

Total Phenolic and Flavonoid Contents, Antioxidant and Antimicrobial Activities of Traditional Unripe Grape Products

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ABSTRACT

Unripe grape products, unripe grape juice (or koruk juice), unripe grape powder and unripe grape piece are common condiments in Kilis traditional cuisine. Besides their taste roles, their probable health benefits were also studied in this work. To evaluate total phenolic and flavonoid contents, antioxidant capacity and antimicrobial activity of unripe grape samples (juice, powder and piece) extracted by various solvents. Samples were extracted separately by ethanol and distilled water (10%). Concentrated samples were suspended in methanol to final concentration 100 mg/mL. Percentage yield in the extracts were observed to range from 4.19 to 17.76. While the total phenolic contents of ethanol and distilled water extracts were revealed to range from 505.682 to 758.523 and 424.432 to 603.409 mg GAE/g; total flavonoid contents of extracts were 0.073 to 0.147 and 0.041 to 0.055 mgRE/g, respectively. Antioxidant capacity of extracts was calculated between 64.07(ethanol) and 82.64 % (distilled water). Studied extracts showed antimicrobial activity against only one test microorganism, *C. albicans*. The highest antibacterial activity (8 mm) was observed in unripe grape piece distilled water extract.

KEY WORDS: Phenolic, Flavonoid, Antioxidant, Antimicrobial, Unripe Grape

INTRODUCTION

Grapes (*Vitisvinifera*) belong to family Vitaceae¹ and are one of the most widely grown fruit in Kilis district. Koruk is locally called as unripen grape and have been processed into various traditional flavoring and condiment products, such as unripe grape juice (or koruk juice), unripe grape powder and unripe grape piece in Kilis located in eastern Mediterranean region of Turkey. Unripe grape is also known as “vertjus” or “verjus” in French, “verjons” or “verjuice” in English, “agraz” in Spanish and German, “agresto” in Italian, “koruk” in Turkish and “abe-ghureh” in Persian^{2,3}. Koruk is the immature form of the grape fruit. It has high acidity, sour taste; low pH, low sugar and high phenolic content⁴. Koruk juice has been used as a food ingredient and as a medicine since

ancient Greeks. In medieval and early modern times koruk juice has been used as an acidifying agent in cooking, as medicine and as digestive agent. Koruk juice has a tart taste and a strong acidity. It is commonly used with salad and vegetables as an acidifying and flavoring agent in Turkey and neighboring countries. It is also consumed as a drink after being sweetened.^{5,6} Koruk and its juice have high antioxidant property because of its phenolic profiles. Moreover, these are sources of organic acid and have antimicrobial effects.⁷ Koruk juice, Verjuice, is used as an alternative to vinegar and lemon juice.² It is produced and consumed locally, particularly in the Mediterranean, Southeastern regions of Turkey, and many different regions of Iran. It has been used to augment

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the flavor of traditional meals, salads, and appetizers, and as an ingredient in the production of various drinks and several sausages such as mustard sausage.³ Unripe grape juice is used to reduce blood pressure and cholesterol as well as maintaining the body weight in traditional medicine.⁸ Ahmadi and Roney⁹ reported that specifically, the effects of verjuice supplementation on serum lipid profile, blood pressure, inflammatory markers, endothelial function, oxidation, glycemic control, and fatty streak formation have been evaluated with *in vivo* and human randomized control trials. Therefore, through these mechanisms, verjuice may be a useful dietary agent for the prevention and treatment of Cardiovascular Disease (CVD). Increasing demand of natural and functional products by consumers has directed research toward to new alternatives. Unripe grape products have gained popularity in the recent years and could be a good alternative with their physicochemical and antioxidant properties. Phenolic compounds in grape juice, grape seed and wine have been investigated by many researchers to show their potent antioxidant, antimutagenic, antibacterial, antiviral, antifungal and antiulcer activities. However, little information is available on the effect of ethanol and distilled water extracts on the percentage yield, total phenolic, total flavonoids, antioxidant activity and antimicrobial activity of unripe grape samples. The purpose of this study was to compare the effects of ethanol and distilled water extracts on the overall qualities in unripe grape samples. The quality criteria evaluated included: studies on the percentage yield, total phenolic, total flavonoids, antioxidant activity and antimicrobial activity.

