

Biological activities of *Vitis vinifera* L. leaves

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Abstract: The fractions of different polarity, namely chloroform, ethylacetate, n-butanol, and remaining water fractions, were fractionated from an aqueous extract of *V. vinifera* leaves. The antibacterial, antifungal, antiviral, antioxidant activities, and total phenolic content of these fractions were investigated. The chloroform fraction showed a promising effect against *Herpes simplex* virus type-1 (HSV-1) and *Parainfluenza* viruses (PIV). The results indicated that all the fractions were more pronounced against gram-positive bacteria (*S. aureus* and *E. faecalis*) than against gram-negative bacteria (*E. coli* and *P. aeruginosa*). On the other hand, the fractions showed equal antifungal activity against the genus *Candida* (*C. albicans* and *C. parapsilosis*). Additionally, the antioxidative potential of different fractions of *V. vinifera* was evaluated using various antioxidant measurements, i.e. 2,2-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging, metal chelating, and superoxide radical scavenging effects. The ethylacetate fraction possessed the highest antioxidant activities except in the case of iron chelation. On the other hand, the chloroform fraction showed the best performance in the iron chelation assay. In our study, the analysis of the relationship between the antioxidant activity of the fractions and their phenolic content showed a positive correlation coefficient. However, such a correlation was not found for the antimicrobial and antiviral properties. In conclusion, the data presented in the study demonstrated that CHCl₃ Fr. produced significant activity against PIV and HSV-1. The results of the antiviral and antioxidant activities supported the utilization of *V. vinifera* leaves in folk medicine.

Key words: Antimicrobial, antiviral, DPPH, metal chelating, superoxide anion scavenging, *Vitis vinifera*

Vitis vinifera L. yapraklarının biyolojik aktiviteleri

Özet: Bu çalışmada, *V. vinifera* yapraklarının sulu ekstresinin fraksiyonlanması ile farklı polaritede fraksiyonlar elde edilmiştir (Kloroform, etilasetat, n-butanol ve kalan su fraksiyonları). Bu fraksiyonların antibakteriyel, antifungal, antiviral, antioksidan aktiviteleri ve toplam fenolik içerikleri incelenmiştir. Kloroform fraksiyonu *Herpes simplex* virüs tip-1 (HSV-1) ve *Parainfluenza* virüslerine (PIV) karşı ümit verici bir etki göstermiştir. Elde edilen sonuçlar, tüm fraksiyonların gram-pozitif bakterilere (*S. aureus* ve *E. faecalis*) gram-negatif bakterilerden (*E. coli* ve *P. aeruginosa*) daha belirgin etki gösterdiğini işaret etmektedir. Diğer yandan, fraksiyonlar *Candida* cinsine (*C. albicans* ve *C. parapsilosis*) karşı eşit antifungal aktivite göstermiştir. Ayrıca, *V. vinifera* fraksiyonlarının antioksidan potansiyeli değişik antioksidan ölçüm yöntemleri (2,2-difenil-2-pikrilhidrazil (DPPH) radikal süpürücü, metal bağlayıcı ve süperoksit radikal temizleyici etki) kullanılarak değerlendirilmiştir. Demir bağlama aktivitesi dışında en yüksek antioksidan aktiviteyi etilasetat fraksiyonu göstermiştir. Diğer yandan, kloroform fraksiyonu en güçlü etkiyi demir bağlama üzerinde göstermiştir. Çalışmamızda, fraksiyonlar ve toplam fenolik içerikleri arasında pozitif bir ilişki görülmüştür. Fakat bu ilişki antimikrobiyal ve antiviral aktivite arasında kurulamamıştır. Sonuç olarak, çalışmada sunulan veriler CHCl₃ Fr.'nün, PIV ve HSV-1'e karşı anlamlı bir aktiviteye sahip olduğunu göstermiştir. Antiviral ve antioksidan aktivite sonuçları, *V. vinifera* yapraklarının bazı folklorik kullanımlarını destekleyebilir.

