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CLASSIFICATION OF FRUIT TREE LEAVES ACORDING TO PHENOLIC PROFILE USING PRINCIPAL COMPONENT ANALYSIS

Milan Mitić[1], Jelena Mitić[1], Jelena Nikolić[1], Pavle Mašković[2]

Abstract: This study aimed to determine and compare a polyphenolic profile of the fruit tree leaves of ten commonly commercially and wildly consumed fruits: apricot, plum, peach, quince, apple, blackcurrant, redcurrant, blackberry, raspberry, and dog rose. The polyphenolic profile was determined using HPLCmethod.Inleaves were identified 17 individual phenolics. To evaluate relationships through various fruit tree leaves, Principal Component Analysis (PCA)was employed. Two statistically significant components were identified, grouping fruit tree leaves based on phenolic compounds content.

Keywords: tree leaves, polyphenolic profile, HPLC, Principal component analysis

Introduction

In recent years, several reports have been published on the use of anatomical parts of these plants other than fruits as a source of active phytochemicals (Ferlemi and Lamari, 2016). The phenolic profile of plant tissues ate known to be affected by many factors such as genotype, environment, growth stage, time of harvesting, process and storage conditions and method of analysis (Pavlović et al., 2019). Studies on the potential of leaves, which are plant waste, demonstrate their usage by "green chemistry" principles.

Large datasets are increasingly widespread in many disciplines. To interpret such datasets, methods are required to drastically reduce their dimensionality in an interpretable way, such that most of the information in the data is preserved. Many techniques have been developed for this purpose, but PCA is one of the oldest and most widely used. Its idea is simple—reduce the

[1]University of Niš, Faculty of Science and Mathematics, Višegradska 33, Niš, Serbia (milan.mitic1@pmf.edu.rs)
[2]University of Kragujevac, Faculty of Agronomy, Cara Dušana 34, Čačak, Serbia

dimensionality of a dataset, while preserving as much 'variability' (i.e. statistical information) as possible (Ian T.Jolliffeand Jorge Cadima, 2016).

In the current study, we investigated the major flavonol glycosides and phenolic acids in the leaves of two of the most important commercial *Grossulariaceae* varieties and eight *Rosaceae* varieties in Serbia, using high-performance liquid chromatography (HPLC) combined with a diode array detection system (HPLC-DAD), as well as their classification using the principal component analysis.

Materials and methods

Plant materials

Ten fruit tree leaves were obtained samples from local growers (Nis, Serbia). All samples were harvested at the same time-2 weeks after full flowering. Immediately after the harvest leaves, were air-dried in shade and ground.

Extract preparation

About 1g of the grounded leaf samples were extracted three times using methanol (purity 99.8%) using an ultrasonic bath for 15 min. The resulting extracts were filtered through Whatman filter paper (black ribbon) and dissolved with methanol to the final volume of 25 mL.

HPLC analysis

Qualitative analysis of phenolic acids, flavonols, and flavonols was performed with the HPLC, employing a direct injection method (Mitić et al., 2012).

Results and discussion

The contents of individual phenolic compounds (mg/g d.w.) of fruit leaves are shown in Table 1 and Table 2. Eight flavonols (quercetin-3-rutinoside, quercetin-3-galactoside, qurcetin-3-glucodide, qurcetin-3-rahmoside, kamferol-3-rutinoside, kamferol-3-glucoside, quercetin and kamferol), two flavonons (luteolin-7-glycoside and apigenin-7-glycoside) and 7 phenolic acids (3-*O*-caffeoylquinic acid, 4-*O*-caffeoylquinic acid, 5-*O*-caffeoylquinic acid, 3,5-*O*-dicaffeoylquinic acid; caffeic acid, p-coumaric acid and elagic acid) were identified and quantified. The results indicate significant differences ($p < 0.05$) in flavonoids between the analyzed species.

Table 1. Polyphenols content and profile in leaves of tested plant species (mg/g d.w.)

Sample	Quince	Apricot	Apple	EU Plum	Peach
3-CQA	0.923±0.063	1.056±0.034	-	0.234±0.021	-
4-CQA	0.018±0.003	0.235±0.005	-	0.153±0.011	-
5-CQA	2.922±0.140	3.032±0.063	1.022±0.085	2.055±0.111	1.532±0.050
3,5-diCQA	0.655±0.045	-	-	-	-
CA	-	0.054±0.008	0.322±0.022	0.140±0.009	0.308±0.014
p-CA	0.010±0.001	0.032±0.001	0.586±0.032	-	0.146±0.005
EA					
Q-3-Rut	0.955±0.054	0.173±0.007	3.736±0.185	1.365±0.104	0.935±0.055
Q-3.Gal	0.909±0.036	1.299±0.025	0.458±0.092	0.280±0.021	-
Q-3-Glu	0.599±0.028	0.468±0.018	-	0.726±0.034	-
Q-3-Rhm	-	-	0.712±0.066	0.105±0.007	-
K-3-Rut	0.306±0.022	0.203±0.015	-	-	0.405±0.019
K-3-Glu	0.197±0.008	-	0.232±0.017	-	1.867±0.047
Q	0.064±0.002	-	0.150±0.008	0.205±0.050	0.682±0.031
K	-	-	-	0.065±0.003	1.015±0.074
L-7-gly	-		0.205	-	
A-7-gly	-		0.092	-	

Table 2. Polyphenols content and profile in leaves of tested plant species (mg/g d.w.)

