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# Proximate Analysis of Seed Extracts and Methanol Content of Juice of Some Grape Varieties in Turkey

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***Abstract—*** *The present study describes the determination and comparison of antibacterial activity, total phenolic content in seeds of seven popular grape cultivars and methanol content of grape juice grown in Turkey. It's called Muskule, Gokuzum, Razaki, Akuzum, Eksi kara, Siyah pekmezlik and Buzgulu in Turkey. The antibacterial activity was evaluated by the microdilution method and the minimum inhibition concentrations of extracts from determined. The phenolic content was determined using spectrophotometer. Pollution from methyl alcohol, which is extremely harmful for human health was determined using GC. The minimum inhibitory concentration (MIC) values of grape extracts with antibacterial activity ranged from 0,312 to 5 µg/ml. The total phenolic content of grape seed of Ekşi kara was found to be about one-half times as mg GAE/g compare to Büzgülü. The highest methyl alcohol ratios are found in Ekşi kara among juice of grape varieties (12.01 g/µl).*

***Keywords—Vitis vinifera L.; Antibacterial activities; Total phenolic content; Methyl alcohol.***

## I. INTRODUCTION

Grape production in our country is a very important fruit species. Seeds of grape varieties contain large amounts of phenolic compounds [1]. These compounds have many favourable effects on human health. For example, human inhibited low-density lipoprotein [2]. Risk of cancer are reduces [3]. The defatted seeds as a rich source of polyphenolic compounds have been largely studied by several research groups [4]. During the chewing, part of the phenolic compounds in grapes are transferred to the human body. Sources of antimicrobial compounds are grape seed extracts [1]. Also, it should be mentioned the importance of phenolics in determining some quality attributes and properties in fresh fruits and vegetables, like the color, texture, taste and flavor. One of the principal roles that have been proposed as part of the actions of phenolics in man is that of an antioxidant [5]. In nature there are a large number of different types of antimicrobial compounds that play an important role in the natural defence of all kinds of living organisms [6].There are high amount linoleic acid in seeed of grape varieties [7]. Linoleic acid consumption may reduce the level of high density lipoprotein cholesterol, increasing the risk for coronary heart disease mortality, increased risk of diabetes, cardiovascular disease and pathophysiological mechanisms impairing insulin activity [8]. Methanol that formaldehyde, and formic acid turned into indicates a toxic effect in the body. Amounts of alcohols depends on grape variety, fermentation conditions, method of distillation and duration of extraction. Due to these compounds is high boiling points during distillation take place in the final product [9]. Studies are announced in alcoholic beverages containing methanol, can cause eye defects, skin cancer and deaths. Methyl alcohol is toxic effect from skin inhaled or absorbed from the gastrointestinal route. Methyl alcohol can cause acidosis, severe abdominal pain, disturbances of consciousness or visual disturbances [10]. For these reasons, grape consumerism is important to know the amount of methanol. Many species of *Vitis* were studied for especially volatile constituents. Some papers have been referred to volatile compounds in endemic grape samples [11]. Although similar studies on some grape seed cultivars in Turkey, a comparative study on methanol content of grape juice and antibacterial activities, phenolic content of seven popular grape seed cultivars in Turkey has not been reported up to now, according to our knowledge. The present study was carried out to determine and compare methanol content of grape juice and the antibacterial activities, phenolic content of them.

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## II. MATERIAL AND METHODS

***Collection of material***

The ripened grape samples were collected from seven different Turkish vineyard producers, Eksikara (black color table and dried grape), Akuzum (white color table grape), Buzgulu (red color table grape), Razaki (white color table grape), Muskule (white color table grape), Gokuzum (white color table and molasses grape) and Siyah pekmezlik (black color molasses grape), in 2011. Seven samples were collected from different grapevines for each cultivar. The seeds were excised from product and air-dried at room temperature under shaded conditions. It was stored at room temperature until analysis.

