ORIGINAL PAPER

UDK: 615.356:577.152.31
616.892.3-085:663.21

DOI: 10.5937/hraish2302025M

Polyphenols from red wine as inhibitors of acetylcholinesterase activity

[1]Tatjana Majkic, [1]Ljiljana Milovanovic, [2]Ljilja Torovic, [3]Mirjana Penic, [1]Ivana Beara

[1]Department of Chemistry, Biochemistry and Environmental Protection, Faculty of Sciences, University of Novi Sad, Novi Sad, Serbia
[2]Department of Pharmacy, Faculty of Medicine, University of Novi Sad, Novi Sad, Serbia
[3]Department of Geography, Tourism and Hotel Management, Faculty of Sciences, University of Novi Sad, Novi Sad, Serbia

Corresponding author:
Ljiljana Milovanovic
Department of Chemistry, Biochemistry and Environmental Protection,
Faculty of Sciences, University of Novi Sad,
Trg Dositeja Obradovica 3, 21000 Novi Sad, Serbia
Phone: +38121-485-2755
E-mail address: ljiljana.milovanovic@dh.uns.ac.rs

Received 14. 11. 2024.
Revised 29. 11. 2024.
Accepted 12. 12. 2024.

Abstract

Alzheimer's disease, the most common type of dementia, impacts more than 55 million individuals globally, with numbers expected to increase significantly. Since acetylcholinesterase (AChE) inhibitors are a significant and promising therapeutic approach in Alzheimer's treatment, identifying natural AChE inhibitors is of great interest. This study investigates the in vitro AChE inhibitory potential of 15 red wines (5 Serbian and 10 European Merlot wines) and analyzes the correlation between AChE inhibition and polyphenolic profiles. All samples demonstrated inhibitory effects, ranging from 9.13 to 23.61 mg equivalents of physostigmine/L of wine. Variation in total phenolics, flavonoids, tannins, and anthocyanins was observed across wines, with French Merlots showing elevated anthocyanin levels. Principal Component Analysis revealed partial clustering based on polyphenolic composition rather than geographic origin. Moderate correlations were found only between AChE inhibitory activity and total anthocyanins (R^2 = 0.518) and malvidin-3-O-glucoside levels (R^2 = 0.674), suggesting anthocyanins may have a more significant role in AChE inhibition than other polyphenol groups. Although limited by sample size and potential in vivo metabolic changes, these findings suggest that Merlot wine polyphenols, particularly anthocyanins, may contribute to AChE inhibition. These results support the potential of natural, polyphenol-rich, products in prevention and treatment of neurodegenerative disorders such as Alzheimer's disease. Nonetheless, additional research involving larger sample sizes is required to confirm these findings and to more thoroughly explore the therapeutic potential of wine-derived polyphenols.

Key words: *neuroprotective activity; red wine; polyphenols; acetylcholinesterase.*

1. INTRODUCTION

Alzheimer's disease represents a predominant form of dementia impacting humanity, particularly among the elderly population. World Health Organization data indicate that over 55 million individuals worldwide are affected by dementia, a figure expected to rise to over 80 million by 2030, with approximately 70% of these cases attributed to Alzheimer's disease [1]. As a neurodegenerative disorder, Alzheimer's disease is marked by a gradual decline in memory and cognitive function, as well as alterations in social and emotional behavior, all of which significantly hinder daily functioning. The primary pathological features of Alzheimer's disease include the accumulation of amyloid-beta (Aβ) plaques, the development of neurofibrillary tangles, and significant neuronal loss. In addition to genetic predispositions, increasing evidence has connected the pathogenesis of Alzheimer's disease to factors including gliosis, neuroinflammation, disbalance between production and neutralisation of reactive oxygen species, impaired mitochondrial function, and abnormal accumulation of metal ions [2]. More than a century has passed since Alzheimer's disease was first documented, yet no cure exists; currently available treatments mostly manage symptoms, and, despite substantial efforts, the majority of clinical trials investigating novel therapeutic compounds for the disease have been unsuccessful [2,3].

One of the most hopeful and common targets in treatment of Alzheimer's disease is acetylcholinesterase (AChE; EC 3.1.1.7), i.e. its inhibition. AChE is a membrane-bound serine hydrolase located in the synaptic cleft, and its primary role is to terminate cholinergic neurotransmission by hydrolyzing the ester bond of acetylcholine into acetate and choline. The enzyme's active site has a catalytic triad: Ser203, Glu334, and His447, positioned at the bottom of a deep and narrow gorge. Additionally, AChE contains a choline-binding pocket (Trp84, Phe330, and Glu199) and an acyl-binding pocket (Phe288 and Phe299) near the active site, along with a peripheral anionic site (Trp84) at the gorge entrance [4,5]. This arrangement facilitates the proper binding and orientation of the substrate, enabling AChE's rapid catalytic reaction, which is essential for the normal functioning of the nervous system. Research suggests that AChE may also serve non-classical roles, including functioning as an adhesion protein involved in the development and maintenance of synaptic structures. Additionally, AChE has been implicated as a component of the bone matrix, as well as playing a role in neurite growth, neuritogenesis, and possibly promoting amyloid fibril aggregation. It is believed that peripheral anionic site has important role in the non-classical function, as well as in the binding of the reversible AChE inhibitors (AChEIs) [6,7].

