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# Antioxidant Activity and Phenolic Content of Organic and Conventional Vine Cane Extracts

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**Abstract**

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Phenolic antioxidants, such as resveratrol and polydatin, occur in grapevine as secondary metabolites responsible for the plants protection against biotic and abiotic stress. The antioxidant profile and content depends on agro-climatic conditions, which may act as stress factors. In order to determine the significance of the use of spraying pesticides on the antioxidant content in pruned canes, we examined samples of white and blue *Vitis vinifera* varieties from conventional and organic vineyards. Phenols from these samples were extracted by 40% ethanol. HPLC was used to determine differences in the stilbenoid composition and the DPPH assay was used to compare the antioxidant activities. While the farming approach did not alter the total polyphenolic content and antioxidant activity of the samples, the resveratrol content was higher in samples from conventional vineyards. These results could be significant for further reusing of winery waste.

**Keywords**: biodynamic; pesticides; polyphenols; resveratrol; *Vitis vinifera*; waste

Grapevine (*Vitis vinifera*) is one of the most commonly grown crops in the world. It is economically important as a source of grapes, both for direct consumption and for the production of wine. Waste and by-products are produced during the growing of grapes as well as winemaking. Every year, vines are pruned, which generates more than 1 ton/ha/year of canes. These canes are considered to be waste and are usually burned, thus offering no direct profit to the winemaker. Since 2009, the European Union strongly encourages winemakers to manage their waste more sustainably (EN 491/2009).

Over this past decade, the chemical composition of canes has been thoroughly investigated (Karacabey & Mazza 2010; Gorena *et al*. 2014, Soural *et al*. 2015) and it was proved that they are a valuable source of phenolic antioxidants. Phenolic compounds are the most widespread plant secondary metabolites with antioxidant activity (Arvanitoyannis *et al*. 2006). The largest groups of phenolic compounds, the flavonoids and stilbenoids, are secondary metabolites produced by plants, in response to stressful conditions (mechanical damage and UV) or to fungal infections. Resveratrol and its glycosidic form, polydatin, are considered to be the most significant stilbenes (Waterhouse 2002).

Resveratrol and its derivatives have been in increasing demand as nutraceuticals, for cosmetic purposes and possibly even pharmaceutical uses, including as dietary supplements. However, there are many factors influencing the production and accumulation of polyphenols in *Vitis vinifera* plants, which makes

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it hard to reuse them because of the variable yields. Therefore, it is important to study which factors influence the total phenolic content, and notably resveratrol content, in canes.

Several studies have proved that the phenolic content in the plants and wine depends on the variety (Cantos *et al*. 2002) and growth conditions. Król *et al*. (2014) studied the effect of drought on polyphenolic content (caffeic acid, *p*-coumaric and ferulic acid) in vine leaves and roots. Previously it was proved that temperature (Spayd *et al*. 2002) and the intensity of solar radiation (Koyama *et al*. 2012) have an impact on the content of phenols in peel berries during their maturation. Furthermore, it was proved that UV light, ozone or anoxia (oxygen-free environment) triggered the synthesis of stilbenoids in *Vitis vinifera* (Teixeira *et al*. 2013). All the previously mentioned studies contributed to a better understanding of the accumulation of polyphenols in various parts of the plant in relation to environmental conditions.

In recent years, several wine companies have moved to organic production of wines (Forbes *et al*. 2009). Organic grapes are usually treated with pesticides such as dry flowable sulphur and copper salts (Mulero *et al*. 2010). Although there seems to be a growing interest in organic wine, to our knowledge, there are no studies dealing with the impact organic approach on the polyphenol content in grape canes.

To address this matter, we studied the polyphenolic content and antioxidant activity of grape cane extracts from conventional as well as organic vineyards. In evaluating the presence of stress-induced phytoalexins, we focused on the presence of resveratrol and polydatin in the tested samples. The results suggest that the use of organic protective spraying did not influence the total polyphenolic content, nor the antioxidant activity of the cane extracts. On the other hand, the resveratrol content was higher in cane extracts from conventional vineyards.

