively. The clone A-23 had higher antioxidant activity than pear genotypes reported by other researchers. A-19 in total phenolic content, A-23 in antioxidant activity and A-18 in total flavonoid content were evaluated more remarkably than others. The findings revealed that these clones might contribute as genetic material to future improvement studies of pear.

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