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# KEMIJSKA KARAKTERIZACIJA I BIOLOŠKI UČINCI POLIFENOLA U VINU

DOKTORSKI RAD

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# CHEMICAL CHARACTERIZATION AND BIOLOGICAL EFFECTS OF POLYPHENOLS IN WINES

DOCTORAL DISSERTATION

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# SAŽETAK

Vina sadrže različite biološki aktivne polifenolne spojeve za koje je utvrđeno da imaju brojne pozitivne učinke na zdravlje ljudi. Polifenolni profil vina često se koristi za procjenu njihove kvalitete i autentičnosti. U okviru doktorskog rada provedena je usporedna kemijska karakterizacija polifenola u dvadeset i pet komercijalnih crnih i bijelih vina proizvedenih u različitim hrvatskim vinogradarskim regijama od osam crnih i devet bijelih sorti grožđa. U tu je svrhu razvijena i validirana metoda visokoučinske tekućinske kromatografije (HPLC-DAD) s direktnim injektiranjem vina. Analizirane su dvadeset i četiri polifenolne sastavnice vina te razvrstane u pet skupina na temelju njihove strukture: fenolne kiseline, flavan-3-ole, antocijane, flavonoide i stilbene. Crna vina sadržavala su značajno veće koncentracije polifenola od bijelih vina te je kod njih uočena velika varijabilnost u sastavu. Specifičnim polifenolnim sastavom i sadržajem posebice su se istaknula vina proizvedena od autohtonih hrvatskih sorti plavac mali i babić. Primjenom multivarijatne analize ustanovljene su sličnosti i razlike u polifenolnim profilima hrvatskih monosortnih vina te detektirani važni diferencijatori. Glavni predstavnici polifenolnih sastavnica koji su bili značajno zastupljeni u istraživanim vinima pokazali su snažno antioksidacijsko djelovanje te sposobnost inhibicije aktivnosti enzima Src-tirozin-kinaze, α-glukozidaze i acetilkolinesteraze. Provedeno istraživanje pružilo je nove spoznaje o polifenolnim profilima hrvatskih vina, dajući doprinos njihovoj sortnoj diferencijaciji, autentifikaciji i kontroli kvalitete. Ustanovljeno je da su analizirana crna vina, prvenstveno ona dobivena iz hrvatskih autohtonih crnih sorti grožđa, vrijedan izvor polifenola sa snažnim antioksidacijskim svojstvima i sposobnošću inhibicije enzima koji predstavljaju važne terapijske mete u prevenciji i liječenju nekih kroničnih bolesti povezanih sa starenjem.



**Ključne riječi:** hrvatska vina, polifenoli, HPLC-DAD, PCA, antioksidacijsko djelovanje, inhibicija enzima, Src-tirozin-kinaza, α-glukozidaza, acetilkolinesteraza

# SUMMARY


## Introduction

Wine is the most traditional and popular alcoholic beverage consumed worldwide. A highly competitive market and consumer expectations force wineries to produce quality wines. Wine quality is determined by various factors, such as the type of grape varieties, geographical, climatic and pedological factors, viticultural practices, winemaking techniques, and aging conditions. Wine quality evaluation is based on both chemical and sensory analyses. The sensory characteristics of wines are significantly influenced by the phenolic profile, which is often used to evaluate the quality and authenticity of wines (Merkytė et al., 2020; Garrido and Borges, 2013). Phenolic compounds are a diverse group of highly bioactive substances present in grapes and wine that can be formed and transformed during the winemaking process. They are crucial for color, flavor, and taste attributes, such as mouthfeel and astringency of wines, especially red wines. The most important phenolic compounds in red wines include anthocyanins and their derivatives, which give the wine its color, flavonols, which are involved in the copigmentation process, and tannins, which are responsible for mouthfeel and astringency (Casassa et al., 2022). The technological process to which the grapes are subjected has a significant impact on the phenolic content and composition of the wines. Since red wines are in contact with all parts of the grapes during maceration, they have a higher polyphenol concentration than white wines, whose polyphenol content comes mainly from the pulp (Gutiérrez-Escobar et al., 2021). During grape treatment and ripening, numerous chemical changes can take place, creating new compounds and/or degrading others. These changes can affect the polyphenolic profiles of wines. The phenolic composition of wines, which determines their organoleptic properties and provides information about their primary characteristics, can be used as a fingerprint to distinguish them according to their origin in terms of region, grape variety, and vintage (Garrido and Borges, 2013). Phenolic compounds most commonly used to evaluate wine quality and authenticity include phenolic acids, flavonoids, tannins, and stilbenes (Merkytė et al., 2020). Polyphenols are not only closely related to wine quality but have also been shown to have health-promoting properties. Numerous studies have shown that moderate consumption of wine, especially red wine, is healthy as it protects against various chronic diseases such as cardiovascular and neurological disorders, metabolic syndrome, cognitive disorders, depression, and some cancers (Gutiérrez-