Anahtar sözcükler: Antimikrobiyal, antiviral, DPPH, metal bağlama, süperoksit anyon süpürücü etki, *Vitis vinifera*

Introduction

Vitis vinifera L. (Vitaceae) is a deciduous woody climber with coiled climbing tendrils and large leaves. It has small, pale, green flowers in the summer followed by bunches of berry fruits that range from green to purple-black (1). The grape has been used in folk medicine for its biological activities since ancient times. The leaves of the plant, which have astringent and haemostatic properties, are used in the treatment of diarrhea, hemorrhage, varicose veins, hemorrhoids, inflammatory disorder, pain, hepatitis, and free radical related diseases and externally for centuries in Anatolia to heal wounds and drain furuncles (2-4). The juice of the leaves has been also recommended as an antiseptic for eye wash (4). Some of above cited diseases could be related to viral or bacterial agents. Furthermore, in recent years, the leaves are used in the formulation of dietary antioxidant supplements (5).

A number of in vivo and in vitro studies were conducted on the plant material and have revealed that *V. vinifera* leaves exert various biological activities including hepatoprotective, spasmolytic, hypoglycemic, and vasorelaxant effects (6-10).

Previous chemical investigations have shown the presence of organic acids (malic, oxalic, fumaric, ascorbic, citric, and tartaric acid), phenolic acids, tannins, anthocyanins, lipid, enzymes, carotenoids, terpenes, and reducing or non-reducing sugars in the leaves of *V. vinifera* (3,11). Most of the therapeutic properties of the plant may be attributed to phenolic compounds. Phenolic compounds have received considerable attention due to their pharmacological effects including antimicrobial and antioxidant activities.

In the present study, we evaluated the phenolic content, antibacterial, antifungal, antiviral activities, and antioxidant abilities of various fractions from *V. vinifera* leaves of Turkish origin. The aim of this study was: i) to investigate the total phenol contents, and antimicrobial, antiviral, and antioxidant activities of the different fractions of *V. vinifera* aqueous leaf extract; ii) to determine the relationship between the content of phenolic compounds and antioxidant activities; iii) to assess the possible antioxidative potency of *V. vinifera* leaves used in the traditional

treatment of numerous diseases. The antimicrobial and antiviral activities were evaluated against different microorganisms, namely gram-positive (*Staphylococcus aureus*, *Enterococcus faecalis*) and gram-negative (*Escherichia coli*, *Pseudomonas aeruginosa*) bacteria, fungi (*Candida albicans* and *Candida parapsilosis*), and DNA virus *Herpes simplex-1* (HSV-1) as well as RNA virus *Parainfluenza* (PI). The antioxidant potential was tested in 3 distinct assays: iron chelation, scavenging of DPPH, and superoxide anion radicals.

Materials and methods

Plant material

The leaves of healthy *Vitis vinifera* were collected before blooming in June 2003 from the Viticultural Research Institute, Faculty of Agriculture, Ankara University (Kalecik). The plant was identified and a voucher specimen (GÜEF 2320) was stored in the Herbarium of Gazi University, Faculty of Pharmacy.

Preparation of extract and fractions

Powdered plant material (500 g) was extracted with distilled water (10 L) by stirring in a 45 °C water bath for 8 h. The extract was filtrated and the filtrates were pooled and then evaporated to dryness under reduced pressure (aqueous extract, yield 19.06%).

The condensed aqueous extract (95.3 g) was dissolved in distilled water (500 mL) and fractionated through successive solvent extractions with chloroform (13 × 500 mL), ethylacetate (18 × 500 mL), and n-butanol saturated with water (28 × 500 mL). Fractions and the remaining aqueous phase were concentrated to dryness under reduced pressure. The yields were obtained as follows: [CHCl₃ Fr.] 1.10%, [EtOAc Fr.] 5.70%, [n-BuOH Fr.] 15.60%, [R-H₂O Fr.] 75.30%.

TLC visualization of phenolics and terpenoids

CHCl₃, EtOAc, n-BuOH, and R-H₂O fractions were submitted to thin layer chromatography (TLC) using ethylacetate:formic acid:water (90:5:5) as the mobile phase (Kieselgel 60F₂₅₄, 0.2 mm, Merck Art. 5554). After spraying the TLC plate with FeCl₃ reagent (3%), the appearance of a green color revealed the presence of the phenolic compounds.

