



Phenolic compound identification, quantification and antioxidant activity in *Malus domestica* Borkh. leaf ethanolic extracts



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Introduction

Phenolic compounds are secondary plant metabolites. They have various biological activities. Phenolic compounds are natural antioxidants which scavenge free radicals, inhibit their formation processes, stimulate production of antioxidative enzymes, therefore prevent oxidative stress that damages structural organism molecules. Antioxidative activity is closely related to other biological effects of phenolic compounds: antimicrobial, anti-inflammatory, anticarcinogenic and cardiovascular protective activity.

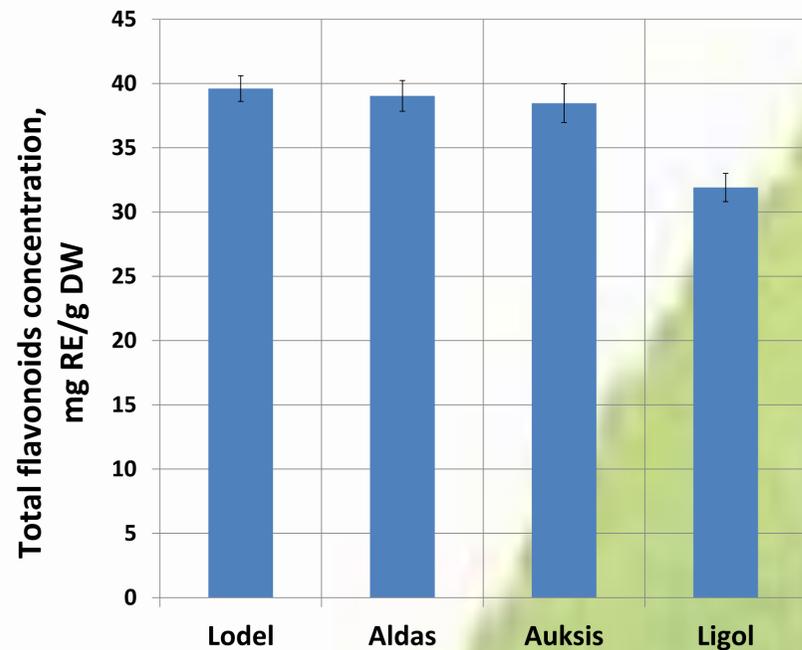


Figure 1. Total flavonoids concentration in ethanol extracts of lyophilised apple leaves

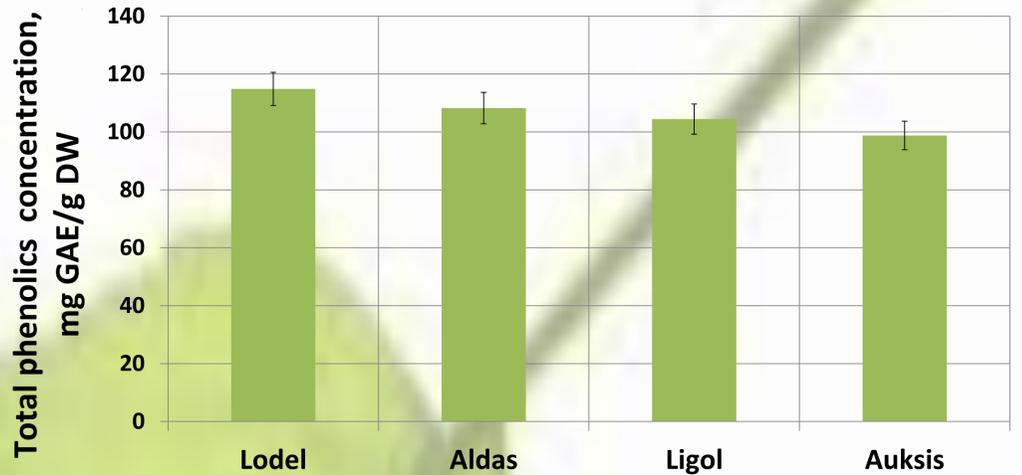


Figure 2. Total phenolics concentration in ethanol extracts of lyophilised apple leaves

Materials and methods

The aim of this research was to evaluate qualitative and quantitative content of phenolic compounds and antioxidant activity of ethanol extracts of apple leaves harvested from the cultivars Aldas, Auksis, Ligol, and Lodel using spectrophotometry and HPLC.

The lyophilized apple leaves were extracted using ultrasonic sound assisted extraction. 70% ethanol was used as extraction solvent. Extraction time was 40 min and temperature was 60°C. Folin-Ciocalteu method was used to determine total concentration of phenolics (expressed as gallic acid equivalent (GAE) mg/g for absolutely dried weight (DW)) in apple leaves extracts. To evaluate total concentration of flavonoids (expressed as rutin equivalent (RE) mg/g for absolutely dried weight) more specific reaction with $AlCl_3$ was used. Two spectrophotometric methods FRAP and DPPH were used to determine antioxidant activity of ethanol extracts. Antioxidant activity was expressed as Trolox equivalent antioxidant capacity (TEAC) μ mol/g DW. HPLC was used to identify individual phenolic compounds and their quantity.

All values in the text and diagrams are means of triplicate analysis. Error bars in the diagrams represent standard deviation (SD).

Results

Total concentration of phenolic compounds in apple leaves ethanol extracts determined by Folin-Ciocalteu method varied from 98,77 mg GAE/g DW to 114,86 mg GAE/g DW (Figure 2). Total concentration of flavonoids in apple leaves ethanol extracts determined by spectrophotometric reaction with $AlCl_3$ varied from 31,91 mg RE/g DW to 39,6 mg RE/g DW (Figure 1).

