J. Fac. Pharm. Ankara / Ankara Ecz. Fak. Derg., 49(4): *early access*, 2025
Doi: 10.33483/jfpau.1773154

ORIGINAL ARTICLE / ÖZGÜN MAKALE

FATTY ACID COMPOSITION AND BIOLOGICAL ACTIVITIES OF *VITIS VINIFERA* L. (ANTEP KARASI) SEED OIL

VITIS VINIFERA L. (ANTEP KARASI) YAĞININ YAĞ ASİDİ BİLEŞİMİ VE BİYOLOJİK AKTİVİTELERİ

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ABSTRACT

Objective: *This study examined the chemical composition and biological activity of Vitis vinifera L. (Antep Karası) seed oil, focusing on its fatty acid profile, phenolic content, and various antioxidant and antimicrobial properties.*
Material and Method: *Seeds of V. vinifera (known as "Antep Karası") were collected from vines in Gaziantep, Türkiye, and the seed oil was extracted using n-hexane in a Soxhlet apparatus. Chemical composition of the oil was studied using GC-FID/MS after transesterification of fatty acids with BF_3 reagent. Standard spectrophotometric and microbiological tests were used to measure total flavonoids, phenolics, antioxidant capacity, enzyme inhibition, and antimicrobial, antifungal, and antibiofilm activities.*
Result and Discussion: *V. vinifera (Antep Karası) seeds yielded 8.2% oil. Total phenolic and flavonoid content was 0.074 ± 0.001 mg GAE/$g_{seed\ oil}$ and 0.023 ± 0.001 mg RE/$g_{seed\ oil}$. DPPH radical scavenging (IC_{50} = 1.423 ± 0.004 mg/ml), β-carotene bleaching inhibition (0.182 ± 0.020 mg/ml), and TEAC value (0.012 µmol trolox/$mg_{seed\ oil}$) were determined. The antibacterial evaluation revealed that the MBC values varied from 7500 -15000 µg/ml. Additionally, antifungal activity by MFC was observed with an MFC value of 7500 µg/ml. The antibiofilm assay revealed an inhibition activity in a dose-dependent manner. Even at a concentration of 1875 µg/ml, biofilm inhibition of 50% and above was observed.*
Keywords: *Antep karası, biological activities, fatty acid composition, GC-MS/FID, Vitis vinifera L.*

ÖZ

Amaç: *Bu çalışma, Vitis vinifera L. (Antep Karası) çekirdek yağının kimyasal bileşimini ve biyolojik aktivitesini incelemiş, özellikle yağ asidi profiline, fenolik içeriğine ve çeşitli antioksidan ve antimikrobiyal potansiyeline odaklanmıştır.*
Gereç ve Yöntem: *Türkiye'nin Gaziantep ilindeki asmalardan toplanan V. vinifera (Antep Karası) çekirdeklerinden, n-hekzan kullanılarak Soxhlet cihazında yağ ekstraksiyonu gerçekleştirilmiştir. Yağ asitlerinin BF_3 reaktifi ile transesterifikasyonundan sonra, yağın kimyasal bileşimi GK-KS/AİD kullanılarak incelenmiştir. Toplam flavonoitler, fenolikler, antioksidan kapasite, enzim inhibisyonu*

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Submitted / Gönderilme : 28.08.2025
Accepted / Kabul : 12.11.2025
Published / Yayınlanma : *early access*

Presented at the 14th International Symposium on Pharmaceutical Sciences (ISOPS-14), 25-28 June 2024, Ankara, Turkey.

ve antimikrobiyal, antifungal ve antibiyofilm aktivitelerini ölçmek için standart spektrofotometrik ve mikrobiyolojik testler kullanılmıştır.
Sonuç ve Tartışma: *V. vinifera (Antep Karası) çekirdeklerinden %8.2 oranında yağ elde edilmiştir. Toplam fenolik ve flavonoid miktarları sırasıyla 0.074 ± 0.001 mg GAE/$g_{çekirdek\ yağı}$ ve 0.023 ± 0.001 mg RE/$g_{çekirdek\ yağı}$ olarak belirlenmiştir. DPPH radikal süpürme (IC_{50} = 1.423 ± 0.004 mg/ml), β-karoten ağarması inhibisyonu (0.182 ± 0.020 mg/ml) ve TEAC değeri (0.012 µmol trolox/$mg_{çekirdek\ yağı}$) belirlenmiştir. Antibakteriyel değerlendirme, MBC değerlerinin 7500 - 15000 µg/ml aralığında değiştiği saptanmıştır. Ayrıca, MFC ile antifungal aktivite değeri 7500 µg/ml olarak belirlenmiştir. Antibiyofilm testi, doz bağımlı inhibisyon etkisi göstermiştir; 1875 µg/ml konsantrasyonda bile %50 ve üzeri biyofilm inhibisyonu gözlenmiştir.*
Anahtar Kelimeler: *Antep karası, biyolojik aktiviteler, GK-KS/AİD, Vitis vinifera L., yağ asidi kompozisyonu*

INTRODUCTION

Vitis vinifera L., a member of the Vitaceae family, is one of the most widely cultivated fruit species worldwide [1]. Grapes are ingested in both their fresh and dried forms and are regarded as a significant source of functional foods and phytotherapeutic compounds. Grape seeds are particularly abundant in oils and bioactive substances, offering substantial nutritional and health benefits [2,3]. Grape seed oil, known as "royal oil," is defined by its elevated levels of fatty acids, phenolic compounds, and tocopherols [4-7]. The food, cosmetic, pharmaceutical, and medical industries have made extensive use of this composition [8-10].