AChE inhibitors enhance acetylcholine availability at the neuromuscular junction, counteracting impaired cholinergic pathways, as seen in conditions like Alzheimer's disease. Since AChEIs increase acetylcholine levels systemically, the side effects of approved treatments often resemble parasympathetic overstimulation, including bradycardia, diarrhea, hypotension, and urinary incontinence. Beyond Alzheimer's disease, reversible AChEIs are used to treat various conditions characterized by disrupted acetylcholine-mediated neurotransmission, such as myasthenia gravis, Parkinson's disease, and glaucoma [3,8]. Extensive research has been dedicated to discovering new agents with strong potential for AChE inhibition. In this effort, extracts from numerous medicinal plants traditionally used for dementia treatment, along with isolated natural products and plant metabolites, have been examined for their AChE inhibitory properties. Findings highlight certain alkaloids as particularly effective, notably galantamine, and huperzine A, already approved as medications. Additionally, compounds from other classes, such as polyphenols, have shown promising AChE inhibitory capabilities [3,9].

Numerous studies suggest that lifestyle and diet may delay age-related diseases, including neurodegenerative disorders. Many epidemiological studies, for instance, have shown that the Mediterranean diet, rich in foods and beverages high in phenolic compounds, offers significant health benefits [10]. A well-known phenomenon, the "French paradox", points to a cardioprotective effect associated with moderate red wine consumption, where French individuals experience lower rates of coronary disease despite a diet high in saturated fats, likely due to regular, moderate wine intake [11]. Moreover, some studies suggest that moderate wine consumption may protect against cognitive decline, linking wine's health benefits to its polyphenol content and associated antioxidant properties [12].

Namely, polyphenolic compounds, which are plant secondary metabolites, exhibit strong antioxidant, anti-inflammatory, antidiabetic, and neuroprotective activities. These compounds transfer from grapes to wine during maceration and fermentation. Due to differences in vinification processes between white and red wine, along with the extended contact of red wine with grape skins and seeds, red wine contains a higher concentration of polyphenolic compounds [13,14].

Continuing our prior investigations of the biological activity of red wines [15,16,17,18], this study aimed to evaluate the *in vitro* acetylcholinesterase inhibitory activity of 15 Merlot wines, including 5 samples from Serbia and 10 samples from other European regions. The overall aim was to explore the relationship between their inhibitory efficacy and polyphenolic composition.

2. MATERIAL AND METHODS

2.1. Merlot wines and reagents

The study included 5 Merlot wines from the Vojvodina region (northern Serbia, M1 to M5) and 10 European Merlot wines: 2 from North Macedonia (M6, M7), 1 from Spain (M8), 3 from France (M9 to M11), 3 from Italy (M12 to M14), and 1 from Slovenia (M15). The wine samples were selected from those produced exclusively from the Merlot grape variety and regularly available on the Serbian market between 2017 and 2019 (**Table 1**). To assess AChE inhibitory activity, the wines were evaporated under vacuum at 45 °C. The resulting dry residue was reconstituted in dimethyl sulfoxide to a concentration of 300 mg of dry residue/mL. All reagents used were of analytical grade.

Table 1. Wine samples.

Sample	Name	Vintage	Origin/Producer locality
M1	Merlo Vino, Patrijaršijsko	2014	Belgrade, Serbia
M2	Merlot, Vinarija Došen	2015	Sremski Karlovci, Serbia
M3	Merlot, Podrum Petrović	2014	Sremski Karlovci, Serbia
M4	Merlot, Mačkov podrum	2015	Rumenka, Serbia
M5	Merlot, Deurić	2015	Mala Remeta, Serbia

M6	Merlot, Tikveš	2016	Skopje, North Macedonia
M7	Merlot, DNV wines		Strumica, North Macedonia
M8	Merlot, Don Luciano La Mancha	2014	Spain
M9	Merlot, La Cacciatora Veneto	2016	Veneto, Italy
M10	Merlot, La Delizia		Udine, Italy
M11	Tenuta San Tome	2013	Italy
M12	Merlot, Grand Sud	2017	Petersbach, France
M13	Merlot, Maison Castel	2016	France
M14	Merlot, JP Chanet	2018	France
M15	Merlot, Goriška Brda	2012	Vinska klet Goriška Brda, Dobrovo, Slovenia

2.2. Acetylcholinesterase inhibition studies

The acetylcholinesterase (AChE) inhibitory activity of the samples was assessed using a modified version of Ellman's method, adapted for a 96-well plate [19,20]. Briefly, 110 µL of 20 mM Tris-HCl buffer (pH 8), 20 µL of AChE (0.5 U/mL, dissolved in 20 mM Tris-HCl buffer at pH 7.5 with 0.01% BSA), and 10 µL of the test samples at various concentrations (dissolved in 20 mM Tris-HCl buffer, pH 8) were incubated for 15 minutes at 37 °C. Subsequently, 40 µL of 3 mM DTNB (dissolved in 50 mM Tris-HCl buffer, pH 8, with 0.1 M NaCl and 0.02 M $MgCl_2 \times 6H_2O$) and 20 µL of 15 mM aq acetylthiocholine were added. After 4 minutes, the absorbance of the yellow 5-thio-2-nitrobenzoate was measured at 412 nm. In the control, the equivalent volume of sample was replaced with the appropriate concentration of DMSO (diluted in 20 mM Tris-HCl buffer, pH 8). In the blank, 20 mM Tris-HCl buffer (pH 7.5) was added instead of the enzyme. As the positive control, physostigmine was applied. All probes (samples, positive control, and controls) were tested in triplicate, and the results were expressed as mg equivalents of physostigmine/L of wine.

2.3. Analysis of polyphenols

The total content of polyphenols, flavonoids, tannins, and monomeric anthocyanins was determined using spectrophotometric methods [18]. HPLC-UV/VIS analysis was performed to quantify selected polyphenols (benzoic, caffeic, chlorogenic, gallic, 4-hydroxybenzoic, *p*-coumaric, syringic, *trans*-cinnamic, and vanillic acid; catechin, hesperetin, kaempferol, naringenin, quercetin, rutin; resveratrol; 3-*O*-glucosides of malvidin, cyanidin, delphinidin, petunidin, and peonidin) following a previously described procedure [18].