The terpenoids were detected by spraying with an anisaldehyde-sulfuric acid solution (Dichloromethane: ethylacetate (9:1) as the mobile phase) with heat at 105 °C for 5 min. The terpenoids turn pink-purple (12). On the other hand, spots of flavonoids in TLC were developed using ammonia vapor (chloroform:methanol:water (61:32:7) as the mobile phase).

Microbiological analysis

Stock solution preparation

The fractions from *V. vinifera* leaves were prepared with dimethylsulphoxide at a final concentration of 1024 and 256 µg mL⁻¹ and sterilized through filtration using 0.22 mm Millipore (MA 01730, USA) and used as the stock solution.

Microorganisms

Standard and isolated strains of bacteria, namely *Escherichia coli* ATCC 35218, *Pseudomonas aeruginosa* ATCC 10145, *Enterococcus faecalis* ATCC 29212, and *Staphylococcus aureus* ATCC 25923 were used in the determination of antibacterial activity, and standard strains of *Candida albicans* ATCC 10231 and *Candida parapsilosis* ATCC 22019 were used in the determination of antifungal activity.

Determination of antibacterial and antifungal activities

Mueller-Hinton Broth and Mueller-Hinton Agar were applied for growing and diluting the microorganism suspensions. The synthetic medium RPMI-1640 with L-glutamine was buffered at pH 7 with 3-[N-morpholino]-propanesulfonic acid (MOPS) and culture suspensions were prepared. Standard and isolated strains of bacteria were grown to exponential phase in the medium (MHB) at 37 °C overnight with aeration. The bacterial suspensions used for inoculation were prepared at 10⁵ cfu mL⁻¹ by diluting fresh cultures at the McFarland 0.5 density (10⁸ cfu mL⁻¹). The fungi suspension was prepared by the spectrophotometric method of inoculum preparation at a final culture suspension of 2.5 × 10³ cfu mL⁻¹ as described previously by Özçelik et al. (13).

The microdilution method was employed for the antibacterial and antifungal activity tests as described previously by Özçelik et al. (13). The lowest

concentration of the fractions that completely inhibit macroscopic growth was determined and minimum inhibitory concentrations (MICs) were reported. Ampicillin, ofloxacin, ketoconazole, and fluconazole were used as the standards.

Cytotoxicity and antiviral tests

Cell line and growth condition

The vero cell line (African green monkey kidney) used was obtained from the Department of Virology, Faculty of Veterinary Science, Ankara University (Ankara, Turkey). The culture of the cells was grown in Eagle's Minimal Essential Medium (EMEM) enriched with 10% fetal calf serum, 100 mg mL⁻¹ of streptomycin, and 100 IU mL⁻¹ of penicillin in a humidified atmosphere with 5% CO₂ at 37 °C. The cells were harvested using trypsin solution.

Test viruses

The *Herpes simplex* virus type 1 (HSV-1) and *Parainfluenza* virus (PIV) were obtained from the Department of Virology, Faculty of Veterinary Science, Ankara University.

Determination of antiviral activity

The antiviral activities of the fractions were determined as previously described by Özçelik et al. (14). Strains of HSV-1 and PIV titers were calculated by the Frey and Liess method as 50% Tissue Culture Infective Dose (TCID₅₀). The cells were evaluated using a cell culture microscope (×400), using treated-untreated control cultures and acyclovir and oseltamivir as the control agent comparisons. Consequently, the maximum cytopathogen effect (CPE) concentrations were determined as the indicator of antiviral activities of the fractions.

Cytotoxicity test

The maximum non-toxic concentration (MNTC) of each sample was determined using the previously described method by Özçelik et al. (13) based on cellular morphologic alteration. Several concentrations of each sample were placed in contact with confluent cell monolayers and incubated in 5% CO₂ at 37 °C for 48 h. The MNTCs were determined by comparing treated and by controlling untreated cultures.