MATERIAL AND METHODS

In this study, unripe grape samples were purchased from in Kilis located in eastern Mediterranean region of Turkey. Samples (unripe grape juice -koruk juice-, unripe grape powder and unripe grape piece) were extracted separately by ethanol and distilled water (10%). Concentrated samples were suspended in methanol to final concentration 100 mg/mL. About 10 g of the powdered materials were extracted in ethanol and distilled water (250 mL) by using the Soxhlet apparatus. Following filtration of the final extracts, they were concentrated under vacuum on a rotary evaporator at 35°C. Dry extracts were dissolved in 5 mL of methanol. Samples prepared in either methanol or ethanol according to the type of the extract. The following terms were used to describe the different treatments in our study: E1 (Koruk juice-ethanol), E2 (Koruk piece-ethanol), E3 (Koruk powder-ethanol), DW1 (Koruk juice-distilled water), DW2 (Koruk piece-

distilled water), DW3 (Koruk powder- distilled water). All treatments and analysis were carried out in triplicates. Samples were stored at 4°C for further use.

Total Phenolic Contents: The concentration of total phenolic in extracts was measured by using spectrophotometric method. 0.5 mL of the methanolic or ethanolic extracts was mixed with 2.5 mL of 10% Folin-Ciocalteu's reagent and 2.5 mL 7.5% NaHCO₃. The reaction mixtures were incubated in a water bath at 45°C for 45 min. Thereafter, the absorbance of samples was spectrophotometrically measured at 765 nm. A standard curve was prepared by using standard Gallic Acid solution in different concentrations. The content of phenolics in extracts was expressed as Gallic Acid Equivalent (mg/L), according to the measured absorbance.¹⁰

Total flavonoid contents: The total flavonoid content of extracts was determined by the aluminum chloride colorimetric method (catechol as a standard). In brief, 1 ml of test sample was diluted (1:6) and mixed with 0.3 ml %5 NaNO₂. Then the mixture was mixed with a vortex and thereafter was incubated for 5 min. At the end of time, 0.6 mL of 10% AlCl₃.6H₂O solution was added and after incubated (5 min), the obtained reaction mixture by adding 2 mL of 1 M NaOH solution was brought to 10 mL with double-distilled water. The mixture was allowed to stand for 15 min, and absorbance was measured at 510 nm with UV-VIS spectrophotometer (Biochrom, Libra S60, B, and England). The total flavonoid content was calculated from a calibration curve and the result was expressed as mg/L catechol.¹¹

DPPH Radical Scavenging Assay: The antioxidant capacity of the extracts was determined by the stable DPPH (2,2-diphenyl 1-picrylhydrazyl) radical scavenging assay. 100 µL of the extracts and 3.9 mL of the DPPH (0.025 g/L in methanol) solution prepared in either methanol or ethanol according to the type of the extract were mixed. The mixtures were incubated in dark at room temperature for 2h. The remaining DPPH amount was determined by measuring at 515 nm absorbance. In test extracts, the inhibition of DPPH was calculated as percent according to the formula $I\% = [(A \text{ blank} - A \text{ sample}) / A \text{ blank}] \times 100$. The blank is the absorbance of the control, which contains all reagents except the extract, and A sample is the absorbance of the test extract or the reference.^{12,13}

Antibacterial Activity: Antibacterial activity of unripe grape sample extracts against three bacteria (*Staphylococcus aureus*, *Candida albicans* and *Escherichia coli*) was investigated by the agar well diffusion method. The standard antibacterial agent, Tetracycline (10 µg/disc) and the pure methanol were used as control groups.

