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Original Article

Investigation and Determination of the Presence of Resveratrol in the Leaves of 99 Grapevine Varieties from the Western Anatolia Region in Turkey

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Abstract

Grapevine (*Vitis vinifera* L.) is one of the main plants of economic importance in the world. The development and adaptation of this plant, which is of great importance economically, to have the desired characteristics, is of great importance. The plant is an extremely important resource not only in terms of its fruit, but also because of the presence of secondary metabolites contained in its cellular structure. One of these secondary metabolites is resveratrol. Resveratrol is a metabolite synthesized in high amounts in grapevine, leaf and grape bark. The aim of the study was to determine the amount and content associated with phenolic content of 99 grapevine varieties protected in the Aegean region in Turkey. Our specific goal with this study was to determine an important parameter for Plant Breeding with data obtained as a result of using ultra-high performance liquid chromatography (HPLC) for the first time in grapevine varieties of the Aegean region. As a result of the analysis, the amount of resveratrol varies between 0-90 mg/kg. At the same time, the total phenol amount analysis was performed to determine the phenolic amount between grapevine types. Compared to plants capable of producing resveratrol, processed or fresh products of the vine are known to be widely consumed by a very large audience. For this reason, it reveals the importance of breeding studies to increase the content of resveratrol.

Keywords: Resveratrol, Grapevine, Phenolic compounds, HPLC, Folin-Ciocalteu analysis

Introduction

There are many studies on plants that are known to have health benefits. There are studies on phytochemicals known as bioactive compounds in foods [1]. Phytochemicals are plant chemicals that have no nutritional value but have protective and disease-preventing properties. Although it is known that the plant produces to protect itself, studies in recent years have proven that it also protects the human body against diseases. Resveratrol, a compound with polyphenolic properties, is also one of them. Resveratrol is an antioxidant substance in polyphenol structure that is found in high amounts in grape skins in nature and protects the plant from fungal infections [2,3]

Resveratrol (trans-3,4',5-trihydroxystilbene) is a compound produced for the purpose of creating a mechanism of resistance to environmental stress and disease during any period of plant growth and development [4]. Resveratrol is one of the most powerful antioxidants and phenolic compound in the stilbene class [5,6]. Phenolic compounds abundant in fruits and vegetables are secondary metabolites of different structures and functions, usually having an aromatic ring containing one or more hydroxyl groups. Secondary metabolites are not components that must be present in the plant in order for all or a certain part of the plant to remain alive, but they can participate in important functions [7]. Biologically, their diversity can be characterized in different ways, such as being synthesized [8].

Resveratrol is found in quite high amounts in grape species (*Vitis* spp.). This compound is a secondary metabolite identified in the genus *Vitis* which is a

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phytoalexin whose health benefits have been proven by many researchers [9,10]. Recently, numerous studies conducted in cellular and animal models have revealed that resveratrol has many biological activities. In this context, resveratrol's antioxidant activity protects against vascular obstruction, regulates the synthesis of liver lipoproteins and fats, cancer tumors prevention and pain relief properties were determined [11]. Although so much research has been done on resveratrol, these studies are carried out only on certain species. Species commonly used in the field of feeding include mulberry, groundnut and grapevines [12,13]

Grapevine is an important plant in Mediterranean region especially in Turkey. In order to increase its economic importance understanding the biological content is very important. When we look at the variety richness the plant has more than 1200 varieties [14,15]. Most of these varieties are not cultivated as they do not bear fruit or they are considered to have no commercial significance due to low fruit quality. However, today, contrary to what farmers think, a plant is not only planted for its fruits. The bio-active compounds contained in plants are used as an important raw material in many areas from the pharmaceutical industry to the cosmetics industry. Although the number of vine varieties grown in the Mediterranean region is over hundreds, there are 99 varieties of lower vine types known to be grown specifically for the Aegean region [16]. These lower grapevine types that are not used in agricultural production because their fruit yield is low, but rich in phenolic content [17,18].