DPPH radical scavenging assay

Radical scavenging activity of the fractions against stable DPPH[•] (2,2-diphenyl-2-picrylhydrazyl hydrate) was determined spectrophotometrically. Radical scavenging activity of fractions was measured by the methods of Brand-Williams et al. and Milliauskas et al. (14,15). The fractions were prepared by dissolving 0.025 g of dry fractions in 10 mL of methanol. R-H₂O Fr. was not fully soluble in methanol (even after treating solutions for 10 min in an ultrasonic bath); therefore, they were filtered and only the soluble part was further analyzed. The DPPH in methanol (6×10^{-5} M) solution was prepared daily, before the UV measurements. Three milliliters of the solution was mixed with 77 μ L of extract solution. The samples were kept in the dark for 15 min at room temperature and then the decrease in absorption was measured at 515 nm on a UV/visible light spectrophotometer (Beckman DU 650 Spectrometer). BHA ($50 \mu\text{g mL}^{-1}$) was used as a reference compound. The experiment was carried out in triplicate. Radical scavenging activity was calculated using the following formula:

$$\% \text{ inhibition} = [(A_C - A_S)/A_C] \times 100,$$

where A_C is absorption of blank sample ($t = 0$ min) and A_S is absorption of tested fraction solution ($t = 15$ min).

Measurement of chelating activity on metal ions

The chelating activity of samples on Fe^{+2} was determined by the method from Dinish et al. (16). The fractions were incubated with 0.05 mL of FeCl_2 (2 mM). The reaction was initiated by the addition of 0.2 mL of ferrozine (5 mM) and the total volume was adjusted to 4 mL with ethanol. After reaching equilibrium (10 min), the absorbance was read at 562 nm. BHA ($50 \mu\text{g mL}^{-1}$) and EDTA (2, 5, and 5 mg mL^{-1}) were used as reference compounds. The percentage of inhibition of the ferrozine- Fe^{+2} complex formation was calculated using the formula given below:

$$\text{Metal chelating activity (\%)} = [(A_C - A_S)/A_C] \times 100,$$

where A_C is absorption of control and A_S is absorption of tested fractions. The control contained FeCl_2 and ferrozine. Analyses were carried out in triplicate and the results were averaged.

Superoxide anion radical scavenging activity

The influence of the fractions on the generation of superoxide radical was achieved through spectrophotometric measurement of the product formed on reduction of nitroblue tetrazolium (NBT) (17). Briefly, the superoxide radical was generated in a non-enzymatic system and the reaction mixture, containing 3.2, 6.4, and 12.8 mg mL^{-1} of the samples in methanol, 1 mL of 60 mM PMS (phenazine methosulfate), 1 mL of 468 mM NADH (b-dihydronicotinamide adenine nucleotide), and 1 mL of 150 mM NBT in phosphate buffer (0.1 M, pH 7.4), was incubated at ambient temperature for 5 min. The absorbance was measured at 560 nm against a blank sample that contained no PMS (18). BHA ($50 \mu\text{g mL}^{-1}$) was used as a reference compound. The scavenging activity on superoxide anion radicals was expressed as:

$$\text{Scavenging activity (\%)} = [(A_C - A_S)/A_C] \times 100,$$

where A_C is absorption of control and A_S is absorption of tested fractions. Analyses were carried out in triplicate and the results were averaged.

Determination of total phenols

The fractions (100 mL) were mixed with 0.2 mL of Folin-Ciocalteu reagent, 2 mL of H_2O , and 1 mL of 15% Na_2CO_3 consecutively. The mixture was measured at 765 nm after 2 h at room temperature. The mean of 3 readings was used and the total phenolic content was expressed in mg of gallic acid equivalents g^{-1} (19). The coefficient of determination was $r^2 = 0.9958$.

Statistical analysis

All values are expressed as the mean $\pm$ the standard error of the mean (SEM); linear regression analyses and correlation coefficients to determine the relationship between 2 variables were calculated using MS-DOS software (GraphPad InStat statistical program).

Results and discussion

V. vinifera leaf fractions were screened for their antibacterial activities against both standard and isolated strains of *E. coli*, *P. aeruginosa*, *E. faecalis*, and *S. aureus*. The MICs of the fractions are shown in Table 1. The effects of the fractions were compared to those of

Table 1. Antimicrobial activities of the fractions of *V. vinifera* aqueous leaf extract expressed as minimum inhibitory concentrations ($\mu\text{g mL}^{-1}$).

