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COMPOSITION OF PHENOLIC COMPOUNDS IN PETAL FLOWERS AND LEAF SAMPLES OF VARIOUS APPLE CULTIVARS

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Abstract

The phenolic compounds of petals and leaf tissue samples were determined in apple cvs. 'Breaburn', 'Golden Reinders', 'Granny Smith' and 'Jonagold ' grafted on M9; 'Summerred ' and 'William's Pride' grafted on M.26. Petals of apple flowers were taken at the pink bloom floral stage in April. Moreover leaves were sampled from the middle part of the annual shoots in July. The phenolic compounds were analyzed by High Pressure Liquid Chromatography (HPLC) technique. The gallic acid, p-hydroxy benzoic acid, eriodictyol, quercetin, ferulic acid, chlorogenic acid, caffeic acid, syringic acid, p-coumaric acid and apigenin-7-glucoside contents were determined in petal samples. The gallic acid, p-hydroxy benzoic acid, eriodictyol, quercetin, ferulic acid and p-coumaric acid were also investigated in leaf tissues. The concentrations of the compounds were influenced by the genotypes as well as by tissue samples. While eriodictyol was the predominant phenolic compound in leaves ranging between 156.75-414.90 µg/g DW, chlorogenic acid was the predominant phenolic compound found only in petals ranging between 7784.60-19293.00 µg/g DW in all cultivars investigated in this research. It was determined that the petals of apples were quite richer than the leaf samples in terms of the phenolic contents. Among the studied cultivars the total concentrations of phenols were higher in both the petal and leaf tissues of 'Granny Smith' apple cultivar.

Key words*: Malus, cultivar, leaves, petal, phenolic.*

INTRODUCTION

Phenolics are aromatic compounds in which one (phenol) or more (polyphenol) hydroxyl groups are bound to a benzene ring and constitute a significant part of the biochemical components of the plant. They represent the most abundant widely spread class of plant biochemicals. They are involved in a number of physiological functions in plants. For example, flavonoids which are a large and diverse group of phenolic compounds, act as attractants for pollination and seed dispersal (Downey et al., 2006) and flavonols also help to facilitate conditional male fertility in pollen by providing pollen tube growth (Pollak et al., 1995). On the other hand phenolic compounds can protect the plant against UV-light, insects and pathogens (Vermerris and Nicholson, 2008) and have a role in mechanical support by lignification and affect the growth of neighboring plants (allelopathy) (Özeker, 1999).

Plant phenolics have positive effects on human health and nutrition (Vermerris and Nicholson. 2008), and are the basis of several plant-derived drugs and cosmetic products. Many researchers have also reported that plants are rich sources of polyphenols which have bioactive effect in human such as antioxidant (Pandey and Rizvi, 2009), antimicrobial activities (Rauha et al., 2000), anti-glycemic (Rizvi and Zaid, 2001) and anti-cancer (Yang et al., 2001), anti-inflammatory (Zhu et al., 2015) etc. Therefore, studies on the definition, extraction and purification of these compounds from different plant organs have been increasing. In this regard determination of the composition and content of phenolic compounds in different plant vegetative organs are important.

Apple (*Malus x domestica* Borkh.) is one of the most produced fruit species in the world. Its fruits are known as rich sources of phenolics, especially catechins, procyanidines, phloretin glycosides and chlorogenic acid (Matthes and Schmitz-Eiberger, 2009). Apple leaves are also

rich in terms of phloridzin in which exhibit antidiabetic activity and quercetin glycosides (Liaudanskas et al., 2014). Although differences in phenolic contents of cultivars have been shown in apple leaves and fruits, there is no research on phenolic compounds of apple flowers. It is known that apple flower tea is recommended for facilitating digestion and for enhancing skin.

The aim of this study was to compare the contents of phenolic components in different apple cultivar petals and leaves to evaluate their potential as sources of bioactive compounds as well as genetic differences.

