Available online: December 10, 2019

Commun.Fac.Sci.Univ.Ank.Series C
Volume 28, Number 2, Pages 211-224 (2019)
ISSN 1303-6025 E-ISSN 2651-3749
https://dergipark.org.tr/en/pub/communc/issue/49312/632636

INVESTIGATION OF THE ANTIMICROBIAL ACTIVITY OF *Vitis vinifera* L. BOĞAZKERE

SEDA ÇİÇEK, TALİP ÇETER

ABSTRACT. In this study, extracts from Boğazkere cultivar of *Vitis vinifera* L. at 10 µL, 50 µL and 100 µL concentrations were tested against 18 different bacterial and fungi strains using disc diffusion (DD) method along with minimum inhibitory concentration (MIC) and minimum bactericidal/fungisidal concentration (MBC/MFC) tests to reveal possible antimicrobial properties. Then, the obtained results were compared with 18 known antibiotics. The results revealed that 7.33-19.66 mm inhibition zones were obtained for 15-different microorganisms at 100 µL concentrations while those obtained 7.33-12.33 mm inhibition zones for 12 microorganims at 50 µL volume, where no inhibition zone was observed at 10 µL volume addition. The extracts of Boğazkere for the tested concentrations showed no antimicrobial capability against *Salmonella kentucky, Enterococcus durans, Salmonella typhimurium* and *Candida ablicans*. MIC tests showed that the extract at 0.039-20 mg/100 mL concentration range was bacteriostatic for the entire tested microorganism. Bactericidal effects of the extract were obtained for *Listeria innocua* at 10 mg /100 mL while that was 20 mg /100 mL for *Pseudomonas fluorescens, Pseudomonas aeruginosa, Enterococcus faecium* and *Staphylococcus aureus*. The findings show that Boğazkere grape species has antibiotic character, what makes them possible preservatives for food products.

1. INTRODUCTION

Humankind has accumulated the knowledge of healing potentials of the plants from the early times of humanity, and has revealed it to the next generations. Phenolic compounds are the basis of curing potentials of the plants. Plants inherently use the phenolic compounds for defense, and those give smell, flavor and color. Antimicrobial properties of the plants derived from the chemicals that have been in use of curing microorganisms-mediated diseases for ages [1].

Received by the editors: October 13, 2019; Accepted: December 09, 2019.
Key word and phrases: Antimicrobial activity, Disc diffusion method, Vitaceae, *Vitis vinifera*, Boğazkere.

There are two characteristic mechanisms that microorganism use to develop resistance for antibiotics, which are natural and acquired resistance. In natural resistance, the microorganisms do not possess compound or system targeted by the antibiotics while the acquired resistance refers to that the microorganism develop a mechanism (e.g. discarding the antibiotic via newly developed proteins) to eliminate antimicrobial agent's effect that inherently kills the microorganism [1, 2].

Antimicrobial agents can trigger a variety of reactions in living organisms. They can either act on cellular membrane or target elements within the cytosol; for example inhibition of nucleic acid synthesis, and cause organelles malfunction and cell wall metabolism problems. Besides, they can cause troubles at organ levels including cardiovascular and urinary system defects [3].

Plants within Vitaceae family are among the oldest plants cultivated worldwide owing to their strong adaptation for different climate and soil types. There are about 1200 cultivated grape types belong to *Vitis vinifera* species. They are used to produce wine, juice, molasses, dried fruit roll-up and as ingredient of cosmetic stuffs, in addition to that they are consumed as dried and fresh fruits. Besides high sugar and vitamin contents, grape carries high amount of antioxidants. Boğazkere grape is one of the most valuable grapes of Anatolia, which is mostly cultivated in Diyarbakır. They are mostly produced in red soils possess gravel, clay and calcareous character. Beads of Boğazkere are dark red colored and mid-sized [4-8].

Baydar et al. (2006) tested the extracts of seeds from Hasandede, Emir and Kalecik Karası grapes (extraction was performed in water/acetone/acetic acid solvent system) on 15 different microorganisms using agar-disc diffusion methods for the concentrations of 1%, 2.5%, 5% and 10%. Among the grape types, Hasandede revealed suppression capacity for all the bacteria at the tested concentrations [4].

Baydar et al. (2004) performed ethyl acetate/methanol/water mediated extraction of grape seeds, whose lipid content was removed beforehand. The extracts were tested for 15 microorganisms using disc diffusion method, where the highest antibacterial activity was observed for *Listeria monocytogenes* while no activity was obtained for *Enterobacter aerogenes* [9].