Grape seed oils are chemically abundant in saturated, monounsaturated, and polyunsaturated fatty acids, notably linoleic and α-linolenic acids, which are necessary for human health [11]. Besides fatty acids, grape seed oils encompass tocopherols, carotenoids, phenolics, and phytosterols, which substantially enhance their antioxidant capacity [12]. These substances have been linked to a decreased risk of chronic illnesses like diabetes, cancer, and cardiovascular diseases since they are known to scavenge free radicals and prevent against oxidative stress [13].

Investigations on grape seed oils from various cultivars have shown that the fatty acid composition and total phenolic/flavonoid contents are closely associated with their health-promoting effects [14]. Antioxidant capacity has frequently been evaluated using assays such as DPPH, ABTS/TEAC, and β-carotene bleaching, which revealed the effective radical scavenging potential of grape seed oils [10]. In addition, several studies have reported that grape seed extracts display antibacterial and antifungal properties [15]. Recent studies have focused on their antibiofilm activity and enzyme inhibitory potential, which are significant for their pharmacological and antidiabetic relevance [16,17].

Antep Karası, a regional cultivar of *V. vinifera* cultivated in Southeastern Anatolia, Türkiye, possesses significant economic and cultural value. However, there has been limited research on the chemical composition and biological properties of its seed oil. Existing research predominantly centers on general grape cultivars, with a notable absence of systematic studies examining Antep Karası seed oil regarding its fatty acid composition, antioxidant capacity, antibacterial/antifungal and antibiofilm properties, and α-amylase inhibitory activity in combined studies. Thus, the current study's purpose was to ascertain the makeup of fatty acids (GC-FID/MS analysis) and total phenolic and flavonoid contents of *V. vinifera* (Antep Karası) seed oil, and to evaluate its antioxidant activities (DPPH, β-carotene bleaching, and TEAC), antibacterial, antifungal, antibiofilm, and α-amylase inhibitory properties, to demonstrate its potential as a useful natural source of bioactive substances.

MATERIAL AND METHOD

Plant Material

The plant material was collected from Gaziantep province of Türkiye in September 2023 during the fruiting period. The identification of the plant material was performed by Dr. Koray Yaylacı (Anadolu University, Faculty of Pharmacy, Department of Pharmaceutical Botany). Seeds were manually separated from the fruits, washed to remove the pulp, and dried at room temperature in the

shade for three days to ensure sufficient dehydration while preserving thermolabile bioactive components [18-20]. The dried seeds were then homogenized using a grinder and stored at +4 °C until further analysis.

Extract Preparation

17.91 grams of seed were weighed into a cartridge and extracted in a Soxhlet extractor with *n*-hexane for 4 hours [7]. After extraction, the *n*-hexane was removed on the rotary evaporator under vacuum at 40°C. The oil was kept at +4°C in the dark until use.

Determination of Fatty Acid Composition by GC–FID/MS

After fatty acid methyl esters were obtained using transmethylation with boron trifluoride (BF_3), the fatty acid composition of *V. vinifera* (Antep Karası) seed oil was examined. After diluting the derivatized samples with 10% v/v hexane, 1 ml of each sample was added to the system at a 40:1 split ratio. For the gas chromatography–mass spectrometry (GC–MS) study, an Agilent 7890B GC system and a 5977B Mass Selective Detector were used. An Agilent HP-Innowax capillary column was used for the separation process. The temperature of the ion source was kept at 230°C, while the injector was set at 250°C. The mass range was scanned between *m/z* and 450 while the electron ionization mode was applied at 70 eV. The oven temperature program ran for 100 minutes, ramping up from 60°C (10 minutes) to 220°C (10 minutes) at a rate of 4°C/minute, then 1°C/minute to 240°C (20 minutes). The carrier gas used was helium, which flowed at a rate of 0.7 ml per minute. The collected spectra were compared to the Wiley 9 and NIST 11 mass spectral libraries to identify the chemicals. Under the same chromatographic circumstances, gas chromatography (GC) with a flame ionization detector (FID) was used for quantification. The temperature of the injector and detector was fixed at 250°C. Peak regions of the FID chromatograms were used to quantify the relative percentages of the fatty acid methyl esters [21-23].