***Antibacterial Activities***

Dried seed of grape varieties material was finely powdered using a laboratory mill. 10g of each sample was exhaustively extracted with 100 ml methanol:acetone:water at room temperature under stirring and the extracts were filtered through a Whatman filter. After evaporation of the solvent at 40°C in rotary evaporator, the residues were stored at 4°C until further analysis. The antibacterial activities of the seeds of grape varieties were assessed against four bacteria species: *Bacillus cereus ATCC 1778, staphylococcus aureus MRSA ATCC 43300, Klebsiella pnömanize ATCC 700603 ESBL (+) and Pseudomonas aeruginosa ATCC 15442*. Ampisid was used as a positive control. All bacteria were grown in the same medium at 37ºC for 22h [12]. Minimum inhibition concentration (MIC) values were determined using 56-well microtiter plates by dissolving the sample in DMSO. Suspensions of standard microorganisms were inoculated onto the microplates. The growth of the microorganisms was observed by using a microplate photometer (Thermo Scientific Multiskan). The MIC values were defined as the lowest concentrations of the seed of grape varieties extracts to inhibit the growth of microorganisms.

***Total phenolic content***

The total phenolic contents of extracts were determined according to the Folin-Ciocalteu method, manually [13]. The extracts were prepared as follows: The seeds (5.0 g) were extracted with a mixture of methanol and water (80:20, v/v, 100 mL) for 18 hours on the 125 rpm rotary shaker. After filtering and evaporating to dryness in vacuo, the crude extracts were obtained. The 100 mL of extract (20 mg/mL) was oxidized with Folin-Ciocalteu reagent (0.2 mL), and the reaction mixture was neutralized with sodium carbonate (1mL, 15%). The absorbance was measured at 760 nm after 60 min on a spectrophotometer (Shimadzu UV-1601). Using gallic acid as standard, total phenolic content was expressed as µg GAE equivalent/g of extract.

***Methanol content***

The methyl and ethyl alcohol were separated and analyzed by Shimadzu 15-A gas chromatograph (GC), equipped with dual flame ionisation detector and a 1.8 m × 3 mm internal diameter packed glass column containing GP Carbopack B60/80 coated with 5% Carbowax 20M. (Cat no: 11766) The injector and detector temperatures were 120 and 130 °C, respectively. Column temperature program was 80 ºC where it was maintained for 7 min. Nitrogen at a flow rate of 0.8 ml/min was used as the carrier gas. Isopropyl alcohol was added as internal standard from each samples [14]. The methyl alcohol were identified by comparison of retention times with known external standard mixtures, quantified by a Shimadzu Class-VP software and the results expressed as percentage distribution of methyl alcohol. All the chemicals used for the gas chromatography analysis procedure were obtained from Supelco Inc. (Bellefonte, PA, U.S.A.). Each of the experiments was repeated three times.

## III. RESULTS AND DISCUSSION

The antibacterial activities of the seed extracts of seven grape varieties were evaluated by using the MIC values in a micro-dilution method. Minimum inhibitory concentrations (MIC) of seed extracts of grape varieties are presented in Table 1. The results show that seeds of seven grape varieties exhibited antibacterial activity. Our findings showed that the ethanol extract from *Vitis vinifera* L. seeds had interesting activity against both gram-negative and gram-positive bacteria.

*Bacillus* species, especially *B. cereus,* are responsible for foodborne diseases [15]. *B. cereus* was inhibited by two of the studied grape cultivars (Eksikara, Akuzum, Buzgulu, Razaki, Muskule, Gokuzum, Siyah pekmezlik (0.312, 0.675, 2.5, 2.5, 2.5, 1.25, 0.675, respectively) with 0.5 µg/ml concentration.

*Staphylococcus aureus* is the cause of many diseases, such as food poisoning, osteomyelitis, polyarthritis, endocarditis [16]. Five studied grape species exhibited a strong antibacterial effect on *S. aureus* and Eksikara had the highest activity on the bacteria with a 0.312 mg/ml concentration. Therefore, Eksikara may be used as an antibiotic for *S. aureus* infections.

