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The Effect of *Rheum ribes* Extract Origin of Elazig Province on Ventilator-Associated Pneumonia and Antioxidant Capacity

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Anahtar Kelimeler

Rheum ribes
Ventilatör ilişkili pnömoni
Antimikrobiyal aktivite
Antioksidan kapasite

Graphical/Tabular Abstract (Grafik Özet)

In contrary to the use of classical antibiotics in ventilator-associated pneumonia, the application of natural herbal agents leads to significant results. / Ventilatör ilişkili pnömoni'nin klasik antibiyotiklerin kullanımının aksine, doğal bitkisel ajanların uygulanması önemli sonuçlar doğurur.

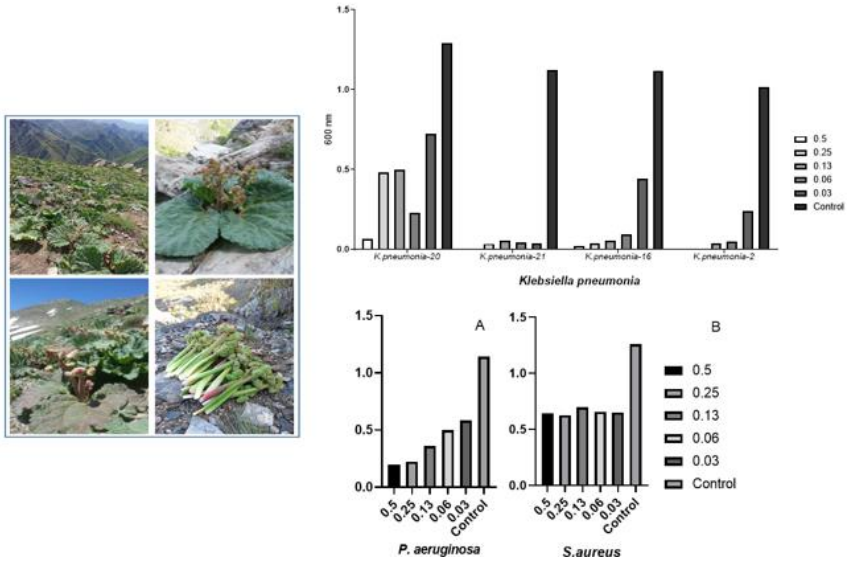

***Figure A**: Effects of R.ribes on VAP pathogens / **Şekil A:**.R.ribes'in VAP patojenlerine etkileri*

Highlights (Önemli noktalar)

- *Ventilator-associated pneumonia may be caused by bacteria whose antibiotic resistance is difficult to break. / Ventilatör ilişkili pnömoni antibiyotik direnci kırılması zor bakteri kökenli olabilir.*
- *Interest in natural agents has increased in cases where antibiotics are inadequate. / Antibiyotiklerin yetersiz kaldığı durumlarda doğal ajanlara olan ilgi artmıştır.*
- *R.ribes is a medicinal plant with many health beneficial effects. / R.ribes birçok sağlığa faydalı etkisi olan tıbbi bir bitkidir.*

***Aim (Amaç):** The possible effects of R. ribes extract as an alternative to classical antibiotic treatment of ventilator-associated pneumonia were investigated. / Ventilatör ilişkili pnömoni'nin klasik antibiyotik tedavisine alternatif olarak R. ribes ekstraktı uygulanıp olası etkileri araştırıldı.*

***Originality (Özgünlük):** R. ribes extract was applied as an alternative to classical antibiotic treatment of ventilator-associated pneumonia, one of the most critical hospital complications of our century./ Çağın en kritik hastane komplikasyonlarından Ventilatör ilişkili pnömoni'nin klasik antibiyotik tedavisine alternatif olarak R. ribes ekstraktı uygulandı.*

***Results (Bulgular):** R.ribes extract showed a strong antimicrobial and antioxidative effect. / R.ribes ekstresi güçlü bir antimikrobiyal ve antioksidatif etki göstermiştir.*

***Conclusion (Sonuç):** R.ribes extract may be used as a supportive treatment for ventilator-associated pneumonia. / R.ribes ekstresi Ventilatör ilişkili pnömoni ile ilgili tedavi yöntemlerini destekleyici olarak kullanılabilir.*