This study focused on resveratrol, which is of great commercial importance. In the literature, it is stated that resveratrol is mostly found in fruits and fruit seeds rather than leaves. The novelty that this study will add to the literature is to reveal the presence of resveratrol in grape leaves. It will also be shown that, contrary to the literature data, it is possible to obtain resveratrol from grape leaves. Another difference revealed in this study is that the leaves of non-fruiting plant varieties are used for resveratrol extraction. Thus, it is planned to bring these grape varieties that are not commercially important to agriculture. In summary, when we look at the literature, it is seen that resveratrol is mostly obtained from the seed of the grape or the peel of the fruit. The resveratrol in its leaves is seen to be too little to be shown and there is no study on it. In this study, it will be shown that when we use the correct mobile phase, resveratrol can also be obtained from the leaves. The 99 grape varieties used in the study are region-specific and are not planted because they do not produce fruit, they are only stored in gene banks. A different bio-economic contribution of these cultivars may emerge when resveratrol has been shown to be present in their leaves. Thus, it will pave the way for the cultivation of these varieties in suitable agricultural areas. In the study, it is aimed to determine the amount and content associated with phenolic content of 99 grapevine varieties protected within the gene resources of the Aegean region of the Directorate of Manisa Viticulture Research Institute in Turkey.

Material and Methods

Collection of Samples

99 grapevine varieties from the grapevine germplasm collection of Manisa Viticulture Research Institute Directorate trial lands were used as plant leave material in the project. Leaves from existing grapevine trees were taken at the end of august and dried in a closed area in the shade for 2 weeks and stored for extraction process. The names of the varieties were shown in Table 1.

Extraction of Active Substance from Grapevine Leaf

The dried grapevine leaves were placed in a mixture of ethyl alcohol and water (70%), after the dried leaves were ground in a mechanical grinder. At this stage, the 1:20 ratio of solid: liquid was used. Plant- alcohol mixture was stirred at room temperature for 2 hours. The mixture was filtered under vacuum and the alcohol in the mixture was removed by the rotary evaporator. Water phase of the mixture was frozen in -20 ℃. The water was removed by using lyophilizer and the extract was obtained.in powder form. The method was used for all 99 grapevine varieties to get the crude extracts. The extracts were stored in black bottle necks at dark.

Determination of Resveratrol from Grapevine Leaf by HPLC

HPLC analysis was performed to determine the amount of the active ingredient resveratrol in the total extract. The Thermo Scientific Ultimate 3000 HPLC device was used. An isocratic analysis was performed. The mobile phase was a mixture of water: methanol: glacial acetic acid (440:550:10, v/v/v). Readings were done through the DAD detector at wavelengths of 306 nm and 288 nm. The column temperature was set to 40° C. The mobile phase flow rate was 0.8 ml/min.

Determination of Phenolic Quantity by Folin-Ciocalteu Method

The total amount of phenol was determined using 96 well plates by the Folin-Ciocalteu method. The experiments were conducted in 3 repetitions. Gallic acid will be used as a standard solution, and the amount of phenol in the extracts was calculated through the gallic acid calibration curve. Folin-Ciocalteu brace was diluted with distilled water in a ratio of 1:10 and brought to working concentration.

