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Table of contents

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In vitro biological effects of clonal red wines

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Abstract: This study aimed to determine the phenolic compound content, *in vitro* antioxidative potential, and cytotoxic effects of four red wine samples: a commercial (V) and three clonal wines (V1, V2, and V3). LC/MS-MS, cyclic voltammetry, and MTT assay techniques were employed for this purpose. Results revealed that all wines were rich in phenolic compounds. Clonal wines outperformed the commercial ones in most phenolic compounds (except myricetin). Notably, V2 and V3 showed the highest levels of gallic acid, catechin, and epicatechin. Among them, V3 exhibited superior antioxidative activity. The MTT assay demonstrated stronger cytotoxic effects of the wine samples on pancreas (Bx-PC3) and colon (HT29) carcinoma cells (47% to 16% and 27% to 7% compared to control, respectively) than on the normal lung fibroblasts (MRC-5) cell line (106% to 77%). It can be concluded that HT29 cells were more sensitive than Bx-PC3 cells. Finally, both clonal and commercial wines serve as valuable sources of polyphenolic compounds, which could have a significant role in preventing cancer and diseases related to oxidative stress.

Keywords: red wine, polyphenols, LC-MS/MS, cyclic voltammetry, cytotoxic activity

1. Introduction

Red wine, an enchanting elixir crafted from dark-colored grape varieties, holds a rich history in human culture, spanning millennia. Within this exquisite beverage lie wine polyphenols, a diverse group of compounds found in grapes and thus, infused into wine. These compounds play pivotal roles in shaping the wine's color, flavor, mouthfeel, and potential health benefits. Major categories of wine polyphenols encompass flavonoids (like anthocyanins and flavonols), phenolic acids, stilbenes (including the renowned resveratrol), and tannins [1]. Their composition varies based on grape variety, winemaking techniques, and aging processes. An important facet of these polyphenols is

their antioxidant function in wine, which aids in countering free radicals and mitigating oxidative stress [2]. This contributes to wine's potential health advantages. Despite advancements in cancer therapies, cancer remains a leading cause of death with limited lifespan extensions and significant side effects. This underscores the urgency of exploring effective cancer prevention strategies, where natural products like red wine have gained attention for their potential in cancer prevention [3]. Research focuses on wine polyphenols, particularly resveratrol, to comprehend their impact on carcinogenesis stages and anticancer efficacy.

The objective of this paper was to examine phenolic content and *in vitro* antioxidative activity of commercial and clonal Vranac red wine, vintage 2010. Furthermore, this paper analyses cytotoxic activity of the mentioned samples against normal lung fibroblasts (MRC5), as well as two carcinoma cell lines, pancreas and colon (Bx-PC3 and HT29, respectively).

2. Materials and methods

The samples of Montenegrin Vranac wines, commercial wine (V) and three clonal wines (V1, V2 and V3) vintage 2010, were analysed. The content of nine phenolic compounds was determined by liquid chromatography − tandem mass spectrometry (LC–MS/MS) using LC system (Waters Acquity UPLC H-Class; WAT176015007; Milford, MA USA) with ultraviolet detector (Waters 2998 PDA), coupled to MS (Waters TQ, WAT-176001263) [4]. Quantification of phenolic compounds was done using appropriate standards. *In vitro* antioxidant capacity was assessed by cyclic voltammetry using a CHI760 B instrument (CHInstruments, Austin, Texas, USA) [5]. Cytotoxic activity of analysed wines on MRC5, Bx-PC3 and HT29 cell lines was determined using MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) tetrazolium reduction assay after 72h of treatment with different wine volume percentage (2.5, 5 and 10%). Absorbance was measured in an ELISA plate reader (Victor2 1420 Multilabel counter, Wallac, Turku, Finland), at a wavelength of 550 nm, and the obtained results were expressed as percentage of control.

2. Results and discussion

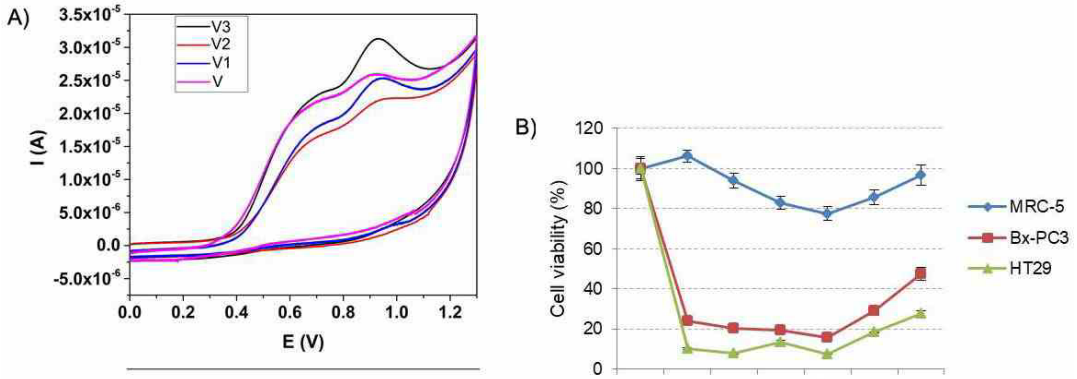
The primary phenolic compound present in all examined wines is gallic acid. As demonstrated in Table 1, the samples labeled as V2 and V3 exhibit the highest concentrations of gallic acid. The observation indicates that, except for myricetin, clonal wines outperform commercial wine as a source of analyzed phenolic compounds. Among these, V2 and V3 samples exhibit the highest concentrations of gallic acid, catechin, and epicatechin. Notably, the cumulative resveratrol content in V1 and V3 wines is nearly twice that found in the commercial wine, as indicated in Table 1.