Determination of Total Phenolic and Flavonoid Contents

According to Singleton, the Folin–Ciocalteu reagent was used to measure the extracts' total phenolic content in gallic acid equivalent (GAE) [24]. Seed oil and gallic acid stock solutions were prepared with 10% DMSO-methanol. Using a 12-channel micropipette, the experiment included mixing 20 µl gallic acid, sample, 1560 µl ultrapure water, and 100 µl FCR (Folin-Ciocalteu Reagent) in 96 deep wells. Add 300 µl of a 20% sodium carbonate solution and stir once more after 1–8 minutes of incubation. The mixture was incubated for 2 hours at 25°C in the dark. After adding 300 µl of the mixture into a 96-well microplate, absorbance values at 760 nm were compared to a 5-point gallic acid calibration curve (0.01-1.0 mg/ml). The experiment was performed in triplicate. The calibration curve was obtained for gallic acid ($y= 0.89243x+0.0477$), $r^2 = 0.9997$. We calculated the mean content value with +/- standard error. The results were calculated as mg $GAE/g_{seed\ oil}$.

In the previous method [25], the extracts' total flavonoid concentration was evaluated as rutin equivalent (RE) using $AlCl_3$ reagent. A 12-channel Eppendorf Xplorer micropipettor was used to mix 80 µl of the extract or rutin, 80 µl of $AlCl_3$, and 1840 µl of pure ethanol in 96 deep wells. For blank samples, 10 µl of 99.8% acetic acid substituted aluminum chloride reagent. Post-40 minutes dark incubation, 300 µl of mixture was transferred to a 96-well microplate and absorbance was read at 415 nm with a microplate reader. The total flavonoids were calculated by using the rutin calibration curve. The 5-point calibration curve ranged from 0.01 to 1.0 mg/ml, and the regression equation was $y=0.7721x-0.0444$ ($r^2= 0.9997$). Average content had a standard error. The reported value was calculated as mg $RE/g_{seed\ oil}$.

Free Radical Scavenging Effect (DPPH)

The samples' free radical scavenging activity against DPPH radicals was assessed utilizing a modified Brand-Williams method [26]. The sample (2 mg/ml) and gallic acid solutions were prepared in methanol containing 10% DMSO. The experiment involved mixing 100 µl of the sample with DPPH solutions (0.08 mg/ml in methanol) in 96 well plate. The mixtures were incubated in darkness for 30 minutes. The reduction in absorbance was measured at 517 nm using a microplate reader. Gallic acid

(0.1 mg/ml) served as a positive control. The experiments were replicated threefold. The equation quantified the samples' free radical scavenging activity as a percentage of inhibition.

$$\% \, Inh = \left(\frac{Abs_{control} - Abs_{sample}}{Abs_{control}}\right) \times 100$$

$Abs_{control}$ refers to the absorbance of the control, while Abs_{sample} refers to the sample containing DPPH. IC_{50} values for substances that showed at least 50% inhibition were determined by gradually diluting the samples and creating a graph of the DPPH scavenging percentage compared to the sample concentration using *SigmaPlot (15.0)* software.

Lipid Peroxidation Inhibition Test (β-Carotene Bleaching Assay)

The extracts' lipid oxidation inhibition was evaluated using a modified β-carotene bleaching assay [27]. An emulsion was created by combining β-carotene (1 mg/ml) in chloroform, 200 mg Tween-20, and 25 µl linoleic acid. Chloroform was evaporated fully at 40°C under vacuum and nitrogen gas. After evaporation, 50 ml of oxygenated ultrapure water was added and violently mixed to produce a stable emulsion. Each experiment began with dark-stored fresh emulsions. The standard antioxidant was BHA. The sample and BHA were dissolved in methanol with 10% DMSO at varied doses. For the test, 60 µl of sample solution and 250 µl of emulsion were added to each 96-well microplate well. At 492 nm and 50°C, a microplate reader measured absorbance every 15 minutes for 105 minutes. The equation for antioxidant activity (AA) was calculated using the following equation:

$$\% \, AA = [1 - \left(\frac{Abs_{0\ sample} - Abs_{120\ sample}}{Abs_{0\ control} - Abs_{120\ control}}\right)] \times 100$$

where $Abs_{0_{sample}}$ and $Abs_{120_{sample}}$ represent sample absorbance at 0 and 120 minutes, respectively, and $Abs_{0_{control}}$ and $Abs_{120_{control}}$ represent control values.

Trolox Equivalent Antioxidant Capacity (TEAC Assay)

The antioxidant potential of *V. vinifera* seed oil was measured using $ABTS^{+\bullet}$ and estimated as Trolox equivalent [28]. At first, a 7 mM ABTS solution was prepared by adding 6.6 mg of potassium persulfate to 10 ml of distilled water. After 16 h incubation without light, the solution was diluted with absolute ethanol to reach an absorbance of 0.700–0.800 at 734 nm. The sample (2 mg/ml), and Trolox (5 dilutions from 3.0 to 0.125 mM) were produced as stock solutions in methanol with 10% DMSO. A 96-deep well plate was used to combine a 10 µl sample or trolox sample with 990 µl $ABTS^{+\bullet}$ solution. A microplate reader showed a decrease in absorbance at 734 nm after 30 min in the dark. The Trolox equivalent antioxidant capacity (TEAC) of the samples was used to measure their ABTS•+ scavenging activity. The linear equation for Trolox is y=-0.2401x+0.9785. The calibration curve's regression coefficient (r^2) was 0.9983. Triplicates were used for all tests.