Table 1 The name of the grape plant varieties used in the study

Name of Varieties	Name of Varieties	Name of Varieties
Abuguş	Bir Çekirdekli	Ergin Çekirdeksizi
Ak Dimrit	Bornova Misketi	Erkenci Beyaz Üzüm
Ak Üzüm	Bostancı	Eski Beyaz Üzüm
Akhisar Razakısı	Bozyaprak	Eski Kara
Al İdris	Bulama	Fahri
Alacalı	Bulkaz Karası	Fazlı Kerim
Alemşah	Buludi Hevenk	Feslehan
Ali Çavuş	Burgunder	Fili
Altı Kulaç	Buza Boku	Gaydura
Alyanak	Bülbül	Gelin Parmağı
Amasya 256/10	Büzgülü	Gökçe Gemre
Analıkız	Cami Üzümü	Gümülcine
Antep Karası	Çavuş	Güvercin Gözü
Askeri	Çekirdeksiz Azmanı	Güzgülü
Asmalık	Çeşme Beyazı	Hacı Balbal
Aydın Karası	Çeşme Pembesi	Hacı Efe Üzümü
Ayvalık Karası	Çilek Üzümü	Hacıoğlu Siyahı
Bağdat Siyahı	Çinili	Hafızali
Balçova Karası	Dam Üzümü	Halis Gemre
Banazı Karası	Denizli Karası	Hasandede
Barbaros	Deve Gözü	Hindistan
Belen	Deve Üzümü	Hurma
Benli Belercen	Dimrit	İnce Kara
Beyaz Çavuş	Dökülgen	İri Beyaz Çekirdekli
Beyaz Gut	Dumalı Gemre	İsimsiz
Beyaz Hevenk	Durif	İslambol
Beyaz İri Üzüm	Efe Püskülü	İstanbul Üzümü
Beyaz Kokulu	Ekse Çubuğu	Kadın Parmağı
Beyaz Marzivat	Eksenaz	Koca Osman
Beyaz Şam	Emir Ali	Nedrebol
Beyaz Şaraplık	Erenci Dimrit	Paşalar Üzümü
Beyaz Şarlak	Erenkö Beyazı	Sarı Üzüm
Beylerce	Erenköy Siyahı	Sulu Üzüm

Sodium carbonate solution will be prepared as 7.5% in distilled water, 100 µl folin solution is added to 20 µl sample for the experiment and left to incubate for 2.5 minutes. After incubation, 80 µl of sodium carbonate solution was added to the samples and left for 1-hour incubation at room temperature and darkness. At the end of the period, samples were taken for photometric reading at 725 nm. The absorption results were converted to gallic acid equivalent by the calibration curve and the total amount of phenol in the extract was calculated.

Results

Grapevine leaves are collected during the summer period. Because environmental stress conditions are effective for the formation of active substances in leaves, the period in which temperatures are especially effective has been selected. Young sprouts were not selected because they would not be exposed to environmental stress, which would allow the formation of bio-active substances in these leaves. Matured leaves were thought to be conducive to gathering. In order to prevent the change in content caused by direct exposure to sunlight in the

leaves and to prevent roasting of the leaves, laying was done in an indoor area. The extraction process was carried out in a total of four stages. These are, respectively, retention in alcohol, removal of solid particles, removal of alcohol, and lyophilization.

Drawing Standard Curve Graphs

A standard curve is a quantitative statistical graph prepared using known concentrations of the relevant substance to determine unknown concentrations of the substance. The resveratrol standard is dissolved in 100% acetonitrile. 6 diluents created by making serial dilutions were studied in the HPLC device and a standard curve was created. As a result of the study, the R-Square value obtained was found to be 98%. The standard curve is shown in Figure-1. Content determination was made by calculating the area below the peak.

Determination of Resveratrol in Grapevine Leaves with HPLC

During the drawing of the standard graph, the retention time of resveratrol was determined as 15 minutes in the program used in HPLC analysis. For this reason, results with a retention time of 15 minutes in the analysis of plant extracts were accepted as resveratrol. During the analysis, the plant extract was loaded into the system at a concentration of 15 mg / ml. In HPLC analyzes of 99 different grape varieties, the resveratrol amounts of the extracts obtained from the leaves were measured in the range of 0 to 90 mg per kilogram of leaf extract. The amount of 90 mg / kg resveratrol contained in the Gaydura variety, which is native to the Aegean region, has the highest value among the analyzes which was shown in Figure-2. As can be seen from Figure-2, resveratrol is observed within 15 minutes. Resveratrol determination was calculated by using the area under this peak in the formula found from the standard curve graph. Another example of grapevine leaves extract can be seen in Figure-3. In the HPLC chromatogram, the amount of resveratrol obtained from the extract of leaves of a grapevine variety called Feslehan, which is native to the Aegean region. The area below the designated peak gave us the amount of resveratrol using the function obtained in the standard curve chart and was calculated as 32 mg/kg.