Statistical Analysis: The software SPSS 20.0 for Windows (SPSS Inc. Chicago, IL, USA) was used for analysis of variance (ANOVA) and Duncan's multiple comparison tests in order to determine significant differences between the samples. Each experiment was repeated at least three times. Statistical analysis was evaluated between samples which obtained from same solvents.

RESULTS AND DISCUSSION

Total phenolic and flavonoid contents, antioxidant activity and antimicrobial activity of unripe grape samples (juice, powder and piece) extracted by ethanol and distilled water solvents were determined in this work and the results were given in the Table 1 and Figure 1. As seen in the Table 1, percentage yields in the extracts ranged from 4.19 to 17.76. Compared to ethanol and distilled water extracts, the highest values were determined in E1 and DW2 as 13.05 and 17.76%, respectively. Jayaprakasha *et al.*¹⁴ reported that extract yield in yield of grape seed extracts for methanol extract were determined as 8.1 (% dry grape seeds). Results obtained for the total phenolic and flavonoid contents and DPPH radical scavenging assay of extracts ethanol and distilled water in unripe grape samples are depicted in Table 1. The phenolic compounds mainly include anthocyanins, flavonols, stablesness (resveratrol) and phenolic acids.¹ While the total phenolic contents of ethanol and distilled water extracts were revealed to range from 505.682 to 758.523 and 424.432 to 603.409 mg GAE/g, the total flavonoid contents of extracts were 0.073 to 0.147 and 0.041 to 0.055 mg RE/g, respectively. Antioxidant capacity of extracts was calculated between 64.07 (ethanol) and 82.64 % (distilled water). For these three analysis,

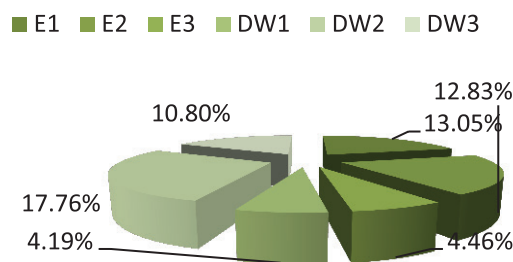


Figure 1: Effects of different extracts on percentage yield of unripe grape samples

the differences of between ethanol and distilled water extracts were found to be statistically significant ($p < 0.05$). In ethanol extracts of unripe grape juice samples, total phenolics were found to be the highest (758.52 mg GAE/g). However, in ethanol extracts of unripe grape pieces samples were found to be the lowest (505.68 mg GAE/g). In distilled water extracts of unripe grape juice samples, total phenolic content were found to be the highest (603.40 mg GAE/g). However; in distilled water extracts of unripe grape pieces samples were found to be the lowest (424.43 mg GAE/g).

Öncül and Karabiyıklı¹⁵ found that total phenol content of koruk (American Rootstock) was 652.13 mg/L. Ahn *et al.*¹⁶ reported that total tannin in grape seed extract were 535.64 mg/100g. Baydar *et al.*¹⁷ reported that total phenolic content extracted by different solvent mixtures ranged from 627.98 to 667.87 mg GAE/g in grape seed extracts. In ethanol extracts of unripe grape juice samples, total flavonoid content was found to be the highest (0.14 mg RE/g). However, in ethanol extracts of unripe grape pieces samples were found to be the lowest (0.07 mg RE/g). In distilled water extracts of unripe grape powder samples, total phenolic content were found to be the highest (0.05 mg RE/g). Jayaprakasha *et al.*¹⁴ reported that total flavonol for methanol extract were determined as 16.0 (in catechin equivalents/100 g extract). In ethanol extracts of unripe grape juice samples, DPPH (% inhibition) were found to be the highest (79.77%). However, in ethanol extracts of unripe grape powder were found to be the lowest (64.07%). When comes to the distilled water, the highest and the lowest values were determined to unripe grape powder (82.64%) and unripe grape juice samples (68.88%), respectively. Ahn *et al.*¹⁶ reported that DPPH (% inhibition) in grape seed extract were found as 86.98%.

