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CHEMOMETRIC INSIGHTS INTO GRAPE STEMS: ANTIOXIDANT CAPACITY, PHENOLIC COMPOSITION, AND MINERAL PROFILE

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ABSTRACT. The primary objective of this investigation was to assess the total polyphenolic content (TPC), identify and quantify individual polyphenols, and evaluate their radical scavenging activity (RSA), as well as determine the mineral composition in stem extracts from nine distinct Vitis vinifera varieties. The total phenolic content in grape stem extracts ranged from 34.87 to 76.95 mg gallic acid equivalents (GAE) per gram of dry weight (d.w.). These extracts exhibited significant free radical scavenging activity, ranging from 0.344 to 0.898 mmol Trolox equivalents (TE) per gram d.w. Stem extracts were predominantly characterized by flavan-3-ols, flavonols, and phenolic acids. Catechin and quercetin-3-glucuronide were identified as the most abundant components, with concentrations of up to 1.858 mg/g d.w. and 1.315 mg/g d.w., respectively. Potassium (K) emerged as the most abundant element in all samples, with content ranging from 7.297 mg/g d.w. to 16.695 mg/g d.w., followed by calcium (Ca), phosphorus (P), and magnesium (Mg).

Keywords: *Vitis vinifera, stem extracts, flavonols, potassium.*

INTRODUCTION

The food processing sector generates a substantial volume of waste, posing environmental threats and causing considerable economic losses.

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Grape stems, a byproduct of the winery industry, are often utilized partially as a source of astringent compounds, primarily comprising proanthocyanidins [1-2]. These stems are typically removed prior to vinification processes to prevent excessive astringency in wine or adverse effects on its sensory properties. Stem quantities typically range from 1.4% to 7.0% of the processed raw material [3]. Presently, grape stems hold low commercial value, primarily serving as animal fodder or soil enhancers. Limited available data on the composition of grape stems suggests their potential as a valuable source of dietary fibber and antioxidants [4-5]. Phenolic compounds represent a significant category of substances due to their well-established health-promoting properties. The phenolic composition of grape stems typically includes flavan-3-ols, hydroxycinnamic acids, monomeric and oligomeric flavonols, and stilbenes [6-7]. It has been reported that phenolics constitute approximately 5.8% of the dry weight of grape stems [2]. While phenolic compounds are commonly found in various plant-based foods, there is a preference for extracting them from agri-industrial by-products [8-9]. Consequently, there has been notable interest in recent years in acquiring polyphenols from plant residues. These polyphenols are sought for applications in the pharmaceutical sector, as food additives and supplements, or in cosmetics.

In addition to the organic constituents, the significance of both major and trace elements in grape stems cannot be overlooked. Findings from Leal et al. [5] underscored that the essential trace elements present in stems offer notable nutritional value. Conversely, concentrations of toxic elements such as arsenic (As), cadmium (Cd), and lead (Pb) were minimal and posed no threat to human health.

This study aims to explore the composition of grape stems to assess their potential utilization in the food and/or pharmaceutical industries. Stems from nine distinct grape cultivars underwent extraction via maceration. The resulting exracts were then subjected to analysis to establish their phenolic profiles using the HPLC method. Additionally, the mineral composition of the stems was assessed utilizing the ICP-OES method.

RESULTS AND DISCUSSION

Mineral contents of grape stems

The data about the mineral contents of the stems are presented in Tables 1-3. Statistical analysis showed that mineral contents of the stems differ significantly among the cultivars ($p<0.05$).

The plant materials with high concentrations of the nutrient elements will play an essential role in maintaining human health when taken at recommended levels [10]. This study determined that grape stems are also useful dietary supplements that can provide K, P, Ca, Mg, Fe, and Zn.

All the grape stems were characterized by high potassium and low sodium contents. The concentration of Na varied and ranged from 2.01 μg/g in the Tamjanika Black cultivar to 4.85 μg/g in the Merlot cultivar.