TEAC values obtained using DPPH method varied from 142,83 μ mol/g DW to 147,94 μ mol/g DW. TEAC values obtained using FRAP method varied from 365,45 μ mol/g DW in to 445,64 μ mol/g DW (Figure 4). Medium strength correlations were observed between DPPH radical scavenging activity and total concentrations of phenolics (0,639) and flavonoids (0,654). Strong correlations were found between FRAP assay and total concentration of phenolics (0,724) and total concentration of flavonoids (0,73).

Using HPLC method several phenolic compounds were identified (Figure 3): quercetin glycosides (hyperoside, isoquercitrin, rutin, avicularin and quercitrin), phenolic acids (chlorogenic acid, caffeic acid), dihydrochalcones (phloridzin and phloretin) and catechins ((+)-catechin and (-)-epicatechin). Phloridzin was a predominant component of phenolic compounds in apple leaves. It accounted for 76.9% to 84.2% of all phenolic compounds identified and quantified in the extracts of apple leaves by the HPLC method.

The quantitative content of different groups of identified phenolic compounds is shown in Table 1.

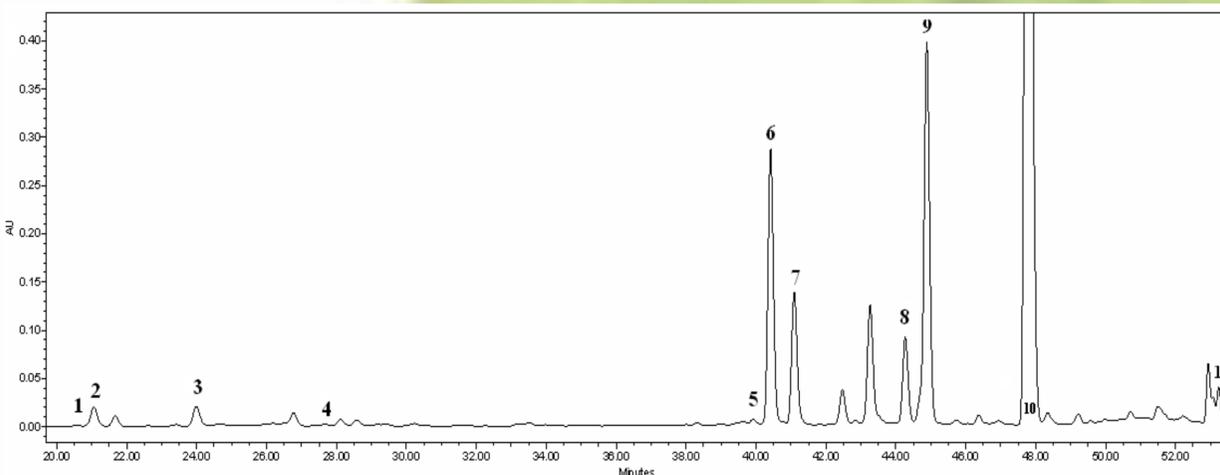


Figure 3. Identified phenolic compounds from apple leaves ethanol extract ($\lambda=320$ nm). 1-(+)-catechin, 2-chlorogenic acid, 3-caffeic acid, 4(-)-epicatechin, 5-rutin, 6-hyperoside, 7-isoquercitrin, 8-avicularin, 9-quercitrin, 10-phloridzin, 11-phloretin

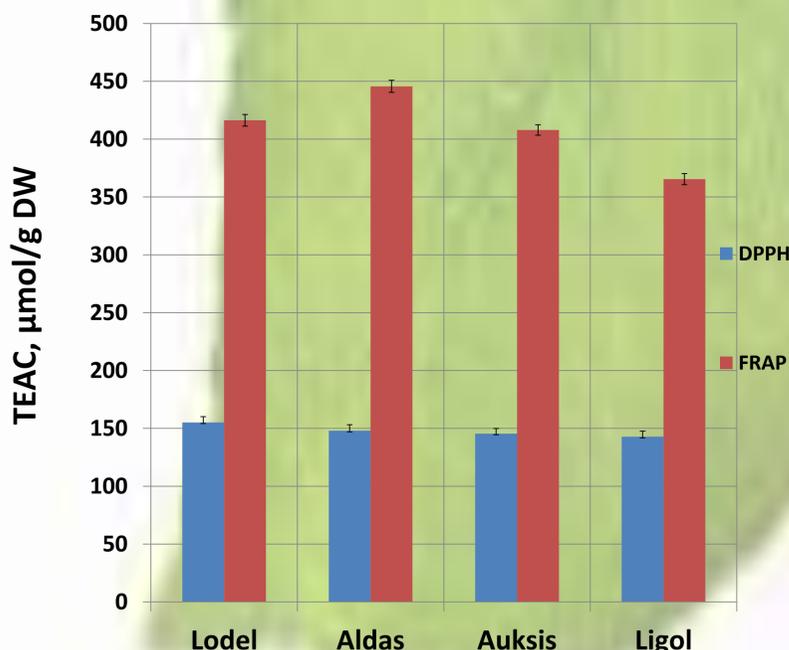


Figure 4. Antioxidant activity of ethanol extracts of lyophilised apple leaves

	Quantitative content of phenolic compounds, mg/g			
	Aldas	Auksis	Lodel	Ligol
Quercetin glycosides	28,60±1,08	22,31±0,89	16,95±0,68	24,78±1,06
Dihydrochalcones	107,81±4,07	110,44±4,63	116,82±4,82	110,90±4,83
Phenolic acids	0,74±0,02	1,61±67,1	1,27±57,0	1,01±0,03
Catechins	0,77±0,03	0,65±0,02	0,57±0,02	0,85±0,04

Table 1. Quantity of different groups of identified phenolic compounds in apple leaves ethanol extracts.

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