Table-1 shows the names of all grape varieties used in the study, and the amount of resveratrol in milligrams. When the table is examined, the results obtained depending on the chromatograms of 99 varieties are extremely promising. When we examine the chromatograms, we see that 99 varieties produce resveratrol. Gaydura is seen as the most promising vine variety producing resveratrol as much as 10% of its dry weight. Apart from Gaydura, 6 more varieties are observed that efficiently produce resveratrol in their leaves per kilogram of dry weight. These are written in bold in Table-2. Among the 99 varieties studied, 20 of them do not produce or produce very little resveratrol.

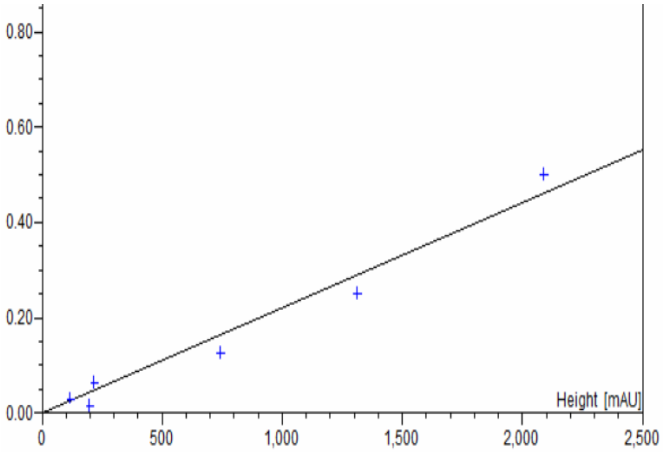

Fig. 1 Resveratrol standard curve. The R-Square value was found to be 98%.

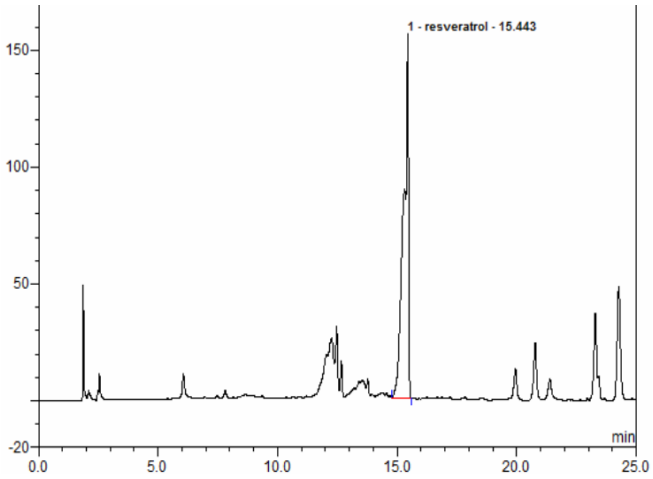

Fig. 2 HLPC Chromatogram of resveratrol found in Gaydura grapevine leaf extract.

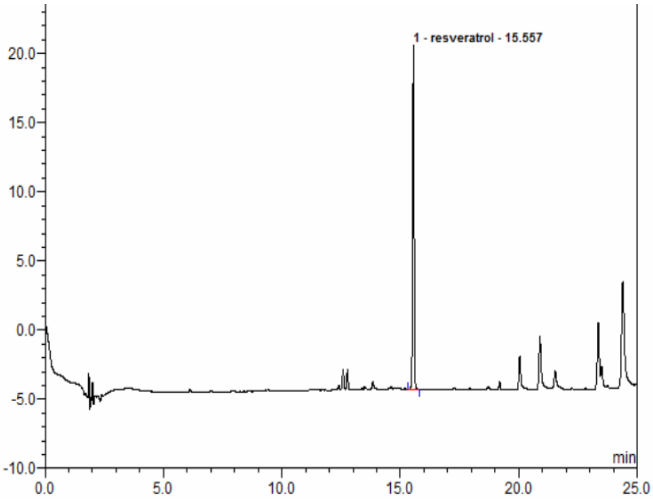

Fig. 3 HLPC Chromatogram of resveratrol found in Feslehan grapevine leaf extract

Table 2 The amounts of resveratrol extracted from 99 different varieties grapevine leaves

