

CHEMICAL COMPOSITION AND ANTIOXIDANT ACTIVITY OF SEVEN DIFFERENT GRAPE EXTRACTS

LAILA RADWAN ABDALLA ELFOGOHI, HAFIZE DILEK TEPE, IDRIS YAZGAN,
TALIP CETER

ABSTRACT. Grape extracts have strong antioxidant potential which can make them promising anticancer and food-supplement. However, one of the main obstacles is that there is not enough data revealing content of the grape extracts. Another important issue is that grape extracts are mostly obtained in methanol and ethanol solvent, which can limit their effective usage. Therefore, in this study, extracts of seven different grape species of white and red grape (which were collected from Kırşehir-Toklumen) were collected using 70:30 water:ethanol, which underwent GC-EI-MS characterization. GC-MS based characterization revealed that the species had key content differences and similarities, which can trigger further research to quantify the content to reveal what exactly brought the differences in the obtained antioxidant potential of the grape species.

1. INTRODUCTION

Plants and their products in treatment of diseases have been used extensively by humans for many years [1]. The World Health Organization estimates that 80% of people in developing countries (65% of the world's population) still rely on traditional medicine. Many higher plants have economically important compounds such as oils, resins, dyes, flavors and fragrances, pharmaceuticals, and pesticides. The antiseptic qualities of aromatic and medicinal plants and their extracts have been recognized since antiquity, while attempts to characterize these properties in the laboratory date back to the early 1900s [2]. However, most species of higher plants have never been described, much less surveyed for chemical or biologically active constituents, and new sources of commercially valuable materials remain to be discovered. More recently, medicinal plant products were gained great importance for use in medicine as natural products [3].

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Plants have been used as a source of natural medicine since antiquity. Today, there is growing interest in the botanist world [4]. *Vitis vinifera* belongs to the Vitaceae family, which comprises about 60 types of fertile wild *Vitis* distributed in Asia, North America, Europa and sub-tropical area. and Mediterranean climate conditions and mild continental weather.

Plant derived drugs are playing an important role in the up growth of cancer therapy [5]. Most of the active compounds in these extracts remain unidentified, and their presence is only detected by biological tests. Most of the identified compounds are products of plant secondary metabolism and belong to the classes of alkaloids, polyphenols, triterpenes, or steroid glycosides [6]. *Vitis vinifera* grape has been the focus of attention around the world because of the nutritional properties of natural product and contain high protein. It also contains content of vitamin E and 10% to 20% of the oils with a high content of vitamin E, which is important for human health. The aim of this study was to determine some chemical properties, fatty acids and minerals from grape seeds in eleven varieties of grapes. wine and the pharmaceutical properties of derivatives, such as seed and seed extracts. For example, grape seed extract [7] (aqueous or alcoholic) has high antioxidant potential. Its beneficial effects include modification of the expression of the antioxidant enzyme, protection against oxidative damage in cells, anti-inflammatory and anti-inflammatory effects, and protection against certain cancers, both in humans and animal models [8]. Grape seed is a byproduct of wine making, and its content of oils is traditionally extracted using either organic solvents or mechanical techniques, which retain more beneficial health ingredients. Therefore, the aim of this study was to determine chemical compositions of eight different grape species of white and red grape were collected from Kırşehir-Toklumen.

2. MATERIALS AND METHODS

2.1 Collection of Samples

Samples of white (i.e. Sauvignon Blanch, Viognier and Narince) and red (i.e. Cot Malbec, Syrah, Kalecik Karası, Öküzgözü and Boğazkere) grapefruits were collected from Kırşehir-Toklumen vineyard of Kavaklıdere Company. Grape samples of each species were collected on September 10, 2017.

2.2 Extraction of Grape Contents

Collected grape samples were first rinsed with 18.2 M Ω pure water, followed by stem was removed using clean blade. The samples were then left for drying under room temperature, followed by mixed with liquid nitrogen. Mortar-pestle system run by hand-power used to crash the samples. The fine-powders were mixed with 96% ethanol, which was then incubated for 3 days at room temperature under 100 rpm continuous shaking. Cell debris and pulp were eliminated via 0.2 μ m filtration. Ethanol in the supernatants were eliminated using rotavapor, for which water-bath temperature was 50 °C. The water content was then vaporized using lyophilizer. The fully dried samples were kept at 4 °C until further experimentations.

