



## Bioactive Compounds, Antimicrobial and Antibiofilm Activity of Two *Verbascum* Species

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### ABSTRACT

Methanol, acetone and ethyl acetate extracts obtained from *Verbascum pinnatifidum* Vahl. and *V. antinori* Boiss. et Heldr were investigated for their bioactive composition, antimicrobial and antibiofilm activity. Antimicrobial and antibiofilm activity was determined with *Escherichia coli* NRRL B-3704, *Pseudomonas aeruginosa* ATCC 27853, *Proteus vulgaris* ATCC 13315, *Acinetobacter baumannii* ATCC 19606), *Bacillus subtilis* ATCC 6633, *Staphylococcus aureus* ATCC 6538P, *Staphylococcus haemolyticus* ATCC 43252 and *Candida albicans* ATCC 10231 by the disk diffusion, minimum inhibitory concentration and minimum bactericidal or fungicidal concentration and microplate biofilm method, respectively. Bioactive compounds analyses reveal the presence of coumarins, cardiac glycosides, quinones, flavanones. It was revealed that extracts of *V. antinori* has more antimicrobial activity than *V. pinnatifidum* extracts against test microorganisms. The lowest MIC value was recorded by *V. pinnatifidum* methanol extract against *A. baumannii* ATCC 19606 (0.3125 ± 0.01). Antibiofilm activities of three extracts of *V. pinnatifidum* and *V. antinori* have been showed an inhibition percentage range of 8.93 - 92.18% and 14.56 - 91.19%, respectively.

### Research Article

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#### Keywords

*Verbascum pinnatifidum* Vahl.

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Bioactive Compounds

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## İki *Verbascum* Türünün Biyoaktif Bileşenleri, Antimikrobiyal ve Antibiyofilm Aktiviteleri

### ÖZET

*Verbascum pinnatifidum* Vahl. ve *V. antinori* Boiss. et Heldr'den elde edilen metanol, aseton ve etil asetat ekstraktları biyoaktif bileşenleri, antimikrobiyal ve antibiyofilm aktiviteleri bakımından araştırıldı. Antimikrobiyal ve antibiyofilm aktiviteleri *Escherichia coli* NRRL B-3704, *Pseudomonas aeruginosa* ATCC 27853, *Proteus vulgaris* ATCC 13315, *Acinetobacter baumannii* ATCC 19606), *Bacillus subtilis* ATCC 6633, *Staphylococcus aureus* ATCC 6538P, *Staphylococcus haemolyticus* ATCC 43252 ve *Candida albicans* ATCC 10231 ile sırasıyla disk difüzyon, minimum inhibitör konsantrasyonu ve minimum bakterisidal veya fungisidal konsantrasyonu ve mikropalak biyofilm metodu ile belirlendi. Biyoaktif bileşik analizleri kumarinler, kardiyak glikozidler, kuinonlar ve flavanonların varlığını ortaya koymuştur. *V. antinori* ekstraktlarının test mikroorganizmalarına karşı *V. pinnatifidum* ekstraktlarından daha yüksek antimikrobiyal aktivite sahip olduğu ortaya çıkmıştır. En düşük MİK değeri *A. baumannii* ATCC 19606 (0.3125 ± 0.01)'ya karşı *V. pinnatifidum* metanol ekstraktı ile kaydedilmiştir. Üç *V. pinnatifidum* ve *V. antinori* ekstraktının antibiyofilm aktiviteleri sırasıyla %8.93 - 92.18 ve %14.56 - 91.19 oranlarında inhibisyon yüzdesi göstermiştir.

### Araştırma Makalesi

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#### Anahtar Kelimeler

*Verbascum pinnatifidum* Vahl.

*V. antinori* Boiss. et Heldr.

Bioaktif Bileşikler

Antimikrobiyal

Antibiyofilm Aktivite

## INTRODUCTION

Antimicrobial agents are substances that are used to stop the growth of microorganisms or to kill them. Antimicrobial medicines have been very effective in treating infections for a long time. However, the treatment possibilities of these agents are limited due to the presence of some resistant pathogens against antimicrobial agents and the occurrence of undesirable side effects. Accordingly, natural or synthetic antimicrobial agents with new active compounds are needed to control infections. Due to toxicity seen in synthetic antimicrobials, there is greater tendency to natural antimicrobial agents (Pulcini et al., 2012).