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Abstract

Ventilator-associated pneumonia (VAP) is one of the most important infections in the intensive care unit (ICU) and contributes to increased mortality and morbidity in patients. In this study, we aimed to evaluate *Rheum ribes* (Elazig/Turkey) extract on bacterial isolates obtained from VAP patients the antimicrobial effects (agar well diffusion, MIC test) and antioxidant capacity (DPPH, FRAP and metal chelation). As a result of the analysis, the highest antimicrobial effect of *R.ribes* was observed in *Klebsiella pneumonia-2 (K.pneumonia)* and *K.pneumonia-21* isolates, with zone diameters of 19.32 and 18.45 mm, respectively. Apart from these, *Staph. aureus., K. pneumonia-20, K. pneumonia-16* and *Pseudomonas aeruginosa (P. aeruginosa)* were detected with zone diameters of 18.32, 15.14, 14.56 and 13.54 mm, respectively. *R. ribes* extract showed 98.3% and 94.88% inhibitory effect at 0.5 ppm in *K.pneumonia* isolates 16 and 20 while, it showed 100% inhibitory effect on the highest isolates 21 and 2. *S. aureus* showed a high inhibition effect of 50.36% at 0.25 ppm, and *P. aeruginosa* isolate at a rate of 82.82% at 0.5 ppm. Besides, DPPH, FRAP and metal chelation analyzes revealed a strong antioxidant effect. DPPH inhibition effect, FRAP analysis and chelating activity values of iron ions (Fe^{2+}) for the antioxidant effects of R.ribes extract were determined as 17.22%, 1.18 and 2.14%, respectively. As a result, strong effect and antioxidant capacity of R. ribes extract on pathogenic bacteria have been determined, and its beneficial properties can be deepened by in vivo and clinical studies.

Elazığ İli Orijinli Rheum ribes Ekstraktının Ventilatör İlişkili Pnömoni ve Antioksidan Kapasite Üzerine Etkisi

Makale Bilgisi

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Anahtar Kelimeler

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Antimikrobiyal aktivite,
Antioksidan kapasite

Öz

Ventilatör ilişkili pnömoni (VİP), yoğun bakım ünitesindeki (YBÜ) en önemli enfeksiyonlardan biridir ve hastalarda mortalite ve morbidite artışına katkıda bulunur. Bu çalışmada *Rheum ribes* (Elazığ/Türkiye) ekstraktının VİP hastalarından elde edilen bakteri izolatları üzerinde antimikrobiyal etkilerini (agar kuyu difüzyonu, MİK testi) ve antioksidan kapasitesini (DPPH, FRAP ve metal şelatlama) değerlendirmeyi amaçladık. Analiz sonucunda *R.ribes*'in en yüksek antimikrobiyal etkisi 19.32 mm zon çapı ile *Klebsiella pneumonia*-2 (K.pneumonia) ve 18.45 mm zon çapı ile *K.pneumonia*-21 izolatında gözlendi. Bunların dışında *Staph. aureus., K. pneumonia*-20, *K. pneumonia*-16 ve *Pseudomonas aeruginosa* (P. aeruginosa) sırasıyla 18.32, 15.14, 14.56 ve 13.54 mm zon çapları ile tespit edildi. *R. ribes* ekstraktı *K.pneumonia* izolatları 16 ve 20'de 0,5 ppm'de %98,3 ve %94,88 inhibitör etki gösterirken, en yüksek izolatlar 21 ve 2'de %100 inhibitör etki göstermiştir. *S. aureus* %50,36 gibi yüksek bir inhibisyon etkisi göstermiştir. 0,25 ppm'de ve *P. aeruginosa* 0,5 ppm'de %82,82 oranında izole edilmiştir. Ayrıca DPPH, metal şelatlama ve FRAP analizleri incelendiğinde güçlü bir antioksidan etki göstermiştir. Ribes ekstraktının antioksidan etkileri için DPPH inhibisyon etkisi, demir iyonlarının (Fe^{2+}) şelatlama aktivitesi, FRAP analizi ve değerleri sırasıyla %17.22, %1.18 ve %2.14 olarak belirlendi. Sonuç olarak, *R. ribes* ekstraktının patojenik bakteriler üzerindeki güçlü etkisi ve antioksidan kapasitesi belirlenmiş olup, *in vivo* ve klinik çalışmalarla faydalı özellikleri derinleştirilebilir.

