POLYPHENOLS AND ANTIOXIDANT ACTIVITY OF DIFFERENT VINEGRAPE LEAVES

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Abstract: The volume of the grape waste produced directly relates to the volume of grapes pressed and in turn depends on the specific climatic conditions of the relevant vintage. The aim of this work is to evaluate vinegrape leaves as a potential source of natural antioxidants – polyphenols for their possible use as dietary supplement or food antioxidants. To this purpose antioxidant activity and contents of phenolic compounds of the leaves extracts of five grape varieties of *Vitis vinifera* L (Vranac, Prokupac, Merlot, Gamay and Italian Rizling), grown in southern Serbia were investigated. The analysis show high content of polyphenols reflecting their high antioxidant activity (R^2 = 0.9819, $p<0.01$). According to the obtained results, the leaves extracts, can be considered rich natural source of phenolic compounds with good antioxidant properties.

Key words: vinegrape leaves, polyphenols, antioxidant activity, tradicional medicine

Introduction

Grapes are the world`s largest fruit crop with more than 60 million metric tons (67.5 x 106 tons during 2009) produced annually (International Organization of Vine and Wine - OIV, 2010, www.oiv.int).. Approximately 71% of world grape production is used for wine, 27% as fresh fruit, and 2% as dried fruit.

However wine making leads to the generation of large quantities of grape waste (around 5-9 million tons per year, worldwide), which considerably increase the chemical oxygen demand (COD) and the biochemical oxygen demand (BOD) due to a high pollution load (high content of organic substance such as sugars, tannins, polyphenols, polyalcohols, pectins and lipids) with detrimental effects on the flora and fauna of dischanged zones (Baydar et al., 2004; Oreopoulou and Tzia, 2007).

Serbia constitutes one of the middle grape and wine producers, with a grape production was 330 000 tonnes of which remains about 7 000 tons of grape by-products (skins, seeds, leaves and stems) which is only used for the production of alcohol, and most of ending up in landfills as organic waste (Statistical Serbian Office – RZS, 2012, www.stat.gov.rs).

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Polyphenols of vine grapes and wines are part of complex mixtures of bioactive compounds that may react with radicals by different mechanisms and often interact synergistically or inhibitory. The species and varieties of the genus *Vitis* probably differ in their ability to synthesize these compounds (Fenglin et al., 2004; Saucier and Waterhouse, 1999).

In Europe, the leaves of *Vitis vinifera* are documented in the literature of traditional medicine for their astringent and homeostatic properties where they are utilized in the treatment of diarrhea, bleeding, hemorrhoids, varicose veins and other circulatory diseases (Balík et al., 2008; Bombardelli and Morazzoni, 1995).

The aim of this study was to determine and compare phenolic composition and antioxidant activity of different varieties of *Vitis vinifera* leaves (Vranac, Prokupac, Merlot, Gamay and Italian Rizling), collected in south Serbia wine region.

Material and methods

Chemicals

Methanol, acetonitrile and formic acid of HPLC-grade were obtained from Merck (Darmstadt, Germany). 2,2`- diphenyl - 1 - picrylhydrazyl (DPPH) free radical, gallic acid, caffeic acid, quercetin, were supplied from Sigma Chemical Co. (St. Louis, MO, USA). The used reagents were of analytical quality.

Plant material and extraction

The study materials were leaves collected from five wine varieties of Vitis *vinifera L.*, mostly growing in South Serbia, 2011. The leaves were from vines of four red varieties Vranac, Prokupac, Merlot and Gamay and one was of white variety Italian Rizling. Immediately after harvesting, leaves were washed and dried at 60^0C.

The grape leaves were crushed in a grinder, extrated with methanol/water/formic acid (70/29.9/0.1, v/v/v%) solution in a magnetic stirrer and then 24 h centrifuged at room temperature (Tehnica LC-320, Železniki, Slovenia) at 4000 rpm for 10 min.

Determination of phenolic compounds

Total phenols, hydroxycinnamoyl tartaric esters and flavonols in grape leaves extracts were determined using by UV/VIS Agilent 8453 spectrophotometer (Mazza et al., 1999; Radovanović et al., 2010). The absorbance (A) at 280 nm was used to estimate phenolics (gallic acid was used as standard), A_{320nm} was used to estimate hydroxycinnamoyl tartaric acids (caffeic acid was used as standard) and A_{360nm} was used to estimate flavonols (quercetin was used as standard).

Determination of antioxidant activity

The antioxidant activity of leaves extracts was analyzed by using DPPH assay (Munoz-Espada et al., 2004). Antioxidant assay are based on measurement of the loss of DPPH color by change of absorbance at 517 nm caused by the reaction of DPPH with tested sample. Antioxidant activity (AA) in percent of each extracts was calculated from the decrease of absorbance according to the relationship:

$$AA\ (\%) = (1 - A_{sample} - A_{blank}/A_{control}) \text{ x } 100$$

Where $A_{control}$ is the absorbance of control reaction, A_{blank} is the absorbance of dilution extrat and A_{sample} is the absorbance of the dilution extract with DPPH radical.

The radical scavenging activity (%) was plotted against the leaves extract concentration (mgg^{-1}) to determine the concentration of extract that reduces activity by 50% (EC_{50}).

Statistical analysis

Three analytical replictes were carried out on each sample. Measurements were averaged, and results are given as mean ± standard deviation (SD).

Results and discussion

The phenolic composition in grape varies widely and is usually determined by several factors, such as: the variety of grape and conditions under which they was grown: soil, geographical location, light exposure, temperature, sun exposure of the clusters and weather (Xu et al., 2011).

Also, the amount and types of phenol compounds present in a particular grape leaves can vary and is greatly influenced by the extraction process, as well as the source, variety and storage of the used grape waste.