2.3 GC-EI-MS Analysis of Grape Contents

Qualitative analysis of the grape extracts was performed using Agilent Technologies 7890 A Gas chromatography (GC) system coupled to 5975C electron-ionization mass spectrometer (EI-MS) as described elsewhere [9]. Agilent HP-5MS capillary column possessing the sizes of 30 m X 0.25 mm X 0.25 mm (length X inner diameter X thickness). Oven temperature was kept at 40 °C for 5 min, followed by heated up to 280 °C at rate of 5 °C min. Helium gas (99.999%) was used as the carrier gas at constant flow rate as 1.5 mL min, where the injector temperature was 250 °C. The extract (which was prepared in ethanol) was injected in the splitless mode with a 1 mL of injection volume. MS were recorded at 70 eV ionization energy in full scan mode in the 35–550 atomic mass unit range. The ionization source and the transfer line temperatures were 230 and 290 °C throughout the study. National Institute Standard and Technology (NIST) database was used to find the possible molecules in the extracts, where 90% similarity was used as the reliable threshold similarity. Mass spectrometry interpretation of GC-MS was performed using the National Institute of Standards and Technology (NIST) database.

2.4 Antioxidant Activity

DPPH (1,1-Diphenyl-2-picrylhydrazyl radical) radical scavenging activity was performed as described elsewhere [10]. Briefly, ascorbic acid (20 μ g/mL- 1 mg/mL range) was used to draw standard graphic using 10 μ g/mL DPPH radical.

The all the samples were used at 20 mg/mL to calculate relative antioxidant capabilities of the samples. All the tests were performed in water in order to have fully dissolved grape samples. Absorbance came from grape extracts at the tested concentrations were normalized to minimize possible biases.

3. RESULTS AND DISCUSSIONS

3.1 Grape Content

Chemical compositions of the fruits play key roles to determine how they contribute human health [11]. Extraction methods determine the chemistry of extracted species [12, 13] such as non-polar organic solvents allow extraction of non-polar compounds while polar solvents provide extraction of the polar ingredients [13]. Due to the fact that we used 96:4, Ethanol:water, common polyphenols were not extracted, rather furan and fatty-acid based compounds were overwhelmingly extracted, what was aimed for the study.

As detailed in the Table 1, all the samples contain similar active ingredients. As it is known that isomerism of compounds alter their biological actions [14], so that those differences are provided in Table 1.

3.2. DPPH Results

Antioxidant potentials of the grape extracts is one of the key determinants how the grapes provide protection against oxidative stress at cell and tissue level [11]. In Figure 1, relative antioxidant potentials of the extracts are provided.

Narince provided the highest antioxidant activity (20 mg can reduce 20 μ g DPPH in 15 min) while the lowest activity was observed for Boğazkere (20 mg can reduce 8.4 μ g DPPH in 15 min), which is related to the content of the grapes. Qualitative content analysis revealed that only Narince species had “9,12,15-Octadecatrienoic acid, ethyl ester” compound, which can be source of the observed high antioxidant capacity. In contrast to this, strong antioxidant molecules (i.e. 2,3-dihydro-3,5-dihydroxy-6-methyl) is common for Narince and Boğazkere. Therefore, it can be speculated that the level of the identified molecules can also be the source of different antioxidant potential of the grape species.

TABLE 1. Chemical composition of the tested grapes.