## MATERIAL AND METHODS

***Plant material***. Grape canes of blue and white *Vitis vinifera* varieties were collected from Czech vineyards with conventional andorganic approaches in the months January and February 2017. The samples were then transported back to laboratory and processed within 24 hours.

The vineyards with an organic approach are referred to as vineyards A, B, and C, while the conventional vineyards are referred to as vineyards D, G, and H. The vineyards A and B had a Demeter biodynamic certification while vineyard C operated as a certified organic vineyard. Vineyards A and B were using plant (such as nettle, St. John's wort) extracts instead of conventional pesticides and vineyard C was only using pesticides approved for integrated farming and two bio certified products against vine mildew (*Oidium tuckeri* or *Erisyphe necator*) and grey mould (*Botrytis cinerea*).

***Chemicals***. Ethanol 96% (v/v) p.a. (Penta, Czech Republic), acetonitrile (VWR Chemicals, USA), *trans*-resveratrol, ≥ 99% GC (Sigma Aldrich, Germany), *trans*-polydatin ≥ 95% HPLC (Sigma-Aldrich, Germany) pinosylvin ≥ 97% HPLC (Sigma-Aldrich, Germany) pterostilbene (Cayman chemical company, USA).

***Sample preparation***. The canes were cut into 2 cm sections and dried in a circulating-air oven (Binder, Germany) at 105°C to constant mass right after cane harvest. Afterwards, the dried canes were ground to the size of 2–3 mm (Zelmer 32Z012; Poland). A static solid-liquid extraction (24 h in dark, laboratory temperature, 1 : 4 ratio) with 40% (v/v) ethanol was applied to obtain an extract containing phenols for further analysis. All extracts were prepared in triplicates.

***HPLC analysis of stilbenes***. HPLC separation and quantification was carried out with an 1100 series HPLC system equipped with a DAD detector (Agilent, USA) and a reversed-phase 125 × 4 mm Watrex, Nucleosil 120-C18 column at 25°C. The samples were analysed after filtration through cellulose acetate membrane filters (0.45 µm) (Sartorius Stedim Biotech, Germany). The compounds in the extracts were identified according to their UV spectra (Figure 1) and retention time by comparison to external standards of *trans*-resveratrol (retention time 12.3 min) , *trans*-polydatin (retention time 8.2), pterostilbene (retention time 26.5 min) and pinosylvin (retention time 30.8) dissolved in 40% (v/v) ethanol. The wavelength of 306 nm was evaluated as the optimum wavelength for detection of resveratrol and its analogues, as it is in near the absorption maxima of these compounds (Kolouchova-Hanzlikova *et al*. 2004).

The concentrations of *trans*-resveratrol, *trans*-polydatin, pterostilbene and pinosylvin were determined by RP-HPLC using a gradient of acetonitrile and demineralized water. The proportion of the acetonitrile in the mobile phase was increased during the time of the analysis from 10% up to 95% (Table 1).

***Determination of antioxidant capacity***. The antioxidant capacity of the samples was measured

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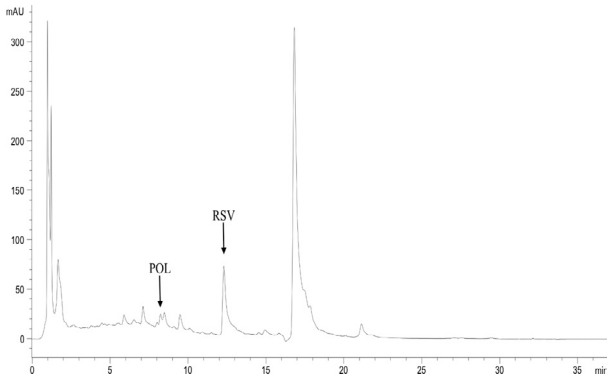