Anastasiadi et al. (2009) analysed chemical contents belong to pomace of four *Vitis vinifera* types and tested anti-*L. monocytogenes* capability using MIC test approach. The findings revealed that the extracts from plant stem and seeds could be introduced to food samples as preservatives [10].

Ege (2015) carried out acetone/water/acetic acid/methanol mediated extraction from seeds of Müşküle (white), Kara dimrit (blue-black) and Öküzgözü grape types, whose lipid content was removed beforehand. Antimicrobial activity of the extracts was tested for 4 fungi and 6

bacteria strains using MIC test. The findings revealed that all the extracts did not show antimicrobial capability against 4 fungi and 4 bacteria strains [11].

Abtahi et al. (2011) extracted dried white, red and black grape samples in 70% alcohol, then which were tested on *Escherichia coli* PTCC1330*, Staphylococcus aureus* PTCC 1431*, Salmonella typhimurium* PTCC1639 and *Pseudomonas aeruginosa* PTCC1310 strains using MIC tests. The findings showed that the extracts gave positive results for all the tested bacteria with showing its highest activity for *S. aureus* strain [12].

Waqar et al. (2014) tested the antimicrobial activity of the extracts from leaves of *V. vinifera* on *E. coli*, *P. aeruginosa, S. aureus* and *Enterococcus faecalis* strains using disc diffusion method. The inhibition zones belong to *S. aureus* as 30 mm, *E. faecalis* as 28.9 mm, *E. coli* as 28 mm and *P. aeruginosa* as 23.7 mm were obtained [13].

Yadav et al. (2015) tested the antibacterial capability of water-, ethanol-, acetone-, and methanol- mediated extracts of grape-peel at three different concentrations for the antibiotic resistant *S. aureus, E. faecalis, Enterobacter aerogenes, Salmonella typhimurium* and *E. coli* using disc diffusion method. The findings revealed the highest antibacterial capacity for methanol mediated extracts. *S. typhimurium* and *E. coli* showed resistance for the tested concentrations. The extract gave the inhibition zones for *S. aureus* as 22 mm, *E. faecalis* 18 mm and *E. aerogenes* as 21 mm [14].

In the present work, antimicrobial activity of Boğazkere belong to *Vitis vinifera* species performed along with evaluating its possible preservative role for food samples.

2. MATERIAL AND METHODS

2.1 Plant Samples and Extraction

Samples of Boğazkere grapes, collected in Kırşehir Toklumen vineyards of Kavaklıdere Company in September of 2017. The collected samples, protected under proper conditions until they reached the laboratory. The samples rinsed thoroughly, followed by the beads were detached from the stems using clean blade. The beads were then grinded in mortar, followed by liquid part, removed with clean cheesecloth. The obtained pomace was mixed with liquid nitrogen, and then grinded into fine particles in mortar. The particles were added to the liquid part. The mixture, was mixed with 96% ethanol solution at 1:1 ratio, which then underwent mixing on orbital shaker at 100 rpm for three days. Followed by the extraction, filtration was performed using whatman paper, where alcohol and water content was eliminated using Rotary evaporator (run in water bath and under vacuum). Samples used for disc diffusion

method was prepared by dissolving 2 gr of dried sample in 8 mL etanol: 2 mL pure water solvent system. In MIC test, 2 gr of dried sample was dissolved in 10 mLof water, followed by filtration through 0.2 µm sterile filter. The prepared stocks were kept under proper conditions until further usage.

2.2 Test Microorganisms

The prepared extracts of Boğazkere were tested for the following standart strains or isolated microorganisms: *Enterobacter aerogenes* (ATCC 13048), *Salmonella infantis, Listeria monocytogenes, Klebsiella pneumoniae*, *Pseudomonas aeruginosa* (DSMZ 50071), *Pseudomonas fluorescens, Salmonella kentucky*, *Enterococcus faecalis* (ATCC 29212), *Listeria innocua, Salmonella enteritidis* (ATCC 13075), *Enterococcus durans, Salmonella typhimurium, Candida ablicans* (DSMZ 1386), *Enterococcus faecium*, *Staphylococcus aureus* (ATCC 25923), *Staphylococcus epidermidis* (DSMZ20044)*, Bacillus subtilis* (DSMZ 1971), and *Escherichia coli* (ATCC 25922), *Saratia marrescens* (ATCC 13048).

