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GC-FID/MS Analysis of Silyl Derivatives and Bioactivities of *Vitis Vinifera* L. (Antep Karası) Seed Methanol Extract: Experimental Study

Vitis Vinifera L. (Antep Karası) Çekirdeği Metanol Ekstresinin Silil Türevlerinin GK-KS/AİD Analizi ve Biyolojik Aktiviteleri: Deneysel Çalışma

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ABSTRACT Objective: This study investigated the phytochemical profile of the methanol extract from *Vitis vinifera* L. (*V. vinifera*) (Antep karası) seeds, focusing on silylated derivatives, phenolic and flavonoid contents, as well as its various biological activities. **Material and Methods:** In this experimental study, the methanolic extract of *V. vinifera* seeds was obtained by ultrasonic extraction in a water bath. The analytical procedures included gas chromatography flame ionization detection/mass spectrometry analysis of silylated derivatives, determination of total phenolic and flavonoid contents, and evaluation of *in vitro* biological activities. Antioxidant potential was assessed the free radical scavenging assay (DPPH), ferric reducing antioxidant power (FRAP), cupric ion-reducing antioxidant capacity (CUPRAC), Trolox equivalent antioxidant capacity (TEAC), oxygen radical absorbance capacity (ORAC), and β-carotene bleaching assay, while antimicrobial and antibiofilm effects were determined by broth microdilution and crystal violet staining methods. **Results:** The extraction yield was 4.7% (w/w). The extract contained 126.11±4.77 mg gallic acid equivalents/$g_{extract}$ of total phenolics and 68.62±0.11 mg quercetin equivalents/$g_{extract}$ extract of total flavonoids. Antioxidant assays showed strong activity [DPPH half maximal inhibitory concentration (IC_{50})=0.032±0.001 mg/mL; FRAP=1.521±0.002 µmol Trolox/$mg_{extract}$; CUPRAC=0.774±0.005 µmol Trolox/$mg_{extract}$; TEAC=0.022±0.001 µmol Trolox/$mg_{extract}$; ORAC=1.372±0.011 µmol Trolox/$g_{extract}$; β-carotene IC_{50}=0.087±0.003 mg/mL]. The antimicrobial tests showed minimum inhibitory concentration values of 468.75 and 234.37 µg/mL for bacterial strains, while *Candida* species required 7,500 µg/mL for inhibition. The antibiofilm test demonstrated a concentration-dependent effect, with more than 50% inhibition still observed at the lowest tested dose of 58.59 µg/mL. **Conclusion:** *V. vinifera* seed methanol extract exhibited strong antioxidant, antimicrobial, and antibiofilm activities, indicating its potential as a valuable natural source of bioactive compounds.

Keywords: Antep karası; *Vitis vinifera* L.; biological activities; silylated derivates; GC-MS/FID

ÖZET Amaç: Bu çalışma, *Vitis vinifera* L. (*V. Vinifera*) (Antep karası) çekirdeklerinden elde edilen metanol ekstresinin fitokimyasal profili; silillenmiş türevleri, fenolik ve flavonoid miktarları ile çeşitli biyolojik aktivitelerini araştırmayı amaçlamıştır. **Gereç ve Yöntemler:** Bu deneysel çalışmada, *V. vinifera* çekirdeklerinin metanol ekstresi, su banyosunda ultrasonik ekstraksiyonu ile metanol ekstresi elde edilmiştir. Uygulanan analitik prosedürler arasında silillenmiş türevlerin gaz kromatografisi alev iyonizasyon dedektörü/kütle spektrometrisi analizi, toplam fenolik ve flavonoid madde miktarları belirlenmiş ve *in vitro* biyolojik aktivitelerin değerlendirilmesi yer almaktadır. Antioksidan potansiyel, serbest radikal süpürme testi (DPPH), ferrik indirgeme antioksidan gücü (FRAP), bakır iyonu indirgeme antioksidan kapasitesi (CUPRAC), Trolox eşdeğeri antioksidan kapasitesi (TEAC), oksijen radikali absorbsiyon kapasitesi (ORAC) ve β-karoten ağartma testi ile değerlendirilmiş; antimikrobiyal ve antibiyofilm etkiler ise sıvı mikrodilüsyon ve kristal viyole boyama yöntemleriyle belirlenmiştir. **Bulgular:** Ekstraksiyon verimi, %4,7 olarak bulunmuştur. Ekstre 126,11±4,77 mg gallik asit eş değerleri/g_{ekstre} toplam fenol ve 68,62±0,11 mg kuersetin eş değerleri/g_{ekstre} toplam flavonoid içermektedir. Antioksidan testleri [DPPH %50 inhibisyon sağlayan konsantrasyon (IC_{50})=0,032±0,001 mg/mL; FRAP=1,521±0,002 µmol Trolox/$m_{gekstre}$; CUPRAC=0,774±0,005 µmol Trolox/mg_{ekstre}; TEAC=0,022±0,001 µmol Trolox/mg_{ekstre}; ORAC=1,372±0,011 µmol Trolox/g_{ekstre}; β-carotene IC_{50}=0,087±0,003 mg/mL] güçlü aktivite göstermiştir. Antimikrobiyal test sonuçları, bakteri suşları için sırasıyla 468,75 ve 234,37 µg/mL minimum inhibitör konsantrasyon değerleri göstermiştir. Buna karşılık *Candida* türlerinin inhibisyonu için 7.500 µg/mL belirlenmiştir. Antibiyofilm testinde ise konsantrasyona bağlı bir etki gözlenmiş ve en düşük konsantrasyon olan 58,59 µg/mL'de dahi %50'nin üzerinde inhibisyon gözlenmiştir. **Sonuç:** *V. vinifera* çekirdeği metanol ekstresi, güçlü antioksidan, antimikrobiyal ve antibiyofilm aktiviteler göstermiş olup, biyoaktif bileşikler açısından değerli bir doğal kaynak olma potansiyeline sahip olduğunu ortaya koymuştur.