Table 2 presents antimicrobial activity against only one test microorganism, *C. albicans* of sample extracts. Sample extracts showed antimicrobial activity against only one test microorganism, *C. albicans*. The highest antibacterial activity (8 mm) was observed in grape piece distilled water extract. The extracts of *Vitis vinifera* seed exhibited

Table 1: Effect of extracts on total flavonoids, total phenolic content and DPPH analysis of unripe grape juice samples

Analysis / Extracts	Total flavonoid (mg RE/g)	Total phenolic content (mg GAE/g)	DPPH (Inhibition %)
Ethanol			
E1	0.14 ^a	758.52 ^a	79.77 ^b
E2	0.07 ^b	505.68 ^c	79.71 ^b
E3	0.076 ^b	596.02 ^b	64.07 ^d
Distilled water			
DW1	0.04 ^d	603.40 ^b	68.88 ^c
DW2	0.04 ^d	424.43 ^d	82.14 ^{ab}
DW3	0.05 ^c	436.36 ^d	82.64 ^a

Values followed by different superscripted letter within the same column are significantly different from each other ($p < 0.05$).

Table 2: Antimicrobial activity against test microorganisms of sample extracts

Extracts	Microorganisms		
	<i>E.coli</i>	<i>S. aureus</i>	<i>C. albicans</i>
Ethanol			
E1	8	10	-
E2	9	9	7
E3	-	-	-
Distilled water			
DW1	9	10	7
DW2	-	10	8
DW3	9	8	-
Methanol	8	10	-
Tetracycline	11	29	-

antimicrobial activity to some pathogens such as *Staphylococcus aureus*, *Escherichia coli* and *Candida albicans*.¹⁸

CONCLUSION

For ethanol extracts, the highest total phenolic and flavonoid contents, antioxidant activity and percentage yield in unripe grape juices were revealed. But in distilled water extracts, while the highest total phenolic content and antioxidant activity were detected in unripe grape powder, the maximum total phenolic content was in unripe grape juice extract. Besides their flavor and condiment uses of the koruk products, it could be said that they are good for human health. As traditional food additives and flavorings, koruk products should be evaluated as natural food flavoring agents in the healthy food industry.

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CONFLICT OF INTEREST STATEMENT

The authors declare that there is no conflict of interests regarding the publication of this paper.

ABBREVIATION USED

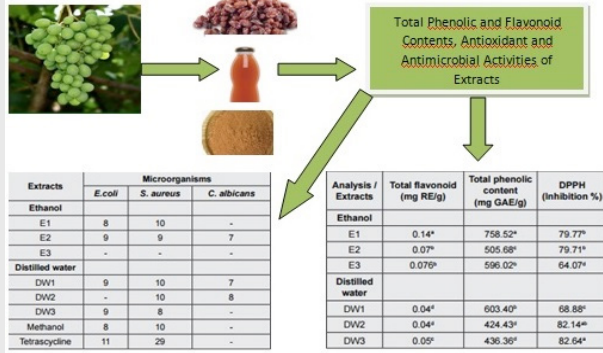
E1: (Koruk juice-ethanol); E2: (Koruk piece-ethanol); E3: (Koruk powder-ethanol); DW1: (Koruk juice-dis-

tilled water); DW2: (Koruk piece distilled water); DW3: (Koruk powder- distilled water).

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PICTORIAL ABSTRACT



SUMMARY

- Unripe grape products are common condiments in Kilis traditional cuisine.
- Samples were extracted separately by ethanol and distilled water (10%).
- Antioxidant capacity of extracts was calculated between 64.07(ethanol) and 82.64 % (distilled water).
- The total phenolic contents of ethanol and distilled water extracts were revealed to range from 505.682 to 758.523 and 424.432 to 603.409 mg GAE/g.
- The total flavonoid contents of extracts were 0.073 to 0.147 and 0.041 to 0.055 mgRE/g
- Studied extracts showed antimicrobial activity against only one test microorganism, *C. albicans*.

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