2.4. Statistical analysis

The experimental data were presented as mean values ± SD from three independent trials. The relationship between AChE inhibitory activity and polyphenol content was examined using Pearson's correlation test, with correlation coefficients considered highly significant at $p < 0.01$. Variability in the polyphenolic composition was analyzed using principal component analysis (PCA). The data were standardized to normalize differences in scale, ensuring that all responses and parameters contributed equally to the variance and principal component calculation. All statistical analyses were conducted using STATISTICA version 14.0.015 (TIBCO Software Inc).

3. RESULTS

3.1. Inhibition of acetylcholinesterase activity

AChE inhibition activity of wine samples is presented in **Figure 1**: all samples expressed modest activity, with the highest rate achieved by samples M3, M5, M8, and M11 (23.61, 21.41, 21.67 and 21.67 mg equivalents of physostigmine/L of wine, respectively).

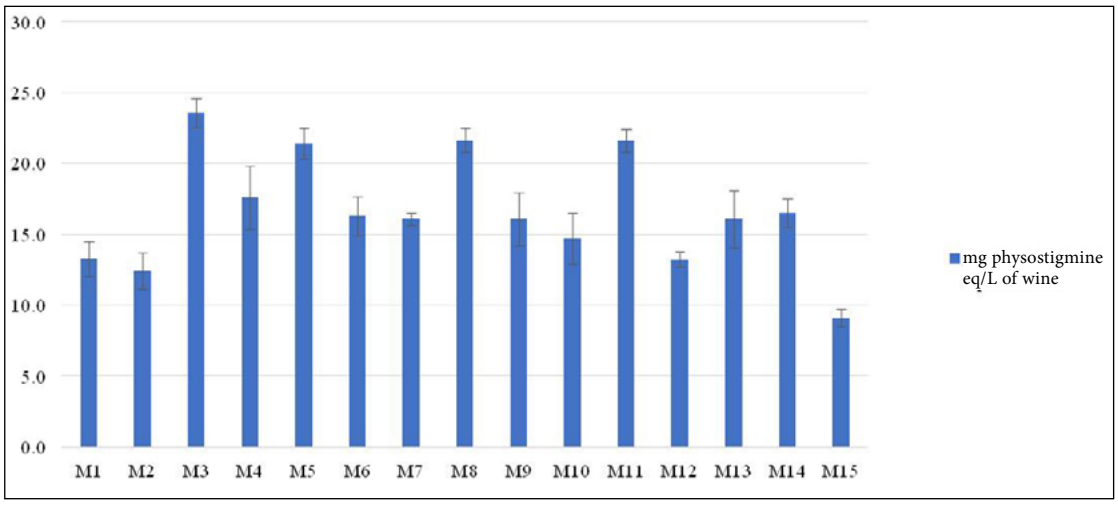

Figure 1. Inhibition of acetylcholinesterase.

3.2. Polyphenolic profile

Figures 2 and **3** illustrate the content of the total polyphenols, flavonoids, tannins, and monomeric anthocyanins in the investigated wines.

Regarding the total polyphenol content (**Figure 2**), the mean concentration was 1726 ± 570 mg GAE/L, with M2 and M14 having the lowest and the highest values, respectively. As illustrated in **Figure 3**, M10 had the highest flavonoid amount, which was approximately six times greater than the lowest concentration found in M3 (17.5 mg QE/L). The mean content of flavonoids was 50.0 ± 24.6 mg QE/L. Following polyphenols quantification, tannins were precipitated using polyvinylpolypyrrolidone and analyzed, revealing an average amount of 833 ± 481 mg CE/L. The average concentration of anthocyanins was determined to be 39.5 ± 26.9 mg M-3-GE/L, with the lowest level observed in sample M7 and the highest in sample M11. **Figures 2** and **3** clearly demonstrate notable variations in the levels of total polyphenols, as well as in the total contents of compounds from particular polyphenol groups.

Figure 4 provides an overview of the polyphenolic profiles of the analyzed samples, although certain findings for some Merlot samples included in this study have been previously reported [16,17,18]. Phenolic acids were the predominant group across all Merlot samples, with gallic acid being the most prominent (mean concentration 37.7 ± 30.1 mg/L). Among flavonoids, catechin was the most abundant (26.2 ± 11.6 mg/L). Resveratrol, a stilbene representative, was found in all samples, with levels reaching up to approximately 5 mg/L. Additionally, notable amounts of malvidin-3-*O*-glucoside were observed (24.4 ± 20.7 mg/L), with the highest concentration in M5 sample.