The content of total phenols, hydroxycinnamoyl tartaric esters and flavonols and radical scavenging activity in five vine leaves extracts are presented in Table 1:

Tabela 1.Sadržaj ukupnih fenola, estara hidroksicimetnih i vinskih kiselina, flavonola i antioksidativna aktivnost ekstrakata vinskih listova, EC_{50}

Table 1. Content of total phenols, hydroxycinnamoyl tartaric esters, flavonols and antioxidant activity of vine leaves extracts, EC_{50}

Variety of vine leaves	Total phenols *($mg_{GAEa}\ g^{-1}$ ± SD)*	Hydroxycinnamoyl Tartaric esters *($mg_{CAEb}\ g^{-1}$ ± SD)*	Flavanols *($mg_{QEc}\ g^{-1}$ ± SD)*	Antioxidant activity, EC50 *(mL g^{-1}± SD)*
Italic Riesling	40.78 ± 1.78	12.24 ± 0.62	15.44 ± 0.88	6.92 ± 0.56
Gamay	26.12 ± 0.98	9.06 ± 0.73	10.12 ± 0.67	15.76 ± 0.93
Merlot	39.07 ± 1.12	11.92 ± 0.45	15.28 ± 0.95	8.28 ± 0.67
Vranac	42.62 ± 2.25	12.73 ± 0.09	15.84 ± 0.68	6.57 ± 0.45
Prokupac	18.32 ± 0.56	12.24 ± 0.62	15.44 ± 0.88	23.74 ± 1.01

[a] Gallic acid equivalent; [b] Caffeic acid equivalent; [c] Quercetin equivalent

The results are shown high concentration of total phenols in all investigation vine leaves extracts of varieties *Vitis vinifera* L., significantly more than in berries, which is in agreement with literature data (Balík et al., 2008; Fernandez-Pachon et al., 2004).

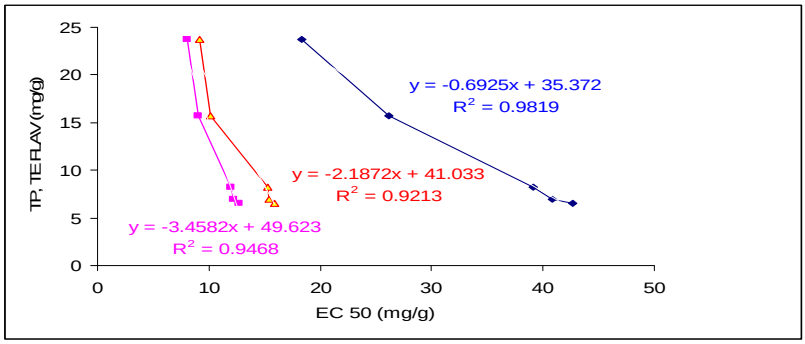
Total phenol content was ranged from 18.32 mg $_{GAE}g^{-1}$ in leaves extract of Prokupac variety to 42.62 $mg_{GAE}g^{-1}$ in Vranac variety; hydroxycinnamoyl tartaric esters ranged from 9.06 $mg_{CAE}g^{-1}$ in Gamay variety to 12.73 $mg_{CAE}g^{-1}$ in Vranac variety and flavonols ranged from 9.11 $mg_{QE}\ g^{-1}$ in Prokupac variety to 15.84 $mg_{QE}g^{-1}$ in Vranac variety, which are higher concentrations than found in leaves extracts of some others

Vitis vinifera L. varieties (Gudej and Tomczyk, 2004; Fenglin et al., 2004; Katalinić et al., 2009).

The antioxidant activity of the tested grape leave extracts was estimated by their ability to scavenge the stable free radical, DPPH· method. The EC_{50} values reflect the equivalent concentration of antioxidant sample able to scavenge 50% of the DPPH-radical. It should be noted that as higher antioxidant activity of the sample is, the lower EC_{50} value is (Table 1).

The leaves of Vranac variety, with the lowest EC_{50} value, indicted the highest DPPH savenging activity, followed by leaves extracts of I.Riesling, Merlot, Gamay and Prokupac varieties (Radovanović et al., 2012; Saucier and Waterhouse, 1999).

The correlations between those phenolic compounds and radical scavenging activity for all investigated vine leaves extracts are presented in Figure 1:



Graf.1. Korelacija između TP (ukupnih fenola), TE (estara hidroksicimetnih i vinskih kiselina), FLAV (flavonola) i antioksidativne aktivnosti, EC_{50} estrakata vinskih listova

Graph.1. Corelation between TP (Total phenols $mg_{GA}E/g$ DW), TE (Hydroxycinnamoyl tartaric esters mg $_{CAE}g$ DW), FLAV (Flavonols mg_{QE}/g DW) and antioxidant activity, EC50 (mg g^{-1}) of vine leaves extracts

The highest correlation occured between anttioxidant activity and total phenols (R^2= 0.9819, $p<0.01$). Also was found significant correlation between total hydroxycinnamoyl tartaric esters followed by total flavonols content and antioxidant activity (R^2= 0.9486 and 0.9213, $p<0.01$, respectively).

The differences in correlation between some polyphenol classes and antioxidant activity can be appearing due to different individual phenolic compounds in vine leaves extracts, which alone and combined with other phenols (synergic properties), shown relatively wide range of the correlation with antioxidant activity.

Conclusions

The contents of the polyphenol compounds and the antioxidant activity were investigated in vine leaves extracts of five *Vitis vinifera* L. varieties. According to the obtained results, *Vitis vinifera* leaves extracts, can be considered rich natural source of polyphenolic compounds with good antioxidant properties.

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