Merlot cultivar had the highest levels of K (16.695 mg/g), whereas cv. Župljanka had the lowest K concentration (0.761 mg/g). K is essential for human health. High-potassium diet lowers blood pressure and reduces cardiovascular disease morbidity and mortality [10]. Since K is necessary for the growth and development of plants, this may be the main reason why it is the most abundant element in grape stems. Romero et al. [11] also analyzed K in stems, and obtained values were in accordance with values obtained in our study. However, Kondi et al. [12] found slightly lower (1.4 mg/g) whereas Leal et al. [5] values were higher (18.10-39.36 mg/g).

The highest P concentration was in the stems of Plovdina (3.349 mg/g) and Merlot (3.017 mg/g) cultivars and it was lowest in the Župljanka cultivar (1.232 mg/g). P can be found most as phosphates in the environment as well as in plant tissues.

The results from the present study also showed that grape stems are rich in Ca. Ca concentrations in grape stems varied from 3.328 to 8.410 mg/g. The average results of Ca (5.016 mg/g) were higher compared to results reported by Kondi et al. [12] (1.92 mg/g), but lower obtained by Romero et al. [11] (14.9 mg/g). Ca is an essential mineral for human health because it is the major component of the bone, assists in tooth development, helps regulate endo- and exo-enzymes, and plays a significant role in regulating blood pressure [13].

Mg is one of the minerals found in high concentrations in grape stems. Mg concentrations varied between 0.546 mg/g (cv. Prokupac) and 2.142 mg/g (cv. Smederevka). Mg is essential to all living cells, where they play a major role in manipulating important biological polyphosphate compounds like ATP, DNA, and RNA. Also, more than 300 enzymes require magnesium ions to function [14].

Merlot Cultivar had the highest concentration of Fe, whereas the Muscat Hamburg cultivar had the highest concentration of Zn (139.82 μg/g and 33.14 μg/g, respectively). The optimum range of the iron content in grapevine petioles ranges from 40 to 180 μg /g [15]. The previous results obtained in grape stems in India, Portugal, and Spain's vineyards were 65.80-98.56 μg/g, 17.51-84.15 μg/g, 19.96-77.62 μg/g for Fe content Fe [12, 5]. Also, the previous results obtained in grape stems in Poland, India and Spain vineyards for Zn

content were 30.8 μg/g, 90.22 μg/g, 21.7 μg/g respectively [11-12]. The deficiency of Zn and Fe in the diet is a widespread problem and a matter og great concern, especially in developing countries where people rely more on vegetarian diets. These essential trace elements are involved with the vital immune system (Zn) and metabolic functions and are intrinsic components of hemoglobin, myoglobin, and cytochrome (Fe) [15]. They are also recognized as potential antioxidants [10].

The most significant levels of manganese were noted in the stems of cv. Merlot (123.07 μg/g), compared with only 10.27 μg/g d.w. in the stems of cv. Tamjanika Blank (Table 2). Generally, manganese shows the most significant variation in stem tissues because root uptake of this nutrient depends on the soil solution concentration of Mn^{2+} [16]. Adequate Mn values for grape petioles are 18 to 100 μg/g. Gastol and Domagala-Swiatkiewicz [17] suggested that the high Mn levels, could be linked to increased availability of Mn in acid soils.

The copper content in grape stems ranged from 5.90 μg/g in stems of Tamjanika Black to 48.80 μg/g in stems of Cabernet Sauvignon. The previous study by Kondi et al. [12] found that Cu content in vine and table grape stems cultivated in India varied from 11.23 μg/g to 29.97 μg/g. The studies of Cu content in grape stems range from 16.00-159.25 μg/g in Portugal [5] and 8.9-21.7 μg/g Spain [11]. Many studies indicated that grapefruit, leaf, and stem are contaminated with Cu worldwide. Copper is generally slightly mobile element in plants as it is strongly bound by nitrogen and proteins. However, some plant species have a great tolerance to increased content of Cu and can accumulate high amounts in their tissues [18]. The content of Cu in the vineyards´ soil usually depends on the age because the long-term use of the same parcels for grapevine growing could cause Cu accumulation in the soil due to the application of Cu-fungicides.