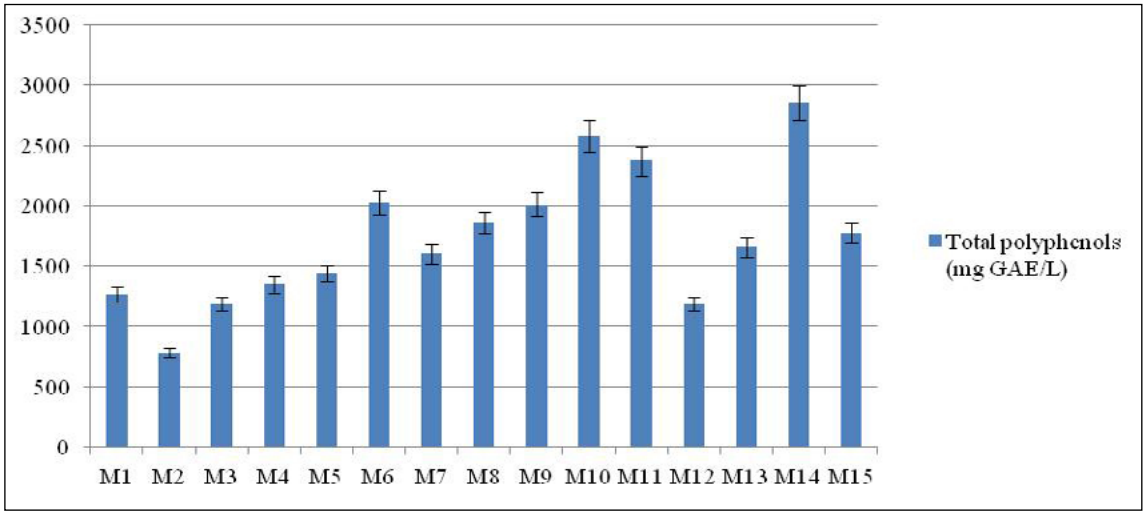

Figure 2. Content of the total polyphenols in analyzed Merlot samples (abbreviation: GAE/L – gallic acid equivalents per liter of wine).

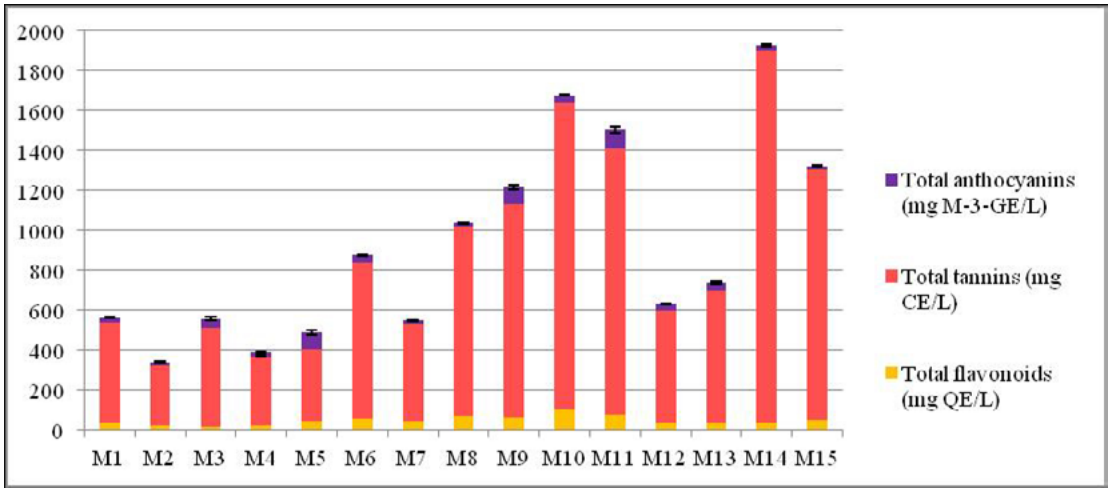

Figure 3. Content of the total flavonoids, tannins, and monomeric anthocyanins in analyzed Merlot samples (abbreviations: QE/L – quercetin equivalents per liter of wine; CE/L – catechin equivalents per liter of wine; M-3-GE/L – malvidin 3-O-glucoside equivalents per liter of wine).

