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Assessment of Elemental Content, Antioxidant Activity, and Total Phenolic Content of Vitis sylvestris Gmelin

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Abstract: In this study, we determined the Cu, Fe, Mn, and Zn contents of Vitis sylvestris Gmelin using flame atomic absorption spectrometry (FAAS), and examined the antioxidant properties of Vitis sylvestris Gmelin using radical scavenging capacities and total phenolic content tests. We found the average elemental contents; Cu, Fe, Mn, and Zn as 1.506 ± 0.042 mg/kg, 0.796 ± 0.020 mg/kg, 2.333 ± 0.033 mg/kg, and 3.191 ± 0.262 mg/kg, respectively. When we examined the antioxidant activity tests applied to different extracts, we determined the highest extraction yield with the methanol extract. DPPH radical scavenging activity, ABTS radical scavenging activity, and total phenolic content values in methanol extract were determined to be respectively 3.957 ± 0.146 mg TEAC/g fw, 9.062 ± 0.273 mg TEAC/g fw, and 2.365 ± 0.028 mg GAE/g fw. When we evaluated the antioxidant activity and total phenolic content results for all extracts statistically, we determined that there was generally a statistically significant difference between each extract ($p < 0.01$). Vitis sylvestris Gmelin has high antioxidant content when considering the data obtained. Also, we determined that it is an effective candidate in the protection against reactive oxygen species.

Keywords: Vitis sylvestris Gmelin, elemental content, antioxidant activity, extraction.

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INTRODUCTION

Copper (Cu), iron (Fe), manganese (Mn), and zinc (Zn) are essential elements for human health and should be taken into the body to maintain many important metabolic activities in the human body (1, 2). These elements play an essential role in the growth, development, and health of the human body (2-5). We can find Cu in the structure of many enzymes; similarly, Zn is defined as a co-factor in some enzymes and is involved in various metabolic activities. For example, if we do not take copper and zinc into the human body in sufficient quantities, then the bodily functions may be adversely affected. Consuming various vegetables, fruits, and food products supply the essential elements needed by the human body.

Antioxidants protect our body against diseases by reacting with almost all classes of compounds in the structure of living organisms, by taking a role in the reduction of reactive oxygen species (ROS), which cause significant damage. Antioxidant compounds have an essential role against diseases such as cancer, diabetes, gastrointestinal diseases, neurodegenerative diseases, and aging caused by excessive reactive oxygen species. Many synthetic antioxidant compounds are available, but it is clear that these compounds have toxic and mutagenic effects. In recent years, we started to prefer

natural antioxidant compounds rather than synthetic ones, and we can take these compounds from various sources, including vegetables and fruits. Fruits and vegetables are rich antioxidant sources concerning phenolic compounds, anthocyanins, vitamins, and mineral elements in the literature (6, 7). Authors state that the adverse effects of free radicals reduce by consuming foods, including antioxidants, because of their positive effects on health (6, 8-10).

Turkey, a country rich in fruit and vegetable varieties, is a potential source of phenolic compounds, and one of them is wild grapes and grape varieties (11, 12). In the literature, there are studies about antioxidant activity, phenolic, vitamin, and elemental content of wild grapes grown in different regions (13-16). As a result of biodiversity, which is emerging due to physical geography characteristics, climate differences, and abundant water resources, it is determined that there is no data about wild edible Vitis sylvestris Gmelin reported (17) to naturally grown in Pertek-Tunceli district (Turkey). In this context, we investigated the element content, antioxidant activity, and total phenolic content of Vitis sylvestris Gmelin.

MATERIAL AND METHOD

Reagents and standards

All chemicals and solvents used in sample and standard solution preparation were of analytically pure grade and obtained from Sigma-Aldrich and Merck. We used ultrapure water (Milli-Q, Millipore 18.2 μΩ cm-1) in the experimental studies. We drew calibration graphs by standard solutions prepared in different concentration levels and used these graphs to evaluate the obtained data.