Anahtar Kelimeler: Antep karası; *Vitis vinifera* L.; biyolojik aktiviteler; silillenmiş türevler; GK-KS/AİD

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Vitis vinifera L. (*V. Vinifera)* is among the most commonly cultivated fruit species worldwide, and its seeds are acknowledged as a significant source of bioactive components, such as polyphenols, fatty acids, sterols, and tocopherols.[1,2] Grape seeds continue to attract significant research interest owing to their potent antioxidant capabilities and documented antibacterial, antifungal, anti-inflammatory, and cardioprotective effects, rendering them viable candidates for nutraceutical and pharmaceutical uses.[3,4] Although substantial study has been conducted on grape seed extracts from various cultivars, the phytochemical and biological characteristics of region-specific types are still inadequately investigated.[5] Antep karası, a traditional grape cultivar grown in Southeastern Anatolia, Türkiye, holds significant economic and cultural value.[6,7] Nevertheless, there is an absence of data regarding the intricate chemical composition and biological efficacy of its seed extracts. Furthermore, to our knowledge, no prior research has examined the silylated methanolic extract of Antep karası seeds utilizing gas chromatography flame ionization detection/mass spectrometry (GC-MS/FID) analysis.

This study sought to perform a thorough assessment of *V. vinifera* seed extract by analyzing its phytochemical constituents using GC-MS/FID, quantifying total phenolic and flavonoid contents, and evaluating its antioxidant, antimicrobial, and antibiofilm activities. Multiple *in vitro* antioxidant assays [free radical scavenging assay (DPPH), ferric reducing antioxidant power (FRAP), cupric ion-reducing antioxidant capacity (CUPRAC), Trolox equivalent antioxidant capacity (TEAC), oxygen radical absorbance capacity (ORAC), and *β*-carotene bleaching] were selected as complementary methods representing different reaction mechanisms, including electron transfer and hydrogen atom transfer. Additionally, antimicrobial and antibiofilm assays were conducted to explore the extract's potential in inhibiting microbial growth and biofilm formation, which are critical factors for food preservation and pharmaceutical applications. These findings are anticipated to provide new insights into the bioactive potential of this indigenous grape variety and underscore its possible applications in pharmaceuticals, nutraceuticals, and food preservation.

MATERIAL AND METHODS

PLANT MATERIAL

The plant material was gathered in Gaziantep province of Türkiye during the fruiting season. Seeds were manually extracted from the fruits, which were subsequently washed to eliminate the pulp, dried at ambient temperature in the shade for 3 days, and stored at +4 °C. Prior to processing, the grinder standardized the sizes of the seeds.

Extract Preparation

20 grams of powdered seed were measured into a container and subjected to extraction with methanol (7 times over 24 hours) via the maceration process on a shaker at ambient temperature. Subsequent to maceration, the mixture was subjected to ultrasonic extraction for 30 minutes in a water bath (40 kHz, 100 W). The materials were agitated for 1 hour before undergoing a repeated 30-minute ultrasonic extraction under the same circumstances. The supernatants were filtered with Whatman No. 1 paper. Methanol was evaporated using a rotary evaporator (Heidolph, Germany) at temperatures below 40 °C under reduced pressure. The dried extract was preserved in amber glass vials at 4 °C until analysis.