Figure 1. HPLC chromatogram of Dornfelder cane extract from the conventional vineyard D (RSV – *trans*-resveratrol; POL – *trans*- polydatin)

by the DPPH assay. A DPPH-methanol solution was prepared by dissolving 0.0125 g DPPH in 500 ml of methanol. For the measurement, 3.9 ml of the DPPH-methanol solution was mixed with 0.1 ml of the properly diluted extract. The reaction mixture was left at standard laboratory conditions in the dark for 15 min. The free radical scavenging activity of the extract was evaluated by measuring the difference in absorbance at 515 nm with and UV-Vis spectrophotometer DU 730 (Beckman Coulter, USA). The antioxidant activity was expressed as a percentage of inhibition of the sample in comparison to a blank sample (0.1 ml ethanol + 3.9 ml DPPH solution) and calculated using the following formula:

$$\%_{inhibition} = [(A_{blank} - A_{sample})/A_{blank}] \times 100$$

***Determination of the total phenolic content***. The amount of total soluble phenols in the extracts was determined using the Folin-Ciocalteu method, in which 0.1 ml of the diluted sample extract was mixed with 0.1 ml of Folin-Ciocalteu reagent. The mixture was homogenized and allowed to equilibrate. After 2 min, 2 ml of the solution of 2% $Na_2CO_3$ was added. Prior to the measurement of the absorbance at 750 nm, the mixture was well mixed and incubated 30 min in darkness at laboratory temperature. The phenolic content was calculated from a calibration curve prepared from the standard solutions of gallic acid.

***Statistical analysis***. The results presented in the tables and figures are average values from at least three replications. All statistical analyses were performed using SigmaStat 3.5 (USA). The statistical significance of differences in mean values of the measured parameters was calculated by one-way ANOVA and compared with Tukey's multiple test at the 5% level of probability.

## RESULTS AND DISCUSSION

Extracts from 44 samples of *Vitis vinifera* L. canes were tested for their polyphenolic content and antioxidant activity. According to our results, grape canes also proved to be a valuable source of stilbenes, such as resveratrol and its analogue polydatin. According to a study by Pawlus *et al*. (2013), grapevine canes from both *vinifera* and non-*vinifera* species are a rich source of multiple stilbenoid monomers, glycosides, and oligomers such as *E*-ampelopsin E, *E*-amurensin B, *E*-piceid, *E*-piceatannol, *E*-resveratrol, *E*-resveratroloside, *E*-ε-viniferin, *E*-ω-viniferin, and *E*-vitisin. Similarly, *E*-ε-viniferin, *E*-resveratrol, *E*-piceatannol, and vitisin B were also detected in 16 *Vitis vinifera* L. cultivars (Lambert *et al*. 2013).

The extraction parameters were based on previous experiments (data not shown). Fourty % ethanol was chosen as the best extraction agent for total polyphenols and *trans*-polydatin. The resveratrol content and antioxidant capacity of extracts prepared with 40% ethanol are lower in comparison to higher ethanol concentrations (Rayne *et al*. 2008; Angelov *et al*. 2016); thus, the results in this study reflect the ratio of these parameters in cane extracts. *Trans*-resveratrol and its glycoside, *trans*-polydatin, were detected in all cane extracts, with the concentration ranging between 4.47–252.79 mg/kg of dry matter (DM) and 4.24–48.73 mg/kg DM, respectively. Even though the extraction parameters were not optimal for resveratrol extraction, the obtained results are in agreement with values previously reported for unstored grape canes (Gorena *et al*. 2014; Houille *et al*. 2015). Pterostilbene was not detected in any of the tested samples and pinosylvin was only detected in Muller Thurgau extracts from vineyard E (conventional vineyards) in small quantities (3.66 ± 0.42 mg/kg DM).

