Research Article

Bioactive compounds of strawberry tree (*Arbutus unedo* L.) genotypes grown in the East Black Sea and Marmara regions

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ABSTRACT

The research was carried out to determine the total phenolics, total flavonoids and antioxidant activity (according to DPPH and FRAP assays) of 21 strawberry tree genotypes grown naturally in Piraziz (Giresun, East Black Sea) and Gebze (Kocaeli, Marmara regions) districts. A wide variation was determined between strawberry tree genotypes in terms of the properties investigated. In the strawberry tree genotypes examined, total phenolics was determined from 528 to 985 mg GAE 100 g⁻¹, while total flavonoids was detected from 21 to 134 mg QE 100 g⁻¹. According to DPPH and FRAP tests antioxidant activity was determined from 2.1 to 15.5 mmol TE 100 g⁻¹ and 20.5 to 50.9 mmol TE 100 g⁻¹, respectively. According to the principal component analysis result, the first two components explained 88.1% of the total variation. PC1 was related to total phenolics and antioxidant activity (both DPPH and FRAP), while PC2 was associated with total flavonoids. As a result, the G-14 genotype had remarkable results in terms of the properties investigated. This genotype, which stands out in terms of beneficial substances on human health, is thought can be used as genetic material in future breeding programs.

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1. Introduction

Strawberry tree (*Arbutus unedo* L.), which belongs to the Ericaceae family, is a fruit species with a wide distribution area in the world. It has found the opportunity to grow in the temperate climatic conditions of Asia, Europe and Africa in the world (Karadeniz et al., 2003). Apart from that, it grows quite extensively in the Mediterranean basin, which covers a large area (Miguel et al., 2014). In Türkiye, it has the opportunity to grow naturally in the Black Sea, Mediterranean, Marmara and Aegean regions (Yaltırık and Erdinç, 2002). In this regard, Türkiye, which has suitable climatic characteristic for the cultivation of strawberry tree, contributes significantly to genotype richness (Anşin and Özkan, 1993).

The strawberry tree is a species with an evergreen plant habitus that can be between 1.5 and 9.0 m tall (Yarılgaç and Pekdemir, 2019) and round or nearly round orange-red fruits (Özcan and Hacıseferoğulları, 2007). Because of the fruit resembles a strawberry, it's also known as the strawberry tree (Anşin and Özkan, 1993). In Türkiye, according to the regions, different names have been given such as "dağ çileği, dağ yemişi, ayı yemişi, kocakarı yemişi, kara yaprak, andrana, andıra, davulga, davulgı üzümü, piridim, yağma, endirek and zefre yemişi" (Şeker, 2004; Erboğa, 2016; Koyu et al., 2019). The strawberry tree plant has many uses, both with its visual and nutritive properties. The evergreen plant forms showy bell-shaped flowers that can be seen on the plant for a long time (Soufleros et al., 2005), with colorful fruits ranging from yellow to red in the period from fruit set to fruit ripening (Gilman and Watson, 1993). It is considered an ornamental plant (Maleš et al., 2006; Celikel et al., 2008; Yıldız, 2014). In addition, shrub-like strawberry tree plants are used as hedge plants (Yarılgaç and Pekdemir, 2019). Fruits are used primarily for fresh consumption, jelly, marmalade, jam, ice cream, pastry, alcoholic beverage production and medical fields (Ayaz et al., 2000; Şeker 2004; Soufleros et al., 2005; Ganhão et al., 2010; Oliveira et al., 2011; Aloğlu et al., 2018).

The fruiting period of the strawberry tree is much longer compared to many other species (Anşin and Özkan, 1993; Şeker, 2004). It provides an alternative food source for people, especially since its ripe fruits are harvested in the winter or close to winter (Soufleros et al., 2005; Yıldız, 2014). In addition, it has been reported that it provides a protective effect against many diseases thanks to its high biochemical content. These have been stated disease such as neurological disorders, cardiovascular diseases, preventing the formation of cancer cells, atherosclerosis, reducing fever, kidney disorders, rheumatism, hypertension, relieving constipation, diuretic and anti-infection (Ziyyat et al., 1997; Jouad et al., 2001; Tuzlacı and Aymaz, 2001; Bnouham et al., 2007; Pallauf et al., 2008; Oliveira et al., 2011; Molina et al., 2011; Erboğa, 2016).