Preparation of Silyl Derivatives of Extracts

The methanol extract of *V. vinifera* seeds underwent silylation for chemical composition analysis via the GC-FID/MS method. The requisite amount of dried extract was diluted in pyridine, thereafter including 80 µL of N,O-bis(trimethylsilyl)trifluoroacetamide (BSTFA) with 1% (trimethylchlorosilane) TMCS. The mixture was vortexed and heated in a sand bath at 100 °C for 1 hour. Subsequent to cooling, 1.0 µL of the silylated solution was injected into the GC-FID/MS apparatus.[8]

Gas Chromatography-Mass Spectrometry and Flame Ionization Detection Analysis

The methanol extract of *V. vinifera* seeds was subject to silylation with BSTFA (1% TMCS) in pyridine and was heated at 100 °C for 1 hour. Following cooling, 1 µL of the silylated solution was subjected to analysis via GC-FID/MS (Agilent 7890A GC cou-

pled with a 5975C MSD; Agilent Technologies, Santa Clara, CA, USA). Separation was accomplished on an HP-5 fused silica capillary column (Agilent Technologies, Santa Clara, CA, USA) using helium as the carrier gas. The oven was set to heat from 100 to 320 °C at a rate of 3 °C/min and maintained at that temperature for 16.7 minutes. The injector and FID temperatures were established at 250 °C and 300 °C, respectively; mass spectra were acquired in electron impact mode (70 eV, *m/z* 35-1,050). Compounds were identified by comparing retention durations and mass spectra with reference standards, the Wiley GC/MS Library, and the Special Özek Silyl Derivative Library. Quantification was conducted using relative peak areas derived from FID chromatograms.[8,9]

Determination of Total Phenolic and Flavonoid Contents

The total phenolic content was determined by the Folin-Ciocalteu method and expressed as mg gallic acid equivalents (GAE)/$g_{extract}$.[10] The total flavonoid content was evaluated using the aluminum chloride colorimetric assay and expressed as mg quercetin equivalents (QE)/$g_{extract}$. Both assays were carried out in triplicate, and calibration curves (0.01-1.0 mg/mL) showed good linearity (r^2>0.99).

Biological Activities

Antioxidant Activities

The ferric reducing ability method

The FRAP of the extract was assessed using the method established by Benzie and Strain, with minor modifications.[11] Absorbance was recorded at 593 nm post-incubation, and antioxidant capacity was determined using Trolox calibration curves. Results were reported as µmol Trolox equivalents (TE)/$mg_{extract}$.

Oxygen radical absorbance capacity (Assay)

The ORAC of the extract was assessed following a previously established method, with some changes.[12] Fluorescence decay was observed at 485/528 nm for 60 minutes at 37 °C, and the data were derived using Trolox calibration curves. Data were presented as TE/$g_{extract}$.

Cupric Ion reducing antioxidant capacity (Assay)

The cupric ion-reducing antioxidant capacity of the extract was assessed using the CUPRAC technique as outlined by Apak et al., with slight modifications.[13] Absorbance was measured at 450 nm after 30 minutes, and findings were derived from Trolox calibration curves, represented as µmol TE/$mg_{extract}$.

Free radical scavenging effect (DPPH Assay)

The free radical scavenging activity was determined using the DPPH assay as described by Brand-Williams et al. with minor modifications.[14] Absorbance was measured at 517 nm after 30 min of incubation in the dark. Results were expressed as percentage inhibition, and half maximal inhibitory concentration (IC_{50}) values were calculated from dose-response curves.

Lipid peroxidation inhibition test (β-carotene bleaching Assay)

The inhibition of lipid peroxidation was assessed using a modified *β*-carotene-linoleic acid bleaching method based on a previously documented procedure.[15] Absorbance was recorded at 492 nm, and the antioxidant activity was quantified as percentage inhibition compared to butylated hydroxyanisole (BHA), the reference antioxidant.

Trolox equivalent antioxidant capacity Assay

The antioxidant capacity of the extract was determined using the 2,2′-azinobis(3-ethylbenzothiazoline-6-sulfonic acid) ($ABTS^{\bullet+}$) radical cation decolorization method.[9] The $ABTS^{\bullet+}$ solution was prepared by reacting ABTS with potassium persulfate and kept in the dark for 16 h. The solution was then diluted with ethanol to obtain an absorbance of 0.700-0.800 at 734 nm. The extract and Trolox standards were prepared in methanol containing 10% dimethyl sulfoxide (DMSO). A mixture of 10 µL sample and 990 µL $ABTS^{\bullet+}$ solution was incubated for 30 min in the dark, and the absorbance decrease was measured at 734 nm. Results were expressed as µmol TE per mg extract. All measurements were performed in triplicate.