Element contents were determined using Perkin Elmer AAnalyst 800 flame atomic absorption spectrometer (FAAS) (Perkin Elmer, Inc., Shelton, CT, USA). The operation conditions are as follows: We set the wavelength and slit width for Cu, Fe, Mn, Zn respectively 324.8-07 nm, 248.3-0.2 nm, 279.5-0.2 nm, 213.9-0.7 nm, and acetylene and air flow rate was 2.0 L/min and 17.0 L/min. We used a Shimadzu 1800 UV-Vis spectrophotometer for antioxidant activity measurements.

Sample preparation

Vitis sylvestris Gmelin (wild grape) naturally grown in Pertek-Tunceli, Turkey was gathered manually on the season, and then samples were mixed, washed with tap water, lastly passed through deionized water. We then stored them in the freezer until the analysis time.

Wet Digestion procedure for elemental analyses: We separated Vitis sylvestris Gmelin samples into small pieces, transferred approximately a 1-g sample into the beakers, and dissolved with 2 mL of concentrated 1:1 HNO_3: H_2O_2 mixture. We evaporated the samples near dryness with occasional stirring and repeated the same procedure once again. We completed the final volumes to 5 mL with 1.0 M HNO_3 and centrifuged to obtain homogeneous solutions. We carried out elemental analyses in the solutions using FAAS. Blank samples and standard reference materials (0.25 g) (NIST-1547 peach leaves) were also prepared using the same procedure and analyzed. We expressed the results as mean values ± standard deviation of three independent analyses based on fresh weight.

Extraction procedure for antioxidant activity: We separated Vitis sylvestris Gmelin samples into small pieces, approximately 5 g samples transferred into the beakers, and acidified 10 mL of extraction solvents such as water, acetonitrile, and methanol with 0.1% HCl to the samples. We then mixed the samples with extraction solvents for 60 min at room temperature and then centrifuged (10 min at 5000 rpm). We passed the clear solutions through a 0.45 μm injection filter. For DPPH and ABTS radical scavenging activities, we applied total phenolic content tests to different sample extracts. We expressed the results as mean values ± standard deviation of three independent analyses based on fresh weight.

Antioxidant Activity Tests

DPPH radical scavenging activities in the extracts were determined using the method applied by Brand-Williams et al. (18). We made the volumes of extracts taken in a certain amount to 2.5 mL with DPPH solution and then incubated for 30 min at room temperature. We used a UV-Vis spectrophotometer for the absorbance measurements of the solutions at 517 nm. Trolox was the standard and stated the results as Trolox-equivalent on fresh weight (mg TEAC/g fw).

We used the method proposed by Re et al. (19) to measure ABTS radical scavenging activity of the sample extracts. The extracts were made up to 2.5 mL with ABTS solution and incubated at room temperature for 30 minutes in the dark. At the end of the incubation time, we measured the absorbance values with a UV-Vis spectrophotometer at 734 nm. Trolox was the standard, and we stated the results as Trolox equivalent on fresh weight (mg TEAC/g fw).

Determination of Total Phenolic Content

We determined the total phenolic contents by measuring the absorbance value of the color formed by adding the Folin-Ciocalteu reactant and 2% sodium carbonate solution to the extracts (20). We determined the absorbance values by

measuring with a UV-Vis spectrophotometer at 755 nm after 30 minutes of incubation at room temperature. The standard was gallic acid and we expressed the experimental data in terms of gallic acid equivalent on fresh weight (mg GAE/g fw).

We used GraphPad Software (version 5.01 for Windows, GraphPad Software, USA) for the statistical analyses for antioxidant activity and total phenolic content. We established the significance between the groups using the one-way analysis of variance (ANOVA) and Tukey's multiple comparison tests. We considered the differences statistically significant when $p < 0.01$.