Escobar et al., 2021; Weaver et al., 2021; Radeka et al., 2022; Krittanawong et al., 2022). The positive role of red wine in oxidative stress (Pavlidou et al., 2018) and in promoting desirable gut bacteria leading to a healthier human body system has also been highlighted (Nash et al., 2018; Nemzer et al., 2022).

Viticulture has a long tradition in Croatia, and there are an estimated 140 autochthonous grape varieties. Although the area under cultivation of foreign varieties has steadily increased over the last 50 years, 14 of the 30 most important varieties in Croatia are still indigenous (Žurga et al., 2019). The geographical position of Croatia is a meeting of continental climate in the eastern and central parts of the country and Mediterranean climate in the southern, coastal areas (Leder et al., 2021). In this context, Croatia is divided into four wine-growing regions (Zakon o vinu (*Law on wine*), 2019). The importance of determining the authenticity and commercial value of a wine, often related to its geographical origin, is recognized in Croatia, where viticulture and wine production play a significant economic role (Leder et al., 2021). In the last two decades, several studies have been conducted to investigate the polyphenolic composition of Croatian wines (Rastija et al., 2009; Šeruga et al., 2011; Lukić et al., 2019). The influence of some viticultural and winemaking conditions on the content and composition of various polyphenols in Croatian wines has also been studied (Jagatić Korenika et al., 2021; Mucalo et al., 2019; Budić-Leto et al., 2008; Budić-Leto et al., 2006; Bubola et al., 2020; Osrečak et al., 2015). In addition, the antioxidant properties of some Croatian wines were studied. A very high correlation was found between antioxidant activity and total polyphenol content (Radeka et al., 2022; Šeruga et al., 2011; Maletić et al., 2009) and consequently better antioxidant properties of the studied red wines than white wines (Radeka et al., 2022; Katalinić et al., 2006; Vinković Vrček et al., 2011). It is worth mentioning that the health benefits of phenolic compounds from wine are often attributed precisely to their antioxidant activity (Radeka et al., 2022; Visioli et al., 2020). Compared to other European and world wines, Croatian wines, especially autochthonous ones, have not been sufficiently researched in terms of the composition of phenolic constituents and their biological properties. Therefore, the main objective of this study was to investigate the phenolic profile of a wide range of wines from all Croatian wine regions and to evaluate possible differences based on the detected polyphenols and multivariate analyses. Twenty-five commercial wines from eight red and nine white grape varieties were studied, with special attention to Croatian autochthonous wines. In addition, the antioxidant properties of the most representative phenolic constituents were evaluated as well as their

ability to inhibit the activity of certain enzymes that play an important role in the development of aging-related chronic diseases and carcinogenesis.

## Methods

The polyphenol content of Croatian wines was determined by high-performance liquid chromatography (HPLC). Wines of the same 2007 vintage coming from different Croatian wine regions have been sampled, filtered, and directly injected in the chromatography system. A total of twenty-four polyphenols were analyzed and classified into five groups based on their structure: phenolic acids, flavan-3-ols, anthocyanins, flavonoids, and stilbenes. Validation of the method used for analysis of phenolic acids, flavan-3-ols, flavonoids and stilbenes was performed according to the recommendations of the guideline ICH Q2 (ICH Harmonised Tripartite Guideline Q2 (R1), 2005). The HPLC analysis of anthocyanins was performed according to the Berente et al. (2000) (Berente et al., 2000). Principal component analysis (PCA) was performed for each type of wine to easily correlate and to understand the relationship between the chemical properties and the wine samples.

Total antioxidant efficiency of the most representative phenolic constituents was determined by a combination of different chemical tests. The ability to scavenge radicals was determined by DPPH and nitric oxide radical scavenging assays, the reducing power was evaluated by ferric reducing antioxidant power assay, while lipid peroxidation assay was used to determine polyphenol ability to prevent lipid peroxidation. The test methods followed have previously been described in the literature (Vladimir-Knežević et al., 2011; Patel et al., 2010; Oyaizu, 1986; Houghton et al., 1995), but were slightly modified during the testing.