Samples	A	A1	B	B1	C	C1	D	D1	E	F
CHCl_3 Fr.	128	256	128	64	64	128	32	64	64	64
EtOAc Fr.	128	256	128	64	64	128	32	32	64	64
n-BuOH Fr.	128	256	128	64	64	128	32	64	64	64
R- H_2O Fr.	128	256	128	64	64	128	32	64	64	64
AMP	2	32	nd	nd	0.25	1	1	16	nd	nd
OFX	0.12	8	1	8	1	8	0.5	nd	nd	nd
KET	nd	nd	nd	nd	nd	nd	nd	nd	2	2
FLU	nd	nd	nd	nd	nd	nd	nd	nd	4	4

A: *E. coli*, A₁: isolated strains, B: *P. aeruginosa* B₁: isolated strains, C: *E. faecalis*, C₁: isolated strains, D: *S. aureus*, D₁: isolated strains, E: *C. albicans*, F: *C. parapsilosis*, OFX: Ofloxacin, AMP: Ampicillin, KET: Ketoconazole, FLU: Fluconazole, nd: Not done

the control agents. According to the results obtained, no significant inhibition was observed with any of the fractions compared to the control agents. However, in this assay, all of the fractions displayed a little more antibacterial activity against gram-positive bacteria than gram-negative bacteria. As also shown in Table 1, none of the fractions showed any significant activity against fungi (*C. albicans* and *C. parapsilosis*).

The data obtained from the antiviral activity screening showed that CHCl_3 Fr. produced significant antiviral activity towards the DNA virus HSV-1 with an MNTC of $16 \mu\text{g/mL}$, similar to that of acyclovir (16 to $<0.0078 \mu\text{g/mL}$) (Table 2). As for PIV, CHCl_3 Fr.

exhibited good inhibition with an MNTC of $16 \mu\text{g/mL}$. The rest of the fractions did not produce any antiviral activity against both DNA and RNA viruses. According to Table 2, the maximum non-toxic concentration value of acyclovir is similar to those of the CHCl_3 , EtOAc, and R- H_2O fractions.

The antioxidant activities of all fractions of the *V. vinifera* leaves were evaluated using a set of in vitro antioxidant assays including the DPPH radical, superoxide anion radical scavenging, and metal chelating activities.

In the DPPH test, among the fractions, EtOAc Fr. showed the most effective DPPH radical scavenger

Table 2. Cytotoxicity and antiviral activity results of the fractions of *V. vinifera* aqueous leaf extract.

Vero cells ($\mu\text{g mL}^{-1}$) Samples	MNTC ($\mu\text{g mL}^{-1}$)	CPE Inhibitory Concentration			
		HSV-1		PIV	
		Max.	Min.	Max.	Min.
CHCl_3 Fr.	16	16	0.0625	16	8
EtOAc Fr.	16	-	-	-	-
n-BuOH Fr.	32	32	-	-	-
R- H_2O Fr.	16	-	-	-	-
Acyclovir	16	16	<0.0078	-	-
Oseltamivir	32	-	-	32	8

MNTC: Maximum non-toxic concentration, CPE: Cytopathogenic effect, - : No activity observed

activity in this assay, with an inhibition of 92.8%. The DPPH radical scavenging activity of the CHCl_3 (41.4%), n-BuOH (22.9%), and R- H_2O (6.3%) fractions was less than that of the EtOAc Fr. (Table 3).

In the PMS/NADH-NBT system, the superoxide anion radical generated by PMS/NADH coupling reaction reduces NBT. The decrease of absorbance at 560 nm with antioxidants thus indicates the consumption of the superoxide anion radical in a reaction mixture (20). Except for the CHCl_3 and R- H_2O fractions, all samples were able to scavenge O_2^- in a concentration-dependent way (Table 3), in which EtOAc Fr. showed the strongest scavenging activity (68.6%-96.0%). The superoxide radical scavenging activity of the samples followed this order: EtOAc Fr. > n-BuOH Fr. > CHCl_3 Fr. > R- H_2O Fr.