RESULTS AND DISCUSSION

We used FAAS to determine Cu, Fe, Mn, and Zn contents in Vitis sylvestris Gmelin. We found the Cu, Fe, Mn, and Zn contents were 1.506 ± 0.042 mg/kg, 0.796 ± 0.020 mg/kg, 2.333 ± 0.033 mg/kg, and 3.191 ± 0.262 mg/kg, respectively. We tested the accuracy of the method with standard reference material (NIST-1547 peach leaves). We determined the certified values for this standard reference material to be 3.75 ± 0.37 mg/kg for Cu, 219.8 ± 6.8 mg/kg for Fe, 97.8 ± 1.8 mg/kg for Mn, and 17.94 ± 0.53 mg/kg for Zn. In the present study, we found the Cu, Fe, Mn, and Zn contents obtained with the used method to be 3.48 ± 0.25 mg/kg, 198.7 ± 7.0 mg/kg, 90.1 ± 2.1 mg/kg, and 17.08 ± 0.48 mg/kg, respectively. We found the recovery values for these elements to be 93% for Cu, 90 % for Fe, 92% for Mn, and 95% for Zn.

A literature study has reported that Cu, Fe, Mn, and Zn concentrations in grapes found from 0.4±0.1 to 2.4±0.1 µg/g, 6.6 ± 0.1 to 14.8 ± 0.3 µg/g, 3.1±0.2 to 41.8±0.2 µg/g, and 2.1±0.1 to 20.4±1.0 µg/g in a study, respectively (21). In another study, researchers determined the element contents in the grape as 2.6±0.1 µg/g for Cu, 3.8±0.2 µg/g for Fe, 3.4±0.2 µg/g for Mn, and 2.2±0.1 µg/g for Zn (22). Researchers examined the elemental contents of some fruits and vegetables in a study. They found Cu and Zn concentrations in the grape to be 3.5 mg/kg and 6.1 mg/kg, respectively (23). In the present study, the data concerning elemental contents were compatible, found with the literature data.

We determined the antioxidant activity of Vitis sylvestris Gmelin using DPPH and ABTS radical scavenging activities and total phenolic content tests, applying these tests to the extracts of the samples processed with water, acetonitrile and methanol, and evaluated the results statistically. There is a mention in a literature report that antioxidant activity results changed significantly depending on the solvent type (24-27). In the current study, we applied the antioxidant activity and total phenolic content test applied to different extracts; we obtained the highest results for all tests with methanolic extracts. As seen in Figure 1, average DPPH radical scavenging activity results were determined to be 0.537 ± 0.060 mg TEAC/g fw for aqueous extracts, 3.785 ± 0.077 mg TEAC/g fw for acetonitrile extracts, 3.570 ± 0.08 mg TEAC/g fw for ethanolic extracts, and 3.957 ± 0.146 mg TEAC/g fw for methanolic extracts. We evaluated the results statistically. We found out that there were statistically significant differences between acetonitrile, ethanolic and methanolic extracts of aqueous extracts, and also between ethanolic and methanolic extracts. On the other hand, we determined that there were no statistically significant differences between ethanol and methanol extracts of acetonitrile extracts ($p < 0.01$).

We found the average ABTS radical scavenging activity results for water, acetonitrile, ethanol, and methanol extracts as 2.285 ± 0.120 mg TEAC/g fw, 7.984 ± 0.355 mg TEAC/g fw, 8.471 ± 0.642 mg TEAC/g fw, and 9.062 ± 0.273 mg TEAC/g fw, respectively (Figure 2). When we examined the data statistically, we determined that there were statistically significant differences between acetonitrile, ethanolic, and methanolic extracts against aqueous extracts, and also between acetonitrile and methanolic extracts. On the other hand, we found that there were no statistically significant differences between acetonitrile and methanol extracts of ethanol extracts ($p < 0.01$). While we found the highest total phenolic content in methanolic extracts (2.365 ± 0.028 mg GAE/g fw), the lowest total phenolic content was in aqueous extracts (0.592 ± 0.045 mg GAE/g fw) (Figure 3). When we statistically compared the data for total phenolic contents, we found that there was a statistically significant difference between every two extracts except for acetonitrile and ethanolic extracts ($p < 0.01$).