The highest content of *trans*-resveratrol was obtained from blue grape canes of Pinot Noir variety

Table 1. The conditions and the composition of the mobile phase gradient

| Time (min) | A | B | Conditions |
|---|---|---|---|
| 0 | 10 | 90 | 25°C |
| 5 | 20 | 80 | sample injection 20 µl |
| 35 | 50 | 50 | flow rate 1 ml/min |
| 40 | 95 | 5 | stop time 46 min |
| 45 | 95 | 5 | post time 5 min |

A – acetonitrile; B – demineralized water

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Table 2. *Trans*-resveratrol, *trans*-polydatin, total polyphenolic content and antioxidant capacity of the tested cane extracts

| Sample | Vineyard | *t*-Resveratrol (mg/kg of DM) | *t*-Polydatin (mg/kg of DM) | Total polyphenolic content (g GA/kg of DM) | Antioxidant activity ($\%_{inhibition}$) |
|---|---|---|---|---|---|
| Riesling | C | 16.89 ± 1.43 | 7.55 ± 0.67 | 6.34 ± 0.30 | 30.43 ± 0.74 |
| | D | 201.36 ± 2.40 | 14.47 ± 0.94 | 13.17 ± 0.34 | 53.60 ± 0.82 |
| | E | 44.79 ± 1.12 | 6.36 ± 0.24 | 11.68 ± 0.10 | 46.53 ± 0.44 |
| | G | 18.34 ± 0.98 | 23.81 ± 1.17 | 11.90 ± 0.33 | 49.67 ± 0.48 |
| | H | 20.85 ± 1.21 | 10.18 ± 0.59 | 13.14 ± 0.11 | 49.87 ± 0.11 |
| Müller Thurgau | A | 14.87 ± 1.09 | 18.40 ± 2.00 | 17.53 ± 0.29 | 64.00 ± 0.47 |
| | B | 14.55 ± 0.77 | 36.16 ± 1.23 | 6.30 ± 0.14 | 29.45 ± 0.59 |
| | D | 28.07 ± 1.11 | 38.69 ± 2.06 | 13.01 ± 0.34 | 53.60 ± 0.08 |
| | E | 23.77 ± 1.45 | 28.38 ± 0.12 | 16.12 ± 0.30 | 62.04 ± 0.19 |
| | F | 132.33 ± 3.71 | 4.92 ± 0.08 | 10.61 ± 0.14 | 45.74 ± 0.18 |
| | G | 198.08 ± 2.19 | 18.96 ± 0.77 | 8.38 ± 0.53 | 38.68 ± 0.82 |
| | H | 31.36 ± 0.83 | 16.63 ± 0.64 | 15.45 ± 0.10 | 56.34 ± 0.59 |
| Gewurztraminer | A | 40.44 ± 1.02 | 25.64 ± 1.17 | 8.55 ± 0.05 | 36.32 ± 0.64 |
| | C | 18.34 ± 0.69 | 11.65 ± 0.85 | 6.15 ± 0.19 | 30.24 ± 0.11 |
| | D | 243.72 ± 3.11 | 32.17 ± 1.12 | 11.89 ± 0.04 | 52.81 ± 0.56 |
| | E | 153.79 ± 2.14 | 7.28 ± 0.44 | 12.56 ± 0.65 | 50.65 ± 0.63 |