MATERIALS AND METHODS

The biological material. The trials were carried out at the experimental apple (*Malus x domestica* Borkh.) orchard of Suleyman Demirel University, Agricultural Research and Application Center located in Isparta, Turkey (37º50'23"N 30º32'02"E) in 2007. All of the trees used as plant material were planted in 2003 with 1x3 m spacing and trained as modified central leader system. The petals of the flower samples were taken at the stage of pink bloom early in the morning in April. Leaf samples were taken from the middle of one year old shoots all around the tree in July. The collected samples were brought to the laboratory immediately, washed under tap water, rinsed with distilled water, put into paper bags, and dried in an air-blowing drying oven set at 65°C. The dried samples were ground to powder with a blender. Trial I: Determining the phenolic compounds of petals and leaves of "Breaburn", "Golden Reinders", "Granny Smith" and "Jonagold" apple cultivars grafted on M9 rootstock. Trial II: Determining the phenolic compounds of petals and leaves of "Summerred" and "William's Pride" apple cultivars grafted on M26 rootstock.

Determination of phenolic compounds. Phenolic compounds were analyzed by the modified procedure of Escarpa and González (1998). 25 ml of acetone-water solution (80 % acetone and 20 % water v/v) was added to 2.5 g of ground samples. The upper phase was taken in a centrifuge tube after the extract was incubated in a water bath at 50°C for 30 min. Then, the extraction was repeated twice using 25 ml of acetone-water solution each time and the extract was incubated in a water bath at 50°C for 30 min and the upper phase was added to the centrifuge tube again. These combined phases were centrifuged at 10.000 rpm for 5 min. The solvent was evaporated at 40°C under vacuum and samples were re-dissolved in 2 ml of methanol. Solutions were filtered by membrane filters with a pore size of 0.45 µm and then 20 µl of the solutions was injected into HPLC (High Pressure Liquid Chromatography). HPLC analysis was performed using a Shimadzu HPLC system with a diode array detector (DAD λmax=278). The column used was an Agilent Eclipse XDB-C_{18} (250x4.60 mm 5 µm) operated at 30°C. Mobile phase: Solvent A (2 % solution of acetic acid in water)—Solvent B (Methanol). Flow rate: 0.8 ml min^{-1}, Injection volume: 10 µl. Peak identification was done according to the standards (*p*-hydroxybenzoik acid, eriodictyol, ferulic acid, *p*-coumaric acid, gallic acid, quercetin, apigenin 7-glucoside, chlorogenic acid, syringic acid, caffeic acid, rosmarinic acid, epicatechin, catechin, rutin, resveratrol, hesperidin, naringenin, luteolin, apigenin and acacetin). The phenolic standards were purchased from Sigma Chemical Co. The concentration of phenolics was expressed as µg g^{-1} dry matter.

Statistical analysis. The data were subjected to the analysis of variance (ANOVA) by using the Minitab software program, and the means were separated by Duncan's Multiple Range Test (5%).

RESULTS AND DISCUSSIONS

Phenolic compounds of petals. The obtained results are presented in Table 1 and Table 2. These data indicated that the petals had the higher phenolic compounds than the leaves of all apple cultivars investigated in this research. Zou et al. (2011) also reported that the petals of loquat were rich in terms of total phenolics and total flavonoids. In our study, a total of 10 kinds of phenolic compounds found in petals of apple flower samples were identified and quantified. The highest amount of phenolic compound determined in petals was the chlorogenic acid followed by apigenin-7-glucoside, quercetin, caffeic acid, eriodictyol, *p*-coumaric acid, syringic acid, gallic acid, *p*-hydroxybenzoic acid and ferulic acid.

Table 1. Average concentration of phenolic compounds in petal samples of four apple cultivars grafted on M9 in Trail 1 (µg/g DW).