Antimicrobial Activities

Determination of antimicrobial activity

The minimum inhibitory concentration (MIC) of *V. vinifera* methanol extract was assessed against standard bacterial strains (*Bacillus subtilis* NRRL B478, *Staphylococcus aureus* ATCC 6538, *Pseudomonas aeruginosa* ATCC 27853, *Salmonella typhimurium* NRRL B4420, *Escherichia coli* ATCC 35218) and yeast strains (*Candida albicans* ATCC 90028, *Candida krusei* ATCC 6258) by broth microdilution method following CLSI M07 (2024).[16,17] Serial dilutions (30000-58.59 µg/mL) were prepared in CAMHB (Cation-Adjusted Mueller Hinton Broth) for bacteria and SDB (Sabouraud Dextrose Broth) for yeasts, with inocula of $5x10^5$ colony-forming unit (CFU)/mL for bacteria and $0.5\text{-}2.5x10^3$ CFU/mL for yeasts. In the experiment, a negative control (microorganisms only), a sterile control (medium only), and a positive control (10% DMSO + methanol as a solvent with standard antimicrobial agents such as erythromycin for Gram-positive bacteria, ciprofloxacin for Gram-negative bacteria, and amphotericin B for yeasts) were used. After 18-20 h incubation at 37 °C, resazurin (0.01%) was added; blue/purple wells indicated inhibition, pink/colorless indicated growth.[18]

Determination of antibiofilm activity

Antibiofilm effects were tested against *S. aureus* and *P. aeruginosa*. Cultures ($5x10^5$ CFU/mL) were incubated in 96-well plates for 24 h to form biofilms. Wells were washed with phosphate buffered saline (PBS), then treated with extract dilutions (937.5-58.59 µg/mL) and incubated for 48 h. After PBS washing, biofilms were fixed with methanol, stained with 0.1% crystal violet, and the dye was solubilized with ethanol. Absorbance was read at 550 nm to quantify biofilm biomass.[19]

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

ETHICAL STATEMENT

This study was conducted in accordance with the principles of the Declaration of Helsinki. Since no human or animal subjects were involved, ethics committee approval was not required.

RESULTS

EXTRACTION YIELD

The extraction yield of Antep karası grape seeds was determined to be 4.7% (w/w).

CHEMICAL COMPOSITION OF SILYLATED EXTRACT

Derivatizing *V. vinifera* methanol extract seeds with BSTFA allowed gas chromatographic characterization of semi-volatile components. Silyl derivatives were separated on an HP-5 FSC capillary column under controlled temperature conditions. To compare retention indices, *n*-alkanes (C_8-C_{40}) were compared. The total area of the GC-FID peaks was used to measure the compounds without altering the values. The volatile components are listed in Table 1.

TABLE 1: Chemical composition of silylated seed methanol extract of *V. vinifera* analyzed by GC-FID/MS

RRI#	RRI¥	Compound	Methanol extract (%)
1506	1512	Malic acid-3TMS	0.1
1529	1540	Erythritol-4TMS	0.1
1662	1667	Tartaric acid-4TMS	0.1
1844	1843	α-Fructofuranose-5TMS	22.9
1852	1854	β-Fructofuranose-5TMS	2.7
1858	1887	β-Fructopyranose-5TMS	0.2
1885	1880	α-Glucofuranose-5TMS	1.7
1929	1930	α-Glucopyranose-TMS	14.7
1971	1973	Mannitol-6 TMS	0.3
1984	1985	Gallic acid-4TMS	0.5
2023	2032	β-Glucopyranose-5TMS	18.8
2127	2129	myo-Inositol-6TMS	0.6
2704	2714	*Sucrose - TMS derivative*	22.8
2813	2814	Palatinose-8TMS (*isomer-1*)	0.2
2844	2849	*neo*-Trehalose-8TMS	0.6
2902	2907	*epi*-Catechin-5TMS	4.7
2935	2936	Catechin-5TMS	4.3
3368	3345	β-Sitosterol-TMS	0.2
3513	3516	1-Kestose-11TMS	0.3
		Total	**95.8**

RRI#: Relative retention indices calculated against n-alkanes for apolar column; RRI¥: Relative retention indices from literature.[20] GC-FID/MS: Gas chromatography flame ionization detection/mass spectrometry; TMS: Trimethylsilyl

TABLE 2: Total phenolic and flavonoid contents of the methanol extract from the seed of *V. Vinifera*

Extract type	Total phenolic content (mg GAEs/$g_{extract}$)	Total flavonoid content (mg QEs/$g_{extract}$)
Methanol extract	126.11±4.77	68.62±0.11

GAE: Gallic acid equivalents; QE: Quercetin equivalents

DETERMINATION OF TOTAL PHENOLIC AND FLAVONOID CONTENTS

The bioactivity of *V. vinifera* seed methanol extracts was assessed by assessing total phenolic and flavonoid content. Flavonoids were quantified in mg QEs/$g_{extract}$ and phenols in mg GAE/$g_{extract}$. Table 2 displays phenolic and flavonoid content.