α-Amylase Inhibitory Activity Assay

A few minor adjustments were made to the I_2/KI reagent used to analyze seed oil for inhibition of α-amylase activity [29]. Acarbose was the positive control. The right proportions of extract solutions are present in methanol with 10% DMSO. A 96-well microplate was filled with 50 µl of α-amylase enzyme solution (0.8 U/ml in 20 mM sodium phosphate buffer, pH 6.9) and 50 µl of sample or acarbose solution. For 10 minutes, the mixture was incubated in the dark at 37°C. Put 50 µl of a 0.05% starch solution into each well, then let it incubate at 37°C in the dark for 10 min. To stop the reaction, 25 µl of a 1 M HCl solution was added. Then, each well received 100 µl of newly made I_2/KI reagent. In blank samples, buffer was used in place of enzyme. All reagents were present in control wells, with the exception of extract and acarbose. Absorbance was measured at 630 nm using a microplate reader. The following formula was used to estimate the proportion of α-amylase inhibition:

$$\% \text{ Inh} = \left(\frac{(Abs_{0\ control} - Abs_{control\ blank}) - (Abs_{sample} - Abs_{sample\ blank})}{(Abs_{0\ control} - Abs_{control\ blank})}\right) \times 100$$

where $Abs_{0\ control}$ = control absorbance at 0 min, $Abs_{control\ blank}$ = control blank absorbance, Abs_{sample} = sample absorbance, and $Abs_{sample\ blank}$ = sample blank absorbance.

Determination of Antibacterial and Antifungal Activities

The MBC (Minimum Bactericidal Concentration) and MFC (Minimum Fungicidal Concentration) values of the *V. vinifera* seed oil against standard bacteria (*Bacillus subtilis* NRRL B478, *S. aureus* ATCC 6538, *P. aeruginosa* ATCC 27853, *S. typhimurium* NRRL B 4420, *E. coli* ATCC 35218) and yeast (*C. albicans* ATCC 90028, *C. krusei* ATCC 6258) strains were determined by the broth microdilution method according to CLSI (Clinical and Laboratory Standards Institute) M07 (2024) and CLSI M27 (2017) guidelines, with resazurin dye as the growth/metabolic indicator. The seed oil was prepared in 96-well microplates using two-fold serial dilutions in 100 µl of cation-adjusted Mueller Hinton broth (CAMHB) for bacterial cultures and RPMI 1640 medium (including L-glutamine, without bicarbonate, buffered to pH 7 with 0.165 M MOPS) for yeast cultures. In this way, concentrations of seed oil ranging from 30000 to 58.59 µg/ml were obtained. Each well was inoculated with 100 µl of standard microorganism suspensions (standardized with the McFarland 0.5 reference) to yield a final inoculum of approximately $5x10^5$ CFU/ml bacteria and $0.5\text{-}2.5x10^3$ CFU/ml yeast cultures**.** Growth/negative control (microorganisms without seed oil), sterility control (medium only), and positive controls (10% DMSO+methanol as a solvent and standard antibiotics (erythromycin for Gram + bacteria, ciprofloxacin for Gram - bacteria and Amphotericin B for yeasts) were included in each plate. Plates were incubated at 37°C for 18-20 h. After the incubation period, 10 µl of resazurin dye (0.01% w/v, filter-sterilized) was added to each well. Wells exhibiting a blue or purple color were considered as no bacterial growth, whereas pink or colorless wells indicated growth [30].

Determination of Antibiofilm Activity

Overnight bacterial cultures (*S. aureus* ATCC 6538, *P. aeruginosa* ATCC 27853) were adjusted to McFarland 0.5 to yield $5x10^5$ CFU/ml in CAMHB. Aliquots (200 µl) were dispensed per well and incubated 24 h at 37°C to allow surface-attached biofilms to develop. To get rid of the planktonic cells, the plates were gently aspirated after a day and then rinsed 3 times with PBS [31]. Two-fold serial dilutions of seed oils were prepared in fresh medium ranging from 30000 to 1875 µg/ml and added to wells (200 µl/well). After 48 h at 37 °C incubation period wells were washed 3 times with PBS**.** After room temperature drying, 200 µl of methanol was applied to each well of the microplates and left for 15 minutes. After washing each well 3 times with 200 µl of distilled water and drying them again, 200 µl of ethanol was added and allowed to incubate for 15 minutes. The optical density (OD) data was then recorded after the plates were measured in a spectrophotometer at a wavelength of 550 nm. OD results were evaluated by comparing them to the optical density of the negative control well, which solely contained microorganism-containing media. The experiment was carried out three times [32]. The percentage of biofilm inhibition was estimated using the formula below:

$$\%\ Biofilm\ Inhibition = \left(\frac{OD_{control} - OD_{sample}}{OD_{control}}\right) \times 100$$

$OD_{control}$ refers to OD of the well containing the biofilm only, while OD_{sample} refers to OD of wells containing different concentrations of seed oil.