*Klebsiella pneumoniae* is a kind of bacteria found in the upper respiratory tract microflora. *K. pneumoniae*, which is very important for human health of the upper respiratory tract infections, urinary tract infections and opportunistic pathogens involved in the formation of wound infections [17]. This bacteria belonging to the type of grapes gathered all of the other samples except for the sample showed the same MIC value.

*Pseudomonas aeruginosa*, is found most in soil and water. *P. aeruginosa*, respiratory and urinary tract in

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patients with immune deficiency, opportunistic pathogen that burns and open wounds. At the same time in the blood can infections. Nosocomial infections, one tenth of the *P. aeruginosa* is due to. *P. aeruginosa* in situations where the dirty bathtubs and Jacuzzis, such as exposure to low water quality can lead to dermatitis. All examples against of this bacteria type showed the 2.5 µg/ml concentration a weak value of the MIC.

The seed extracts of grape varieties was found to have antibacterial action against four strains of bacteria. These varieties were Akuzum, Eksikara and Siyah pekmezlik. Due to values are very close the other varieties does not mean there is strong antibacterial. However, a lower concentration of ampisid (0.5 µg/ml) had a strong antibacterial effect on the bacteria.

According to study, antibacterial effects of seeds of grape varieties are different. Differences of antibacterial activities reported that resulted from the difference in methodology, chemical composition, type of microorganism and climatic conditions.

Gokturk et al., 2004 [18] also determined that the grape seed extracts had antibacterial activities against fourteen bacteria. Both the ethyl acetate:methanol:water (60:30:10) and Acetone:water:acetic acid (90:9.5:0.5) extracts of *Vitis vinifera* L. inhibited the growth of 13 bacteria and the MIC values of the acetate:methanol:water (60:30:10) extracts were determined as 30 µg/ml for *K. pneumoniae*, and 27.5 µg/ml for *P. aeruginosa.* According to the results, all examined *Vitis vinifera* L. varieties showed antibacterial activity the ethyl acetate:methanol:water (60:30:10) grape seed extract on growth of different bacteria [6]. However, our results demonstrated that some studied grape varieties were active against other bacteria (Table 1).

***Total phenolic content***

The phenolic contents are shown in Table 1. The total oil contents were ranged from 7.17 to 14.29%. The variation depends largely on the variety. As shown in the table, grape seeds are a moderate source of oil and protein. These findings were supported by Tangolar et al., 2009, [19]. The highest phenolic content was determined in seed of Eksikara (121.3 mg GAE/g), followed by Razaki (103.84 mg GAE/g), Siyah pekmezlik (90.16 mg GAE/g), Muşkule (81.23 mg GAE/g), Göküzüm (76.35 mg GAE/g), Akuzum (74.81 mg GAE/g) and Buzgulu (66.29 mg GAE/g). The results of similar studies for other cultivars follow; 50.66-58.91 mg GAE/g [20], 79.20-154.60 mg GAE/g [21], 11.17-24.43 mg GAE/g [22] and 25.03-48.03 mg GAE/g [23]. The phenolic content in seeds of Eksikara is relatively higher than those of the above-mentioned literature data. The results also revealed that total phenolic content of seed of Eksikara was found to be one-half times of that of Buzgulu. It is thought that the higher phenolic contents from Eksikara and Razaki (the station with the milder climatic conditions) than those of Akuzum and Buzgulu (the harder climatic conditions) are result from different latitude.