IN VITRO BIOLOGICAL ACTIVITY

Antioxidant Activities

The evaluation of antioxidant activity was conducted through a series of *in vitro* assays, including DPPH, CUPRAC, FRAP, TEAC, ORAC, and *β*-carotene peroxidation inhibition. The results from the CUPRAC, FRAP, TEAC, and ORAC tests were shown as mg TE/$g_{extract}$, while the results for DPPH, and *β*-carotene peroxidation inhibition were reported as IC_{50} values (µg/mL). Table 3 summarizes the results of all biological activity.

In this study, the antimicrobial effects of *V. vinifera* methanol extract against various bacterial and fungal strains were investigated (Table 4).

When examining the MICs, the *V. vinifera* methanol extract showed stronger activity against Gram-negative bacteria, with inhibition at 234.37 µg/mL for *P. aeruginosa*, *S. typhimurium*, and *E. coli*. In contrast, higher concentrations (468.75 µg/mL) were required to inhibit *B. subtilis* and *S. aureus*. The extract displayed limited antifungal activity, with *C. albicans* and *C. krusei* inhibited only at markedly high concentrations (7,500 µg/mL).

Determination of Antibiofilm Activity

This study assessed the capacity of *V. vinifera* extract to eliminate biofilm formation against *S. aureus* and *P. aeruginosa* (Table 5).

Upon analysis, *V. vinifera* extract demonstrated a clear concentration-dependent antibiofilm effect against both *P. aeruginosa* and *S. aureus*. At 937.5 µg/mL, inhibition reached 95.45% and 98.54%, re-

TABLE 3: Antioxidant activities of the methanol extract from the seed of *V. vinifera*

Extract type	DPPH[a] IC_{50}, mg/mL	FRAP[b] µmol trolox/ $mg_{extract}$	CUPRAC[b] µmol trolox/ $mg_{extract}$	TEAC[b] µmol trolox/ $mg_{extract}$	ORAC[b] µmol trolox/ $g_{extract}$	β-Carotene peroxidation inhibition[a] IC_{50}, mg/mL
Methanol extract	0.032±0.001	1.521±0.002	0.774±0.005	0.022±0.001	1.372±0.011	0.087±0.003
Gallic acid	0.002±0.000	-	-	-	-	-
Ascorbic acid	0.011±0.000	-	-	-	-	-
Butylated hydroxyanisole	0.117±0.009	-	-	-	-	0.009±0.000

[a]IC_{50} values are half maximal inhibitory concentrations; [b]ORAC, FRAP, CUPRAC values are determined for extracts at 0.1 mg/mL. DPPH: Free radical scavenging assay; FRAP: Ferric reducing antioxidant power; CUPRAC: Cupric ion-reducing antioxidant capacity; TEAC: Trolox equivalent antioxidant capacity; ORAC: Oxygen radical absorbance capacity

TABLE 4: MIC values of *V. vinifera* methanol extract (µg/mL).

Samples	MIC (µg/mL) *B. subtilis*	*S. aureus*	*P. aeruginosa*	*S. typhimurium*	*E. coli*	*C. albicans*	*C. krusei*
Extract	468.75	468.75	234.37	234.37	234.37	>7,500	>7,500
Erythromycin	1.25	1.25	-	-	-	-	-
Ciprofloxacin	-	-	1	0.0156	0.0156	-	-
Amphotericine B	-	-	-	-	-	0.5	1

MIC: Minimum inhibitory concentration

TABLE 5: % Biofilm inhibition values of *V. vinifera* methanol extract.

Sample concentrations	*P. aeruginosa* (%)	*S. aureus* (%)
937.5 µg/mL	95.45±1.36	98.54±0.96
468.75 µg/mL	83.44±0.93	77.60±0.68
234.37 µg/mL	72.26±0.87	73.75±0.96
117.18 µg/mL	62.44±1.30	64.50±1.40
58.59 µg/mL	52.58±1.35	53.56±1.15

spectively, while at the lowest tested concentration (58.59 µg/mL) it decreased to 52.58% and 53.56% (Figure 1). These results indicate that grape-derived polyphenols can effectively suppress biofilm formation in both Gram-negative and Gram-positive bacteria.