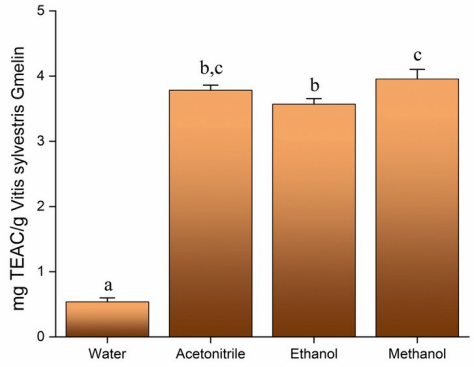

Figure 1: DPPH radical scavenging activity of Vitis sylvestris Gmelin.

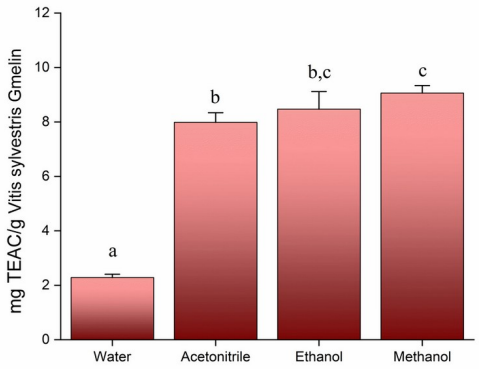

Figure 2: ABTS radical scavenging activity of Vitis sylvestris Gmelin.

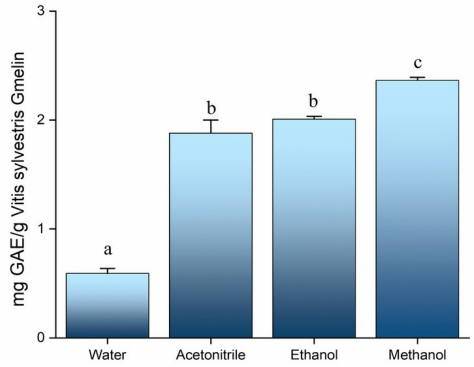

Figure 3: Total phenolic content of Vitis sylvestris Gmelin.

In a study, researchers examined phenolic profile and antioxidant activity of different grape varieties and reported total phenolic content results in a range from 0.44 ± 0.02 to 7.94 ± 0.19 mg gallic acid equivalents/g grape sample (28). Antioxidant activity of black grape obtained applying different drying methods determined with DPPH and ABTS assay, total phenolic content tests. Literature reports that antioxidant activity results changed depending on different drying methods. The highest value for total phenolic content found to be 20.21 mg/g DW in fresh grapes, but this value in the freeze-, oven- and sun-dried samples continued to decrease. The values of DPPH and ABTS antioxidant activities in fresh grapes determined as 66.07 mmol TE/kg DW and 137.65 mmol TE/kg DW (29). Total phenolic compounds of red grape varieties examined by Correia and

Jordão found to be average 1341.0 mg/L, and antioxidant capacities determined varying from 3.96 to 32.96 mm/L Fe(II) (30). The results in the presented study were compatible with those found in the literature. Researchers determined that the data changed importantly depending on different conditions such as drying method, extraction method, and solvent type, as stated in the literature (24-27, 30).

CONCLUSIONS

In this study, we investigated the elemental content and antioxidant activity of Vitis sylvestris Gmelin. Elemental analysis results carried out as applied to the wet digestion method were determined to be 1.506 ± 0.042 mg/kg for Cu, 0.796 ± 0.020 mg/kg for Fe, 2.333 ± 0.033 mg/kg for Mn, and 3.191 ± 0.262 mg/kg for Zn, respectively. We have found that DPPH and ABTS radical scavenging activity, total phenolic content results tested with different polar solvents are 3.957 ± 0.146 mg TEAC/g fw, 9.062 ± 0.273 mg TEAC/g fw, 2.365 ± 0.028 mg GAE/g fw for methanol extracts determined as the best solvents. Vitis sylvestris Gmelin naturally grown in Pertek, Tunceli; according to the obtained results can be evaluated as an antioxidant food, especially, we will add new data with this study about the fruit to the literature.

CONFLICT OF INTEREST

The author declares no conflict of interest.

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