RESULT AND DISCUSSION

Extraction Yield

The extraction yield of Antep Karası grape seeds was calculated as 8.2% (w/w). This result is consistent with the average range for grape seed oils, which generally spans from 6% to 20%, influenced by cultivar, cultivation circumstances, and extraction technique [33]. A further extensive examination indicates that the oil content in grape seeds generally ranges from 6% in certain dark kinds to 20% in sweet white grape varieties, with 5-13 kg of dried grape seeds necessary to provide 1 liter of oil [3]. The

reported yield demonstrates the value of this native cultivar as a significant source of seed oil for subsequent phytochemical and biological research.

Chemical Composition of Fatty Acids

The fatty acid composition of *V. vinifera* (Antep Karası) seed oil was determined by GC-FID/MS analysis. The representative GC-FID chromatogram of the fatty acid methyl esters is shown in Figure 1, while the detailed results are in Table 1.

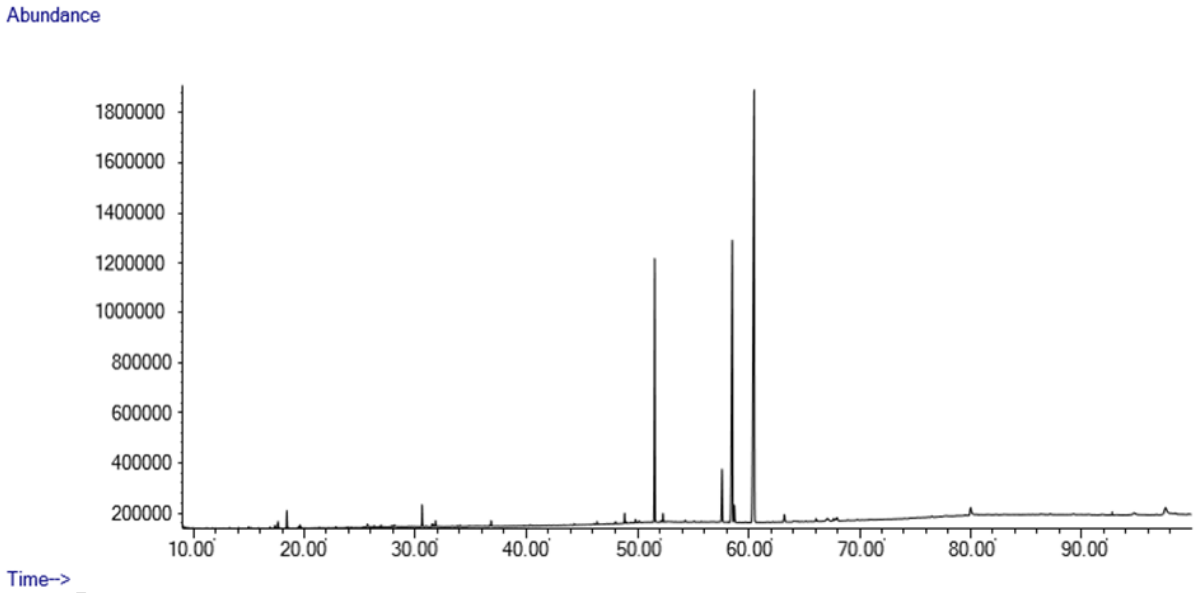

Figure 1. GC-FID chromatogram fatty acid methyl esters obtained from *V. vinifera* (Antep Karası) seed oil

Table 1. Relative percentages of fatty acids identified in *V. vinifera* (Antep Karası) seed oil by GC-FID/MS analysis

No	Compound	Relative percentage (%)
1	Hexadecanoic acid (=*Palmitic acid*); (16:0)	15.3
2	(*Z*)-9-Hexadecenoic acid (=*Palmitoleic acid*); (16:1); ω-7	0.5
3	Octadecanoic acid (=*Stearic acid*); (18:0)	4.2
4	(*Z*)-9-Octadecenoic acid (=*Oleic acid*); (18:1); ω-9	26.8
5	(*E*)-9-Octadecenoic acid (=*Elaidic acid*); (18:1); ω-9	1.2
6	(*Z,Z*)-9,12-Octadecadienoic acid (=*Linoleic acid*); (18:2); ω-6	51.3
7	(*Z,Z,Z*)- 9,12,15-Octadecatrienoic acid (=*a-Linolenic acid*); (18:3); ω-3	0.7
Total		**100.0**
Total ω-3		0.7
Total ω-6		51.3
Total ω-7		0.5
Total ω-9		28.0
Total saturated fatty acids		**19.5**
Total unsaturated fatty acids		**80.5**