| | F | 39.28 ± 0.99 | 12.22 ± 0.63 | 14.56 ± 0.81 | 54.18 ± 0.97 |
| Dornfelder | D | 43.52 ± 1.25 | 8.57 ± 0.84 | 10.06 ± 0.15 | 43.78 ± 0.58 |
| | F | 12.76 ± 0.34 | 18.53 ± 1.15 | 6.50 ± 0.27 | 30.63 ± 0.37 |
| | H | 147.52 ± 2.22 | 27.45 ± 1.03 | 15.23 ± 0.35 | 59.88 ± 0.24 |
| Pinot Noir | A | 21.38 ± 1.87 | 28.17 ± 1.67 | 13.37 ± 0.26 | 54.38 ± 0.06 |
| | B | 16.62 ± 1.66 | 34.11 ± 1.46 | 18.57 ± 0.09 | 71.26 ± 0.78 |
| | C | 10.43 ± 0.57 | 10.43 ± 1.37 | 9.33 ± 0.14 | 40.84 ± 0.13 |
| | D | 51.7 ± 1.07 | 11.59 ± 0.87 | 14.39 ± 0.21 | 57.33 ± 0.25 |
| | E | 42.15 ± 1.93 | 20.69 ± 1.73 | 9.33 ± 0.23 | 39.66 ± 0.35 |
| | F | 37.69 ± 1.99 | 23.09 ± 1.79 | 8.87 ± 0.16 | 40.05 ± 0.61 |
| | G | 37.96 ± 1.57 | 23.39 ± 1.37 | 19.15 ± 0.42 | 71.46 ± 1.41 |
| | H | 252.79 ± 3.63 | 25.93 ± 3.43 | 19.15 ± 0.41 | 68.32 ± 0.33 |
| Pinot Gris | A | 16.93 ± 1.70 | 34.14 ± 1.05 | 10.33 ± 0.15 | 44.96 ± 0.36 |
| | B | 39.79 ± 0.97 | 48.73 ± 1.12 | 18.54 ± 0.12 | 70.48 ± 0.76 |
| | C | 14.68 ± 0.44 | 8.92 ± 0.77 | 20.44 ± 0.16 | 65.96 ± 0.07 |
| | D | 35.82 ± 1.11 | 6.35 ± 0.02 | 11.55 ± 0.06 | 49.28 ± 0.22 |
| | E | 58.87 ± 1.00 | 29.73 ± 1.36 | 10.71 ± 0.38 | 42.41 ± 0.17 |
| | F | 105.76 ± 2.35 | 36.22 ± 1.10 | 10.24 ± 0.61 | 44.37 ± 0.47 |
| | G | 47.85 ± 1.04 | 21.37 ± 0.55 | 16.48 ± 0.34 | 59.09 ± 0.72 |
| Hibernal | B | 21.20 ± 0.35 | 35.91 ± 0.98 | 7.04 ± 0.08 | 31.81 ± 0.35 |
| | F | 21.30 ± 0.10 | 31.75 ± 0.20 | 7.42 ± 0.37 | 34.16 ± 0.71 |
| Solaris | A | 8.31 ± 0.20 | 23.93 ± 0.66 | 13.45 ± 0.40 | 49.47 ± 0.11 |
| | B | 14.30 ± 0.09 | 34.20 ± 0.15 | 14.84 ± 0.17 | 59.29 ± 0.85 |
| | D | 19.52 ± 0.24 | 27.46 ± 0.52 | 13.88 ± 0.24 | 54.97 ± 0.49 |
| St. Laurent | C | 19.32 ± 1.00 | 4.24 ± 0.37 | 9.36 ± 0.10 | 38.48 ± 1.07 |
| | G | 52.67 ± 1.07 | 19.09 ± 0.99 | 12.62 ± 0.51 | 51.04 ± 0.89 |
| Neronet | F | 4.47 ± 0.21 | 24.79 ± 1.32 | 11.22 ± 0.39 | 45.15 ± 0.09 |
| Zweigeltrebe | G | 30.35 ± 1.11 | 21.63 ± 0.85 | 11.80 ± 0.21 | 48.69 ± 0.33 |