However, can find other elements in the vineyard soils, and consequently, in the vines, namely toxic or heavy metals (As, Al, Cd, Pb, and Hg). These elements appear in the soil, mainly due to the fertilizers and chemical pesticides used, and due to industrial activities or traffic. Thus, in the last years there has been a growing concern by the population about the increase in the quantity of toxic elements in plants [19], since these metals, when accumulated in the human body, can have negative effects, causing damage, for example, in the kidneys, nervous and immune systems, and even having carcinogenic effects [20]. For this reason, maximum levels of toxic metals in food have been set in most countries to prevent possible poisoning [22]. In this way, to verify the possibility of using grape stems in distinct industry areas, their content toxic metals were determined, if this by-product can be used safely in new and innovative products on food, cosmetic, and pharmaceutical industries.

The contents of toxic elements Al, Cd, and Pb in grape stems are also presented in Table 3. The most abundant toxic element in grape stems was Al, with concentrations ranging from 3.74 μg/kg dw (cv. Župljanka) to 48.13 μg/kg d.w. (cv. Cabernet Sauvignon). Pb content, an element with no known function in human organism, varied between 0.56 μg/g (cv. Cabernet Sauvignon) and 1.70 μg/g (cv. Plovdina).

Cd was detected only in three cultivars in range 0.01 (cv. Italian Rizling) to 0.04 μg/g (cv. Merlot), whereas As was not detected.

Concerning the literature, Bustamante et al. [22] also quantified Cd and Pb in grape stems, obtaining average concentrations of 0.80 μg/g d.w. and 26.2 μg/g d.w., respectively. Compared to the present study, these concentrations are much higher, since the average value obtained for the nine cultivars analysed were 0.08 μg/g DW for Cd and 0.99 μg/g d.w. for Pb. These differences between studies can be justified by the above mentioned, namely, genetic characteristics, pollution, and de-stemming process.

Table 1. The content of essential macroelements ±SD[a] (μg/g) in grape stems

Sample	K	Mg	Na	P	Ca
Žu	7612±35	645±3	2.48±0.02	1232±13	7287±45
TB	14800±60	1194±11	2.01±0.01	2099±30	4477±18
MH	11836±60	771±5	2,92±0.01	2656±26	5571±20
Sm	9905±40	2142±19	3.73±0.06	2194±21	8410±55
Pr	11344±20	547±4	2.08±0.01	1402±12	3329±20
Pl	7297±40	1832±13.	3.80±0.01	3350±27	4534±27
IR	9685±45	1492±13	4.37±0.06	2727±5	4197±15
CS	10412±180	665±6	2.07±0.02	2757±7	3515±27
Me	16695±132	823±9	4.85±0.05	3017±17	4232±30

[a]SD-standard deviation

Table 2. The content of essential trace and probably essential elements ±SD[a] (μg/g) in grape stems

Sample	Mn	Ni	V	Zn	Fe	Cu
Žu	29.40±0.06	2.60±0.01	1.04±0.02	11.61±0.04	35.38±0.3	8.67±0.07
TB	10.27±0.04	1.72±0.02	2.04±0.05	11.03±0.06	75.3±0.1	5.90±0.09
MH	80.3±0.4	2.04±0.02	2.28±0.02	33.1±0.1	49.7±0.2	18.59±0.06
Sm	16.61±0.05	1.93±0.01	3.81±0.02	29.75±0.08	58.6±0.1	9.34±0.04
Pr	45.60±0.09	3.03±0.00	1.01±0.02	17.18±0.06	70.3±0.2	57.2±0.3
Pl	45±0.2	3.04±0.02	3.40±0.06	13.5±0.1	115.5±0.2	9.19±0.04
IR	37.3±0.1	2.23±0.00	1.41±0.06	11.87±0.01	44.1±0.3	72.7±0.2
CS	72.9±0.4	1.09±0.00	1.3±0.1	10.68±0.06	53.80±0.07	48.7±0.1
Me	123±2	1.81±0.01	1.31±0.08	30.7±0.4	139.8±0.8	43.6±0.3

[a]SD-standard deviation

Table 3. The content of toxic and probable toxic elements ±SD[a] (μg/g) in grape stems