2.3. Preparation of Inoculation

All the tested microorganisms were grown in Nutrient agar, where the identical colonies were collected using a sterile disposable swabing tool and placed into 10 mL of 0.9 % steril NaCl solution. Based on 0.5 McFarland turbiditiy standard, bacterial colonies were prepared at 10^8 cfu.mL^{-1} while that was 10^7 cfu.mL^{-1} for *C. albicans* [15-17].

2.4. Loading Extract to Empty Disks

In disc diffusion method, sterile empty antibiogram discs were used to evaluate antimicrobial activity of the extracts.

The stock Boğazkere extracts at 10 µL, 50 µL and 100 µL volumes, placed on the empty discs, followed by incubated at 30 ℃for overnight under sterile condition. The dried samples, kept at +4 ℃until further usage.

2.5 Disc Diffusion Method (DD)

0.1 mL of inoculum was evenly spread over the Mueller Hinton Agar (MHA) using swab stick. Antimicrobial vulnerability test was performed in accordance with Bauer-Kirby method [18].

For each MHA, empty, 10 µL, 50 µL and 100 µL extract impregnated discs were added onto the surface of MHA. Bacterial samples and fungus sample were incubated at 37 °C and 27 °C for 24 h. Right after 24 h incubation period, the inhibition zones were measured in millimeter (mm). The tests were performed in three parallel.

2.6. Determination of minimum inhibitory concentration (MIC)

All the microorganisms showed vulnerability for the Boğazkere extracts from the disc diffusion tests were included into MIC test. MIC test was performed in sterile 96-well plate. MIC value was accepted as the concentration at which bacterial growth was not visually observed [19-20].

100 µL of sterile Muellller Hinton Broth was added to each well of 12-well microplate. 100 µL from the stock Boğazkere extract was added to the first well of the 12-well microplate (Number 1 well). 2-times serial dilution was then applied to dilute the extract from number well 1 to well 10 (Number 10 well). 100 µL solution was then discarded from number 10 well. 10 µL from each of the selected microorganisms were added from Number 1-Number 11 wells. Wells from 1-10 were used to evaluate MIC of Boğazkere while Number 11 well was used for microbial positive control and Number 12 well was used as system control. Bacterial samples and fungi sample were incubated at 37°C and 27°C for 24 h to explore MIC values for each microbial strain. The tests were performed in three parallel.

2.7. Determination of Minimum Bactericidal/Fungicidal Concentration (MBC/MFC)

Minimum bactericidal/bacteriostatic concentration (MBC) test is to find out whether any bacterial development takes place in the wells where no bacterial growth was observed in MIC test. The findings of this test clarifies whether the MIC concentrations are bactericidal or bacteriostatic.

Followed by the MIC test, samples from the wells (where no growth was visually observed) were transferred onto a fresh Nutrient Agar, followed by incubated at 37°C for overnight. In

the case of no growth on the fresh agar, the MIC concentration was accepted as *minimum bactericidal concentration* while that was accepted, as *minimum bacteriostatic concentration* in the case growth was clear. The tests were performed in three parallel.

2.8. Controls

In disc diffusion method, sterile empty discs were used as negative control while 18-standard antibiotic discs were used for 19 microorganisms as positive control.

3. RESULTS AND DISCUSSIONS

Antimicrobial activity of the extract from *Vitis vinifera* cv. Boğazkere was tested for 18 bacterial strains and one fungus species using disc diffusion method. The findings based on triple parallel examination with inhibition zone diameters are given in Table 1. The findings revealed that Boğazkere extract possessed antimicrobial activity for 15 microorganisms by giving inhibition zones between 7.33 and 19.66 mm.

Treatment with 10 µL of the preprared stock extract did not show any antimicrobial activity for the tested microorganisms while at 50 µL treatment antibacterial activity was observed for *S. infantis, L. monocytogenes, K. pneumoniae, P. fluorescens, P. aeruginosa, E. faecalis, L. innocua, E. faecium, S. aeureus, S. epidermidis, B. subtilis* and *E. coli* with causing inhibition zones in the range of 7.33-12.33 mm.