In cells, free Fe^{+2} is toxic because it is able to catalyze the decomposition of H_2O_2 into the extremely reactive hydroxyl (OH) radical. This is known as the Fenton reaction. The resulting Fe^{+3} can be reduced back to Fe^{+2} by the superoxide radical (O_2^-), regenerating Fe^{+2} and allowing it to continue. In other words, in the presence of trace amounts of iron ion, superoxide and hydrogen peroxide will create the destructive hydroxyl radical, and initiate the oxidation of organic substrates (21, 22). It is therefore important to determine the iron binding activity of the natural antioxidants. The percentage of the metal chelating capacity of the 12.8 mg mL^{-1} concentrations of CHCl_3 , EtOAc, n-BuOH, and R- H_2O fractions were found to be 75.9, 61.3, 39.2 and 22.5%, respectively. CHCl_3 Fr. showed the strongest chelating activity on Fe^{+2} ions.

Table 3. Total phenolic content, DPPH absorption inhibition, metal chelating and superoxide anion scavenging activities of the fractions of *V. vinifera* aqueous leaf extract.

Samples	Total phenolic content (mg GAE g ⁻¹ Fr. $\pm$ SEM)	DPPH inhibition (%)	Samples	Metal chelating activity (%)	Samples	Superoxide anion scavenging activity (%)
CHCl_3 Fr.	126.2 $\pm$ 2.4	41.4 $\pm$ 2.4	CHCl_3 Fr. 12.8 mg mL^{-1} 6.4 mg mL^{-1} 3.2 mg mL^{-1}	75.9 $\pm$ 0.9 54.5 $\pm$ 1.4 21.3 $\pm$ 1.3	CHCl_3 Fr. 3.2 mg mL^{-1} 2.24 mg mL^{-1} 0.96 mg mL^{-1}	59.4 $\pm$ 1.9 67.5 $\pm$ 2.1 56.9 $\pm$ 1.1
EtOAc Fr.	205.8 $\pm$ 8.9	92.8 $\pm$ 0.9	EtOAc Fr. 12.8 mg mL^{-1} 6.4 mg mL^{-1} 3.2 mg mL^{-1}	61.3 $\pm$ 1.2 62.1 $\pm$ 2.6 59.3 $\pm$ 0.8	EtOAc Fr. 3.2 mg mL^{-1} 2.24 mg mL^{-1} 0.96 mg mL^{-1}	96.0 $\pm$ 0.7 89.6 $\pm$ 1.7 68.6 $\pm$ 0.9
n-BuOH Fr.	57.2 $\pm$ 6.5	22.9 $\pm$ 1.5	n-BuOH Fr. 12.8 mg mL^{-1} 6.4 mg mL^{-1} 3.2 mg mL^{-1}	39.2 $\pm$ 7.7 24.8 $\pm$ 1.3 12.4 $\pm$ 0.2	n-BuOH Fr. 3.2 mg mL^{-1} 2.24 mg mL^{-1} 0.96 mg mL^{-1}	81.8 $\pm$ 1.2 70.1 $\pm$ 2.2 56.9 $\pm$ 0.9
R- H_2O Fr.	nd	6.3 $\pm$ 1.1	R- H_2O Fr. 12.8 mg mL^{-1} 6.4 mg mL^{-1} 3.2 mg mL^{-1}	22.5 $\pm$ 0.2 5.5 $\pm$ 3.3 nd	R- H_2O Fr. 3.2 mg mL^{-1} 2.24 mg mL^{-1} 0.96 mg mL^{-1}	35.0 $\pm$ 0.7 51.3 $\pm$ 4.2 31.1 $\pm$ 0.2
BHA 50 $\mu\text{g mL}^{-1}$		67.6 $\pm$ 0.9	BHA 50 $\mu\text{g mL}^{-1}$ EDTA 2.5 mg mL^{-1} EDTA 5 mg mL^{-1}	9.9 $\pm$ 0.7 76.7 $\pm$ 0.4 94.8 $\pm$ 0.9	BHA 50 $\mu\text{g mL}^{-1}$	17.6 $\pm$ 0.6

GAE: Gallic acid equivalent, values are mean $\pm$ SEM of 3 replications. nd: not determined