The strawberry tree has been used for centuries both as a food ingredient and in folk medicine. Today, it still has an important position with its similar uses. So much so that with the increase in the world population, the importance of foods with high nutritional value has increased even more. In addition, people's perspectives on healthy life have changed due to epidemics that have been common in recent years. In this case, people have directed the consumption of fruits and vegetables that increase their quality of life, are more economical, more accessible and have high bioactive content. The strawberry tree is one of these fruit species with its rich nutritional content.

This study aimed to determine the bioactive components that support human health in strawberry tree genotypes grown naturally in two different regions.

2. Materials and methods

2.1. Plant materials

The research was carried out in Piraziz district (Giresun, Türkiye), located in the Eastern Black Sea region and Gebze district (Kocaeli, Türkiye), located in the Marmara region. The research material consisted of 4 strawberry tree genotypes grown in Piraziz district and 17 strawberry tree genotypes grown in Gebze district, a total of 21 genotypes. Each plant selected in the study was accepted as a genotype.

2.2. Methods

In the selection of the strawberry tree genotypes, fruit size was taken into account. Approximately 250 g of fruit samples were collected from the genotypes at harvest time. The fruits were stored at -20°C until biochemical analysis. Total phenolics, total flavonoids and antioxidant activity (according to DPPH and FRAP assays) were determined as biochemical properties.

2.2.1. Bioactive compounds

Spectrophotometric measurements for biochemical properties were performed in a UV-Vis spectrophotometer (UVmini-1240, Shimadzu, Japan). Total phenolics were measured according to the method described by Aglar et al. (2019). Total flavonoids were determined according to defined Chang et al. (2002). Total phenolics and flavonoids were expressed as mg GAE (gallic acid equivalent) 100 g⁻¹ fw and mg QE (quercetin equivalent) 100 g⁻¹ fw, respectively. The antioxidant activity of strawberry tree genotypes was detected according to two different procedures of DPPH (Blois, 1958) and FRAP (Benzie and Strain, 1996) assays. According to both DPPH and FRAP, the antioxidant activity was expressed as mmol trolox equivalent (TE) 100 g⁻¹ fw.

2.2.2. Statistical analysis

To evaluate the data were used Minitab 17 and JMP 14 (trial) statistical package programs. The difference between the genotypes in terms of the properties investigated was determined according to the Tukey multiple comparison method at the 5% significance level. Principal component analyzes and hierarchical clustering analysis were performed using biochemical properties.

3. Results and discussion

3.1. Total phenolics

It has been reported that many diseases such as cancer and cardiovascular diseases, especially chronic diseases that significant impact on human health, can be prevented thanks to the biochemicals found in fruits and vegetables (Doré, 2005). In this sense, antioxidants have been stated to contribute significantly to the body's defense system by preventing the harmful effects of irregular reactive oxygennitrogen species and free radicals known to cause diseases (Ratnam et al., 2006). However, it has been stated that the antioxidant produced in the human body has a limited protective effect, and the antioxidants taken from food have a higher protective capacity (Thomas et al., 2010). This situation increases the importance of consuming edible wild fruit species with high biochemical content. In this sense, the strawberry tree has an important place with its rich nutritional content.

Significant differences were determined between the strawberry tree genotypes in terms of total phenolics (p<0.05). While the highest total phenolics was detected in the G-14 genotype with 985 mg 100 g⁻¹, the lowest was determined in the G-6 genotype with 528 mg 100 g⁻¹. The genotypes G-14, G-8, and G-15 with the highest total phenolic content were statistically grouped (Table 1). Total phenolics was reported as 1428 mg 100 g⁻¹ in Arbutus unedo specie grown in Lapseki (Çanakkale) region (Isbilir et al., 2012), from 483 to 627 mg 100 g⁻¹ in strawberry tree genotypes grown in the Muğla province (Colak, 2019), from 567 to 818 mg 100 g⁻¹ in strawberry tree genotypes grown in Akçabat (Trabzon) region (Sagbas et al., 2020), from 479.62 to 850.02 mg 100 g⁻¹ in strawberry tree grown in Croatia (Sic Zlabur et al., 2020). In terms of total phenolics, the findings obtained are compatible with the results of many researchers, while Isbilir et al. (2012) were lower than the findings. Differences in total phenolics are thought to be due to genotype, ecological conditions, and fruit ripeness.