Microbial biofilms are important virulence factors enabling the microorganism to live in a complex matrix (Sánchez et al., 2016). Biofilm production in microorganisms and resistance to antibiotics required new biofilm control strategies. In alternative searches for microbial control, especially plant-derived products are widely used. It is known that plants are potential sources as bioactive agents. They are widely accepted in alternative antimicrobial agent research due to their reliability, use in the treatment of diseases and infections, and their long history in traditional medicine (Packiavathy et al., 2012).

The knowledge that plants have therapeutic agents dates back to ancient times. According to a research conducted by the World Health Organization (WHO) based on the pharmacopoeia of 91 countries and some

publications on medicinal plants, the total amount of medicinal plants used for therapeutic purposes is approximately 20000 (Mahindru, 1992). *Verbascum* plants also are used medicinally in folk medicine especially for respiratory problems, anodyne, sedative, diuretic, sudorific, expectorant and antidiarrheal (Baytop, 1999; Georgiev et al., 2011). Biological and medical (antimicrobial, antioxidant, anticholinesterase etc.) activities of these plants have been also previously reviewed (Tatli and Akdemir, 2006; Dulger and Hacıoğlu, 2009; Kahraman et al., 2010; Kahraman et al., 2011; Kozan et al., 2011; Ozcan et al., 2011; Boga et al., 2016). However, there is no any literature about *Verbascum* plant species antibiofilm activity, except Moghaddam et al. (2015).

The objective of this study was to evaluate chemical composition, antimicrobial and antibiofilm activities of methanol, acetone and ethyl acetate extracts of two *Verbascum* plants including *Verbascum pinnatifidum* Vahl. and *V. antinori* Boiss. et Heldr.

## MATERIALS and METHODS

### Plant materials

The specimens belong to *V. pinnatifidum* and *V. antinori* were collected from Canakkale in 2018, respectively (Table 1). Two plants were described with the aid of Flora of Turkey (Davis et al., 1988) and other relevant publications by Dr. Ersin KARABACAK.

Table 1 Plant specimens used in this study  
*Çizelge 1 Çalışmada kullanılan bitki örnekleri*

Species (Türler)	Collected area (Toplanan alan)	Identification by (Tanımlayan)
<i>Verbascum pinnatifidum</i>	Çanakkale: Kumkale, near the lantern, d.s. sandy area, 40.008101 N, 26.203127 E	<i>E. Karabacak</i>
<i>Verbascum antinori</i>	Çanakkale: Ayvacık, Balabanlı, Sivrice village way, 300 m, volcanic cliffss, 39.495868 N, 26.218705 E, 4 viii 2018	<i>E. Karabacak</i>

### Plants extraction

The plants samples were air-dried. Each dry powdered plant material 15 g was extracted with 150 mL of methanol, acetone and ethyl acetate (Merck, Darmstadt, Germany) for 24 h by using Soxhlet equipment. Filtered extracts were evaporated under reduced pressure and dried using a rotary evaporator at 55°C and stored in labeled sterile screw-capped bottles at -20°C (Khan et al., 1988).

### Qualitative phytochemical screening

*V. pinnatifidum* and *V. antinori* plant extracts were standardized phytochemical tests (coumarins, cardiac glycosides, phlobotannins, quinones, flavanones, anthocyanins and proteins) in order to evaluate their chemical composition for different active constituents

(Harborne and Baxter, 1999; Raaman, 2006; Evans, 2009).

Coumarins: To 1 mL of plant extract, 1 mL of 10% NaOH was added. The formation of yellow color indicates the presence of coumarins (Raaman, 2006).

Cardiac glycosides: To the plant extract, few mL of glacial acetic acid, ferric chloride (FeCl<sub>3</sub>) and conc. H<sub>2</sub>SO<sub>4</sub> were added. Green color indicates the presence of cardiac glycosides (Harborne and Baxter, 1999).

Phlobotannins: Plant extract was dissolved in distilled water. The filtrate was boiled with 2% HCl. Red precipitate indicates the presence of phlobotannins (Raaman, 2006).

Quinones: To 1 mL of extract 1 mL of conc. H<sub>2</sub>SO<sub>4</sub> were added, formation of red color indicates the presence of quinones (Raaman, 2006; Evans, 2009).

Flavanones: To few mL plant extract, 10% of few drops of NaOH was added. Yellow color indicates the presence of flavanones (Harborne and Baxter, 1999; Evans, 2009).