Grape species	Content
Öküzgözü	2-Furanmethanol (96%), Furfural (95%), 2-Furaldehyde (90), Furoil (90%), 2-Furancarboxaldehyde (91%), 5-methyl-2-Furancarboxaldehyde (91%), Linoleic acid ethyl ester (97%), and (9,12-Octadecadienoic acid, ethyl ester 97%),
Narince	2-Furancarboxaldehyde,5-methyl-Furancarboxaldehyde (91%), 2-Furancarboxaldehyde- 5-(hydroxymethyl) (93 %), 4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl (91%), n-Hexadecenoic acid (96%), Hexadecenoic acid, ethyl ester (96%), Octadecanoic acid (90%), 9,12,15-Octadecatrienoic acid, ethyl ester (97%),
Syrah	Furfural (94 %), 3-Furaldehyde (91%), 2-Furanmethanol (94 %), 3-Furanmethanol (93 %),2-Furancarboxaldehyde, 5-methyl- 91 4H-Pyran-4-one, 2,3-dihydro-3,5-di 19887 028564-83-2 91hydroxy-6-methyl- 2-Furancarboxaldehyde,5 (hydroxymethyl) (97%), Hexadecenoic acid, ethyl ester (99), Linoleic acid ethyl ester (99), 9,12-Octadecadienoic acid, ethyl ester (99), Ethyl Oleate (92), 9-Octadecenamide, (Z)- (87)
Boğazkere	Furfural (93%), 3-Furaldehyde (90%), 2-Furanmethanol (93%) 4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl (91%) 2-Furancarboxaldehyde, 5-(hydroxymethyl) (97%), n-Hexadecanoic acid (96%), Hexadecanoic acid, ethyl ester (98%) Octadecanoic acid (90%)
Kalecik Karası	4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl (97%) 2-Furancarboxaldehyde, 5-(hydroxymethyl) (95%), n-Hexadecanoic acid (96%) Hexadecanoic acid, ethyl ester (90 %) Octadecanoic acid (94 %), 9-Octadecenamide, (Z) (95 %)
Viognier	2-Furanmethanol (93 %) 4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl (87%), n-Hexadecanoic acid (96 %) Hexadecanoic acid, ethyl ester (99 %) 9,12-Octadecadienoic acid (Z,Z) (93 %) Linoleic acid ethyl ester (99 %) 9,12-Octadecadienoic acid, ethyl ester (99 %) 9,12,15-Octadecatrienoic acid, ethyl ester, (Z,Z,Z) (90 %), 9-Octadecenamide, (Z) (87 %)
Sauvignon Blanc	Furfural (94 %), 3-Furaldehyde (91 %) 2-Furanmethanol (95 %), 4H-Pyran-4-one, 2,3-dihydro-3,5-di hydroxy-6-methyl (87 %) 2-Furancarboxaldehyde, 5-(hydroxymethyl) (93 %), Octadecanoic acid (98 %) 9-Octadecenamide, (Z) (91 %)

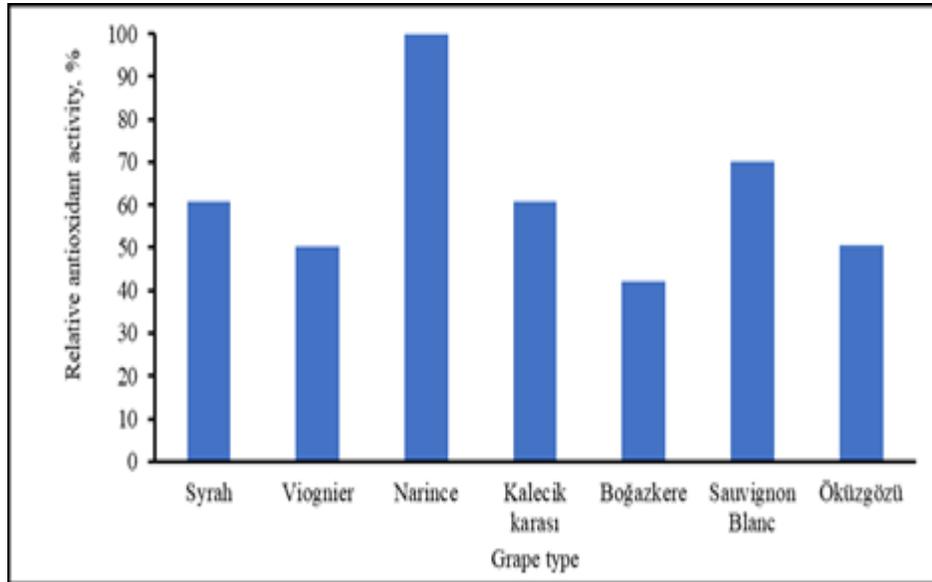


FIGURE 1. Relative antioxidant capabilities of grape types.

4. CONCLUSION

The findings revealed that ethanol-water extracts of the grape species revealed strong antioxidant potential based on DPPH radical scavenging test. Due to the fact that the species possess similar key antioxidant ingredients, further research to evaluate percentage of the antioxidant species in the grape extracts is emerging need to find out exact antioxidant potential of the grape species.