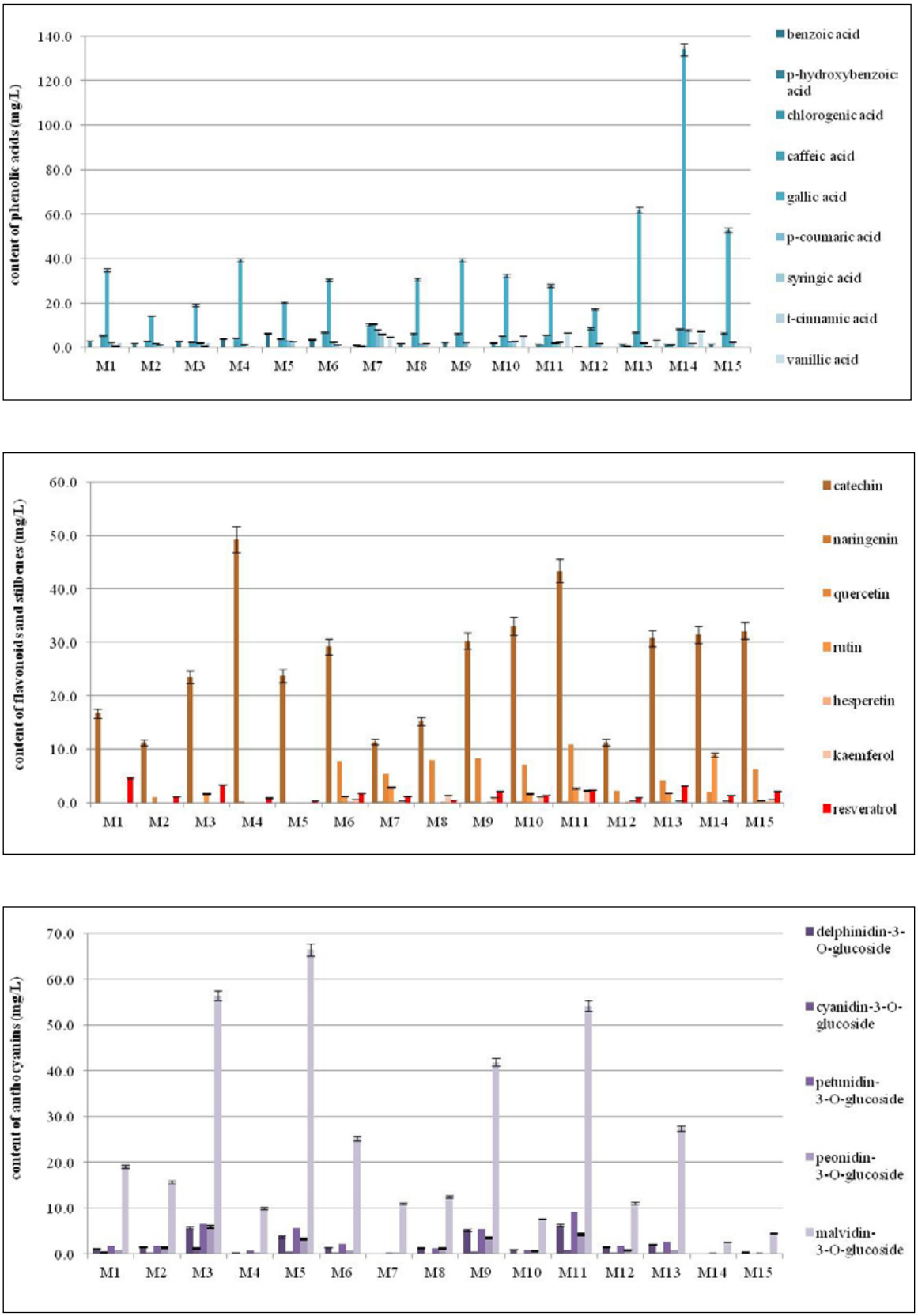

Figure 4. Polyphenolic profile of examined Merlot samples.

To gain deeper insight into the variations among the analyzed Merlot samples, Principal Component Analysis (PCA) was performed using the identified polyphenolic compounds (**Figure 5**). The first two principal components explained a total of 56.23% of the variance, with F1 accounting for 30.73% and F2 for 25.50%. Partial clustering was observed for approxi-

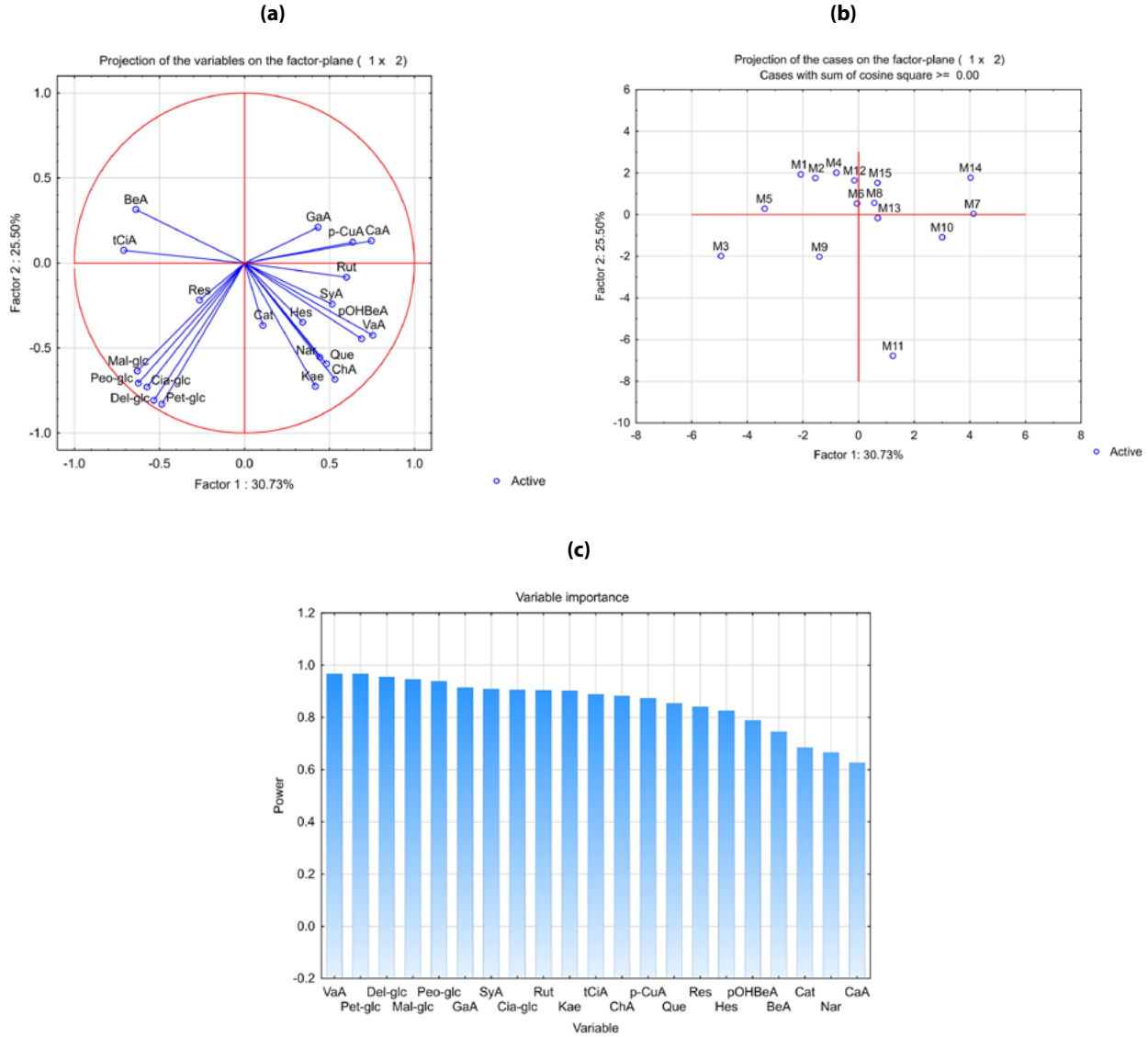

Figure 5. PCA of polyphenolic profile. (a) Distribution of variables on loadings plot, (b) distribution of wine samples on scores plot, (c) variable importance. Abbreviations: BeA: benzoic acid; Cya-glc: cyanidin 3-*O*-glucoside; CaA: caffeic acid; ChA: chlorogenic acid; Cat: catechin; Del-glc: delphinidin 3-*O*-glucoside; GaA: gallic acid; Hes: hesperetin; Kae: kaempferol; M-3-G: malvidin 3-*O*-glucoside; Nar: naringenin; *p*-CuA: p-coumaric acid; Peo-glc: peonidin 3-*O*-glucoside; *p*-OH-BeA: *p*-OH benzoic acid; Pet-glc: petunidin 3-*O*-glucoside; Que: quercetin; Res: resveratrol; Rut: rutin; SyA: syringic acid; tCiA: *t*-cinnamic acid; VaA: vanillic acid;

mately half of the samples in the central and upper regions of the plot, while the remaining samples were more widely distributed, reflecting variability in the concentrations of specific polyphenols.