Anthocyanins: To the plant extract, 10% NaOH was added; blue color indicates the presence of anthocyanins (Harborne and Baxter, 1999).

Proteins: To few mL plant extract, 1 mL of 40% NaOH solution and 2 mL of 1% CuSO<sub>4</sub> were added. Violet color indicates the presence of proteins (Evans, 2009).

### Test microorganisms

Gram-negative bacteria (*Escherichia coli* NRRL B-3704, *Pseudomonas aeruginosa* ATCC 27853, *Proteus vulgaris* ATCC 13315, *Acinetobacter baumannii* ATCC 19606), Gram-positive bacteria (*Bacillus subtilis* ATCC 6633, *Staphylococcus aureus* ATCC 6538P, *Staphylococcus haemolyticus* ATCC 43252) and yeast (*Candida albicans* ATCC 10231) were used for determining the antimicrobial and antibiofilm activity of two *Verbascum* plant extracts.

### Screening for antimicrobial activities

Disc diffusion method was used for qualitative analyses of two *Verbascum* species extracts (Collins et al., 1989). Studies were performed in triplicate. Treatments with Penicillin (P10), Streptomycin (ST) and Nystatin (NYS100) served as positive controls and treatments with methanol, acetone and ethyl acetate without plant materials served as negative controls.

For quantitative antimicrobial analyses, minimum inhibitory concentration (MIC) and minimum bactericidal/fungicidal concentration (MBC or MFC) values of all samples were determined. MIC and MBC or MFC were investigated as recommended instruction of the Clinical and Laboratory Standards Institute (Barry, 2007). The lowest concentration of extracts inhibiting visible growth of each test microorganisms was taken as the MIC. The medium, 0.1% (w/v) Streptomycin (ST), NYS100 and 10% DMSO were used as the non-treated, positive and negative controls, respectively.

To support each MIC and to define the MBC and MFC, 10 µL of the following dilutions were inoculated into dishes with Mueller Hinton Agar (MHA) to evaluate microbial growth. Each experiment was repeated for three times (Teapasian et al., 2017).

### Biofilm inhibition assay

Microplate biofilm method (Merritt et al., 2005) was used to evaluate the inhibition of biofilm formation by two *Verbascum* extracts against test microorganisms. All experiments were repeated thrice in triplicate. The measurement of the antibiofilm effect of the

extracts was made by the percentage reduction formulation.

$$\% \text{ Inhibition} = (A_{\text{control}} - A_{\text{sample}} / A_{\text{control}}) \times 100 \quad (1)$$

A<sub>control</sub>: Absorbance of the control (containing 100 µL Mueller Hinton Broth instead of plant extract) reaction

A<sub>sample</sub>: Absorbance of the test compounds

### Statistical analysis

The results of antimicrobial and antibiofilm activity assays were mean ± SD of three parallel measurements. The statistical significance was estimated using a Student's *t*-test, *p* values < 0.05 were regarded as significant.

## RESULT and DISCUSSION

The extract yields obtained from each extraction are provided in Table 2. With reference to, extracts yields were obtained methanol > ethyl acetate > acetone > acetone > ethyl acetate > methanol for *V. pinnatifidum* and *V. antinori* plants, respectively. Alimpić et al. (2016), showed that the largest yield was obtained from the water extract, followed by methanol, hexane and ethyl acetate extracts. Findings in this study have some differences about *V. antinori* extracts yield rates. This case may be related to the dissolution of different contents of plant samples by different solvents.

Table 2 Yield (%) from three extracts of *V. pinnatifidum* and *V. antinori*  
Çizelge 2 *V. pinnatifidum* ve *V. antinori* elde edilen üç ekstrektinin verimi (%)

Solvent of extracts (Ekstrektlerin çözücüsü)	Yield of extract (%) (Ekstrekt verimi (%))	
	<i>V. pinnatifidum</i>	<i>V. antinori</i>
Methanol (Metanol)	1.1370±0.1	0.3908±0.17
Acetone (Aseton)	0.197±0.14	4.408±0.4
Ethyl acetate (Etil asetat)	0.2915±0.02	1.994±0.12

Phytochemical compounds of the plant extracts are given in Table 3. Coumarins, cardiac glycosides, quinones, flavanones were found in all two plants extracts. Similar phytochemicals have been reported in different species of these plants like, where the presence of glycosides, flavonoids and saponins has been reported (Tatli and Akdemir, 2006; Kahraman et al., 2010; Georgiev et al., 2011; Boga et al., 2016). Flavonoids cause bacterial death by inhibiting DNA or RNA synthesis (Sánchez et al., 2016). Therefore, there may be a correlation between the antimicrobial activities and phytochemical compounds of two plants extracts.