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REFERENCES

- [1] P. Suppakul, J. Miltz, K. Sonneveld and S.W. Bigger, Antimicrobial properties of basil and its possible application in food packaging. *Journal of Agricultural and Food Chemistry*, 51, (2003) 3197–3207.
- [2] R. Mickiene, B. Bakutis and V. Baliukoniene, Antimicrobial activity of two essential oils. *Annals of Agricultural and Environmental Medicine*, 18 (1), (2011) 139-44.
- [3] M.F. Balandrin, J.A. Klocke, E.S. Wurtele, W.H. Bollinger, Natural plant chemicals: sources of industrial and medicinal materials. *Science*, 228, (1985) 1154–1160.
- [4] M.J. Tuorkey, Cancer therapy with phytochemicals: present and future perspectives. *Biomedical and Environmental Sciences*, 28, (2015) 808–819.
- [5] F. Conforti, G. Ioele, G.A. Statti, et al Antiproliferative activity against human tumor cell lines and toxicity test on Mediterranean dietary plants. *Food and Chemical Toxicology*, 46, (2008) 3325–3332.
- [6] B. Dzhambazov, S. Daskalova, A. Monteva, and N. Popov, In vitro screening for antitumour activity of *Clinopodium vulgare* L.(Lamiaceae) extracts. *Biological and Pharmaceutical Bulletin*, 25, (2002) 499–504.
- [7] S. Bail, G. Stuebiger, S. Krist, H.Unterweger, and G.Buchbauer, Characterisation of various grape seed oils by volatile compounds, triacylglycerol composition, total phenols and antioxidant capacity. *Food Chemistry*, 108, (2008) 1122–1132.
- [8] C. Pérez, M.L.R. del Castillo, C. Gil, G.P. Blanch, and G. Flores, Supercritical fluid extraction of grape seeds: extract chemical composition, antioxidant activity and inhibition of nitrite production in LPS-stimulated Raw 264.7 cells. *Food & Function*, 6, (2015) 2607–2613.
- [9] H. Atmaca, E. Bozkurt, M. Cittan, and H. Dilek Tepe, Effects of Galium aparine extract on the cell viability, cell cycle and cell death in breast cancer cell lines. *Journal of Ethnopharmacology*, 186, (2016) 305–310.
- [10] O.P. Sharma and T.K. Bhat, DPPH antioxidant assay revisited. *Food Chemistry*, 113, (2009) 1202–1205.
- [11] P. Iacopini, M. Baldi, P. Storchi and L. Sebastiani, Catechin, epicatechin, quercetin, rutin and resveratrol in red grape: Content, in vitro antioxidant activity and interactions. *Journal of Food Composition and Analysis*, 21, (2008) 589–598.

- [12] V.M. Burin, N.E. Ferreira-Lima, C.P. Panceri and M.T. Bordignon-Luiz Bioactive compounds and antioxidant activity of *Vitis vinifera* and *Vitis labrusca* grapes: Evaluation of different extraction methods. *Microchemical Journal*, 114, (2014) 155–163.
- [13] E.A. Hayouni, M. Abedrabba, M. Bouix and M. Hamdi The effects of solvents and extraction method on the phenolic contents and biological activities in vitro of Tunisian *Quercus coccifera* L. and *Juniperus phoenicea* L. fruit extracts. *Food Chemistry*, 105, (2007) 1126–1134 .
- [14] N.S. Kelley, N.E. Hubbard and K.L. Erickson, Conjugated linoleic acid isomers and cancer. *The Journal of Nutrition*, 137, (2007) 2599–2607.

Current address: LAILA RADWAN ABDALLA ELFOGOHI: Kastamonu University, Arts and Sciences Faculty, Department of Biology, Kastamonu, Turkey.

Email: lailaabdalla03@gmail.com.

ORCID: <https://orcid.org/0000-0003-4962-5960>

Current address: HAFIZE DILEK TEPE: Celal Bayar University, Arts and Sciences Faculty, Department of Biology, Manisa, Turkey.

E-mail : hafize.dilek@hotmail.com

ORCID: <https://orcid.org/0000-0002-6035-6901>

Current Adress: IDRIS YAZGAN: Kastamonu University, Arts and Sciences Faculty, Department of Biology, Kastamonu, Turkey.

E-mail : idrisyazgan@gmail.com

ORCID: <https://orcid.org/0000-0002-0264-1253>

Current Adress: TALIP CETER: (*Corresponding author*). Kastamonu University, Arts and Sciences Faculty, Department of Biology, Kastamonu, Turkey.

E-mail : talipceter@gmail.com

ORCID: <https://orcid.org/0000-0003-3626-1758>