Sample	Si	Ba	Cr	Co	Al	Pb	Cd
Žu	3.80±0.01	24.5±0.1	3.16±0.01	0.06±0.01	3.7±0. 8	0.61±0.03	-[b]
TB	35.5±0.2	6.17±0.02	2.88±0.02	0.05±0.01	27±1	1.41±0.04	-
MH	7.4±0.2	7.01±0.03	2.86±0.02	0.03±0.00	16.8±0.5	0.72±0.04	-
Sm	10.9±0.2	17.4±0.1	2.97±0.01	0.04±0.01	14.7±0.5	1.18±0.02	-
Pr	25.7±0.4	42.4±0.3	3.00±0.05	0.04±0.01	21.8±0.3	1.40±0.01	-
Pl	24.2±0.1	16.45±0.05	3.30±0.01	0.06±0.01	62.1±0.8	1.70±0.04	-
IR	17.44±0.08	7.78±0.06	0.05±0.00	0.21±0.01	24.96±0.07	0.68±0.05	0.010±0.001
CS	31.80±0.05	17.8±0.1	0.24±0.06	0.20±0.01	48.1±0.4	0.56±0.03	0.021±0.002
Me	62.5±0.7	29.45±0.05	0.14±0.02	0.34±0.02	40.0±0.7	0.64±0.04	0.042±0.010

[a]SD-standard deviation; [b]-<LOD (<limit of detection)

Total phenolic content

According to the results for total phenolic content and (Table 4), noticeable difference among investigated grape stem samples is observed. The highest TPC was identified in cv. Merlot stem extract (76.95 mg GAE/g d.w.). The lowest value for TPC was obtained in the sample of Plovdina (34.87 mg GAE/g d.w.). The results of TPC for different grape stems obtained here in agree with those reported in another publication ([7]- 47.04/115.25 mg GAE/g d.w.; [5]- 30.91-96.12 mg GAE/g d.w.). However, Spigno et al. [23] observed total phenolic values considerably lower than those reported in the present research (3.30 mg GA/g d.w. in Barbera variety). All these differences may be attributed to the different vintage, geographical origin, and viticultural conditions of the samples and to the solvent used during the polyphenol extraction process [24].

Phenolic profile

It is also important to note that, these colorimetric methods, namely the Folin-Ciocalteu assay, enclose some constraints related to the limited information provided, the overestimation of the phenolic concentration and the lack of qualitative information on individual bioactive phenolics [5]. However, this assay, and others spectrophotometric methods employed, do not require expensive equipment, are easy to use and are all used to complement the total phenolics determination. Furthermore, also determined the identification of the individual phenolic compounds of these stem samples by HPLC. A total of 13 polyphenols were quantified using the available

standards in the grape stem extracts, mainly phenolic acids, flavan-3-ols and flavonols (Table 4).

Table 4. Phenolic Profile of Grape Stems (mg/g d.w.), TPC- total phenolic content (mg GAE/g d.w.), RSA- radical scavenging activity (mmol TE/g d.w.)

Sample	Žu	TB	MH	Sm	Pr	Pl	IR	CS	Me
(+)catechin	0.584	0.612	0.702	0.898	0.855	0.654	1.125	1.080	1.858
(-)epicatechin	0.011	0.014	0.020	0.032	0.018	0.014	0.028	0.022	0.018
Total flavan-3-ols	0.595	0.626	0.722	0.930	0.873	0.668	1.153	1.102	1.876
Q-3-rutinoside	0.034	0.110	0.012	0.110	0.205	0.005	0.044	0.017	0.022
Q-3-glucuronide	0.414	0.508	0.607	0.547	0.478	0.405	0.573	0.630	1.315
Q-3-glucoside	0.111	0.119	0.151	0.196	0.256	0.132	0.117	0.167	0.449
Quercetin	0.009	0.010	0.037	0.040	0.025	0.046	0.015	0.092	0.018
Kaemferol	0.008	0.009	0.013	0.011	0.020	0.018	0.017	0.015	0.016
Total flavonols	0.576	0.756	0.820	0.904	0.984	0.606	0.766	0.921	1.820
L-7-glucoside	0.055	-[a]	0.114	0.261	0.168	-	0.382	0.097	0.448
Total flavonon	0.055	-	0.114	0.261	0.168	-	0.382	0.097	0.448
t-caftaric acid	0.024	0.055	0.023	0.016	0.094	0.014	0.086	0.135	0.106
Caffeic acid	0.023	0.065	0.020	0.387	0.028	0.010	0.262	0.093	0.092
Syringic acid	0.008	-	0.049	0.061	0.021	0.019	0.115	0.065	0.036
p-Coumaric acid	0.014	0.463	0.083	0.184	0.176	0.012	0.235	0.198	0.213
Ferulic acid	-	-	-	-	0.014	0.016	0.026	0.030	0.028
Total phenolic acids	0.069	0.583	0.175	0.648	0.333	0.071	0.724	0.521	0.475
Total polyphenols	**1.157**	**1.957**	**1.821**	**2.717**	**2.344**	**1.326**	**3.060**	**2.721**	**4.611**
TPC	40.65	52.13	39.15	49.69	43.52	34.87	67.84	78.53	76,95
RSA	0.433	0.547	0.497	0.668	0.523	0.344	0.732	0.766	0.898