	Cultivars				
Phenolic compound	Breaburn	Golden Reinders	Granny.Smith	Jonagold	Average
Phenolic acids					
clorogenic acid	12208.00 b*	9840.10 c	19293.00 a	12877.00 b	13554.53
caffeic acid	160.65 d	203.25 c	400.50 a	349.30 b	278.43
p-coumaric acid	28.26 d	99.77 b	146.60 a	95.49 c	92.53
syringic acid	40.19 c	100.02 b	111.75 ab	116.45a	92.10
gallic acid	89.70 b	108.40 a	83.07 c	63.98 d	86.29
p-hydroxybenzoik acid	71.50 b	29.11 c	60.34 b	141.90 a	75.71
ferulic acid	25.27 b	nd	48.54 a	nd	36.91
Flavonoids					
apigenin-7-glucoside	1272.60 c	1728.10 b	1383.90 c	2222.40 a	1651.75
quercetin	307.50 b	894.20 a	303.35 bc	236.50 c	435.39
eriodictyol	116.65 b	103.15 bc	457.25 a	95.58 c	193.16
Total	14320.32	13106.10	22288.30	16198.60	16496.78

*Means with different superscripts in the same line are statistically significantly different ($p<0.05$), nd: not detected.

Table 2. Average concentration of phenolic compounds in petal samples of two apple cultivars grafted on M26 in Trail 2 (µg/g DW).

	Cultivars		
Phenolic compound	Summerred	William's Pride	Average
Phenolic acids			
clorogenic acid (phenolic acid)	7784.60 b*	7997.60 a	7891.10
caffeic acid	191.50 b	197.15 a	194.325
p-coumaric acid	130.25 a	44.07 b	87.16
syringic acid	81.00 a	61.54 b	71.27
gallic acid	90.91 b	94.11 a	92.51
p-hydroxybenzoik acid	110.30 a	77.60 b	93.95
ferulic acid	276.95 b	343.30 a	310.125
Flavonoids			
apigenin-7-glucoside	1735.80 a	1132.80 b	1434.30
quercetin	214.25 a	137.50 b	175.88
eriodictyol	16.99 b	23.17 a	20.08
Total	10632.55	10108.84	10370.70

*Means with different superscripts in the same line are statistically significantly different ($p<0.05$), nd: not detected.

These results revealed that the petals of apple flowers have a strong antioxidant capacity. Likewise a positive relationship was detected between the phenolics and antioxidant capacities of loquat flowers (Liaudanskas et al., 2014). To our knowledge there is no literature on the phenolic compounds of petals of apple flowers. Therefore, this research is also important in terms of being the first in its field. The results indicated that the contents of each phenolic component of petals were affected by the cultivars. Similarly Zhou et al. (2011) found differences between the contents of flavonoids and phenolics of the flowers of five loquat cultivars. "Granny Smith" had the highest values for total amount of the phenolic components detected in the petals, whereas the lowest value was found in "Golden Reinders" in trail 1 (Table 1). "Granny Smith" is a self-fertile, good pollinator and high productive apple cultivar. The high phenolic contents of this cultivar can be the reason for its high fertility. Likewise, it is reported that attraction may occur through secondary phenolic compounds (flavonoids) in the petals (Shirley, 1996; Özeker, 1999). Thus phenolics may increase the fruit set and yield by playing a role in pollination as well as pollen tube growth. The contents of clorogenic acid, caffeic acid, *p*-coumaric acid, ferulic acid and eriodictyol were the highest in "Granny Smith" (Table 1). While the contents of gallic acid and quarcetin were the highest in "Golden Reinders", the contents of *p*-hydroxybenzoik acid, syringic acid and apigenin-7-glucoside were the highest in "Jonagold". In trial 2, total amount of the phenolic components of "Summerred" and "William's Pride" cultivars were found close to each other and the lowest than the cultivars investigated in trail 1. The contents of ferulic acid of both cultivars were quite higher than the other cultivars evaluated in trail 1, while the

contents of eriodictyol and quercetin were quite lower. The results of this research emphasized that ferulic acid, eriodictyol and quercetin found in the petal are affected by the rootstocks as well as cultivars.