Name of Varieties	Amount of Resveratrol (mg/kg)	Name of Varieties	Amount of Resveratrol (mg/kg)	Name of Varieties	Amount of Resveratrol (mg/kg)
Abuguş	10,00	Bir Çekirdekli	6,00	Ergin Çekirdeksizi	2,00
Ak Dimrit	4,00	Bornova Misketi	2,00	Erkenci Beyaz Üzüm	4,00
Ak Üzüm	2,00	Bostancı	18,00	Eski Beyaz Üzüm	2,00
Akhisar Razakısı	0,00	Bozyaprak	10,00	Eski Kara	8,00
Al İdris	2,00	Bulama	26,00	Fahri	2,00
Alacalı	4,00	Bulkaz Karası	14,00	Fazlı Kerim	0,00
Alemşah	8,00	Buludi Hevenk	6,00	Feslehan	32,00
Ali Çavuş	8,00	Burgunder	2,00	Fili	8,00
Altı Kulaç	24,00	Buza Boku	2,00	Gaydura	90,00
Alyanak	68,00	Bülbül	12,00	Gelin Parmağı	4,00
Amasya 256/10	38,00	Büzgülü	10,00	Gökçe Gemre	4,00
Analıkız	6,00	Cami Üzümü	6,00	Gümülcine	18,00
Antep Karası	42,00	Çavuş	10,00	Güvercin Gözü	8,00
Askeri	10,00	Çekirdeksiz Azmanı	4,00	Güzgülü	18,00
Asmalık	4,00	Çeşme Beyazı	16,00	Hacı Balbal	0,00
Aydın Karası	22,00	Çeşme Pembesi	4,00	Hacı Efe Üzümü	6,00
Ayvalık Karası	4,00	Çilek Üzümü	16,00	Hacıoğlu Siyahı	2,00
Bağdat Siyahı	10,00	Çinili	4,00	Hafızali	2,00
Balçova Karası	8,00	Dam Üzümü	22,00	Halis Gemre	10,00
Banazı Karası	38,00	Denizli Karası	6,00	Hasandede	22,00
Barbaros	4,00	Deve Gözü	12,00	Hindistan	2,00
Belen	6,00	Deve Üzümü	36,00	Hurma	6,00
Benli Belercen	4,00	Dimrit	12,00	İnce Kara	12,00
Beyaz Çavuş	2,00	Dökülgen	10,00	İri Beyaz Çekirdekli	6,00
Beyaz Gut	10,00	Dumalı Gemre	2,00	İsimsiz	4,00
Beyaz Hevenk	20,00	Durif	10,00	İslambol	8,00
Beyaz İri Üzüm	22,00	Efe Püskülü	12,00	İstanbul Üzümü	8,00
Beyaz Kokulu	2,00	Ekse Çubuğu	10,00	Kadın Parmağı	6,00
Beyaz Marzivat	8,00	Eksenaz	6,00	Koca Osman	2,00
Beyaz Şam	2,00	Emir Ali	4,00	Nedrebol	2,00
Beyaz Şaraplık	0,00	Erenci Dimrit	6,00	Paşalar Üzümü	8,00
Beyaz Şarlak	6,00	Erenkö Beyazı	6,00	Sarı Üzüm	8,00
Beylerce	4,00	Erenköy Siyahı	6,00	Sulu Üzüm	14,00

The grape varieties other than these two groups appear as resveratrol-producing cultivars that do not have high productivity.

Total Phenol Analysis in Grapevine Leaf

A total phenolic substance was determined by a spectrophotometric method using Folin Ciocalteau agent. The absorption values obtained were replaced in the calibration curve prepared with gallic acid and the results were expressed as gallic acid equivalent. Gallic acid calibration curve used for determination of total phenolic substance.