[a]-<LOD (<limit of detection)

In this study, only (+)-catechin and (-)-epicatechin were identified in grape stems. This finding aligns with prior research by Souquet et al. [3], where similar constituents were reported. Apostolou et al. [4] identified additional compounds such as procyanidin B3, procyanidin B2, and epicatechin gallate in grape stem extracts from various Greek Vitis vinifera varieties. Similarly, Silva et al. [9] found catechin, epicatechin, gallocatechin gallate, and catechin gallate in grape stem extracts from Portuguese red grape varieties: Touriga Nacional and Preto Martinho. Conversely, Leal et al. [5] detected only catechin. These discrepancies contributed to establishing distinct flavan-3-ol profiles for stems of each grape variety, focusing on quantification of individual compounds [7].

Catechin emerged as the most abundant compound in all examined grape stems. Its concentration ranged from 1.858 mg/g in Merlot to 0.584 mg/g in Župljanka stems. Previous studies reported catechin content in grape stems from Portugal, Spain, and Mexico as 0.55-2.03 mg/g dw, 0.093-1.339 mg/g d.w., and 0.034 mg/g d.w., respectively [7,5]. Interestingly, the concentration of (+)-catechin in grape stems surpassed that found in skins and seeds [25]. This compound exhibits high lability under oxidative conditions, providing protective effects against free radicals [26]. Epicatechin concentrations in the stem extracts of nine cultivars ranged from 0.011 to 0.032 mg/g d.w. This aligns with findings from other studies indicating lower epicatechin concentrations compared to catechin in stems of grape cultivars from Spain (0.006-0.111 mg/g d.w.) [7] and Greece (0.012-0.099 mg/g d.w.) [27].

Although some European studies reported higher catechin concentrations than epicatechin, others found the opposite trend. For instance, Barros et al. [28] found epicatechin to be the most abundant, representing 68% and 75% of total proanthocyanidins in red and white grape cultivars' stems, respectively. These variations may arise from genetic differences between cultivars, light intensity, soil composition, and regional factors [29].

Flavonol content in grape stems ranged from 0.576 to 1.820 mg/g dw. Quercetin, presented in both aglycon and glycoside forms, was consistently detected in all stem extracts. The average amount of aglycon quercetin was 0.032 mg/g d.w., ranging from 0.009 to 0.092 mg/g d.w. Kaempferol was found in relatively low amounts, ranging from 0.008 to 0.020 mg/g d.w. The most abundant glycoside forms of quercetin were quercetin-3-glucuronide and quercetin-3-glucoside, ranging from 0.405 to 1.315 mg/g d.w. and 0.111 to 0.449 mg/g d.w., respectively. Quercetin-3-glucuronide was the major flavonol, representing 48.57% to 74.80% of the total. Luteolin-7-glucoside was also detected in stem samples, albeit in extremely low concentrations compared to major flavonols such as catechin.