Phenolic compounds of leaves. The obtained results are presented in Table 3 and Table 4. The phenolic contents of the leaf samples were found lower than the petals of the apple cultivars. Totally 6 kinds of phenolic compounds were evaluated in this research. The highest amount of phenolic compound obtained in leaf samples was eriodictyol followed by ferulic acid, quercetin, *p*-hydroxybenzoic acid, gallic acid and *p*-coumaric acid. Similarly gallic acid, ferulic acid and *p*-coumaric acid were found in leaf samples of apple by Tao et al. (2008) and Petkovsek et al. (2009). Eriodictyol, the highest amount of phenolic compound obtained in this research from the leaf samples of apples, is known as a flavanone and has long been considered as an antioxidant and anti-inflammatory agent. Thus, apple leaves may be considered as a potential antioxidant for human diseases such as acute lung injury (Zhu et al., 2015). The results of this research indicated that the content of each phenolic component obtained from the leaf samples of apples were affected by cultivar. Likewise, many researchers reported that the phenolic contents of the apple vary by cultivar (García et al., 2004; Mikulič-Petkovšek et al., 2004; Usenik et al., 2004; Petkovsek et al., 2009). According to the total results, the highest amount of phenolic components were found in "Granny Smith" followed by "Golden Reinders", "Jonagold" and "Breaburn" in trail 1 (Table 3), while "William's Pride" had the highest amount of these components in trail 2. The highest amount of *p*-hydroxybenzoic acid was found in "Granny Smith" and *p*-coumaric acid was found only in this cultivar. The highest amount of eriodictyol, quercetin, and ferulic acid were found in "Golden Reinders", while the amount of gallic acid was the highest in "Jonagold". The highest amount of *p*-hydroxybenzoik acid, quercetin and ferulic acid were found in "William's Pride", while the amount of gallic acid was the highest in "Summerred" in trail 2.

Table 3. Average concentration of phenolic compounds in leaf samples of four apple cultivars grafted on M9 in Trail 1 (µg/g DW).

	Cultivars				
Phenolic compound	Breaburn	Golden Reinders	Granny Smith	Jonagold	Average
Phenolic acids					
p-coumaric acid	nd	nd	17.58	nd	17.58
gallic acid	56.82 b*	47.19 bc	38.72 c	71.38 a	53.53
p-hydroxybenzoik acid	35.88 c	nd	131.20 a	48.20 b	89.70
ferulic acid	63.08 c	96.13 a	90.05 a	87.40 ab	84.17
Flavonoids					
quercetin	60.46 c	77.97 a	70.67 ab	63.42 b	68.13
eriodictyol	197.90 b	414.90 a	338.90 a	156.75 b	277.11
Total	378.26	636.19	687.12	427.15	590.22

*Means with different superscripts in the same line are statistically significantly different ($p<0.05$), nd: not detected.

Table 4. Average concentration of phenolic compounds in leaf samples of two apple cultivars grafted on M26 in Trail 2 (µg/g DW).

	Cultivars		
Phenolic compound	Summerred	William's Pride	Average
Phenolic acids			
gallic acid	49.92 a*	39.33 b	44.63
p-hydroxybenzoik acid	34.26 b	43.85 a	39.06
ferulic acid	53.71 b	91.18 a	72.45
Flavonoids			
quercetin	65.51 b	97.63 a	81.57
Eriodictyol	239.90	238.25	239.08
Total	443.30	510.24	572.12

*Means with different superscripts in the same line are statistically significantly different ($p<0.05$).

CONCLUSIONS

As a result, according to the results obtained from this research, the phenolic compounds of the apple vary by the cultivar and the parts of the plant. It was determined that the petals of an apple cultivar were quite richer than the leaf samples in terms of the phenolic contents. While chlorogenic acid was the predominant phenolic compound and found only in petals, eriodictyol was the predominant phenolic compound of leaf samples of apple. Among the studied cultivars, the total concentration of phenols was found higher in both of the tissues of "Granny Smith" apple.

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