Total phenol and gallic acid measurements were taken for evaluation. As shown in Table-2, the results show a profile close to the normal distribution. Experiments were conducted in three repetitions. When the results are examined, we can say that the grapevine leaves contain a high amount of phenol. Table-2 shows gallic acid equivalents of grapevine leaves. Gallic acid results were calculated according to the gallic acid calibration curve. These measurements showed different phenol amounts

between grapevine leaf species compared to each other. Looking at the Table-2 and Table-3 shows that there is a distribution between the amount of resveratrol and gallic acid equivalents.

Discussion

Control of phenolic substance content in grapevine plant and determination of quantity is important for their use in plant breeding. Considering that a large number of grapevine varieties found in the world are not produced due to insufficient fruit yield, the contribution of breeding and production of fertile species in terms of the active substance found by the study to the economy of countries is an amount that cannot be ignored. As a result of HPLC studies, it was observed that the amount of phenolic content produced by each grapevine variety was different from each other, especially as a result of resveratrol quantity analysis and HPLC chromatogram studies.

Table 3 The amounts of total phenols extracted from 99 different varieties grapevine leaves

Name of Varieties	Gallic Acid Equivalents (GAE)	Name of Varieties	Gallic Acid Equivalents (GAE)	Name of Varieties	Gallic Acid Equivalents (GAE)
Abuguş	21,50	Bir Çekirdekli	34,85	Ergin Çekirdeksizi	26,87
Ak Dimrit	30,78	Bornova Misketi	26,22	Erkenci Beyaz Üzüm	20,85
Ak Üzüm	18,24	Bostancı	31,76	Eski Beyaz Üzüm	27,04
Akhisar Razakısı	18,24	Bozyaprak	31,76	Eski Kara	27,36
Al İdris	27,20	Bulama	45,12	Fahri	19,22
Alacalı	22,48	Bulkaz Karası	46,74	Fazlı Kerim	18,57
Alemşah	77,36	Buludi Hevenk	24,92	Feslehan	56,35
Ali Çavuş	32,09	Burgunder	25,57	Fili	46,58
Altı Kulaç	37,13	Buza Boku	23,94	Gaydura	95,44
Alyanak	167,11	Bülbül	32,25	Gelin Parmağı	25,73
Amasya 256/10	27,20	Büzgülü	32,90	Gökçe Gemre	56,52
Analıkız	54,56	Cami Üzümü	39,09	Gümülcine	35,51
Antep Karası	115,96	Çavuş	141,70	Güvercin Gözü	25,41
Askeri	120,69	Çekirdeksiz Azmanı	16,12	Güzgülü	63,85
Asmalık	53,75	Çeşme Beyazı	45,28	Hacı Balbal	22,64
Aydın Karası	47,07	Çeşme Pembesi	21,17	Hacı Efe Üzümü	42,18
Ayvalık Karası	28,67	Çilek Üzümü	23,78	Hacıoğlu Siyahı	21,99
Bağdat Siyahı	30,95	Çinili	28,18	Hafızali	19,38
Balçova Karası	73,62	Dam Üzümü	72,15	Halis Gemre	33,71
Banazı Karası	31,11	Denizli Karası	14,82	Hasandede	37,13
Barbaros	26,55	Deve Gözü	36,97	Hindistan	18,57
Belen	60,10	Deve Üzümü	70,85	Hurma	21,50
Benli Belercen	28,34	Dimrit	53,26	İnce Kara	65,64
Beyaz Çavuş	23,13	Dökülgen	21,82	İri Beyaz Çekirdekli	30,13
Beyaz Gut	41,53	Dumalı Gemre	30,94	İsimsiz	40,56
Beyaz Hevenk	76,71	Durif	36,32	İslambol	50,49
Beyaz İri Üzüm	39,41	Efe Püskülü	73,13	İstanbul Üzümü	47,23
Beyaz Kokulu	19,71	Ekse Çubuğu	49,84	Kadın Parmağı	25,90
Beyaz Marzivat	24,92	Eksenaz	46,58	Koca Osman	17,92
Beyaz Şam	43,81	Emir Ali	50,16	Nedrebol	25,73
Beyaz Şaraplık	38,11	Erenci Dimrit	36,32	Paşalar Üzümü	21,17
Beyaz Şarlak	36,16	Erenkö Beyazı	32,09	Sarı Üzüm	41,53
Beylerce	32,09	Erenköy Siyahı	27,36	Sulu Üzüm	43,16