The antimicrobial activities of the plants extract against different test microorganisms were showed in Table 4-5. All extracts obtained from both plants

showed higher antibacterial effect against *P. aeruginosa* ATCC 27853 than comparison antibiotic P10. *V. antinori* also showed antibacterial activity against *A. baumannii* ATCC 19606 (extracts of methanol, acetone and ethyl acetate), *P. vulgaris* ATCC 13315 and *B. subtilis* ATCC 6633 (extracts of ethyl acetate) higher than comparison antibiotic P10. It can be seen in Table 4, no significant activity was found against *E. coli* NRRL B-3704, *A. baumannii*

ATCC 19606, *S. aureus* ATCC 6538P, *S. haemolyticus* ATCC 43252, *C. albicans* ATCC 10231 (except acetone) all extracts of *V. pinnatifidum*. It was found that extracts of *V. antinori* had more antagonistic activity than *V. pinnatifidum* extracts against test microorganisms. The lowest MIC value was recorded by *V. pinnatifidum* methanol extract against *A. baumannii* ATCC 19606 (0.3125 ± 0.01).

Table 3 Phytochemical screening results of two *Verbascum* plant extracts  
 Çizelge 3 İki *Verbascum* bitki ekstraktının fitokimyasal tarama sonuçları

Phytochemical compounds (Fitokimyasal bileşikler)	<i>V. pinnatifidum</i>			<i>V. antinori</i>		
	E1	E2	E3	E1	E2	E3
Coumarins ( <i>Kumarinler</i> )	++	+	+	+	+	+++
Cardiac glycosides ( <i>Kardiak glikozitler</i> )	++	++	++	+	++	+++
Phlobotannins ( <i>Filobotanninler</i> )	+	-	-	-	-	-
Quinones ( <i>Kuinonlar</i> )	++	++	++	+	+++	++
Flavanones ( <i>Flavonlar</i> )	+	+	+	+	+	+
Anthocyanins ( <i>Antosiyaninler</i> )	+	-	-	+	-	-
Proteins ( <i>Proteinler</i> )	-	+	+	-	-	+

[+: low intensity reaction; ++: medium intensity reaction; +++: strong intensity reaction; -: Not Present; E1: Methanol extract, E2: Acetone extract, E3: Ethyl acetate extract]

There are many reports regarding to *Verbascum* species antimicrobial activity (Meurer-Grimes et al., 1996; Dulger et al., 2002; Guarino, 2002; Dulger et al., 2005; Dulger and Hacıoğlu, 2008; Prakash et al., 2016; Dulger and Dulger, 2018). It was shown that *Verbascum* L. species showed antimicrobial activity against the Gr (+) bacteria and yeasts, but no activity was observed against the Gr (-) bacteria by disc-diffusion method (Meurer-Grimes et al., 1996; Dulger et al., 2002; Dulger and Gonuz, 2004; Dulger and Hacıoğlu, 2008). As a result of these studies *S. aureus* and the yeast cultures appear to be more susceptible to plant extracts. There is no data about *V. pinnatifidum* and *V. antinori* antimicrobial activities except Dulger and Dulger (2018). According to this investigation *V. antinori* has a strong antagonistic effect against Gram (+) bacteria; but it was ineffective on gram (-) bacteria.

Obtained different results in this research with antibacterial activity of *V. pinnatifidum* and *V. antinori* against *P. aeruginosa* ATCC 27853 and *A. baumannii* ATCC 19606 (Gram (-) bacteria). It was thought that the *Verbascum* species gave different results on the same test microorganisms was due to differences of plant collected localization, ecological status, seasonal differences and variety of extraction methods.

The results indicated that three extracts *V. pinnatifidum* and *V. antinori* reduced metabolic activity of cells in biofilm all test microorganisms, showing an inhibition percentage range of 8.93-92.18% and 14.56-91.19%, respectively (Figure 1-2).

All extracts compared, *V. pinnatifidum* and *V.*

*antinori* methanol extracts were the most effective in inhibiting formation and growth of *B. subtilis* ATCC 6633 biofilm by 92.18% and 91.19%. The lowest antibiofilm activity was obtained against *E. coli* NRRLB-3704 by two plants all extracts. Inhibition biofilm activity was performed with MIC concentrations of plant extracts.