The findings in Table 1 indicate that the fatty acid profile of *V. vinifera* (Antep Karası) seed oil was primarily composed of unsaturated fatty acids. The primary component was *(Z,Z)*-9,12-octadecadienoic acid (linoleic acid, C18:2, ω-6), constituting 51.3% of the total fatty acids. Following

that was *(Z)*-9-octadecenoic acid (oleic acid, C18:1, ω-9) at 26.3% and hexadecanoic acid (palmitic acid, C16:0) at 15.3%. Linoleic acid was the main ω-6 fatty acid, while unsaturated fatty acids comprised 80.5% of the total fatty acid profile. In the literature, grape seed oil contains a lot of unsaturated fatty acids, and the profile is essentially identical across different *V. vinifera* cultivars. Linoleic acid (C18:2, ω-6) is said to be the most common fatty acid, accounting for up to 66-75% of the total fatty acids. However, values between 53.6% and 83.1% have also been observed, depending on the grape type and extraction method [33-37]. Oleic acid (C18:1, ω-9) constitutes the second predominant component, with documented concentrations ranging from 15% to 31%. Palmitic acid (C16:0), a saturated fatty acid, is typically present in concentrations of 6–13%, whereas stearic acid (C18:0) is generally observed at levels of 1–10% [38,39]. Alongside these primary components, trace fatty acids such as α-linolenic acid (C18:3, ω-3), palmitoleic acid (C16:1), myristic acid (C14:0), and arachidic acid (C20:0) are found in minimal quantities, typically under 1% [40,41].

The fatty acid profile of Antep Karası seed oil showed both similarities and certain deviations when compared with previously reported data for grape seed oils. While linoleic acid (51.3%) was identified as the major fatty acid, its proportion was slightly lower than the commonly reported ranges (66–75%). Oleic acid (26.3%) was consistent with the literature values (15–31%), whereas palmitic acid (15.3%) was found to be somewhat higher than the typical range (6–13%). Notwithstanding these differences, the comprehensive profile, with unsaturated fatty acids constituting 80.5% of the total fatty acids, aligns with the typical attributes of *V. vinifera* seed oils, hence affirming the nutritional significance of the Antep Karası cultivar.

Total Phenolic, Flavonoid Contents

The oil of *V. vinifera* (Antep Karası) seeds were evaluated for bioactivity by measuring total phenolic and flavonoid content. Phenolic content was measured in gallic acid equivalents (mg GAE/$g_{seed\ oil}$) and flavonoid content in rutin equivalents (mg RE/$g_{seed\ oil}$). Table 2 presents the total content of phenolics and flavonoids.

Table 2. Quantification of total phenolic and flavonoid concentrations in *V. vinifera* (Antep Karası) seed oil

Sample	Total phenolic content (mg GAE/$g_{seed\ oil}$)	Total flavonoid content (mg RE/$g_{seed\ oil}$)
***V. vinifera* (Antep Karası) seed oil**	0.074 ± 0.001	0.02 3± 0.001

The total phenolic content of grape seed oils documented in the literature exhibits significant variability based on grape cultivar, extraction process, and analysis approach, with values spanning from 0.59 to 154 mg GAE/g [42]. The total phenolic content of Antep Karası seed oil was measured at 0.074 ± 0.001 mg GAE/$g_{seed\ oil}$, significantly lower than most previously reported values. Various factors, such as individual cultivar traits, regional and environmental cultivation conditions, and technical discrepancies in extraction and quantification, may contribute to these disparities. Notwithstanding the reduced phenolic content, the antioxidant capacity demonstrated in this study indicates that other bioactive components, especially unsaturated fatty acids and trace chemicals, may substantially enhance the overall efficacy of Antep Karası seed oil.

The total flavonoid concentration in grape seed oils is typically determined as exceedingly low, frequently measured in micrograms per gram (μg/g) [2]. Our findings aligned with this pattern, since the total flavonoid content of Antep Karası seed oil was determined to be 0.023 ± 0.001 mg RE/$g_{seed\ oil}$, indicating that flavonoids are present solely in minimal quantities. This discovery suggests that phenolic chemicals, as opposed to flavonoids, are the primary contributors to the antioxidant capacity of Antep Karası seed oil.

Biological Activities

Antioxidant and Enzyme Inhibitory Activities

The assessment of antioxidant activity was performed using a series of *in vitro* tests, including DPPH, TEAC, and β-carotene bleaching. The inhibitory potential of α-amylase was also evaluated. The result from the TEAC was shown as Trolox equivalents (mg TE/$g_{seed\ oil}$), while the results for DPPH, β-carotene bleaching, and α-amylase inhibition were reported as IC_{50} values (µg/ml). Table 3 shows the results of all biological activity.

Table 3. Antioxidant and α-amylase inhibitory activities of *V. vinifera* (Antep Karası) seed oil