DISCUSSION

EXTRACTION YIELD

The extract yield of *V. vinifera* seeds has been determined at 8.2%. Prior research indicated that methanolic extracts of grape seeds typically range from 2.6 to 7.6 mg/g (0.26 to 0.76%) to 10 to 21.6 mg/g (1 to 2.1%).[21-23] The observed results are much inferior to the current extract yield, attributable to variations in solvent polarity, extraction techniques, and the characteristics of the raw material.

CHEMICAL COMPOSITION OF SILYLATED EXTRACT

This is the first study on the silylated methanolic extract of *V. vinifera* seeds, evaluated via GC-MS/FID. In our investigation, the predominant compounds were α-fructofuranose-5TMS (22.9%), sucrose-TMS derivative (22.8%), *β*-glucopyranose-5TMS (18.8%), and α-glucopyranose-5TMS (14.7%), with 95.8% of the extract's composition revealed.

Prior GC-MS analyses of various *V. vinifera* cultivars identified a variety of bioactive metabolites, predominantly comprising fatty acids [9-octadecenamide, (9E,11E)-octadeca-9,11-dienoate], sterols (γ-sitosterol, stigmasterol), and tocopherol derivatives including γ-tocotrienol and α-tocopherol acetate. Other compounds such as paromomycin and 4,6-cholestadienol were identified in particular extracts.[24,25] Furthermore, liquid chromatography-tandem mass spectrometry (LC-MS/MS) and ultra-high-performance liquid chromatography-quadrupole time-of-flight (UHPLC-QTOF) analyses have revealed a variety of polyphenolic chemicals, including gallic acid, caffeic acid, quercetin, isoquercetin, resveratrol, and pterostilbene, demonstrating that grape seeds are abundant in phenolic acids, flavonoids, stilbenes, and fatty acids.[26-28]

In contrast to these previous reports, the present study primarily detected silylated sugar derivatives rather than lipophilic or phenolic components, suggesting that the methanol extract of the Antep karası seeds possesses a distinct metabolite profile. This difference may arise from the extraction solvent, plant part used, or the varietal characteristics of this local Turkish cultivar. The obtained data thus provide the first GC-MS/FID-based compositional reference for

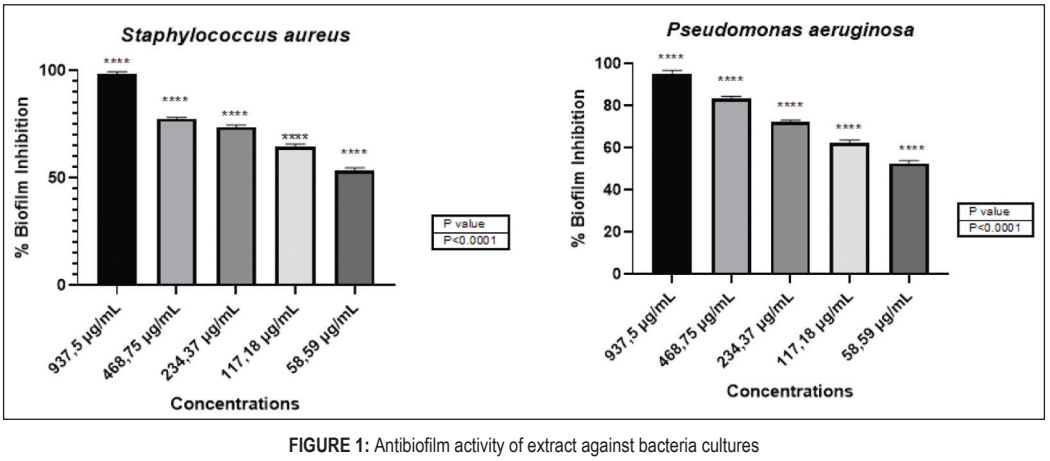

FIGURE 1: Antibiofilm activity of extract against bacteria cultures

the silylated methanolic extract of *V. vinifera* (Antep Karası) seeds.