Treatment with 100 µL of the stock antibacterial activity for *E. aerogenes*, *S. infantis*, *L. monocytogenes*, *K. pneumonia*, *P. fluorescens*, *P. aeruginosa*, *E. faecalis, L. innocua, S. enteritidis, E. faecium, S. aeureus, S. epidermidis, B. subtilis, E. coli* and *S. marcescens* was observed with causing inhibition zones between 7.33 and 19.66 mm diameters. However, *S. kentucky, E. durans, S. typhimurium* and *C. ablicans* did not show any vulnerability towards the extract at the tested concentrations.

Followed by the disc diffusion method, MIC tests were performed for all the microbial strains that showed vulnerability for the extract. Microbial resistance gradually increased upon decreases in the applied extract concentration. The extract between 0.039 and 20 mg/100 mL concentrations gave MIC values for the selected bacterial strains, which was given in Table 2. Further studies on MIC tests revealed that most of the MIC values were bacteriostatic while such high concentrations were more of bactericidal doses (Table 2).

TABLE 1. Antimicrobial acitivity of Boğazkere extract based on disc diffusion method (Negative test result equal to empty disk diameter = 6 mm).

Mikroorganisms	Zone Diameter (mm)											
	10 µL				50 µL				100 µL			
	A	B	C	Mean	A	B	C	Mean	A	B	C	Mean
B. subtilis	-	-	-	-	10	9	10	**9.66**	12	12	12	**12**
C. albicans	-	-	-	-	-	-	-	-	-	-	-	-
E. aerogenes	-	-	-	-	-	-	-	-	7	7	8	**7.33**
E. coli	-	-	-	-	9	7	10	**8.66**	10	9	10	**9.66**
E. durans	-	-	-	-	-	-	-	-	-	-	-	-
E. faecalis	-	-	-	-	12	12	14	**12.66**	12	17	17	**15.33**
E. faecium	-	-	-	-	12	12	13	**12.33**	20	19	20	**19.66**
K. pneumoniae	-	-	-	-	7	8	7	**7.33**	10	10	10	**10**
L. innocua	-	-	-	-	12	10	13	**11.66**	16	15	16	**15.66**
L. monocytogenes	-	-	-	-	9	10	11	**10**	12	14	16	**14**
P. aeruginosa	-	-	-	-	7	7	8	**7.33**	9	8	9	**8.66**
P. fluorescens	-	-	-	-	10	14	14	**11.33**	14	15	17	**15.33**
S. aeureus	-	-	-	-	11	7	10	**9.33**	13	10	12	**11.66**
S. enteritidis	-	-	-	-	-	-	-	-	8	8	10	**8.66**
S. epidermidis	-	-	-	-	7	7	8	**7.33**	8	8	7	**7.66**
S. infantis	-	-	-	-	9	7	7	**7.66**	10	12	12	**11.33**
S. kentucky	-	-	-	-	-	-	-	-	-	-	-	-
S. marcescens	-	-	-	-	-	-	-	-	9	9	10	**9.33**
S. typhimurium	-	-	-	-	-	-	-	-	-	-	-	-

TABLE 2. Results for Minimum inhibition concentration (MIC) test and Minimun Bactericidal Concentration (MBC). *(*Bcd: Bactericidal effect, Bst: Bacteriostatic effect*)*. (Initial concertation of the extract was 10 mg/100 µL).

BACTERIA	MIC	MMC (Bcd)	MBC (Bst)
E. aerogenes	1.25	-	1.25
S. infantis	5	-	5
L. monocytogenes	10	-	10
K. pneumoniae	20	-	20
P. fluorescens	10	20	10
P. aeruginosa	0.625	20	0.625
S. kentucky	-	-	-
E. faecalis	20	-	20
L. innocua	0.625	10	0.625
S. enteritidis	0.039	-	0.039
E. durans	-	-	-
S. typhimurium	-	-	-
C. albicans	-	-	-
E. faecium	10	20	10
S. aeureus	0.625	20	0.625
S. epidermidis	2.5	-	2.5
B. subtilis	5	-	5
E. coli	10	-	10
S. marrescens	5	-	5

Antibiogram tests revealed that Lincomycin (L2) posed antibacterial activity only for *P. aeruginosa*, *S. aereus*, *B. subtilis* among the tested 18 different bacterial strains with 9 mm, 25 mm and 16 mm diameter zone inhibitions. Meropenem (MEM 10), Gentamicin (CN10), Neomycin (N30) and Ciprofloxacin (CIP5) showed antibacterial activity nearly all of the tested bacteria. The highest activities were observed for *B. subtilis* (46 mm zone diameter) and *S. marcescens* (43 mm zone diameter) with Ampicillin 10 mcg and Ciprofloxacin 5mcg treatment. Similarly Meropenem at 10 mcg. Caused 40 mm zone diameter formation for *B. subtilis* (Table 3).