The phenolic acid composition, including t-caftaric, caffeic, syringic, p-coumarin, and ferulic acids, varied significantly among different cultivars. T-caftaric acid ranged from 0.014 mg/g d.w. to 0.135 mg/g d.w., consistent with previous reports [27]. Grape stem cultivars Smederevka and Italian Riesling exhibited richness in caffeic acid, while syringic acid content varied across cultivars. P-coumaric acid content was highest in cv. Tamjanika Black and lowest in t-caftaric and caffeic acids.

Radical scavenging activity

The DPPH• test results for grape stem extracts are presented in Table 4, indicating notable antiradical activity in Merlot and Cabernet Sauvignon stem extracts (0.898 mmol TE/g d.w. and 0.766 mmol TE/g d.w., respectively), with the lowest RSA value observed in Plovdina stems (0.344 mmol TE/g d.w.).

Correlating total phenolics, flavan-3-ols, flavonols, flavonones, and phenolic acids contents with DPPH•, yielded corresponding coefficients of determination R^2 of 0.8707, 0.7857, 0.5813, 0.6319, and 0.5466, respectively.

The exact mechanism by which phenolic compounds scavenge free radicals remains uncertain. However, it is evident that the structural composition, particularly the aromatic OH groups, notably the 3',4'-dihydroxy catechol group, play a crucial role, with their activity potentially enhanced by the electron-donating effects of other substituents [30]. For flavonoid compounds like quercetin and rutin, antioxidant activity is associated with O-dihydroxy groups in the B-ring, the presence of a C 2-3 double bond in conjunction with 4-oxo in the C-ring, and 3- and 5-hydroxy groups, along with the 4-oxo function in the A and C-rings.

Pearson's Correlation Analysis

Pearson correlation analysis was conducted to examine the relationship between metals in grape stem samples. Strong correlations ($r \geq |0.5|$) were observed between certain metals (Mn, Fe, Cd, Si, and Co), while others (Cr, Zn, Ba, and Mg) showed poor correlation. The distribution of trace metal ions generally follows a pattern of roots > stems > leaves > fruit > seeds. Metal uptake by plants depends on soil content and plant affinity for specific metals. Antagonistic interactions between ions, particularly mono- and divalent cations, affect uptake, transport, and accumulation in plants. For instance, a strong positive correlation was found between Mg and V, Si and Cd, and Al and P. Vanadium concentrations above certain levels can be toxic to animals and plants, causing oxidative stress and nutrient disruption. Conversely, synergistic interactions between V and mineral elements may occur. Excessive phosphorus reduces the uptake of cationic micronutrients like Ni, Ba, and Cr.

Potassium shows a negative correlation with Ca and Mg due to competitive binding strengths. Lead exhibits negative correlations with elements like K, Na, Ca, Mn, Zn, Cu, and Co, indicating potential atmospheric or soil origins and translocation into plant tissues. Excessive calcium reduces copper uptake, leading to decreased Ca content in stems. These findings suggest complex interactions influencing metal uptake and distribution in grape stems.

In this research, the correlation of all individual polyphenolic compounds in selected types of stems were also determined based on the Pearson correlation coefficient. A high positive correlation was observed between catechin and Q3 glucuronide, catechin and Q3 glucoside, catechin and L7 glucoside, epicatechin and Caffeic acid, Q3 glucuronide and Q3 glucoside, Q3 glucuronide and L7 glucoside. The highest correlation coefficient occurs between catechin and Q3 glucuronide.

Cluster Analysis

Ward's method was employed to classify stem types based on the content of selected elements. The dendrogram presented illustrates the association of wine types based on the elemental content (K, Mg, Na, P, Ca, Mn, Ni, V, Zn, Fe, Cu, Si, Ba, Cr, Co, Al, Pb, and Cd) therein. Through cluster analysis, the analyzed stems were delineated into two significant clusters.