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1. INTRODUCTION (GİRİŞ)

Infectious diseases, from past to present, are one of the most important factors that threaten human health. Lower respiratory tract infections are a common cause of death in developing countries and represent an important source of morbidity worldwide [1]. A significant health issue, pneumonia is characterized by substantial morbidity and both short- and long-term mortality. Additionally, it is the major cause of infectious diseases that affect people of all ages globally. It is an acute respiratory infection that impairs breathing by causing edema in the lungs' alveoli, pus accumulation, and fluid accumulation [2-3]. For empirical treatment, choosing antibiotics that cover the main bacterial causes of disease is the main focus. Antimicrobial resistance poses a challenge to both these temporary and permanent treatments, indicating that they will be more and more at risk over the next few decades [4]. The majority of bacterial pathogens that cause pneumonia such as *Staphylococcus aureus*, *Streptococcus* pneumoniae, *Escherichia coli*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, and Acinetobacter species. The most prevalent organisms in the globe that cause community-acquired pneumonia are *S. pneumonia* and *S. aureus* [2].

In the context of ICU-acquired pneumonia, VAP is characterized as lung parenchymal infection in patients subjected to invasive mechanical ventilation for at least 48 hours. Atelectasis, aspiration, venous thromboembolic event, pulmonary edema, acute respiratory distress syndrome and delirium are other adverse events that might potentially lengthen hospital stays and increase morbidity and mortality [5]. VAP is just one of several adverse events. Although microbiological technologies have recently advanced, the epidemiology and diagnostic standards for VAP are still debatable. making treatment, prevention, and outcome studies difficult to interpret [6-7].

The main goal in the treatment of diseases aims to create a medication that has a lower effective dose and greater efficacy. The development of synthetic drugs often results in side effects such as angioedema, gastric irritation, ulceration, headache, hyperglycemia, hemolytic anemia, liver failure, and problems with immunodeficiency as well as others. Therefore, it is increasingly being thought about using natural medicines, which are typically regarded as safe, as an alternative therapeutic modality with little pharmacological response and side effects. There are numerous reports of the use of natural compounds extracted from herbs as successful antibiotics [8]. These compounds, such as curcumin, resveratrol, epigallo-catechin-3-gallate (EGCG), lycopene, capsaicin, genistein, sulforaphane are among the most researched [9-10].

Rheum ribes grows wild in China, India, Iran and Turkey. In Turkey, it grows especially around Southern and Eastern Anatolia. *R. ribes* it is the only rheum species growing wild in Turkey [11]. *R. ribes* is called “uçkun, usgun or iskin” in Turkey. In the GC-MS component analyzes of *R.ribes*, important chemical contents such as palmitic acid (67.7%), 1-octadecanol (4.3%), vitamin E (3.85%) were determined [12-13]. In addition, approximately 30 major components were detected in the analysis of *R. Ribes* stem methanol extracts by HPLC-ESI-QTOF-MS. Among these components, the most researched health-related ones are gallic acid (m/z 169.0157), salicylic acid (m/z 137.026), epigallocatehin (m/z 305.0681), epigallocatechin gallate (m/z 457.083), cis-resveratrol (m/z 227.0707).), genistein (m/z 271.0608), quercetin (m/z 303.0499), rutin (m/z 609.1495), kaempferol-7-O-glucoside (m/z 449.1086) and emodol anthraquinones (m/z 271.0608) [14]. Many plant species grown in Turkey are consumed by the people of the region to alleviate or eliminate the symptoms of various diseases [14]. The studies investigating the therapeutic efficacy of *R. ribes* are mainly concerned with its antimicrobial and antiradical properties [15-16]. Although the therapeutic properties of *R. ribes* grown in many regions have been investigated, there is no adequate study on ventilator-associated pneumonia of *R. ribes* originating from Aricak district of Elazig province. Previously, our team conducted research on gram-negative and positive bacteria related to *R. ribes* [17] and *Lavandula angustifolia* [18]. The beneficial effects of herbal materials led to the investigation of pathogenic pneumonia bacteria and antioxidant structures of *R. ribes* plant. Therefore, in this study, it was aimed to determine the antimicrobial effects of methanol-chloroform extract of *R. ribes* obtained from VAP patients by agar well diffusion test and antioxidant capacity with different techniques on some pneumonia pathogens from Elazig province Aricak district.

2.MATERIALS AND METHODS (MATERYAL VE METOD)

2.1. *R.ribes* Extracts Obtaining (R.ribes Ekstraktlarının Elde Edilmesi)

3. *Rheum ribes* plant, (38°32'52.7"N-40°05'29.9"E) coordinates of was collected in Elazig Province of Turkey (Saman Town,

Aricak County). The collected *R.ribes* was immediately taken to –80ºC cold chain, untill analysis done. The area where the plant was collected and the images of the plant are given in Figure 1.