According to the total phenol contents of the test samples, EtOAc Fr. possessed a $205.8 \pm 8.9 \text{ mg g}^{-1}$ fraction of total phenol amount as a gallic acid equivalent, followed by the CHCl_3 and n-BuOH fractions (Table 3). The total phenolic contents of the fractions were highly correlated with the DPPH activities ($r = 0.974$), superoxide anion scavenging ($r = 0.766\text{-}0.925$) and metal chelating activities ($r = 0.808\text{-}0.961$). However, no correlation was observed between the total phenol content and antiviral activity of the fractions. On the other hand, the TLC analysis of the fractions indicated that CHCl_3 Fr. is rich in flavonoids and terpenoids, while EtOAc Fr. consists of mainly phenolic compounds.

In this study, we tested the fractions against standard and isolated strains of *E. coli*, *P. aeruginosa*, *E. faecalis*, and *S. aureus* bacteria, as well as *C. albicans* and *C. parapsilosis* fungi by using the microdilution method. All of the fractions displayed a little more antibacterial activity against gram-positive bacteria than gram-negative bacteria. Oskay and Sarı (23) reported that the ethanol extract of *V. vinifera* leaves showed broad-spectrum antimicrobial activity against gram-positive and gram-negative bacteria, using the agar-well diffusion method. Conflicting results may be due to variations in experimental conditions.

To our knowledge, the antiviral activity of *V. vinifera* leaves has not yet been reported. This study is the first report about the antiviral activity of the different fractions of the plant against PIV and HSV-1. CHCl_3 Fr. displayed much higher antiviral activity towards PIV than oseltamivir. However, the results regarding the antioxidant activity demonstrated that CHCl_3 Fr. possessed strong metal chelating activity whereas EtOAc Fr. had the highest DPPH and superoxide anion scavenging activities. Metal chelating compounds (flavonoids, β -diketones, and polyethers) can inhibit virus-induced enzymes in infected cells by coordinating with metal ions at their active sites (24). Consideration of the coordinating properties of ligands can explain the antiviral activity of these compounds. In light of our findings, the metal chelating activity of CHCl_3 Fr. may explain the observed antiviral activity.

In contrast, many authors described that some terpenoids (moronic, maslinic, ursolic, oleanolic, pomolic, and 1- β -hydroxy-2-oxopomolic acids) have produced significant activity against RNA and DNA

viruses (25-27). It is known that *V. vinifera* also contains various terpenoids such as 16- β -hydroxyoleanolic acid, α and β amylene, ursolic acid, oleanolic acid, cycloartenol, and taraxasterol (3). In this study, CHCl_3 Fr. exhibited strong antiviral activity against PIV. Although the highest amounts of total phenolics were found in EtOAc Fr., this fraction did not exhibit any antiviral activity against PIV (RNA virus) and HSV-1 (DNA virus). Therefore, the terpenoids in CHCl_3 Fr. may be responsible for the antiviral activity, along with phenolic compounds.

Doshi et al. (28) studied the phenolic composition and antioxidant potential of different parts of the commercially popular variety Kishmish Chornyi (Sharad Seedless). As a result, a correlation was found between the phenolic composition and antioxidant potential of different grapevine parts. Similarly, in our study, the analysis of the relationship between the antioxidant activity of the fractions and their phenolic content showed a positive correlation coefficient. Therefore, total phenolic components can be used as an indicator in assessing the antioxidant activity of *V. vinifera* leaves.

In conclusion, the fractionating solvent significantly affected the total phenolic content, antiviral and antioxidant activities of *V. vinifera* leaves. EtOAc Fr. had the highest total phenolic content as well as the highest antioxidant activity when determined by the DPPH and superoxide anion scavenging assays, while CHCl_3 Fr. showed the highest antioxidant activity when evaluated by the metal chelating assay. Additionally, the data presented in the study demonstrated that CHCl_3 Fr. produced significant activity against PIV and HSV-1. The antiviral and antioxidant activity results supported the utilization of *V. vinifera* leaves in folk medicine. Therefore, further research should be performed on the active principle(s) of CHCl_3 Fr. (terpenoids, flavonoids and other phenolic compounds) with each in isolation.

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