DETERMINATION OF TOTAL PHENOLIC AND FLAVONOID CONTENTS

Prior research on grape seed varieties has shown total phenolic contents between 111 and 149 mg GAE per 100 g of fresh seeds, although alternative studies have revealed values ranging from 79.2 to 154.6 mg/g, corresponding to 7,920 to 15,460 mg per 100 g.[29] The reported range of GAE in Karaerik grape seeds was 1.45-235.05 mg GAE/g. The total phenolic content of *V. vinifera* seed extract was quantified as 126.11±4.77 mg GAE/$g_{extract}$ in the current study. Additionally, the total flavonoid content of *V. vinifera* seed extract was quantified as 68.62±0.11 mg QE per gram of extract. Values between 2.46 and 704.36 mg QE/g have been documented in Karaerik grape seeds. The flavanol subgroup for various Turkish grape seed varieties was reported to range from 89.2 to 179.4 mg/g.[30,31] This result aligns with the heterogeneity documented in the literature for several grape seed cultivars and may indicate disparities in cultivar type, environmental circumstances, and extraction methods.

IN VITRO BIOLOGICAL ACTIVITY

Antioxidant Activities

The antioxidant potential of *V. vinifera* seed extract was evaluated using six different tests. The DPPH radical scavenging experiment showed a low IC_{50} value of 0.032±0.001 mg/mL. Lower IC_{50} values imply stronger radical scavenging capacity than previous investigations, which found EC_{50} values of 3.55-5.76 mg Trolox equivalents/$g_{extract}$ for grape seed extracts. The FRAP value (1.521±0.002 µmol Trolox/$mg_{extract}$) showed strong reducing power, consistent with the wide range (3.96-135 µmol of ascorbic acid equivalents per gram or mmol Fe^{2+} per gram) found in grape seeds. The CUPRAC (0.774±0.005 µmol Trolox/$mg_{extract}$) and TEAC (0.022±0.001 µmol Trolox/$mg_{extract}$) results confirmed considerable electron-donating ability and broadly matched published ranges. After normalizing the extraction yield, the ORAC value (1.372±0.011 µmol Trolox/$g_{extract}$) was much lower than the average 1,000-2,000 µmol Trolox/$g_{dry\ matter}$ for grape seed extracts. In the *β*-carotene/linoleic acid assay, the extract had an IC_{50} of 0.087±0.003 mg/mL. Lower IC_{50} values imply increased activity, demonstrating a significant lipid peroxidation inhibitory impact. Despite most studies presenting *β*-carotene results as a percentage inhibition (65-90%), the IC_{50} value suggests moderate-to-strong antioxidant activity. Results show Antep karası seed extract has strong radical scavenging activity in DPPH, FRAP, CUPRAC, and TEAC assays but is less effective in ORAC and *β*-carotene models. This highlights the assay-dependent nature of antioxidant assessment and the complex mechanisms influencing this cultivar's antioxidant capabilities.

Compared with previous studies on other Turkish grape cultivars, the notably lower IC_{50} and higher FRAP values observed in the present work indicate a stronger overall antioxidant efficiency of the Antep karası seed extract. This difference may be attributed to the unique phenolic and flavonoid composition of this local variety, making this the first detailed antioxidant characterization of Antep Karası seeds in the literature.

Determination of Antimicrobial Activity

The findings highlight the selective effects of grape polyphenols on different microbial groups. Consistent with earlier reports, polyphenolic extracts from grape seeds and skins show stronger activity against Gram-negative bacteria, likely due to their interaction with the lipopolysaccharide layer and disruption of membrane permeability.[32] By contrast, the higher MIC values for Gram-positive bacteria agree with previous studies, as *S. aureus* and *B. subtilis* are protected by their thick peptidoglycan walls. Interestingly, several studies also suggest synergistic effects between grape phenolics and antibiotics, where polyphenols enhance antibiotic efficacy. The weak antifungal activity observed against *Candida* is likewise supported by literature, which indicates that *V. vinifera* polyphenols generally exert only partial inhibition at high concentrations. Thus, while these extracts may have limited standalone antifungal potential, they could be useful in combination therapies with conventional antifungals.

Previous studies have reported that *V. vinifera* methanolic extracts obtained from seeds, leaves, and fruits exhibit broad-spectrum antimicrobial and antifungal properties. For instance, grape seed methanolic extracts showed MIC values of 128 µg/mL against *Staphylococcus aureus* and 256 µg/mL against *E. faecalis* and *C. albicans*, indicating strong inhibitory effects on both Gram-positive and Gram-negative bacteria as well as fungi. Moreover, methanolic extracts have been found to inhibit biofilm formation by *S. aureus* and *S. haemolyticus*, and leaf or fruit extracts also demonstrated significant inhibition against *E. coli*, *B. subtilis*, *K. pneumoniae*, *P. aeruginosa*, *A. niger*, and *C. albicans*.[33]

In comparison, the *V. vinifera* (Antep karası) seed methanolic extract evaluated in this study exhibited MIC values of 468.75-234.37 µg/mL for bacterial strains and 7,500 µg/mL for *Candida* species, along with a notable antibiofilm effect. Although the antifungal effect was relatively weaker, the antibacterial activities were comparable to or slightly stronger than those previously reported for other *V. vinifera* varieties. This higher antibacterial performance may be associated with the distinctive phenolic and flavonoid composition of the Antep karası cultivar. Therefore, this study provides the first comprehensive evaluation of the antibacterial potential of Antep karası seed extract in the literature.