A, B, C – organic vineyards; D, E, F, G, H – conventional vineyards

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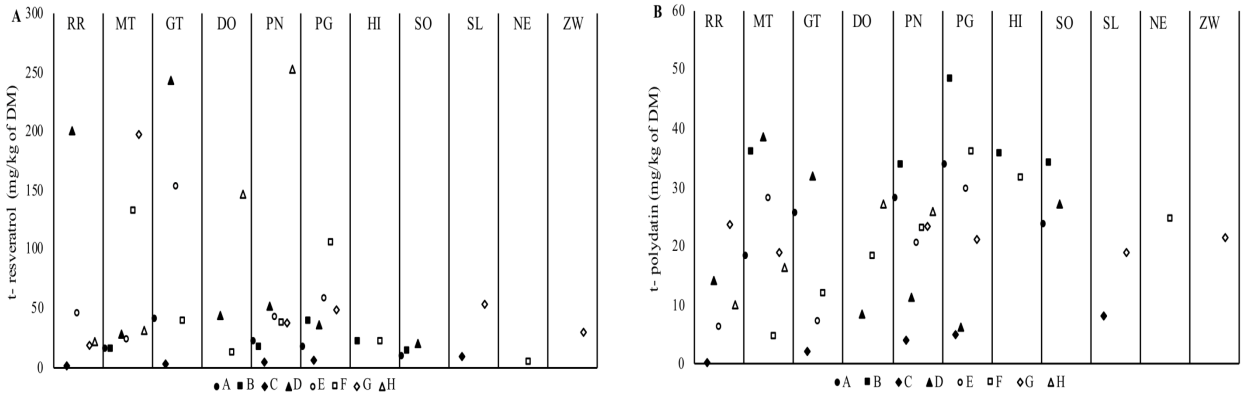


Figure 2. (**A**) *Trans*-resveratrol and (**B**) *trans*-polydatin content of cane extracts from obtained from organic vineyards (A-C) and from conventional vineyards (D-H)

RR-Rheinriesling; MT–M**ü**ller Thurgau; GT–Gewurztraminer; DO–Dornfelder; PN–Pinot Noir; PG–Pinot Gris; HI–Hibernal; SO–Solaris; SL–St. Laurent; NE–Neronet; ZW–Zweigeltrebe

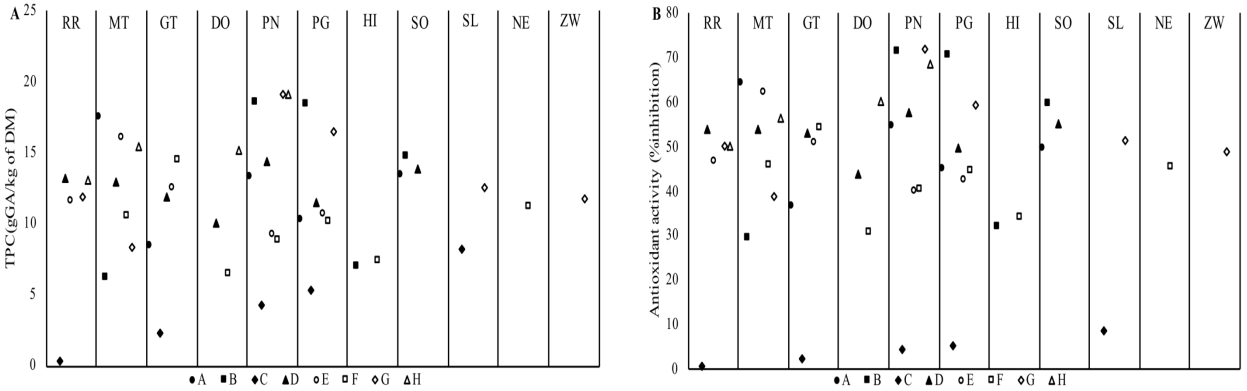


Figure 3. (**A**) total polyphenolic content (TPC) and (**B**) antioxidant activity of cane extracts from obtained from organic vineyards (A-C) and from conventional vineyards (D-H)

RR-Rheinriesling; MT–M**ü**ller Thurgau; GT–Gewurztraminer; DO–Dornfelder; PN–Pinot Noir; PG–Pinot Gris; HI–Hibernal; SO–Solaris; SL–St. Laurent; NE–Neronet; ZW–Zweigeltrebe

from vineyard H (Table 2 and Figure 2). The differences among the same variety are understandable since stilbenoid concentrations depend on environmental and microclimatic conditions, plant disease or soil type (Sellappan *et al*. 2002). A study by Tříska *et al*. (2017) showed that the difference in stilbenes among most varieties was varietal, regardless of the vineyard location. Only two interspecific hybrids (Laurot and Hibernal) differed significantly and their stilbene content was dependent on the area of cultivation.