3.3. Correlation factors

Correlation coefficients between AChE inhibitory activity and polyphenolic contents were calculated (**Table 2**). Considerable correlations were observed between total anthocyanin content (R^2 = 0.518) and malvidin-3-*O*-glucoside concentration (R^2 = 0.674) and achieved inhibitory activity.

Table 2. Pearson's linear correlation coefficients between inhibition of AChE activity and polyphenolic contents.

Polyphenols	AChE inhibition
Total polyphenols	0.110
Total flavonoids	0.034
Total tannins	-0.082
Total anthocyanins	0.518
Gallic acid	-0.172
Catechin	0.169
Resveratrol	-0.130
Malvidin-3-*O*-glucoside	0.674

4. DISCUSSION

Previous studies have demonstrated that polyphenol-rich foods, such as grapes, grape skin extracts, and various red wines, can inhibit AChE activity [21,22]. In general, the precise mechanisms by which polyphenols influence different cellular processes, including modulation of AChE activity, remain incompletely understood. Polyphenolic compounds are known to interrelate with amino acid residues at the AChE active site through H-bonds, hydrophobic, and π–π interactions. The presence of multiple hydroxyl groups in these compounds is thought to enhance AChE inhibition due to increased binding strength. While this mode of action accounts for the inhibitory potential of many phenolic compounds, it does not apply universally to all [23]. For example, flavonoids possess an aromatic B-ring with hydroxyl groups that form bonds with the anionic, peripheral site of the enzyme. Flavonoids do not appear to alter the enzyme's tertiary structure; instead, their inhibitory effect results from obstructing access to the active site [9]. Although phenolic acids have demonstrated AChE inhibitory activity, some studies revealed that pairs of phenolic acids generally exhibited lower inhibitory activity than the sum of their individual effects. Similarly, combinations of phenolic acids and flavonoids in most cases showed reduced inhibitory activity compared to the calculated effects of each compound alone. This lack of synergy among paired inhibitors suggests a limited binding site that prevents simultaneous accommodation of both inhibitors in close proximity [24]. Regarding anthocyanins, studies on structure-activity relationships suggest that the presence of hydroxyl groups on all three rings is essential for their inhibitory activity against AChE. Glycosylation at the 3-position (C ring) or 5-position (A ring) reduces their inhibitory potency, whereas hydroxyl groups at the 3′ and 5′ positions on the B ring enhance AChE inhibition [25]. However, it is important to consider some limitations of these types of *in vitro* studies and conclusions: most polyphenolic compounds undergo changes during absorption in the gastrointestinal tract and later in the liver, resulting in *O*-methylated, sulfated, and glucuronidated metabolites. These metabolites, along with those produced by bacterial activity in the large intestine, are chemically distinct from the forms found in foods and those primarily tested for AChE inhibitory activity thus far [9].

As shown in **Figure 1**, all examined samples exhibited AChE inhibitory effects, with levels of activity ranging from 9.13 to 23.61 mg equivalents of physostigmine/L of wine for Slovenian Merlot (M15) and Serbian Merlot (M3), respectively. Also, compared with our previous studies about grape juice, young wine, and wine from Fruška Gora, based on which we have chosen Merlot for our further investigation, Merlot wines analyzed in these studies had slightly weaker activity [20]. Nevertheless, one previous [21] research showed lower level of AChE activity in some Merlot wines from France (0.086 mmol physostigmine/L, i.e. 23.680 mg physostigmine/L). Overall, no clear correlation was found between enzyme inhibition levels and the wines' geographic origin: besides the best-ranked M3, another Serbian Merlot (M5), along with Spanish (M8) and French Merlot (M11), showed the highest inhibitory activities. These findings enhance the understanding of the biological activity of Serbian Merlot wines, potentially supporting their increased recognition and promotion in the global market.

The analysis of data (**Figures 2 and 3**) regarding the total levels of polyphenols, flavonoids, tannins, and monomeric anthocyanins in the examined wines indicates variability among the samples, with overall concentrations being lower in Serbian wines (M1–M5) compared to those from other European regions. This finding can be partially explained by the influence of various environmental factors, including soil composition and climate, on grapevine development. Notably, wines produced in regions with higher sunlight exposure, such as those from certain foreign Merlot vineyards, often exhibit elevated concentrations of polyphenolic compounds [26].

Given that spectrophotometric methods are regarded as semiquantitative and subject to certain limitations impacting result accuracy, a more precise approach, HPLC-UV/VIS analysis, was employed to characterize the polyphenolic profiles of the analyzed Merlot samples. The findings reported in **Figure 4** are consistent with those of prior studies on Merlot and red wines in general [18,27]. PCA (**Figure 5**) depicted clear separation of French Merlot samples (M9-11) from the central group, primarily attributed to elevated levels of the determined monomeric anthocyanins. Conversely, the separation of M14 (Italian Merlot) and M7 (Macedonian Merlot) was predominantly associated with substantial quantities of caffeic and *p*-coumaric acids, while positioning of M3 and M5 was influenced by the highest content of benzoic and *t*-cinnamic acids, respectively. Overall, only partial grouping was observed among wines from the same geographic origin.