*V. pinnatifidum* and *V. antinori* extracts have not been investigated in terms of antibiofilm activity. Moghaddam et al. (2015) showed that ethanol extract of *V. thapsus* had inhibitory effect on biofilm formation of *Streptococcus mutans*, *S. sanguinis*, and *S. salivarius*. The current findings indicated that biofilm forming of bacteria and yeast could be potentially being managed using *V. pinnatifidum* and *V. antinori* plant extracts.

Microbial biofilms were thought to be important virulence factors due to their high resistance properties to chemotherapeutics and host immune system (Grant and Hung, 2013). Bacteria in biofilms had higher antibiotic resistance than planktonic cells owing to it was preserved in the exopolysaccharide. This necessitated the screening of new and natural antibiotic sources, such as medicinal plants, in the fight against biofilm. Biofilm formation was controlled by quorum sensing, a bacterial communication mechanism (Vuong et al., 2004; Pratiwi et al., 2015; Erdonmez et al., 2018). Therefore, in this study the reason for the antibiofilm activity of *V. pinnatifidum* and *V. antinori* extracts in MIC and sub-MIC concentrations, is thought to be the inhibition of the quorum sensing mechanism. Further studies need to be performed to confirm the actual

mode of action of antibiofilm activity from these extracts. Therefore, detailed studies are needed to

reveal the mechanism of antibiofilm activity of both plants.

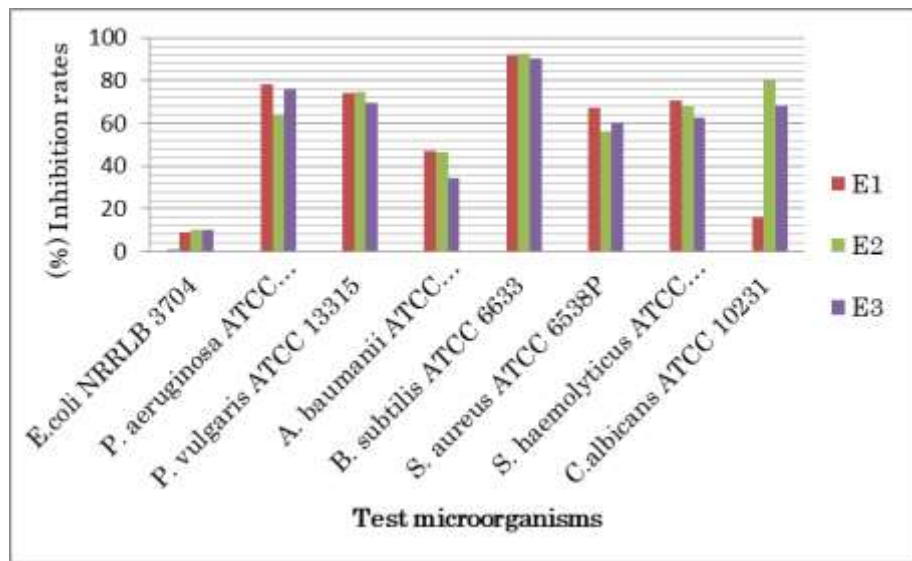


Figure 1 Antibiofilm activity of *V. pinnatifidum* extracts (E1: Methanol, E2: Acetone, E3: Ethyl acetate)  
Şekil 1 *V. pinnatifidum* ekstraktlarının antibiyofilm aktivitesi (E1: Metanol, E2: Aseton, E3: Etil asetat)