Table 1. Content of phenolic compounds in analyzed wine samples determined by LC-MS/MS

Phenolic compound	V	V1	V2	V3
	Concentration (mg/L)			
Gallic acid	16,41 ± 0,41 ^{A}	20,81 ± 0,38 ^{B}	26,48 ± 0,37 ^{C}	25,87 ± 0,41 ^{C}
Catechin	7,41 ± 0,22 ^{A}	9,27 ± 0,14 ^{B}	12,74 ± 0,10 ^{D}	11,24 ± 0,15 ^{C}
Epicatechin	2,96 ± 0,04 ^{A}	5,69 ± 0,15 ^{B}	7,09 ± 0,15 ^{C}	7,16 ± 0,15 ^{C}
trans-Resveratrol	0,46 ± 0,02 ^{A}	0,74 ± 0,05 ^{B}	0,65 ± 0,02 ^{B}	0,51 ± 0,04 ^{A}
cis-Resveratrol	0,11 ± 0,01 ^{A}	0,36 ± 0,01 ^{C}	0,43 ± 0,02 ^{D}	0,26 ± 0,02 ^{B}
trans-Piceid	2,08 ± 0,07 ^{A}	4,36 ± 0,01 ^{C}	3,11 ± 0,02 ^{B}	3,04 ± 0,15 ^{B}
cis-Piceid	1,84 ± 0,04 ^{A}	3,31 ± 0,03 ^{C}	2,89 ± 0,02 ^{B}	4,81 ± 0,15 ^{D}
Myricetin	1,32 ± 0,05 ^{C}	0,58 ± 0,03 ^{A}	0,92 ± 0,05 ^{B}	0,99 ± 0,02 ^{B}
Quercetin	0,82 ± 0,03 ^{C}	0,27 ± 0,01 ^{A}	0,40 ± 0,03 ^{B}	1,06 ± 0,03 ^{D}

Comm – commercial wine; CI/II/III – clonal wines. All values are represented as mean ± SD (in triplicate). Different letters within each column show statistically significant differences at the level of $p < 0.05$, according to HSD Tukey's test.

In Figure 1(A), the cyclic voltammograms depict the characteristics of the examined wine samples. The cyclic voltammogram of the commercial wine sample displays two distinct anodic peaks occurring at approximately +0.67 and +0.92 V, with a Q600 factor of 2.871. The voltammograms of V1 and V3 wines show an increase in the oxidation current of the second anodic peak with a slight displacement of the peak toward more positive values.



Sample	Q_{600}	ACI (%)
V	2.871	68.15
V1	2.671	63.40
V2	0.787	18.68
V3	4.217	100.00

Figure 1. A) Cyclic voltammograms obtained for the analysed wine samples (V, V1, V2 and V3) and **B)** Cell viability after treatment with different wine volume ratio of commercial wine (V) and clonal wine (V3).

For sample V1, a slight decrease in both the Q600 factor and the ACI index is noticeable. In contrast, the voltammogram of sample V3 reveals a notable increase in

these two parameters, indicating an enhanced antioxidant activity when compared to samples V and V1.

Cytotoxic effect of V and V3 wines was the lowest on MRC-5 cells, ranging between 106% to 77% compared to control cells (Figure 1B). Conversely, the cytotoxic impact on the two cancer cell lines demonstrated higher percentages, reaching 47% to 16% on Bx-PC3, and 27% to 7% on HT29 cells. Notably, the HT29 cells displayed greater sensitivity when compared to Bx-PC3 cells. Additionally, as the volume percentage of V3 wine increased, a corresponding rise in its cytotoxic effect on cancer cells was observed, suggesting a dose-dependent relationship between the volume percentage and cytotoxicity.

3. Conclusions

In summary, both commercial and clonal wines are rich sources of polyphenols that exhibit antioxidative properties *in vitro*. A particularly important result is that the cytotoxic effect of analysed wine on normal cells is far less than on cancer cell lines. The presence of powerful antioxidants in this natural product holds promise for developing effective and low-risk strategies for cancer prevention. By understanding the mechanisms and potential benefits of red wine polyphenols, researchers aim to pave the way for innovative and safer approaches to combatting cancer and improving patient outcomes.

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