Figure 1. Aricak / Saman town localization of *Rheum ribes* (Rheum ribes'in Arıcak / Saman köyü lokalizasyonu)

The collected *R. ribes* samples were extracted by modifying the Tufekci [19] method. The residues were cleaned by washing with distilled water. Then, 150 grams of plant stem was weighed and kept in an oven at 60 ºC for 24 hours. Then, IKA (A10) was ground in the mill with the help of mechanical grinder. 100 grams of the ground sample was taken and 500 mL of Methanol-Chloroform (1:1 v/v) was added to it and kept in a desiccator for 3 days. At the end of the 3rd day, extracts Whatman No. It was filtered with 2 filter papers and evaporated at 35 °C. Thus, the entire solvent was removed and the extract was obtained. As a result, it was prepared from this extract with distilled water at a final concentration of 10 ppm [19].

2.2. Pathogenic Microorganisms (Patojen Mikroorganizmalar)

Pseudomonas aeruginosa and *Klebsiella pneumoniae* and *Staphylococcus aureus* isolates grown in tracheal aspiration cultures (TAK) of patients receiving mechanical ventilation therapy in Çukurova University Faculty of Medicine Reanimation unit (respectively 21 Pseudomonas, 21 Klebsiella, 6 Staphylococcus were identified in Çukurova University Hospital Central Laboratory) It was sent to the Microbiology Laboratory for 8 months (November 2016 to June 2017). All samples were first investigated for morphological and biochemical features, including gram staining, motility, and catalase. After adding 15% glycerol to a 1.5 mL microtube containing tryptic soy broth, the isolated strains were transferred there and kept at -80°C. This study was approved by the local ethics committee (protocol no: 54/2016). All critically ill patients diagnosed with VAP who needed invasive ventilation for at least 48 hours were included in the study. The diagnosis of pneumonia was made using new or progressive infiltration on a chest X-ray with at least two of the following criteria: fever leukocytosis (WBC>12000 cells/mL), (T>38°C) or hypothermia (T35.5°C) or leukopenia (WBC4000) cells/mL, or positive tracheal culture [20-21].

Bacterial strain identification and antibiotic susceptibility tests were performed using conventional methods and the VITEK 2 system (bioMérieux SA, France). Acquired resistance profiles of pathogenic bacterial strains such as

Staphylococcus aureus, *Klebsiella pneumonia*, *Pseudomonas aeruginosa*, and were determined as MDR according to the Centers for Disease Control and Prevention (CDC) and European Center for Disease Prevention Control (ECDC) definition. Absence of (at least) sensitivity to an antibiotic in three or more antibacterial categories was defined as MDR. MDR pathogens were included in this study.

2.3. Antimicrobial Activity: strains and media (Antimikrobiyal Aktivite: suşlar ve ortamlar)

The antimicrobial effect of *R.ribes* on indicator microorganisms was investigated by well diffusion agar and MIC assay method were performed according to the methods designed by Bauer et al. [22], and Ericsson and Sherris [23]. The effect of the *R.ribes* was evaluated for pneumoni bacteria *K.pneumonia* (20, 21, 16 and 2 isolates), *S.aureus* and *P.aeruginosa*. MRS Broth (*Lactobacillus* Broth acc. to de Man, Rogosa and Sharp: MRS) Merck 1.10661**,** MRS Agar (*Lactobacillus* Agar acc. to De Man, Rogosa and Sharpe) Merck 1.10660 were used for growth of lactic acid bacteria, Tryptic Soy Broth (TSB) Merck 1.05459) for pathogenic microorganisms, Mueller Hinton Agar Merck 1.05437 for agar test, Mueller Hinton Broth (Merck 1.10293) for MIC test.

2.3.1. Agar well diffusion test (Agar kuyu difüzyon testi)

Indicator microorganisms were stored at temperature below 5°C. They were removed andreactivated in tryptic soy broth at 37°C for 18 hour. 12 ml of Mueller-Hinton agar (cooled 50 °C after otoclave) was poured into 90 mm Petri dishes and added 1ml fresh culture indicator bacteria with a density of 0.5 Mcfarland left to dry at 37 °C for 30 minutes. 6 mm diameter wells were drilled in frozen agars. 100 µL of *R. ribes* extract was added to the wells and incubated overnight at +4 °C for diffuse [24-25].