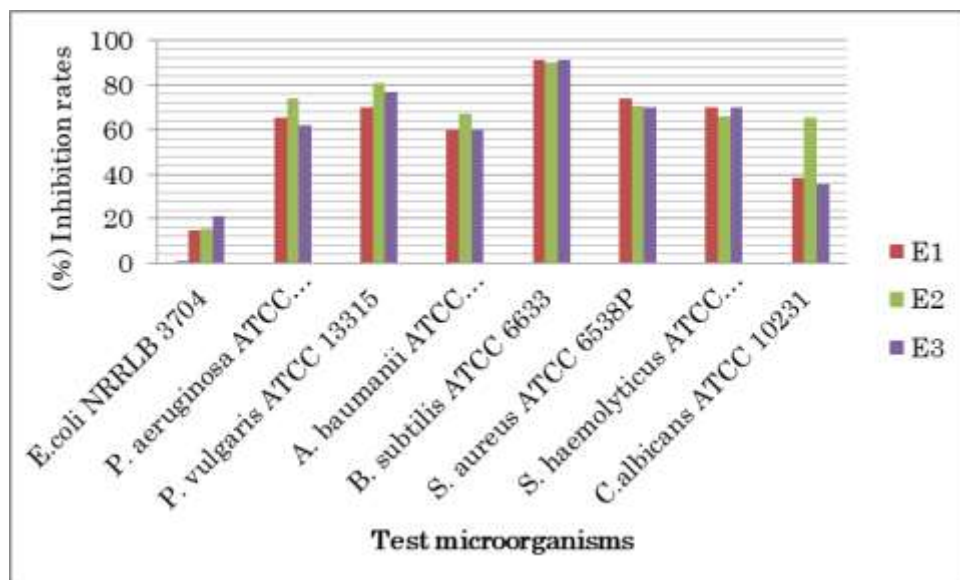


Figure 2 Antibiofilm activity of *V. antinori* extracts (E1: Methanol, E2: Acetone, E3: Ethyl acetate)  
Şekil 2 *V. antinori* ekstraktlarının antibiyofilm aktivitesi (E1: Metanol, E2: Aseton, E3: Etil asetat)

## CONCLUSION

Natural products are important source of new drugs which are very vital in modern medicine. The most important feature of this study is that all of the extracts had significant antibiofilm activity that inhibits serious bacterial and fungal pathogens. Comprehensive pharmacological studies are required to determine the components of these plants that have antimicrobial and antibiofilm effects and their use in treatment. This is the first report on the bioactive compounds and antibiofilm activity of *V. pinnatifidum* and *V. antinori* extracts. This approach may also allow new kind of research in medicinal usage or development of drug research.

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## Statement of Conflict of Interest

Authors have declared no conflict of interest.

Table 4 Disc Diffusion, MIC, MBC or MFC ratios of the *V. pinnatifidum* extracts  
 Çizelge 4 *V. pinnatifidum* ekstraktlarının disk difüzyon, MİK, MBK veya MFK oranları

Test microorganisms (Test mikroorganizmaları)	Plant extracts (Bitki ekstraktları)													
	*Disc Diffusion <sup>z</sup> (mm) (*Disk Difüzyon <sup>z</sup> (mm))						MIC (µg mL <sup>-1</sup> ) (MİK)					MBC or MFC (MBK veya MFK)		
	Extracts (Ekstraktlar)			Control(Kontrol)			Extracts(Ekstraktlar)			Control(Kontrol)		Extracts (Ekstraktlar)		
	E1	E2	E3	P10	NY100	E1	E2	E3	ST	NY100	E1	E2	E3	
<i>E.coli</i> NRRL B-3704	8.0±0.21	8.3±0.31	8.0±0.02	16.0	NT	5.0±0.01	5.0±0.03	5.0±0.03	4.0	NT	10.0±0.01	20.0±0.34	20.0±0.24	
<i>P. aeruginosa</i> ATCC 27853	10.3±0.10	12.0±0.12	10.0±0.14	8.0	NT	5.0±0.02	20.0±0.03	10.0±0.01	1.0	NT	10.0±0.02	20.0±0.24	20.0±0.01	
<i>P. vulgaris</i> ATCC 13315	11.0±0.01	9.3±0.23	9.3±0.14	13.0	NT	2.5±0.01	10.0±0.01	20.0±0.01	4.0	NT	10.0±0.04	20.0±0.28	20.0±0.15	
<i>A. baumannii</i> ATCC 19606	7.6± 0.32	7.0±0.35	7.3±0.43	12.0	NT	0.3125±0.01	5.0 ± 0.03	5.0 ± 0.03	2.0	NT	20.0±0.10	20.0±0.64	20.0±0.58	
<i>B. subtilis</i> ATCC 6633	11.3±0.24	11.3±0.35	11.3±0.32	14.0	NT	0.3125±0.01	20.0±0.01	5.0 ± 0.01	4.0	NT	20.0±0.27	20.0±0.47	20.0±0.01	
<i>S. aureus</i> ATCC 6538P	7.3±0.51	7.0±0.21	7.6± 0.01	15.0	NT	2.5±0.03	20.0±0.03	10.0±0.01	4.0	NT	20.0±0.15	20.0±0.15	20.0±0.01	
<i>S. haemolyticus</i> ATCC 43252	8.0±0.03	7.0± 0.12	7.0± 0.04	14.0	NT	5.0±0.01	20.0±0.01	5.0 ± 0.01	5.0	NT	10.0±0.17	20.0±0.17	20.0±0.01	
<i>C.albicans</i> ATCC 10231	7.0±0.11	10.3±0.02	8.3±0.12	NT	16.0	2.5±0.01	10.0±0.01	1.25±0.01	NT	5.0	20.0±0.18	20.0±0.15	20.0±0.01	