Yadav et al. (2015) tested the antibacterial capability of water-, ethanol-, acetone-, and methanol- mediated extracts of grape-peel at three different concentrations (i.e. 260 mg/TAE/ml, 540 mg/TAE/ml and 1080 mg/TAE/ml) for the antibiotic resistant *S. aureus, E. faecalis, E. aerogenes, S. typhimurium* and *E. coli* using disc diffusion method. The findings revealed the highest antibacterial capacity was from the methanol-mediated extracts. *S. typhimurium* and *E. coli* showed resistance for the tested concentrations while the rest gave vulnerability for all the extracts performed in different solvents ($P < 0.05$). The extract gave the inhibition zones for *S. aureus* as 22 mm, *E. faecalis* 18 mm and *E. aerogenes* as 21 mm [15], for which zone inhibitions were obtained as 11.66 mm, 15.33 mm and 7.33 respectively upon treatment with Boğazkere extract in the present work. The difference in the findings might be related to the difference of the plant species and chemistry of the extraction solvent.

TABLE 3. Disc diffusion test results of positive control antibiotics

BACTERIA	L2	OFX5	ME M10	TE30	CZ30	VA 30	AM 10	K 30	CN 10	S 10	S 300	NA 30	SH 100	SXT 25	N30	IP 5	AM C30	C30
E. aerogenes	-	27	28	18	13	-	10	24	25	-	23	24	30	28	20	32	10	30
S. infantis	-	24	37	9	15	-	20	-	20	10	-	-	12	-	10	30	21	30
L. monocytogenes	-	19	25	-	18	-	22	-	20	11	-	-	12	25	10	25	25	27
K .pneumoniae	-	30	30	17	-	-	-	25	24	20	25	24	18	-	20	35	11	30
P. fluorescens	-	23	25	18	-	-	-	-	20	13	16	-	17	-	12	33	-	-
P. aeruginosa	9	19	14	20	-	21	30	12	15	-	-	-	20	25	12	24	30	23
S. kentucky	-	32	33	15	-	-	25	23	14	11	-	23	-	26	21	32	26	30
E. faecalis	-	20	21	10	-	22	30	18	15	-	20	-	19	28	17	23	30	25
L. innocua	-	17	26	22	-	20	28	25	25	26	35	17	21	26	12	22	30	24
S. enteritidis	-	32	32	20	19	-	23	22	21	20	15	25	24	25	18	30	25	28
E. durans	-	17	27	21	12	-	-	25	20	22	22	24	24	26	18	21	10	30
S. typhimurium	-	32	32	15	15	-	25	26	27	-	-	25	33	23	22	35	30	33
E. faecium	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
S. aereus	25	28	35	25	-	21	40	24	25	20	17	-	21	28	22	30	37	25
S. epidermidis	-	30	32	15	10	-	-	25	24	18	24	28	30	30	21	37	10	33
B. subtilis	16	28	40	33	9	24	46	28	30	20	35	23	30	35	34	35	10	33
E. coli	-	-	36	-	-	-	-	20	25	21	23	-	30	16	22	-	18	25
S. marcescens	-	38	38	18	24	-	-	30	27	25	25	39	33	30	22	43	10	32

(-) No effect, Lincomycin: L2, Ofloxacin: OFX 5, Meropenem: MEM 10, Tetracycline: TE 30, Ceftazidime: CAZ 30, Vancomycin: VA 30, Ampıcillin: AM10 Kanomycin: K 30, Gentamicin: CN 10, Streptomycin: S10, Compound Sulphonamides: S 3 300, Nalidixic acid: NA 30, Spectonomycine: SH 100 Sulphamethoxazole trimethaprim: SXT 25, Chloramphenicol: C 30, Neomycin: N 30, Cıprofloxacin: CIP 5, Amoxycillın clavulanic acıd: AMC30