The inhibitory effect of the investigated polyphenols on Src kinase activity was determined by the LANCE® Ultra test (Jelić et al., 2016). Determination of α-glucosidase enzyme inhibition rate by the selected polyphenolic compounds was performed in microtiter plates according to the slightly modified method previously described by Bljajić et al. (2017) (Bljajić et al., 2017), with minor modifications. The ability to inhibit the acetylcholinesterase activity was tested according to the procedure described by Conforti et al. (2007) (Conforti et al., 2007), with minor modifications.

## Results

The content of twenty-four different phenolic compounds in selected Croatian wines was determined by the HPLC-DAD methods and divided into five classes based on their structure. The red wines contained the highest concentrations of phenolics, while the white wines had lower values. Three hydroxybenzoic acids and five hydroxycinnamic acids were identified in all red wines studied, while some white wines did not contain syringic acid, *o*-coumaric acid, or chlorogenic acid. Gallic acid was the most abundant phenolic acid in the red wines; its content ranged from 11,8 mg/L to 90,3 mg/L. The red wines also contained significant amounts of syringic acid and hydroxycinnamic acids, such as *p*-coumaric acid and caffeic acid, whose concentrations ranged from 3,0 mg/L to 21,8 mg/L. In the wines studied, the proportion of the above-mentioned acids varies, which indicates their importance for varietal differentiation of Croatian wines. Plavac mali and Babić wines, produced from the autochthonous Croatian grape varieties of the same name, contained the highest levels of phenolic acids (113,0-139,7 mg/L), with gallic acid being the most representative (72,5-90,3 mg/L). As far as we are aware, this study is the first to provide detailed polyphenol composition in Babić wine. Teran is an autochthonous variety widely grown on the Istrian Peninsula. The tested sample from Croatian Istria contained 77,9 mg/L phenolic acids, of which 53,0 mg/L was gallic acid. Three wine samples coming from Dalmatian Hinterland produced from non-native grape varieties (Cabernet Sauvignon, Merlot and Syrah) contained highest proportions of *p*-hydroxybenzoic acid (8,0-11,2 mg/L), syringic acid (6,9-9,2 mg/L), chlorogenic acid (0,6-1,0 mg/L) and *o*-coumaric acid (0,5-0,6 mg/L).

Istrian Malvasia is an autochthonous Croatian white grape variety grown mainly on the Istrian Peninsula. The percentage of quantified phenolic acids in the sample of Istrian Malvasia from western Istria was 6,3 mg/L, by dominated of *p*-hydroxybenzoic acid, *p*-coumaric acid and caffeic acid. Malvasia Bianca Lunga (Maraština) sample from southern Dalmatia is characterized by a low content of hydroxycinnamic acids and a higher content of hydroxybenzoic acids, especially gallic acid (2,3 mg/mL). Other white wines contained significant amounts of caffeic acid (1,5-8,7 mg/L) and *p*-coumaric acid (1,5-4,4 mg/L). In addition, white wine samples of Sauvignon Blanc, Traminer and Rhine Riesling contained gallic acid at concentrations of 2,4-4,0 mg/L. The content of *p*-hydroxybenzoic acid was also significant in most of the tested wines, except of Sauvignon Blanc, Traminer and Welschriesling from the Slavonia and Croatian Danube regions. Welschriesling (Italian Riesling) is the most widely cultivated grape variety in Croatia, where it is also known as

Graševina. Samples studied in this work were from western and eastern mainland Croatia. They were characterized by a higher proportion of hydroxycinnamic acids compared to hydroxybenzoic acids. In addition, gallic acid was present in traces (below the calculated limit of quantification).