[NT: Not Tested; E1: Methanol, E2: Acetone, E3: Ethyl acetate; \*Inhibition zone (mm); z includes diameter of disk (6 mm); P10 = Penicillin (10 ug/disc); ST: Streptomycin (10 ug/disc); NY100 Nystatin (100 ug/disc)]

Table 5 Disc Diffusion, MIC, MBC, and MBC or MFC ratios of the *V. antinori* extracts  
 Çizelge 5 *V. antinori* ekstraktlarının disk difüzyon, MİK, MBK veya MFK oranları

Test microorganisms ( <i>Test mikroorganizmaları</i> )	Plant extracts ( <i>Bitki ekstraktları</i> )													
	*Disc Diffusion <sup>z</sup> (mm) ( <i>*Disk Difüzyon<sup>z</sup> (mm)</i> )						MIC ( $\mu\text{g mL}^{-1}$ ) ( <i>MİK</i> )					MBC or MFC ( <i>MBK yada MFK</i> )		
	Extracts ( <i>Ekstraktlar</i> )			Control ( <i>Kontrol</i> )			Extracts ( <i>Ekstraktlar</i> )			Control ( <i>Kontrol</i> )		Extracts ( <i>Ekstraktlar</i> )		
	E1	E2	E3	P10	NY100	E1	E2	E3	ST	NY100	E1	E2	E3	
<i>E. coli</i> NRRL B-3704	11.0±0.34	12.0±0.28	11.0±0.04	16.0	NT	10.0±0.01	10.0±0.03	20.0±0.03	4.0	NT	20.0±0.01	20.0±0.37	20.0±0.24	
<i>P. aeruginosa</i> ATCC 27853	9.0±0.15	10.0±0.14	12.0±0.18	8.0	NT	10.0±0.03	5.0±0.06	10.0±0.01	1.0	NT	20.0±0.12	10.0±0.24	10.0±0.01	
<i>P. vulgaris</i> ATCC 13315	10.0±0.03	12.0±0.26	15.0±0.16	13.0	NT	10.0±0.01	20.0±0.06	20.0±0.01	4.0	NT	20.0±0.04	20.0±0.27	20.0±0.15	
<i>A. baumannii</i> ATCC 19606	13.33±0.16	15.3±0.48	13.0±0.34	12.0	NT	10.0±0.05	20.0±0.05	20.0±0.03	2.0	NT	20.0±0.10	20.0±0.64	20.0±0.58	
<i>B. subtilis</i> ATCC 6633	11.0±0.12	12.0±0.37	16.0±0.36	14.0	NT	5.0 ±0.14	10.0±0.07	10.0±0.01	4.0	NT	10.0±0.29	10.0±0.47	10.0±0.01	
<i>S. aureus</i> ATCC 6538P	12.0±0.42	12.0±0.25	11.0±0.12	15.0	NT	10.0±0.03	10.0±0.08	10.0±0.01	4.0	NT	20.0±0.17	20.0±0.15	10.0±0.01	
<i>S. haemolyticus</i> ATCC 43252	11.0±0.13	11.0±0.16	11.0±0.04	14.0	NT	10.0±0.08	20.0±0.11	20.0±0.01	5.0	NT	20.0±0.19	20.0±0.27	20.0±0.01	
<i>C. albicans</i> ATCC 10231	10.0±0.13	10.3±0.01	10.0±0.11	NT	16.0	5.0±0.07	10.0±0.10	10±0.01	NT	5.0	10.0±0.18	20.0±0.17	20.0±0.01	

[NT: Not Tested; E1: Methanol, E2: Acetone, E3: Ethyl acetate; \*Inhibition zone (mm); z includes diameter of disk (6 mm); P10 = Penicillin (10 ug/disc); ST: Streptomycin (10 ug/disc); NY100 Nystatin (100 ug/disc)]

## Author's Contributions

The contribution of the authors is equal.

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