Baydar et al. (2006) tested the extracts of seeds from Hasandede, Emir and Kalecik Karası grapes (extraction was performed in water:acetone:acetic acid solvent system, 90:9.5:0.5) on *Aeromonas hydrophila* ATCC 7965, *Bacillus cereus* FMC 19, *Enterobacter aerogenes* CCM 2531, *Enterococcus faecalis* ATCC 15753, *Escherichia coli* DM, *E. coli* O157:H7 KUEN 1461, *Klebsiella pneumoniae* FMC 5, *Mycobacterium smegmatis* RUT, *Proteus vulgaris*

FMC 1, *Pseudomonas aeruginosa* ATCC 27853, *Pseudomonas fluorescens* EU, *Salmonella enteritidis*, *Salmonella typhimurium*, *Staphylococcus aureus* Cowan 1 and *Yersinia enterocolitica* EU using agar-disc diffusion methods for the concentrations of 1%, 2.5%, 5% and 10%. Among the tested grape types, Hasandede revealed suppression capacity on all the bacteria at the tested concentrations [4]. Hasandede extract at 10% concentration gave the highest antibacterial activity for *Aeromonas hydrophila* ATCC 7965 with 30.67 mm inhibition zone. Extracts from all the tested grape types at 0.5% and 1% concentrations gave bacteriostatic effect for *Escherichia coli* DM and *E. coli* O157:H7 KUEN 1461 strains while all the grape types showed bacteriostatic effect for *S. aureus* Cowan 1 strain. In our study, extracts of Boğazkere caused 9.33-11.66 mm inhibition zone formations for *S.aureus* while it was between 12.66 and 15.33 mm for *E. faecalis*. However, the extract did not show any inhibitory effect on *E. aerogenes* for 10 µL and 50 µL treatment while only for 100 µL treatment 7.33 mm zone inhibition was observed. Similar to the Baydar et al. study, our MIC findings were more of bacteriostatic.

Baydar et al. (2004) performed ethyl acetate/methanol/water mediated extraction of grape seeds, whose lipid content was removed beforehand. The extracts were tested for *Aeromonas hydrophila, Bacillus amyloliquefaciens, Bacillus brevis, Bacillus cereus, Bacillus megaterium, Bacillus subtilis, Enterobacter aerogenes, Enterococcus faecalis Escherichia coli, Klebsiella pneumoniae Listeria monocytogenes, Mycobacterium smegmatis, Proteus vulgaris, Pseudomonas aeruginosa* and *Staphylococcus aureus* using disc diffusion method [9].

The findings revealed that the highest antibacterial activity of methanol: water: asetic acid mediated extracts was for *L. monocytogenes* with 33.5 mm zone inhibition diameter at 20 % concentration. Similarly for acetone: acetic acid: water mediated extract gave the highest antibacterial capability for *L. monocytogenes* strain at 4 % concentration. Ethyl acetate: methanol: water mediated extract did not show any activity towards *E. aerogenes* at 4% concentration. However, certain extracts showed antibacterial activity for such bacterial strains even at 4% concentrations. In our study, the Boğazkere extract caused 9.33-11.66 mm zone inhibition for *S. aureus* strain at 50 µL and 100 µL volume application while at 10 µL concentration no activity was observed. Similarly, the extract caused 12.66-15.33 mm zone inhibition for *E. faecalis* strain at 50 µL and 100 µL volume application while at 10 µL concentration no activity was obtained. However, the extract gave anti-*E. aerogenes* activity at 100 µL treatment with 7.33 mm zone diameter.

Anastasiadi et al. (2009) analysed chemical contents belong to fruit, seed, stem and pomace of four *Vitis vinifera* types (i.e. Mandilaria, Voidomato, Asyrtiko and Aidani) and tested anti-*L. monocytogenes* capability using MIC test approach. The findings revealed that extracts from plant stem and seeds were very effective, and they seem a possible preservative could be introduced to food samples [10]. In our study, the Boğazkere extract gave anti- *L.*

monocytogenes activity at 50 µL and 100 µL volume treatment with causing 10.00 and 14.00 mm inhibition zone formation while at 10 µL no activity was observed.