Samples	**DPPH** IC_{50}, mg/ml	**TEAC** µmol trolox/$mg_{seed\ oil}$	**β-Carotene peroxidation inhibition** IC_{50}, mg/ml	**α-Amylase inhibition** IC_{50}, mg/ml
Seed oil	1.423 ± 0.004	0.012 ± 0.000	0.182 ± 0.020	273.32 ± 0.10 [a)]
Gallic acid	0.002 ± 0.000	-	-	-
Ascorbic acid	0.012 ± 0.000	-	-	-
Butylated hydroxyanisole	0.113 ± 0.008	-	0.009 ± 0.000	-
Acarbose	-	-	-	0.011 ± 0.000

a): IC_{50} was not calculated; the value is 0.625 mg/ml

The radical scavenging capacity of Antep Karası seed oil was evaluated by the DPPH assay, yielding an IC_{50} value of 1.423 ± 0.004 mg/ml. In comparison, cold-pressed grape seed oils from Türkiye have previously been reported to exhibit DPPH scavenging activities ranging between 31.00% and 45.31% [43]. TEAC was 0.012 µmol TE/$mg_{seed\ oil}$ substantially exceeding literature values reported for grape seed oils (0.09–1.16 µg TE/g), most likely due to different reporting bases and assay normalization [44]. β-carotene peroxidation inhibition values for *V. vinifera* seed oils are not always reported as a percentage, and the available studies generally indicate that grape seed oils exhibit only moderate antioxidant activity, which is usually lower than that observed for grape seed extracts [2,3]. In the present study, the Antep Karası seed oil showed an IC_{50} value of 0.182 ± 0.020 mg/ml, supporting these earlier observations. The α-amylase inhibitory activity of *Vitis vinifera* seed oils has generally been reported as weak in the literature. For instance, one study reported an IC_{50} value of 257.03 ± 2.4 µg/ml, indicating that grape seed oil is not a strong inhibitor of this enzyme [45]. The IC_{50} value of Antep Karası seed oil in this investigation was established at 273.32 ± 0.10 µg/ml, closely aligning with previously reported findings. Notwithstanding the comparatively low levels of phenolics and flavonoids, these findings indicate that the antioxidant and enzyme inhibitory capabilities of Antep Karası seed oil are primarily ascribed to its unsaturated fatty acids and other lipophilic bioactive components, thereby endorsing its potential as a natural reservoir of biologically active compounds.

Antimicrobial and Antibiofilm Activities

Determination of Antibacterial and Antifungal Activities

This study assessed the antibacterial efficacy of *V. vinifera* seed oil against several microorganisms, with results detailed in Table 4.

Table 4. Antibacterial and antifungal activities of *V. vinifera* (Antep Karası) seed oil (µg/ml)

Samples	***B. subtilis***	***S. aureus***	***P. aeruginosa***	***S. typhimurium***	***E. coli***	***C. albicans***	***C. krusei***
Seed oil	15000	30000	15000	7500	7500	7500	7500
Erythromycin	1.25	1.25	-	-	-	-	-
Ciprofloxacin	-	-	1	0.015	0.0156	-	-
Amphotericin B	-	-	-	-	-	0.5	1

The results showed that the oil provided moderate inhibition against Gram-positive bacteria *B. subtilis* and *S. aureus* with MBC values of 15000 and 30000 µg/ml, respectively. For Gram-negative bacteria *P. aeruginosa*, *S. typhimurium*, and *E. coli*, the MBC values ranged from 7500 to 15000 µg/ml, with particularly lower MBC values against *S. typhimurium* and *E. coli*, suggesting that the oil exhibits relatively higher activity against these species. The MFC values for the yeast species *C. albicans* and *C. krusei* were determined to be 7500 µg/ml, indicating that the oil also has an antifungal spectrum. This result indicates that plant oils have a weaker but broader spectrum of inhibition potential compared to standard antibiotics, and that their efficacy may be related to the phenolic compounds, tocopherols, and unsaturated fatty acids they contain. The literature also reports that grape seed oil exhibits antimicrobial and antioxidant properties due to its polyphenolic content, but that high MBC values require higher amounts for effective concentrations *in vitro* conditions [46]. These results suggest that *V. vinifera* seed oil may not be a strong antimicrobial agent on its own but may have potential for use as a natural preservative or supportive component in food, cosmetic, or pharmaceutical formulations.

The antimicrobial properties of seed oil derived from the Balkan native variety *V. vinifera* were studied, reporting MBC values of 15.400 µg/ml for *S. aureus* and *P. aeruginosa* [40]. These findings are consistent with the current results, indicating that microbial inhibition requires relatively high oil concentrations, likely due to the complex lipid composition and low water solubility of the extract. Additionally, MIC values ranging from 20.000 to 40.000 µg/ml for antifungal activity were also reported in the same study [40]. Therefore, the fungicidal effect found in this study provides to understanding of the range of activity of grape seed oil by indicating that lipid components like oleic and linoleic acids, along with trace amounts of bioactive substances like tocopherols and phenolic residues, may be involved in fungal cell membrane disruption and metabolic inhibition. These results showed the grape seed oil contains bioactive compounds capable of exerting direct antimicrobial action. This activity is likely attributed to the combined effects of minor phenolic constituents such as catechins, epicatechins, and proanthocyanidins, as well as the high proportion of polyunsaturated fatty acids, particularly linoleic acid, which has been reported to disrupt cytoplasmic membrane integrity and interfere with essential metabolic processes [47].

Determination of Antibiofilm Activity

The efficacy of *V. vinifera* seed oil in eliminating biofilm formations of *S. aureus* and *P. aeruginosa* at concentrations of 1875, 3750, 7500, 15000, and 30000 µg/ml was assessed, with the findings detailed in Table 5. Representative graphical data are also shown in Figure 2 for *P. aeruginosa* and *S. aureus*, illustrating the antibiofilm activity of the seed oil against these microorganisms.