3.2. Total flavonoids

The difference between the total flavonoids of the strawberry tree genotypes investigated was significant (p<0.05). Total flavonoids varied between 21 (G-10) and 134 (P-4) mg 100 g⁻¹. The P-4 genotype with the highest total flavonoids was followed by P-1 (129 mg 100 g⁻¹), G-3 (120 mg 100 g⁻¹) and G-8 (114 mg 100 g⁻¹) genotypes, respectively (Table 1). Sic Zlabur et al. (2020) determined the total flavonoids between 235.39 and 466.88 mg 100 g⁻¹ in strawberry tree genotypes grown in Croatia. The findings obtained in terms of total flavonoids were found to be lower than the results of Sic Zlabur et al. (2020). It can be stated that the observed differences may be due to the genetic structure and ecological conditions, as well as the sunshine period and overnight temperature during the maturing period.

| Genotypes | Total phenolics (mg GAE 100 g ⁻¹) | Total flavonoids (mg QE 100 g ⁻¹) | Antioxidant activity (mmol TE 100 g ⁻¹) | |
|-----------|---|--|--|-------------------------|
| | | | DPPH | FRAP |
| G-1 | 685±30.8 cde* | 88±4.0 ^{ef} | 9.2±0.5 ^{ef} | 34.8±1.7 de |
| G-2 | 555±24.9 ^f | $60{\pm}2.7^{\text{ jk}}$ | 2.1±0.1 ¹ | 22.0±1.1 ^{hi} |
| G-3 | 670±30.1 ^{cde} | 120±5.4 ^{bc} | $8.5{\pm}0.4$ ef | 42.0±2.1 bc |
| G-4 | 602±27.1 def | 72±3.2 ^{gh1} | 3.5±0.2 ^{hi} | 25.7±1.3 ^{gh1} |
| G-5 | 680±30.6 cde | 66 ± 3.0 hij | 10.7±0.5 cde | 37.6±1.9 cde |
| G-6 | 528±23.7 ^f | 46±2.1 ^{lm} | 2.7±0.1 ¹ | 20.5±1.0 ¹ |
| G-7 | 701±31.5 ^{cd} | 63±2.8 ^{ijk} | 9.5±0.5 ^{ef} | 32.9±1.6 ef |
| G-8 | 968±43.5 ª | 114±5.1 ^{cd} | 12.1±0.6 bc | 50.9±2.5 ª |
| G-9 | 667±30.0 cde | 78 ± 3.5 fg | 9.2±0.5 ^{ef} | 34.8±1.7 de |
| G-10 | 697±31.3 ^{cd} | 21±1.0 ⁿ | 13.1±0.7 ^b | 38.9±1.9 ^{cd} |
| G-11 | 628±28.2 def | 53±2.4 ^{kl} | 11.8±0.6 bc | 38.1±1.9 cde |
| G-12 | 589±26.5 ef | 69±3.1 ^{g-j} | 4.3±0.2 ^h | 27.5 ± 1.4 fgh |
| G-13 | 835±37.5 ^b | 72±3.2 ^{gh1} | 9.6±0.5 ^{ef} | 35.2±1.8 de |
| G-14 | 985±44.3 ª | 84±3.8 ^{ef} | 15.5±0.8 ^a | 40.0±2.0 bcc |
| G-15 | 963±43.3 ^a | 35±1.6 ^m | 10.1±0.5 de | 41.0±2.1 bc |
| G-16 | 760±34.2 bc | 77 ± 3.5 fgh | 9.5±0.5 ^{ef} | 45.1±2.3 ^b |
| G-17 | 611±27.4 def | 41±1.8 ^m | 6.9±0.3 ^g | $28.4{\pm}1.4$ fg |
| P-1 | 700±31.5 ^{cd} | 129±5.8 ^{ab} | 11.5±0.6 ^{cd} | 38.0±1.9 cd |
| P-2 | 693±31.1 ^{cd} | 90±4.0 ° | $8.2{\pm}0.4$ fg | 26.4±1.3 ^{gh} |
| P-3 | 834±37.5 ^b | 103±4.6 ^d | 11.6±0.6 bc | 42.7±2.1 bc |
| P-4 | 762±34.2 bc | 134±6.0 ^a | 11.9±0.6 bc | 37.6±1.9 ^{cde} |

 Table 1. Total phenolics, total flavonoids and antioxidant activity (according to DPPH and FRAP assays) of strawberry tree genotypes investigated

*The differences among mean values shown on the same line with the same letter is not significant (p<0.05).