Ege (2015) carried out extraction from seeds of Müşküle (white), Kara dimrit (blue-black) and Öküzgözü grape types, whose lipid content was removed beforehand. The extractions, performed in dedicated solvents systems of acetone, water, acetic acid and methanol. The extract stocks were prepared at 65.536 mg/mL concentration. Antimicrobial activity of the extracts was tested *Alternaria alternata, Aspergillus niger, Botrytis cinerea, Penicillium expansum, Escherichia coli 35218, Pseudomonas aeruginosa 27853, Klebsiella pneumonia 700603, Enterococcus faecalis 51299, Streptococccus pneumonia 49616* and *Staphylococcus aureus 44300* using MIC test. The findings revealed that all the extracts did not show antimicrobial capability against Alternaria *alternata, Aspergillus niger, Botrytis cinerea, Penicillium expansum, Escherichia coli 35218, Pseudomonas aeruginosa 27853, Klebsiella pneumoniae 700603* and *Enterococcus faecalis 51299*. In contrasto to this, Müşküle exract at 32.768 mg/mL and Öküzgözü at 65.536 mg/mL concentraions showed anti *S. aureus* activity. Similarly, Kara dimrit, Müşküle and Öküzgözü posssed anti- *S. pneumonia* activity at 2.048 mg/mL, 4.096 mg/mL and 32.768 mg/mL concentrations [11]. In our study, the Boğazkere extract did not show antimicrobial activity against *K. pneumonia, E. aerogenes, Candida ablicans, L. monocytogenes*, *P. fluorescens* ve *S. marcescens*. However, the extract at the range of 0.039 and 20 mg/100 µL gave antibacterial activity against *S. infentis, P. aeruginosa, S. Kentucky, E. faecalis, E. coli, L. innocua, S. enteritidis, S. typhimurium, E. faecium, S. aeureus, S. epidermidis* and *B. subtilis* strains. The difference between our study and the mentioned literature could be related to the different extraction solvent usage.

Abtahi et al. (2011) extracted dried white, red and black grape samples in 70% alcohol, which were then tested on *E. coli* PTCC1330*, S. aureus* PTCC 1431*, S. typhimurium* PTCC1639 and *P. aeruginosa* PTCC1310 strains using MIC tests. The findings showed that the extracts gave positive results for all the tested bacteria with showing the highest activity for *S. aureus* strain [12]. The obtained MIC values for *E. coli, S. aureus, S. typhimurium* and *P. aeruginosa* strains were 125, 32, 125 and 250 µg/mL, respectively. In our study, the Boğazkere extract gave 10 mg/100 µL and 0,625 µg/mL MIC values for *E. coli* and *S. aureus*, respectively. In contrast to this, it did not give any effect on *S. typhimurium*. In the present work, Boğazkere extract showed antimicrobial effect against other microorganisms with MIC values range between 0.039 and 20 mg/µL. As it was seen from the results, different grape types have different effect on the same microorganisms.

Waqar et al. (2014) tested the antimicrobial activity of the extracts from leaves of *V. vinifera* on *E. coli*, *P. aeruginosa, S. aureus* and *E. faecalis* strains using disc diffusion method. In the study, 5 mg of leaf extract was dissolved in 70% ethanol, followed by 3 discs were treated with 3 mg/0.1 mL of the dissolved extract. The inhibition zones belong to *S. aureus* as 30 mm, *E. faecalis* as 28.9 mm, *E. coli* as 28 mm and *P. aeruginosa* as 23.7 mm were obtained [13]. In our study, the Boğazkere extract gave antibacterial capability against

S. aureus, *E. faecalis*, *E. coli* and *P. aeruginosa* with 9.33-11.66 mm, 12.66-15.33 mm, 8.66-9.66 mm and 7.33-8.66 mm zone inhibitions, respectively. However, the extract did not show any effect on these bacteria at 10 µL concentration. The obtained difference between the two studies came from utilization of different grapes and different parts of the plant.

The findings of this study along with the literature provide strong insight into that leaves, fruits and seeds of *Vitis vinifera* L. can provide antibacterial activity depending on the extraction method. Even at low concentrations of *Vitis vinifera* L. Boğazkere extracts can pose antimicrobial effect for the tested bacterial strains. Our findings are endorsing the literature revealing that the plant extracts can be alternative and viable tools to fight against microbial development, which has been under investigation for a long time.

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Current Address: SEDA ÇİÇEK: *Kastamonu University, Arts and Sciences Faculty, Department of Biology, Kastamonu, Turkey*
E-mail: seda_biolog@hotmail.com
https://orcid.org/0000-0001-8965-119X

Current Address: TALİP ÇETER: *Kastamonu University, Arts and Sciences Faculty, Department of Biology, Kastamonu, Turkey*
E-mail: talipceter@gmail.com
https://orcid.org/ 0000-0003-3626-1758