***Table 1***

**Methanol content**

The alcohol compositions of varieties of the grape juice and are presented in Table 2. It was identified methyl and ethyl alcohol for the grape juice varieties and evaluated their compositions for species. The highest alcohol ratios are as follow; methyl alcohol; Eksikara, Razaki, Buzgulu, Göküzüm, Muşkule Akuzum, and Siyah pekmezlik, 12.01 g/µl, 8.7 g/µl, 3.21 g/µl, 2.72 g/µl, 1.02 g/µl, 0.21 g/µl and 0.03 g/µl, respectively. The highest ethyl alcohol ratios are as follow; 0.021 g/µl, 0.011 g/µl, 0.009 g/µl, 0.007 g/µl, 0.0001 g/µl, 0.0001 g/µl, 0.00 g/µl, same line, respectively. The results showed that methyl alcohol and ethyl alcohol level of varieties of the grape juice from Eksikara were higher than that of other grape cultivars. Similarly, Yilmaz and Toledo, 2006 [4] reported there are water, methanol, ethanol, acetone, and ethyl acetate in seeds of grape varieties.

***Table 2***

According to the analysis of juice of 35 grape varieties: average of methyl alcohol was 3.96 g/µl. It is not harmful. In our country, alcohol limit is 50 ml/dl [14]. This regard, the results is suitable Turkish Food Codex. Some researchers reported that there was a relationship between the chemical structures of the most abundant compounds in the tested products and the antimicrobial activity [24].

## IV. CONCLUSION

Consequently, the methanol content of grape juice and phenolic contents, antibacterial activities of seven cultivars were determined and compared. The results clearly indicate that there are differences in phenolic contents and antibacterial activities between them. These differences are thought to genetic features and different latitudes. In general, the present study revealed that *Vitis vinifera* L. extracts possess antibacterial activity against several tested microorganisms. Therefore, the extracts can be used as a source of natural antibiotics. According to the results, Eksikara, in terms of the phenolic content and antibacterial effect was found to be the best grape variety. But, methanol amount of this variety is the highest level. According to these results, seeds of grape varieties which is the highest antibacterial effect can be used as the type of wine.

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**TABLES**

*Table.1: Phenolic compound contents and minimum inhibitory concentrations (MIC) of of extracts of seeds of grape varieties grown in Turkey*

| | gram (+) | | gram (-) | | |
|---|---|---|---|---|---|
| **Grape Verieties** | ***Bacillus cereus*** | ***Staphylococcus aureus*** | ***Klebsiella pnömanize*** | ***Pseudomonas aeruginosa*** | ***Phenolic comp. (µg GAE/g)*** |
| Eksikara | 0,312 µg/ml | 0,312 µg/ml | 1,25 µg/ml | 1,25 µg/ml | 121.3 |
| Akuzum | 0,675 µg/ml | 1,25 µg/ml | 2,5 µg/ml | 2,5 µg/ml | 74.81 |
| Buzgulu | 2,5 µg/ml | 1,25 µg/ml | 5 µg/ml | 5 µg/ml | 66.29 |
| Razaki | 2,5 µg/ml | 1,25 µg/ml | 2,5 µg/ml | 2,5 µg/ml | 103.84 |
| Muskule | 2,5 µg/ml | 0,675 µg/ml | 2,5 µg/ml | 5 µg/ml | 81.23 |
| Gokuzum | 1,25 µg/ml | 1,25 µg/ml | 2,5 µg/ml | 2,5 µg/ml | 76.35 |
| Siyah pekmezlik | 0,675 µg/ml | 1,25 µg/ml | 2,5 µg/ml | 2,5 µg/ml | 90.16 |
| Ampisid | 0,5 µg/ml | 1 µg/ml | 64 µg/ml | 128 µg/ml | |

*Table.2: Value of methyl and ethyl alcohol of juice of some grape varieties grown in Turkey*

| **Sample(g/µl)** | **Methyl alcohol** | **Ethyl alcohol** |
|---|---|---|
| Eksikara | 12.01±0.00 | 0.021 |
| Akuzum | 0.21±0.00 | 0.011 |
| Buzgulu | 3.21±0.00 | 0.009 |
| Razaki | 8.7±0.00 | 0.007 |
| Muşkule | 1.02±0.00 | 0.0001 |
| Gokuzum | 2.72±0.00 | 0.0001 |
| SiyahPekmezlik | 0.03±0.00 | 0.00 |