To evaluate the relationship between the demonstrated inhibitory activity and the polyphenol contents, correlation coefficients were determined (**Table 2**). However, levels of correlation were modest, and only observed between measured inhibitory activity and contents of total anthocyanin ($R^2 = 0.518$), as well as malvidin-3-*O*-glucoside ($R^2 = 0.674$), suggesting a greater contribution of anthocyanins to this activity compared to other polyphenol groups. Although this study has certain limitations, including the relatively small sample size, the results suggest that wine polyphenols, particularly anthocyanins, may contribute to AChE inhibition. These findings support the potential of natural products as a strategy for managing neurodegenerative disorders, such as Alzheimer's disease.

5. CONCLUSION

The objective of this study was to assess the acetylcholinesterase inhibitory activity of 15 Merlot wines and correlate this activity with the wines' polyphenolic profiles. The results revealed that all examined wines demonstrated AChE inhibition, although to varying extents. Serbian Merlot (sample M3) exhibited the highest inhibitory activity, although even the most active wines required significantly higher concentrations than the standard inhibitor, physostigmine. Notably, no clear relationship was found between inhibitory potential and the geographic origin of the wines. Polyphenolic analysis showed marked variability in total phenolic content, flavonoids, tannins, and anthocyanins across samples. While French wines (samples M9-M11) stood out due to higher anthocyanin levels, certain Italian and Macedonian wines (M14 and M7) exhibited significant quantities of caffeic and *p*-coumaric acids. Principal Component Analysis further highlighted the unique polyphenolic profiles of each wine.

Moderate correlations were observed between total anthocyanin content and malvidin-3-*O*-glucoside concentration with AChE inhibitory activity, implying that anthocyanins might contribute more significantly to AChE inhibition than other polyphenol groups. Despite limitations such as sample size and potential metabolic transformations of polyphenols *in vivo*, the findings suggest that Merlot wine polyphenols, particularly anthocyanins, may contribute to the inhibition of AChE. These results emphasize the role of polyphenol-rich natural products as potential candidates in the prevention of neurodegenerative disorders, including Alzheimer's disease. Further research involving larger sample sets and more diverse wine types is warranted to strengthen these findings.

ACKNOWLEDGEMENTS

The authors gratefully acknowledge the financial support of the Ministry of Science, Technological Development and Innovation of the Republic of Serbia (Grants No. 451-03-66/2024-03/ 200125 & 451-03-65/2024-03/200125).

REFERENCES

1. World Health Organization (WHO), 2024. https://www.who.int/news-room/fact-sheets/detail/dementia. Accessed on 30.10.2024.
2. Monteiro AR, Barbosa DJ, Remião F, Silva R. Alzheimer's disease: Insights and new prospects in disease pathophysiology, biomarkers and disease-modifying drugs. Biochem Pharmacol. 2023;211:115522.
3. Thangwaritorn S, Lee C, Metchikoff E, Razdan V, Ghafary S, Rivera D, Pinto A, Pemminati S. A review of recent advances in the management of Alzheimer's disease. Cureus. 2024;16(4):e58416.
4. Kryger G, Silman I, Sussman JL. Structure of acetylcholinesterase complexed with E2020 (Aricept®): implications for the design of new anti-Alzheimer drugs. Structure. 1999;7(3):297-307.
5. Cheung J, Gary EN, Shiomi K, Rosenberry TL. Structures of human acetylcholinesterase bound to dihydrotanshinone I and territrem B show peripheral site flexibility. ACS Med Chem Lett. 2013;4(11):1091-1096.
6. Silman I, Sussman JL. Acetylcholinesterase:'classical'and 'non-classical'functions and pharmacology. Curr Opin Pharmacol. 2005;5(3):293-302.
7. Fisher SK, Wonnacott S. Acetylcholine. In Basic neurochemistry, Academic Press 2012;258-282.
8. Čolović MB, Krstić DZ, Lazarević-Pašti TD, Bondžić AM, Vasić VM. Acetylcholinesterase inhibitors: pharmacology and toxicology. Curr Neuropharmacol. 2013;11(3):315-335.
9. Roseiro LB, Rauter AP, Serralheiro MLM. Polyphenols as acetylcholinesterase inhibitors: Structural specificity and impact on human disease. Nutr Aging. 2012;1(2):99–111.
10. Gardener H, Caunca MR. Mediterranean diet in preventing neurodegenerative diseases. Curr Nutr Rep. 2018;7(1):10-20.
11. Renaud S, de Lorgeril M. Wine, alcohol, platelets, and the French paradox for coronary heart disease. The Lancet. 1992;339:1523–1526.
12. Reale M, Costantini E, Jagarlapoodi S, Khan H, Belwal T, Cichelli A. Relationship of wine consumption with Alzheimer's disease. Nutrients. 2020;12(1):206.
13. Gris EF, Mattivi F, Ferreira EA, Vrhovsek U, Filho DW, Pedrosa RC, Bordignon-Luiz, MT. Phenolic profile and effect of regular consumption of Brazilian red wines on in vivo antioxidant activity. J Food Compos Anal. 2013;31(1):31–40.
14. Guilford JM, Pezzuto JM. Wine and Health: A Review. Am J Enol Vitic. 2011;62(4):471-486.
15. Milovanović Lj, Majkić T, Torović Lj, Božović P, Kalajdžić M, Ivanišević D, Beara, I. Hybrid variety Dionis (Cabernet Franc E11× Panonia) vs. Cabernet Franc wines: Comparative study of chemical profile, in vitro biological activity, sensory quality and potential health risk. J Food Compos Anal. 2024;131:106229.
16. Milovanović Lj, Majkić T, Torović Lj, Svirčev E, Penić M, Beara I. In vitro study on hypoglycaemic activity of Serbian and some European Merlot wines. Hrana i ishrana. 2023;64(1): 28-37.
17. Majkić T, Torović Lj, Milovanović Lj, Molnar N, Beara I. Red wine concentrates as inhibitors of lipid oxidation in compound butter. Hrana i ishrana. 2022;63(1):26-32.
18. Majkić T, Torović Lj, Lesjak M, Četojević-Simin D, Beara I. Activity profiling of Serbian and some other European Merlot wines in inflammation and oxidation processes. Food Res Internat. 2019;121:151–160.
19. Ellman G L, Courtney KD, Andres JV, Featherstone RM. A new and rapid colorimetric determination of acetylcholinesterase activity. Bio pharm. 1961;7(2):88-95.
20. Beara IN, Torović LD, Pintać DĐ, Majkić TM, Orčić DZ, Mimica-Dukić NM, Lesjak MM. Polyphenolic profile, antioxidant and neuroprotective potency of grape juices and wines from Fruška Gora region (Serbia). Int J Food Prop. 2017;20:S2552-S2568.
21. Stój A, Szwajgier D, Baranowska-Wójcik E, Domagala D.. Gentisic acid, salicylic acid, total phenolic content