Determination of Antibiofilm Activity

The literature indicates that polyphenolic compounds from grape skins and seeds proanthocyanidins and resveratrol can inhibit biofilm development by interfering with bacterial adhesion, quorum sensing, and extracellular polymeric matrix production. Grape seed extracts have been shown to disrupt quorum sensing pathways in *S. aureus* biofilms, while in *P. aeruginosa*, phenolic compounds reduce biofilm density by limiting extracellular polysaccharide synthesis.[34]

Previous studies have shown that grape extracts are effective not only against planktonic bacteria but also in preventing biofilm formation, including antibiotic-resistant S*taphylococcus* strains. In particular, methanolic extracts of grape seeds have been reported to significantly inhibit biofilm formation by *S. aureus* and *S. haemolyticu*s, while leaf and fruit extracts demonstrated inhibitory effects against *E. coli*, *B. subtilis*, *K. pneumoniae*, *P. aeruginosa*, *A. niger*, and *C. albicans*. These inhibitory effects are mainly attributed to polyphenolic constituents such as proanthocyanidins and resveratrol, which disrupt bacterial adhesion and quorum sensing processes.[35]

In the present study, the *V. vinifera* (Antep karası) seed methanolic extract exhibited a concentration-dependent suppression of biofilm formation, with almost complete inhibition observed at higher concentrations. This level of inhibition is comparable to or slightly stronger than the 60-80% inhibition previously reported for other *V. vinifera* cultivars. The pronounced antibiofilm effect of the Antep karası extract could be associated with its unique phenolic and sugar-derived silylated components, which may interfere with the formation of extracellular polymeric substances in the biofilm matrix. These findings represent the first comprehensive demonstration of antibiofilm activity in the Antep karası variety, expanding the current understanding of biofilm inhibition mechanisms in grape seed extracts.

CONCLUSION

This study provides the first comprehensive evaluation of the silylated methanolic extract of *V. vinifera* (Antep karası) seeds, combining GC-FID/MS-based phytochemical profiling with *in vitro* antioxidant, antimicrobial, antibiofilm, and *β*-carotene/linoleic acid assays. The extract demonstrated notable radical scavenging and reducing capabilities, strong *β*-carotene bleaching inhibition, and significant antibiofilm activity, while exhibiting moderate antimicrobial efficacy, particularly against Gram-negative bacteria.

These findings highlight the unique phytochemical and biological profile of the Antep karası variety compared to previously studied *V. vinifera* cultivars. The results underscore its potential as a natural source of multifunctional bioactive compounds with promising applications in pharmaceutical, nutraceutical, and food preservation fields. Future research should focus on the isolation and structural elucidation of active constituents, mechanistic exploration of their antiox-

idant and antibiofilm effects, and formulation of synergistic combinations with conventional antimicrobial agents to optimize their practical use.

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Conflict of Interest

No conflicts of interest between the authors and / or family members of the scientific and medical committee members or members of the potential conflicts of interest, counseling, expertise, working conditions, share holding and similar situations in any firm.

Authorship Contributions

Idea/Concept: *Kübra Öğüt, Pervin Soyer, Gülmira Özek, Temel Özek;* ***Design:*** *Kübra Öğüt, Pervin Soyer, Gülmira Özek, Temel Özek;* ***Control/Supervision:*** *Kübra Öğüt, Pervin Soyer, Gülmira Özek, Temel Özek;* ***Data Collection and/or Processing:*** *Kübra Öğüt, Pervin Soyer, Gülmira Özek, Temel Özek;* ***Analysis and/or Interpretation:*** *Kübra Öğüt, Pervin Soyer, Gülmira Özek, Temel Özek;* ***Literature Review:*** *Kübra Öğüt, Pervin Soyer, Gülmira Özek, Temel Özek;* ***Writing the Article:*** *Kübra Öğüt;* ***Critical Review:*** *Kübra Öğüt, Pervin Soyer, Gülmira Özek, Temel Özek;* ***References and Fundings:*** *Kübra Öğüt, Pervin Soyer, Gülmira Özek, Temel Özek;* ***Materials:*** *Kübra Öğüt, Pervin Soyer, Gülmira Özek, Temel Özek.*

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