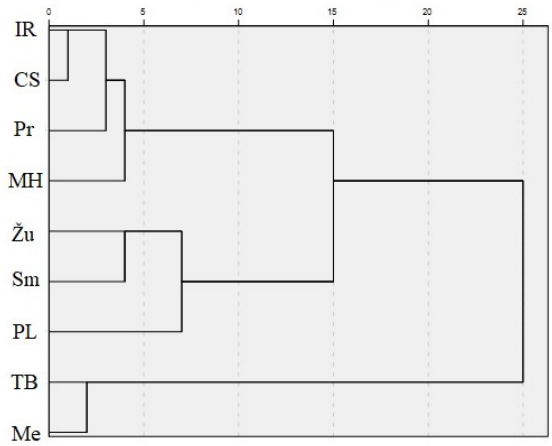

Figure 1. Dendrogram of stems type grouping according to mineral contents

The first cluster encompasses the stems of varieties: Žu, MH, Sm, Pr, Pl, IR, and CS, divided into two subclusters. The first subcluster comprises IR, CS, Pr, and MH, while the second subcluster comprises Žu and Sm along

with Pl. Through cluster analysis, based on the Euclidean distance, it's evident that the greatest similarity exists between wine types CS and IR due to their similar elemental content. Subsequently, Žu and Sm species are grouped together owing to their analogous elemental composition. The second cluster comprises wine types TB and Me, distinguished from other analyzed wines by their notably higher potassium (K) content.

The correlation between wine types regarding their phenol content (catechin, epicatechin, Q-3 - rutinoside, Q-3-glucuronide Q-3-glucoside, Quercetin, Kaemferol, L-7-glucoside, t-caftaric acid, and Caffeic acid) is depicted on Dendrogram 2. Through cluster analysis, the wine types are classified into two significant clusters.

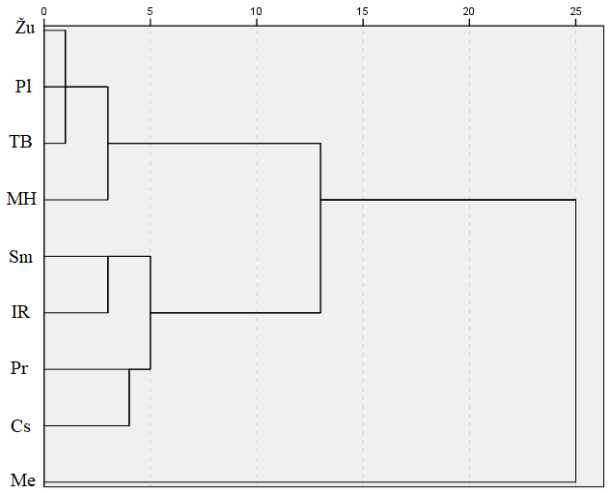

Figure 2. Dendrogram of stems type grouping according to individual polyphenols

The initial cluster comprises: Žu, TB, MH, Sm, Pr, Pl, IR, and CS. Within this cluster, there are two subclusters: the first includes Žu, Pl, TB, and MH, while the second subcluster comprises Sm, IR, Pr, and CS. Utilizing the Euclidean distance obtained from cluster analysis, it becomes evident that the greatest similarity exists among the stem types Žu, Pl, and TB due to their similar element content. The second cluster is represented by the Me stem, distinguished from others by significantly higher levels of catechins, Q-3-glucuronide, and Q-3-glucoside.

CONCLUSIONS

Grape stems, as a by-product of the wine industry, could be further used to make the wine industry eco-friendlier and more sustainable. Results of the present study showed that grape stems have a high content of essential minerals, as the most represented Na, Mg, Ca, and K, as well as phenol compounds, as the most represented Q-3-glucuronide and catechin. In fact, the stems are richer in phenol compounds and minerals, in some cases higher than some food matrices consumed in our diet, whereby this by-product can be a good pledge in the production of value-added products.

EXPERIMENTAL SECTION

Samples

Well known international white and red wine varieties 'Cabernet Sauvignon' (CS), 'Merlot' (Me), 'Italian Riesling' (IR), 'Muscat Hamburg' (MH), together with Serbian autochthonous varieties 'Prokupac' (Pr), 'Plovdina' (Pl), and 'Smederevka' (Sm), Tamjanika Black (TB) and 'Župljanka' (Žu) were studied.

A total of 9 grapevine stem samples were collected in the south-east region of Serbia. For twenty days, samples were washed with water and dried on air, in the dark, and at room temperature. The dry plant material was then packed in paper bags and kept in the dark, dry, and cool place. Before being used, the plant material was comminated by a hammer mill and sieved through a 6 mm sieve.