3.3 Antioxidant activity (DPPH and FRAP)

Significant differences were determined in terms of antioxidant activity among strawberry tree genotypes investigated (p<0.05). According to the DPPH test, the highest antioxidant activity was determined as 15.5 mmol TE 100 g⁻¹ (G-14), while the lowest was determined as 2.1 mmol TE 100 g⁻¹ (G-2). The G-14 genotype with the highest antioxidant activity was followed by the G-10 (13.1 mmol TE 100 g⁻¹), G-8 (12.1 mmol TE 100 g⁻¹) and P-4 (11.9 mmol TE 100 g⁻¹) genotypes, respectively. According to the FRAP test, the antioxidant activity was determined between 20.5 (G-6) and 50.9 mmol TE 100 g⁻¹ (G-8). The G-8 genotype, in which the highest antioxidant activity was determined, was followed by the G-16 (45.1 mmol TE 100 g⁻¹), P-3 (42.7 mmol TE 100 g⁻¹) and G-3 (42.0 mmol TE 100 g⁻¹) genotypes, respectively (Table 1).

Serce et al. (2010) reported as 2.7 mmol TE 100 g⁻¹ the antioxidant activity in the *Arbutus andrachne* specie according to the FRAP assay, while Isbilir et al. (2012) found 4.4 mmol TE 100 g-1 in the *Arbutus unedo* specie according to the DPPH assay.

In addition, according to the TEAC assay, antioxidant activity was determined from 1.6 to 2.9 mmol TE 100 g⁻¹ in strawberry tree genotypes grown in Akçabat region (Sagbas et al., 2020) and 1.8 to 3.3 mmol TE 100 g⁻¹ in strawberry tree genotypes grown in the Muğla province (Colak, 2019). When the antioxidant activity results determined according to the DPPH and FRAP assays by different researchers were

examined, the findings obtained from the FRAP assay were high; in contrast, the results obtained from the DPPH assay were low. Some differences observed are thought to be due to genetic structure, ecological conditions, and fruit ripeness.

According to principal component analysis results, the first two components (PC1 and PC2) explained 88.1% of the total variation. PC1 was related to total phenolic and antioxidant activity (both DPPH and FRAP), explaining 66.9% of the total variation. PC2 was associated with total flavonoids and accounted for 21.2% of the total variation (Figure 1).



Figure 1. Biplot of the first two principal components (PC1 and PC2) in the strawberry tree genotypes based on bioactive compounds

Two main groups (A and B) were formed in the dendrogram, which was created using the biochemical properties of the strawberry tree genotypes investigated. The first main group (A) was divided into 2 subgroups (A-1 and A-2) and consisted of 16 genotypes. The first subgroup (A-1) included 7 genotypes (G-1, G-9, G-7, P-2, G-5, G-11 and G-10), while the second subgroup (A-2) consisted of 9 genotypes (G-3, P-1, P-4, G-8, G-14, G-13, G-16, P-3 and G-15) were formed. The second main group (B) included 5 genotypes (G-2, G-6, G-4, G-12 and G-17). When evaluated generally, the genotypes in the first main group had higher values than the genotypes in the second main group in terms of biochemical properties investigated. The G-14 in the first main group gave remarkable results in terms of total phenolics and antioxidant activity (according to DPPH assay). Also, the P-4 stood out with regards to the total flavonoid, while G-8 was remarkable in terms of antioxidant activity (according to FRAP assay) (Figure 2).



Figure 2. Dendrogram grouping of strawberry tree genotypes based on bioactive compounds

4. Conclusion

In the research examining the total phenolics, total flavonoids and antioxidant activity (DPPH and FRAP) properties of strawberry tree genotypes grown in two different regions (Piraziz and Gebze), a wide variation was determined among genotypes in terms of these properties. Among the genotypes investigated, G-14 gave remarkable results with regard to total phenolics and antioxidant activity (according to DPPH assay), while the P-4 stood out in terms of total flavonoids. As a result, G-14, which stands out in terms of beneficial substances on human health, can be used as genetic material in breeding programs. In addition, more detailed research is recommended carried out on the examined genotypes in the future.

Compliance with Ethical Standards

Conflict of Interest

The authors declare that they have no conflict of interest.

Authors' Contributions

Umut Ates: Investigation, Conceptualization, Validation, Writing - original draft, Visualization, Review and editing. **Orhan Karakaya:** Investigation, Conceptualization, Writing - original draft, Visualization, Validation, Review and editing. **Süleyman Muhammed Çelik:** Methodology, Formal analysis, Data curation. **Ahmet Haseb Faizy:** Formal analysis, Data curation

Ethical approval

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Data availability

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Consent for publication

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