The highest amount of *trans*-resveratrol among the grape varieties was found in extracts from Gewurztraminer (white variety) canes, although the differences between the vine varieties were not significant at a 5% level of probability.

There were no notable differences between the *trans*-polydatin levels of the sample extracts grown organically (24.15 ± 13.33 mg/kg of dry matter) or conventionally (20.40 ± 9.32 mg/kg of dry matter). However, the *trans*-resveratrol levels in the cane extracts from organic vineyards (19.20 ± 9.19 mg/kg DM) were statistically significantly ($P < 0.05$) lower in comparison to the conventional ones (65.91 ± 64.25 mg/kg DM) (Table 2). Similarly, Vian *et al*. (2006) observed higher anthocyanin levels in berry skins of grapes grown conventionally. This might be explained by the response of the vine to certain synthetic chemical pesticides used by conventional vineyards, which show similarities with the vines reaction to fungal attack (Iriti *et al*. 2004). Henceforth, the accumulation of certain types of stilbenoids, such as resveratrol, could also be a response to chemical stress caused by spraying pesticides. In the case of vine canes, pesticides are typically not used by winemakers in winter

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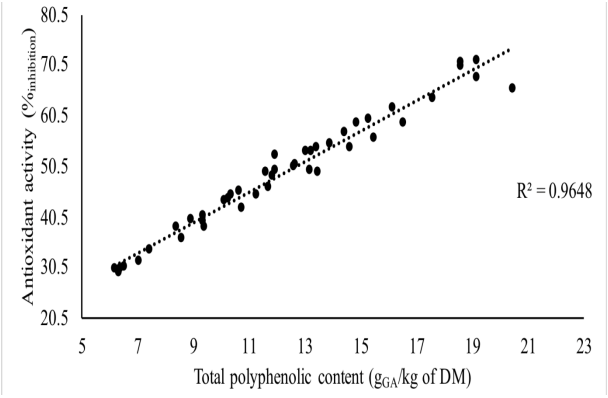


Figure 4. The relationship between total polyphenolic content and antioxidant activity of the tested cane extracts

when the canes are pruned. Our results suggest that conventionally grown vines might be more stressed compared to organically grown ones, which are used to a smaller level of protection during the growing season.

As expected, there was a strong correlation (0.96) between the antioxidant capacity and total polyphenolic content of the tested samples (Figure 4). Similar correlations were also determined in other published studies (Tabart *et al*. 2007, Karacabey et al. 2010).

The antioxidant activity of the samples ranged between 29.46–71.46% inhibition and the total polyphenolic content varied between 6.30–20.44 mg GA/g of DM. These results are comparable to already published results (Cetin *et al*. 2011). The lowest antioxidant capacity was observed in a Muller Thurgau from vineyard B (Table 2 and Figure 3), whereas Pinot Noir from vineyard G exhibited the highest antioxidant capacity.

Our results showed that the antioxidant activity and total polyphenolic content were not dependent on the agro-technical interventions (e.g. the use of chemical pesticides during vegetative growth). Comparably, Mulero *et al*. (2010) reported that the antioxidant activity and polyphenolic content were similar in organic wine, in conventional wine and in grape skins.

## CONCLUSIONS

Our result revealed that the agro-technical approach did not have an effect on the total polyphenolic content and antioxidant activity of the studied grape cane extracts. The canes from the grapevines grown organically had lower levels of *trans*-resveratrol while the *trans*-polydatin levels did not differ in the two studied groups. Still, cane extracts are an important source of polyphenolic antioxidants and a better understanding of the antioxidant and polyphenolic content could help with their future reuse on an industrial scale.

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