Sample	Blackcurrant	Redcurrant	Blackberry	Raspberry	Dog rose
3-CQA	0.465±0.017	0.443±0.012	-		0.287±0.009
4-CQA	-	-	-		0.105±0.003
5-CQA	2.234±0.020	1.125±0.029	0.278±0.010	0.263±0.009	0.943±0.096
3,5-diCQA	-	-	-	-	-
CA	0.206±0.015	1.086±0.017	0.432±0.024	0.782±0.053	0.126±0.004
p-CA	0.297±0.011	0.107±0.005	1.020±0.065	0.936±0.031	0.045±0.002
EA			3.012±0.082	2.922±0.154	3.054±0.098
Q-3-Rut	2.227±0.014	3.872±0.206	0.381±0.015	0.952±0.075	0.735±0.022
Q-3.Gal	1.123±0.052	2.162±0.183	-	0.245±0.026	0.958±0.028
Q-3-Glu	4.322±0.266	1.622±0.018	12.096±0.956	4.083±0.121	1.345±0.019
Q-3-Rhm	-	-	-	-	1.985±0.032

K-3-Rut	0.123±0.008	0.755±0.016	0.636±0.028	0.032±0.005	0.865±0.018
K-3-Glu	0.237±0.008	0.410±0.009	-	1.118±0.053	1.866±0.026
Q	0.165±0.005	0.299±0.011	-	0.211±0.006	
K	-	0.028±0.003	-		
L-7-gly	-	-	0.182±0.013	0.389±0.033	0.353±0.017
A-7-gly	-	-	0.012±0.002	-	

The content of phenolic compounds in 10 fruit tree leaves samples were subjected to PCA analysis to better understand the relationship between analyzed samples (Figure1). PCA was applied to standardized data, so each parameter contributes equally to the data set variance and carries equal weight in the principal component calculation.

Six principal components were obtained (PC1, PC2, PC3, PC4, PC5 and PC6) accounted for 30.75%, 18.72%, 15.14%, 13.31%, 9.29% and 6.66% of the total variance, respectively. This statistical analysis resulted in a six-component model that explains 89.161% of the total variance between the results obtained.

L-7-gly (0.798), EA (0.785), p-CA (0.772), CA (0.530), Q-3-Rhm (0.317), K-3-Glu (0.289), Q-3-Glu (0.552), K-3-Rut (0.289) and A-7-gly (0.366) are strongly positive correlated and grouped on the plot (Fig. 1). In contrast, 5-CQA (-0.985), 3-CQA (-0.823), Q-3-Gal (-0.402), 3.5di-CQA (-0.470) and 4-CQA (-0.519) are on the negative side of the plot.

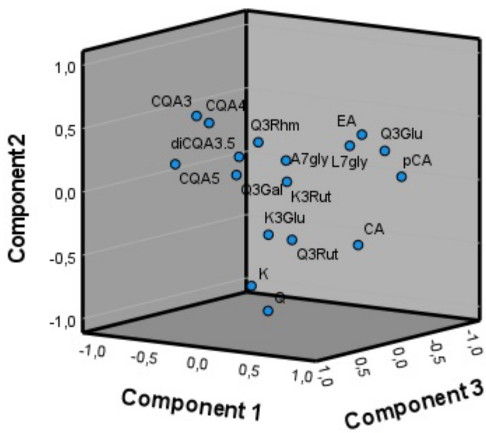

Figure 1. Plot obtained by principal component analysis

The first principal component distinctly separates wild blackberry (1.31) and wild raspberry leaves (1.32) from all other fruit tree leaf samples, because they have the highest positive loadings on PC1.The second principal component distinctly separates dog rose leaves from all others because dog rose leaves (1.43) have the highest positive loadings on PC2.

The other hand, The Q-3-Rhm (0.76) and K-3-Glu (0.76), have the highest positive loadings on PC3 and contribute the most to the third component.PC4 was loaded by positively correlated redcurrant and apple leaves (1.2 and 2.0) and negatively correlated peach leaves (-1.4) and explained 13.31 % of the total variance.PC5 component explained 9.29 % of the total variance, strongly influenced by the positively correlated variable, redcurrant leaves (1.5). PC6 explained 6.66 % of the total variance, and it is made up of apricot (-1.4) and quince leaves (2.3).

Conclusion

The results demonstrate that fruit tree leaves are promising sources of phenolic compounds, especially wild tree leaf species. Principal component analysis grouped analyzed fruit tree leaf species regarding their phenolic composition. The study, therefore, presents data that attest to the importance of fruit tree leaves in providing the much-needed dietary important sources of phenolic compounds. Fruit tree leaves are a significant and rich source of these compounds.

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