Due to the difference in their genetic structure, the mechanisms of plants to protect themselves against external factors also differ. Even if they are in the same species, it is inevitable that they will gain the ability to synthesize different metabolic at different rates as a result of their crossover, which leads to the formation of different grapevine varieties. The study also showed a supportive result.

In the literature review, Zareei *et al*., 2019 and Ji *et al*., 2014 [19,20], quantity determination analyses of resveratrol extracted by different methods from grapevine varieties were examined. In the Gutierrez-Escobar *et al*., 2021 [21] study, analyses were performed on the pure stilbene extract from grapevine shoots and it was determined that 0.5 (w/w) of the total stilbene content belonged to resveratrol. Compared to our experiments, there were similarities in resveratrol ratios. This paper is a larger-scale study and includes an analysis of numerous grapevine species.

As a result of total phenol analysis, phenol amounts of 99 different grapevine leaves were determined. All of the grapevine samples collected and measured were taken during the full ripening period of the plant. They have been exposed to extreme heat and rain. Phenolic compounds are secondary metabolites, and variability in their phenolic amounts can be observed due to the environmental factors they are exposed to. We can say that there are also differences in phenolic amounts of grapevine leaves according to the species differences between each other.

It has been noted that the product efficiency is low in the selected 99 types of grapevine. Along with the results obtained, the content of grapevine species that have low crop yield but are rich in phenolic content was determined. It is aimed to have a return to the country's economy with the use of grapevine species that are not used in agricultural production but are rich in phenolic content in breeding programs.

Conclusion

In this study, the presence of resveratrol in the leaves of 99 grape varieties belonging to the Aegean region and the change in total phenol amounts were investigated. When the results of the research are examined, it has been shown that there are different rates of resveratrol in the leaves of 99 varieties. This is considered to be an extremely important finding for the grape plant, which is generally reported to have a high amount of resveratrol in the skin or seed of the fruit in the literature. In addition, it has been shown that the amount of phenolic content among these cultivars differs from each other. In the light of these findings, it is exciting to think that these varieties can be grown in agricultural lands suitable for their cultivation due to their different bio-economic contributions. It can be used as an alternative to grapes grown only for their fruit in the cosmetics, pharmaceutical and food industries.

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References

1. Villano D, Fernveez-Pachon S, Troncoso A.M, Garcia-Parrilla M.C. Comparison of antioxidant activity of wine phenolic compounds and metabolites in vitro**.** Analytica Chimica Acta. 2005;538:391-398.
2. Hershoff A, Rotelli A. Herbal Remedies: A Quick and Easy Guide to Common Disorders and Their Herbal Treatments, Penguin. 2001.
3. Kaul N, Siveski-Iliskovic N, Hill M, Slezak J, Singal P K. Free radicals and the heart. J Pharma Toxicological Methods. 1993;30:55-67.
4. Detenchuk E.A, Trebse P, Marjanovic A, Kosyakov D.S, UIyanovskii N.V, Kralj M.B, Lebedev A.T. Transformation of resveratrol under disinfection conditions. Chemosphere. 2020;260:127557.
5. Martinez J, Moreno J.J. Effect of Resveratrol, a Natural Polyphenolic Compound, on Reactive Oxygen Species and Prostaglandin Production. Biochemical Pharmacology. 2000;59:865-870.
6. Krol A, Amarowicz R, Weidner S. Changes in the composition of phenolic compounds and antioxidant properties of grapevine roots and leaves (*Vitis vinifera* L.) under continuous of long-term drought stress. Acta Physial Plant. 2014; 36:1491-1499.
7. Limmongkon A, Janhom P, Amthong A, Kawpanuk M, Nopprang P, Poohadsuan J, Somboon T, Saijeen S, Surangkul D, Srikummool M, Boonsong T. Antioxidant activity, total phenolic, and resveratrol content in five cultivars of peanut sprouts. Asian Pacific J Trapical Biomedicine. 2017;7:332-338.
8. Robards K, Prenzler P.D, Tucker G, Swatsitang P, Glover W. Phenolic compounds and their role in oxidative processes in fruits. Food Chemi. 1999;66:401-436.
9. Siemann E.H, Creasy L.L. Concentration of the phytoalexin resveratrol in wine. American J Enology Viticulture. 1992;43:49-52.