Catechin, epicatechin and their dimers (type B proanthocyanidins) were highly represented polyphenols in red wines. As for phenolic acids, Croatian wines of Babić and Plavac mali grape varieties were the richest in monomeric and dimeric flavan-3-ols (97,9-140,0 mg/L), while Cabernet Sauvignon wine from the Croatian Danube subregion contained the lowest amount of flavan-3-ols (27,2 mg/L). In addition to the autochthonous wines from the Croatian coastal region, another red wine of the Pinot Noir variety from the continental part of Croatia, more precisely from the subregion of Slavonia, stood out for its content of flavan-3-ols (101,2 mg/L). Considering the individual proportions, the highest contents of catechin, epicatechin and procyanidin B1 were found in Babić, while procyanidin B2 was the most abundant in Pinot Noir. In addition to gallic acid, *p*-coumaric acid and caffeic acid, individual flavan-3-ols also appear to be of great importance for the differentiation of Croatian wines, as their proportions vary greatly in the wines studied. Cabernet Sauvignon wines from Dalmatia, Istria, and Croatian Danube regions were characterized by large amounts of procyanidin B1 (13,7-44,6 mg/L) and catechin (10,5-19,3 mg/L), while epicatechin and procyanidin B2 contents were much lower (<LOQ-11,0 mg/L). A very similar composition of flavan-3-ols was also found in Merlot samples from the Croatian coastal area. Flavan-3-ols composition of studied Syrah, Teran and Blauer Portugieser wines were also very similar.

As expected, the white wines had significantly less flavan-3-ols. In the majority of the white wines tested, their individual contents were below the limit of quantification. Epicatechin was detected in several samples but could not be quantified in any of them. Of the white wines studied, Pinot Blanc from the Croatian Uplands contained the most flavan-3-ols (13,0 mg/L), while Sauvignon Blanc from Slavonia, Istrian Malvazija and Malvasia Bianca Lunga (Maraština) sample from southern Dalmatia contained the least (< LOQ). No epicatechin was detected in Rhine Riesling from Slavonia and Malvasia Bianca Lunga samples, and no procyanidin B1 was detected in Istrian Malvazija, Malvasia Bianca Lunga (Maraština) sample from southern Dalmatia and Sauvignon Blanc from Slavonia, while Rhine Riesling had the highest amount of procyanidin B2 (8,1 mg/L). Nine out of the twelve white wines contained more procyanidin B2 than procyanidin B1. The highest individual levels of catechin,

epicatechin, and procyanidin B1 were detected in Pinot Blanc from the Croatian Uplands region. Looking at the composition of flavan-3-ol in the Chardonnay samples studied, only one difference can be noted, namely that the sample from Istria contained more catechin than the sample from the Croatian Danube region.

No tested anthocyanins were detected in white wines, while their content varied widely in red wines. The content of delphindin-3-glucoside was low, reaching a maximum of 0,5 mg/L in Cabernet Sauvignon from southern Dalmatia. The same wine also contained a high concentration of 20,2 mg/L malvidin-3-glucoside, which was not the case in other Cabernet Sauvignon wines from Istria, Dalmatia and the Croatian Danube. A slightly higher malvidin-3-glucoside content than in previously mentioned Cabernet Sauvignon was found in Blauer Portugieser from the Croatian Uplands at 21,4 mg/L. Other red wines contained 1,4-6,0 mg/L malvidin-3-glucoside. As the most abundant anthocyanin in grapes, malvidin-3-O-glucoside was predominant in all red wine samples, but its content in the wines studied was significantly lower compared to most previously published results. It is interesting to note that not only the examined non-autochthonous but also the Croatian autochthonous red wines had unexpectedly low anthocyanin contents.

Red wines contained significantly more stilbenes than white wines, and the proportion of *trans*-resveratrol was generally much higher than that of *cis*-resveratrol in all the wines studied. In addition to anthocyanins, the Blauer Portugieser from Croatian Uplands contained the highest levels of *trans*-resveratrol (6,8 mg/L) and *cis*-resveratrol (1,6 mg/L). Differences in resveratrol content found in samples analysed are not only due to differences in grape variety, but could also be because of lower temperatures, higher humidity, and precipitation in the vineyard leading to the synthesis of stilbenes in grapes in response to fungal attack triggered by these environmental conditions. Also, in the production of red wine, maceration with skins and seeds during fermentation results in higher resveratrol concentrations in red wines than in white wines. Our results also support this fact. *trans*-Resveratrol was detected only in one white wine sample (Welschriesling from Croatian Uplands).