Samples preparation for HPLC analysis

The dried and ground stem (2.5 g) was macerated in 60%, v/v acetone/water for 24 h (at room temperature, in the shade). After the incubation, the extracts were filtered using the Whatman No. 1 filter paper. The residues were extracted twice with the same fresh solvent and extracts combined. The combined extracts were concentrated and freed of solvent under reduced pressure at 45 °C, using a rotary evaporator (BUCHI Rotavapor R-200). The dried crude concentrated extracts were dissolved using extraction solvent and kept in refrigerator until analyses.

Samples preparation for ICP-OES analysis

Homogenized dried stems (1.0 g) were digested in a solution containing HNO_3 and water (2:1). The samples were heated at 200 ^oC.

Then 1 ml 70% $HClO_4$ was added and reheated. The residue was taken up in 25 ml of 1 % HCl.

Determination of total polyphenolic content (TPC)

Total polyphenolic content in the stem extracts was determined using Folin-Ciocalteu method [31].

Determination of radical scavenging activity using DPPH test, RSA

Radical scavenging activity was determined spectrophotometrically using DPPH (1,1-diphenyl-2-picrylhydrazyl) method [32].

HPLC method

Quantification of individual phenolic compounds was performed using reversed-phase HPLC analysis according to research Mitić et al. 2012 [33]. The equipment used was an HPLC Agilent-1200 series with a UV–Vis DAD detector for multi-wavelength detection. The calibration curve, coefficient of correlation (R^2), the limit of detection (LOD) and the limit of quantification (LOQ) are shown in Table 5. The content of phenolic compound was expressed as micrograms per gram of dried plant material (μg/g).

Table 5. Analytical parameters for hydroxycinnamic acids used for HPLC-DAD analysis

Compound	Calibration curve	(R^2)	LOD[a] (μg/ml)	LOQ[b] (μg/ml)
Catechin	$y = 4628.36x + 0.97$	0.9996	0.33	1.10
Epicatechin	$y = 4785.17x - 0.18$	0.9998	0.30	1.00
Q-3-rutinoside	$y = 4879.79x - 5.55$	0.9996	0.39	1.18
Q-3-glucoside	$y = 5209.08x - 1.05$	0.9996	0.48	1.45
Quercetin	$y = 8143.54x + 5.62$	0.9999	0.52	1.57
Kaemferol	$y = 18921.26x + 1.82$	0.9999	0.55	1.67
Luteolin	$y = 3542.67x + 1.81$	0.9997	0.63	1.93
Caffeic acid	$y = 33621.18x - 0.67$	0.9998	0.30	1.00
Syringic acid	$y = 20540.50x + 0.98$	0.9997	0.29	0.97
p-coumaric acid	$y = 32964.76x - 2.39$	0.9992	0.52	1.57
Ferulic acid	$y = 18346.18x + 1.18$	0.9998	0.48	1.45

[a]LOD-limit of detection, [b]LOQ-limit of determination

ICP-OES analysis

For the elemental analysis, an iCAP 6000 inductively coupled plasma optical emission spectrometer (Thermo Scientific, Cambridge, United Kingdom) with an Echelle optical design and a charge injection device (CID) solid-state detector was used under the operating conditions as follows: flush pump rate – 100 rpm; analysis pump rate – 50 rpm; RF power – 1150 W; nebulizer gas flow – 0.7 L min-1; coolant gas flow – 12 L min-1; auxiliary gas flow – 0.5 L min 1; plasma view – axial; time of rinse – 30 s; measurement in three repetitions. All measurements were carried out in triplicate.

The precise method was optimized for each element. The choice of wavelength was performed based on the relative intensity of the signal as a measure of sensitivity, defects in response to the standards, and the extent of interference in the real sample. All calibration curves were prepared with four standard solutions, including the blank.

Statistical analysis

All measurements and analyses were performed in three replicates in the present study. The data in tables and graphs are presented as mean ± standard deviation. Differences were considered statistically significant at $p < 0.05$.

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