and cholinesterase inhibitory activities of red wines made from various grape varieties. S Afr J Enol Vitic. 2019;40(1):1-12.
22. Pervin M, Hasnat MA, Lee YM, Kim DH, Jo JE, Lim BO. Antioxidant activity and acetylcholinesterase inhibition of grape skin anthocyanin (GSA). Molecules. 2014;19(7):9403-9418.
23. Jabir NR, Khan FR, Tabrez S. Cholinesterase targeting by polyphenols: A therapeutic approach for the treatment of Alzheimer's disease. CNS Neurosci Ther. 2018;24(9):753-762.
24. Szwajgier D. Anticholinesterase activity of selected phenolic acids and flavonoids – interaction testing in model solutions. Ann Agric Environ Med. 2015;22(4):690-694.
25. Zaa CA, Marcelo ÁJ, An Z, Medina-Franco JL, Velasco-Velázquez MA. Anthocyanins: Molecular aspects on their neuroprotective activity. Biomolecules. 2023;13(11):1598.
26. Spayd SE, Tarara JM., Mee DL, Ferguson JC. Separation of sunlight and temperature effects on the composition of Vitis vinifera cv. Merlot berries. Am J Enol Vitic. 2002;53:171–182.
27. Heras-Roger J, Díaz-Romero C, Darias-Martín J. A comprehensive study of red wine properties according to variety. Food Chem. 2016;196:1224–1231.

Polifenoli iz crvenog vina kao inhibitori aktivnosti acetilholinesteraze

[1]Tatjana Majkić, [1]Ljiljana Milovanović, [2]Ljilja Torović, [3]Mirjana Penić, [1]Ivana Beara

[1]Departman za hemiju, biohemiju i zaštitu životne sredine, Prirodno-matematički fakultet, Univerzitet u Novom Sadu, Novi Sad, Srbija

[2]Katedra za farmaciju, Medicinski fakultet, Univerzitet u Novom Sadu, Novi Sad, Srbija

[3]Departman za geografiju, turizam i hotelijerstvo, Prirodno-matematički fakultet, Univerzitet u Novom Sadu, Novi Sad, Srbija

Kratak sadržaj

Alchajmerova bolest, najčešći oblik demencije, trenutno pogađa preko 55 miliona ljudi širom sveta, a predviđa se i značajan porast ovog broja. Budući da su inhibitori acetilholinesteraze (AChE) važan i obećavajući terapijski pristup u lečenju Alchajmerove bolesti, identifikacija prirodnih proizvoda kao inhibitora AChE je od velikog značaja. Ova istraživanja uključuju in vitro ispitivanje potencijala 15 crvenih vina (5 Merlo vina iz Srbije i 10 Merlo vina iz Evrope) kao inhibitora AChE i analizu korelacije polifenolnog profila i nivoa inhibicije AChE. Svi uzorci su pokazali aktivnost, sa vrednostima u rasponu od 9,13 do 23,61 mg ekvivalenata fizostigmina/L vina. Uočena je varijacija u ukupnom sadržaju fenola, flavonoida, tanina i antocijanina među vinima, pri čemu su francuska Merlo vina imala više nivoe antocijanina. Analizom glavnih komponenti (PCA) utvrđeno je delimično grupisanje uzoraka na osnovu polifenolnog profila, ali ne i geografskog porekla. Umerene korelacije uočene su samo između nivoa inhibitorne aktivnosti AChE i ukupnih antocijanina (R^2 = 0,518) i sadržaja malvidin-3-O-glukozida (R^2 = 0,674), što sugeriše da antocijanini mogu imati značajniju ulogu u inhibiciji AChE u poređenju sa drugim grupama polifenola. Iako ograničena brojem uzoraka i potencijalnim metaboličkim in vivo promenama, ova istraživanja ukazuju na to da polifenoli iz Merlo vina, posebno antocijanini, mogu uticati na inhibiciju AChE. Ovi rezultati podržavaju potencijal upotrebe prirodnih proizvoda bogatih polifenolima u prevenciji i tretmanu neurodegenerativnih poremećaja, poput Alchajmerove bolesti. Međutim, neophodna su dalja istraživanja sa većim brojem uzoraka kako bi se potvrdili ovi rezultati i detaljnije istražio terapeutski potencijal polifenola iz vina.

Ključne reči: *neuroprotektivna aktivnost; crveno vino; polifenoli; acetilholinesteraza.*