Eight flavonoids were analyzed in selected commercial Croatian wines. White wines contain almost no flavonoids. Only quercitrin was detected in Welschriesling and Pinot Blanc from the Croatian Uplands. In contrast to the white wines, the red wines contained different types of flavonoids in amounts ranging from 3,3 mg/L to 37,6 mg/L. Again, as for some of

hydroxybenzoic and hydroxycinnamic acids, the highest amount of flavonoids tested was present in three wine varieties from the same wine region. Merlot, Cabernet Sauvignon and Syrah originated from the Dalmatian Hinterland contained 32,4-37,6 mg/L flavonoids. They were followed by the Croatian autochthonous wine varieties Plavac Mali and Babić from Dalmatia with flavonoid content of 16,9-29,6 mg/L and 26,1 mg/L, respectively. These Plavac Mali wines are from the same subregion, but from different Croatian vineyard locations. Cabernet Sauvignon from Croatian Danube subregion, a red wine originating from a continental wine region is characterized by high flavonoid content (19,3 mg/L), which, however, is significantly higher than that of the sample of the same variety from Croatian Istria (6,6 mg/L) and from the extreme south of Croatia (7,1 mg/L). Likewise, the Merlot from Croatian Istria contained much less flavonoids (7,9 mg/L) than the sample from the Dalmatian Hinterland. Among tested flavonoids, the flavonols quercetin (< LOQ-21,7 mg/L) and myricetin (2,9-12,1 mg/L) stood out in terms of quantity in all studied red wines. As far as the content of quercetin is concerned, the order of the wines from the richest in quercetin to the poorest in quercetin is completely identical to that which we described when considering the contents of total flavonoids tested. Since the flavonols accumulation is induced by solar radiation, especially UV-B, Croatian wines from the coastal region, where solar radiation is significantly higher compared to the mainland, were richer in flavonols. Among the studied red wines, Merlot, Cabernet Sauvignon and Syrah from the Dalmatian Hinterland stood out for their content. Although these wines were obtained from different grape varieties, they had very similar flavonol composition. The reasons for this are most likely the same location of the vineyard, exposed to high and uniform solar radiation.

Because of the obvious difference between red and white wines, a separate Principal Component Analysis (PCA) was performed. Analysis of the PCA score plot for the monitored values showed that 61,02 % and 53,97 % of the total variance in the data could be described by F1 and F2 for red and white wines, respectively. Three well-separated clusters for the red wines studied can be differentiated. Croatian autochthonous wines Plavac mali and Babić form the separate group. The mentioned differences are due to the different content of gallic acid, *p*-coumaric acid, ferulic acid, catechin and procyanidin B1 compared to other red wines. A group of non-native wines Merlot, Cabernet Sauvignon and Syrah, all coming from the same vineyard location in Dalmatian Hinterland, were also separated. According to F1, this group shows similarity with Croatian autochthonous red wines from the same wine-growing region, and the

differences present can be attributed mostly to higher levels of quercetin and rutin compared to Plavac Mali and Babić wines. One of the possible reasons for this difference is the better adaptation of autochthonous species to local conditions and the resulting lower stress, which leads to a lower accumulation of quercetin. Seven other wines formed a third distinctive group, also comprising other Cabernet Sauvignon samples from different wine-growing regions. Although the white wines did not differ as much as the red wines, the multivariate analysis revealed more detailed relationships among them. Malvasia Bianca Lunga wine from the Northern Dalmatia showed to be significantly different from all other white wines, including wine from the same grape variety coming from the same wine region. Possible reasons for this lie not only in the different locations, but also in the winemaking process. Namely, wine from Northern Dalmatia was produced using a special technique based on ageing on the lees (*sur lie*). Pinot Blanc originated from the Croatian Uplands and Chardonnay from Istria were distinguished from the other white wines by their high content of catechin. The results of the HPLC analysis and corresponding PCA can be used for evaluation of Croatian wines according to their polyphenolic profiles.

Apart from sensory properties and wine quality, polyphenols have also been shown to be highly beneficial to human health. The antioxidant capacity of a wine depends largely on its phenolic profile, since different compounds have different levels of activity that are closely related to their chemical structure. The most representative phenolic components in the studied Croatian wines showed a strong antioxidant effect, mostly exceeding the effect of Trolox as a reference antioxidant. Gallic acid proved to be the most potent DPPH and NO scavenger, reaching $IC_{50}$ values of 0,33 µg/mL and 12,36 µg/mL, respectively. The same phenolic acid showed the highest reducing power ($IC_{50}$ = 1,21 µg/mL). Of the seven polyphenols tested, resveratrol had the weakest potential to inhibit the production of free DPPH ($IC_{50}$ = 7,56 µg/mL) and NO radicals ($IC_{50}$ = 35,08 µg/mL) and showed the lowest antioxidant capacity in the ferric-reducing power test ($IC_{50}$ = 14,66 µg/mL) but showed a much better ability to inhibit lipid peroxidation ($IC_{50}$ = 5,02 µg/mL). The strongest inhibitor of lipid peroxidation was myricetin ($IC_{50}$ = 1,68 µg/mL), followed by quercetin ($IC_{50}$ = 2,06 µg/mL), while caffeic acid showed the weakest effect, achieving 50 % inhibition of lipid peroxidation at a concentration of 141,76 µg/mL. The selected polyphenols were found to be potent DPPH and NO radical scavengers with significant reducing power and lipid peroxidation inhibitors.