Table 5. Antibiofilm activity of *V. vinifera* (Antep Karası) seed oil against *S. aureus* and *P. aeruginosa*

Sample concentrations	***P. aeruginosa***	***S. aureus***
30000 µg/ml	86.55 ± 0.91 %	73.33 ± 1.40 %
15000 µg/ml	78.43 ± 0.85 %	66.67 ± 1.26 %
7500 µg/ml	73.69 ± 1.08 %	62.23 ± 1.20 %
3750 µg/ml	69.34 ± 0.99 %	58.10 ± 1.05 %
1875 µg/ml	57.43 ± 1.21 %	51.56 ± 1.33 %

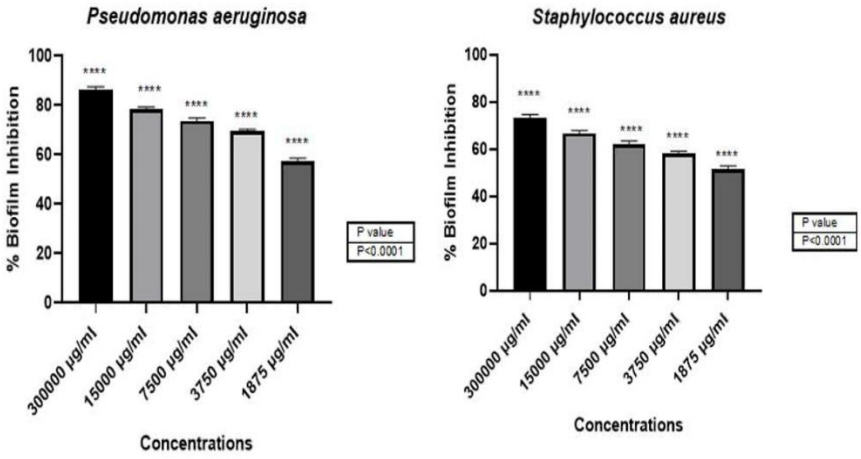

Figure 2. Antibiofilm activity of the extracts against *P. aeruginosa* and *S. aureus*

The results revealed that the oil exhibited a dose-dependent antibiofilm effect against both microorganisms. At the highest concentration of 30000 µg/ml, inhibition rates of 86.55 ± 0.91% in *P. aeruginosa* biofilms and 73.33 ± 1.40% in *S. aureus* biofilms were achieved. As the concentration decreased, a gradual decrease in inhibition rates was observed; at the lowest concentration (1875 µg/ml), the inhibition rate was 57.43 ± 1.21% for *P. aeruginosa* and 51.56 ± 1.33% for *S. aureus*. Even at the lowest dose of 1875 µg/ml, the biofilm inhibition value did not fall below 50%. In general, it was observed that the inhibition percentages obtained against *P. aeruginosa* were higher than those against *S. aureus* at all concentrations, suggesting that the oil may be more effective against Gram-negative bacterial biofilms. In the literature, it has been reported that the phenolic compounds, tocopherols, and unsaturated fatty acids contained in grape seed oil can inhibit biofilm formation by disrupting cell membrane permeability and affecting quorum sensing mechanisms [48,49]. The results obtained indicate that *V. vinifera* seed oil possesses a strong biofilm inhibition capacity at high concentrations, suggesting that it could be considered as a natural agent in areas where biofilm control is critical, such as food safety, medical device coatings, or topical formulations.

This study demonstrated that *V. vinifera* (Antep Karası) seed oil possesses a similar fatty acid profile to other grapeseed oils dominated by unsaturated fatty acids and contains measurable amounts of phenolic and flavonoid compounds. Notwithstanding the comparatively low phenolic content, the oil demonstrated antioxidant, antibacterial, antifungal, antibiofilm, and enzyme inhibitory effects, indicating that many types of bioactive compounds enhance its overall biological efficacy. The findings suggest that Antep Karası seed oil may serve as a significant natural resource for functional culinary, medicinal, and nutraceutical uses. Further investigations, particularly focusing on the isolation of active compounds and *in vivo* studies, are warranted to better understand its mechanisms of action and potential health benefits.

AUTHOR CONTRIBUTIONS

Concept: K.Ö.; Design: K.Ö.; Control: K.Ö., P.S.; Sources: K.Ö., P.S., G.Ö.; Materials: K.Ö., G.Ö., P.S., T.Ö.; Data Collection and/or Processing: K.Ö., G.Ö., P.S., T.Ö.; Analysis and/or Interpretation: K.Ö., G.Ö., P.S., T.Ö.; Literature Review: K.Ö., P.S.; Manuscript Writing: K.Ö., P.S.; Critical Review: K.Ö., G.Ö., P.S., T.Ö.; Other: -

CONFLICT OF INTEREST

The authors declare that there is no real, potential, or perceived conflict of interest for this article.

ETHICS COMMITTEE APPROVAL

The authors declare that the ethics committee approval is not required for this study.

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