10. Dzhambazova T, Kondakova V, Tsvetkov I, Batchvarov R. Grape Secondary Metabolites – Benefits for Human Health. In Chang, R.C.-C. (ed.), Advanced Understanding of Neurodegenerative Diseases, In Tech, 2011, pp. 265-298.
11. Cleophas T.J. Wine, beer and spirits and the risk of myocardial infarction: a systematic review. Biomedicine Pharmaco. 1999;53:417-423.
12. Atanacković M, Petrović A, Jović S, Gojković-Bukarica L, Bursać M, Cvejić J. Influence of winemaking techniques on the resveratrol content, total phenolic content and antioxidant potential of red wines. Food Chem. 2012;131:513-518.
13. Sukovic D, Knezevic B, Gasic U, Sredojevic M, Ciric I, Todic S, Mutic J, Tesic Z. Phenolic Profiles of Leaves, Grapes and Wine of Grapevine Variety Vranac (*Vitis vinifera* L.) from Montenegro. Foods. 2020;9:1-24.
14. Maia M, Ferreia A.E.N, Laureano G, Marques A.P, Torres V.M, Silva A.B, Matos A.R, Cordeiro C, Figueiredo A, Silva M.S. *Vitis vinifera* 'pinot noir' leaves as a source of bioactive nutraceutical compounds. Food Function. 2019;10:3803-4456.
15. Brigheti E, Casagrande K, Cardoso P.Z, Pasa M.S, Ciotta M.N, Brighenti A.F. Total polyphenols content in different grapevine varieties in highlands of southern brazil. BIO Web of Conferences 9, 40th World Congress of Vine and Wine. 2017. doi: 10.1051/bioconf/20170901024.
16. Akyurt B, Başyiğit B, Çam M. Phenolic Compounds Content, Antioxıdant and Antidiabetic Potentials of Seven Edible Leaves. The J Food, 2018;43:876-885.
17. Ince E. Türkiye'de Meyve ve Asma Fidanları Üretim Materyallerinde Tarım Bakanlığı Bitki Sağlığı Uygulama Çalışmaları. Inter J Agric Natural Sci. 2018;1:14-18.
18. Amarowicz R, Narolewska O, Karamac M, Kosinska A, Weidner A. Grapevine Leaves as a Source of Natural Antioxidants. Polish J Food and Nutrition Sci. 2008;58:73-78.
19. Ji M, Li Q, Ji H, Lou H. Investigation of the distribution and season regularity of resveratrol in *Vitis amurensis* via HPLC-DAD-MS/MS. Food Chem. 2014;142:61-65.
20. Zareei E, Zaare-Nahandi F, Oustani S, Hajilou J. Effects of magnetic solutions on some biochemical properties and production of some phenolic compounds in grapevine (*Vitis vinifera* L.). Sci Hortic. 2019;253:217-226.
21. Gutierrez-Escobar R, Fernandez-Martin M.I, Richard T, Fernandez-Morales A, Carbu M, Cerbarian-Tarancon C, Torija M.J, Puertas B, Cantos-Villar E. Development and characterization of a pure stilbene extract from grapevine shoots for use as a preservative in wine. Food Control. 2021;121:107684.