The role of Src tyrosine kinase, α-glucosidase and acetylcholinesterase in development and progression of several diseases is well studied. Our results suggest that selected polyphenols, present in wines, show a significant inhibitory effect against these enzymes. Myricetin proved to be the most significant inhibitor of Src tyrosine kinase ($IC_{50}$ = 29 μM) among the tested compounds, while flavan-3-ols showed to be the weakest ($IC_{50}$ values ranged from 338 to > 2000 μM). Resveratrol was the strongest inhibitor of α-glucosidase ($IC_{50}$ = 22,82 μg/mL) with a significantly stronger effect compared to the reference inhibitor acarbose ($IC_{50}$ = 617,94 μg/mL). The flavonols quercetin ($IC_{50}$ = 158,37 μg/mL) and myricetin ($IC_{50}$ = 188,98 μg/mL) also had a stronger effect on α-glucosidase than the reference inhibitor. Tested representatives of flavonols (quercetin and myricetin) and phenolic acids (gallic and caffeic acid) showed an inhibitory effect on acetylcholinesterase, but significantly weaker ($IC_{50}$ vales ranged from 84,17 to 167,49 μg/mL) than galantamine that was used as a reference compound ($IC_{50}$ = 0,12 μg/mL).

## Conclusion

In this study, twenty-five commercial Croatian wines from eight red and nine white grape varieties were characterized according to their phenolic composition. To our knowledge, this is one of the few comparative studies of wines from all four wine-growing regions of Croatia. Also, for the first time, some data on the phenolic composition of specific wines were reported in detail. The twenty-four polyphenols analyzed were classified as phenolic acids, flavan-3-ols, anthocyanins, flavonoids, and stilbenes. In contrast to the white wines, the red wines contained higher concentrations of polyphenols with a wide variability. Gallic acid, *p*- and *o*-coumaric acid, caffeic acid, ferulic acid, catechin, epicatechin, procyanidins B1 and B2, quercetin, rutin, and myricetin proved to be important differentiators among the Croatian monovarietal wines. Red wines formed three well-divided groups, of which the group of Croatian autochthonous wines Plavac Mali and Babić differed the most in terms of polyphenolic composition. Plavac Mali and Babić wines were the richest sources of gallic acid and catechin. Babić wine also contained the highest levels of epicatechin and myricetin. Merlot, Cabernet Sauvignon and Shiraz wines from the Dalmatian Hinterland were the richest sources of quercetin.

The main representative phenolic components in the studied Croatian wines showed strong antioxidant activity, including gallic acid as the most effective DPPH$^{\cdot}$ and NO$^{\cdot}$ scavenger and the strongest $Fe^{3+}$ reducing agent, while myricetin proved to be the strongest inhibitor of lipid peroxidation. When the effect of selected polyphenols on the enzymes Src tyrosine kinase, α-glucosidase and acetylcholinesterase was studied, a significant inhibitory activity was found. Among the selected polyphenols, myricetin proved to be the most significant inhibitor of Src tyrosine kinase, followed by the other flavonol quercetin, although both were much weaker than the reference compound staurosporine. A similar effect of flavonols on acetylcholinesterase activity was found, but in this case, quercetin showed stronger effect. Resveratrol and the flavonols myricetin and quercetin showed strong inhibitory activity against α-glucosidase with $IC_{50}$ values lower than those of the standard inhibitor acarbose.

Our study provided new insights into the phenolic profiles of selected red and white wines from different Croatian regions, contributing to their characterization and varietal differentiation. It also highlighted the wines rich in polyphenols with significant biological effects and nutraceutical potential. The red wines studied, especially those from Croatian autochthonous red grape varieties, proved to be a valuable source of polyphenols with strong antioxidant properties and the ability to inhibit enzymes that are important therapeutic targets in the prevention and treatment of some chronic diseases associated with aging.