The petri dishes were then incubated under optimum conditions. The diameters of the inhibitory zones were measured in millimetres. Mueller Hinton Agar standard method procedure developed by Bauer et al. [22] and Stella and Marin [26] was used to eliminate or reduce variability in this test method. The procedure was adopted by the Clinical and Laboratory Standards Authority (CLSI, former NCCLS) as a consensus standard [27]. In addition, bacterial isolates, *Acinetobacter baumannii*, *Klebsiella pneumoniae* and *Pseudomonas aeruginosa*, were tested for antibiotic resistance/sensitivity (Table 1–3).

2.3.2. MIC test (Minimum inhibisyon konsantrasyonu testi)

MICs obtained with spectrophotometric microdilution method for *R. ribes* against indicator microorganisms were measured at 600 nm in a 96-well plate reader (Multiskan™ FC Microplate Photometer, USA), 150 µL of dual-strength MHB and Dual-strength *R. ribes* were added to the first wells, dilutions (1:1 v/v) were then added to the other wells. Then, 30 µL of bacterial suspension of fresh culture indicator bacteria with a density of 0.5 Macfarland was mixed into prepared plates and incubated at optimum temperatures for 18-24 hours. Then, samples density were detected by plate reader MIC was expressed as the highest dilution inhibiting growth (turbidity max in tube is low). Among these MIC dilutions, those with positive detection of nonviable cells > 99% in the medium are considered as MBC [28].

2.4. Antioxidant Analysis Methods (Antioksidan Analiz Yöntemleri)

DPPH, ethanol, trolox, butyl hydroxytoluene **(BHT)**, $NaCH_3COO$, HCl, 2,4,6-tripyridyl-s-triazine (TPTZ), Ferrozin, $FeCl_2$ were purchased from Sigma-Aldrich GmbH (Sternheim, Germany). BHT and Throlox were used as standard in antioxidant analysis methods. Since the extract was concentrated, it was diluted 1:25 v/v.

2.4.1. DPPH Free Radical Scavenging Activity (DPPH Serbest Radikal Temizleme Etkinliği)

Basic data on the extracts' antiradical activity is provided by the DPPH assay. Using a modified version of Blois [29] approach, the radical scavenging capacity of the *R. ribes* extract was assessed spectrophotometrically by observing the elimination of DPPH at 517 nm. In the presence of the sample, the bleaching rate of DPPH, a stable free radical, is observed at a distinctive wavelength. DPPH absorbs at 517 nm while it is a radical, but when it is reduced by an antioxidant or another radical species, its absorption drops. In a nutshell, 1.5 mL of *R. ribes* extract in ethanol at various concentrations (15-45 g/mL) were mixed to 0.5 mL of a 0.1 mM solution of DPPH (10^{-3} M) in ethanol. These solutions underwent accurate vortexing and dark incubation. At 517 nm, the absorbance was measured against the blank samples after 30 minutes. The reaction mixture's lower absorbance

denotes a higher level of DPPH free radical scavenging activity.

Standarts (BHT and Trolox) and *R.ribes* extract were compared against the absorbance of blank and then calculated of % inhibition values (Figure-2.A).

2.4.2. Chelating activity of ferrous ions (Fe^{2+}) (Demir iyonlarının (Fe2+) şelatlama aktivitesi)

By using *R. ribes* extract and the standards, ferrous ions were chelated using the Dinis et al. [30] method. The reaction took place in an aquatic environment. Briefly, 0.4 mL *R.ribes* extract (10 µg/mL) was added to a solution of 0.2 mL $FeCl_2$ (2 mM). The reaction was initiated by the addition of 0.4 mL ferrozine (5 mM) and the total volume was adjusted to 4 mL with ethanol. Then the mixture was shaken vigorously and left at room temperature for 10 min. Standarts (BHT and Trolox) (10 µg/mL) and *R.ribes* extract were compared against the absorbance of blank (contains $FeCl_2$ and ferrozine) at 562 nm (Figure-2.B).

2.4.3. FRAP (FRAP testi)

FRAP method (suitable to determine hydrophilic and lipophilic antioxidants) was introduced to determine the total amount of antioxidants by the reduction capacity of iron (III). The oxidant in the FRAP assay was prepared by mixing 2.5 mL 10 mM TPTZ dissolved in 40 mM HCl, 25 mL acetate buffer, 20 mM 2.5 mL $FeCl_3$ and water. This mixture is called as FRAP reagent. The final solution contained 1.67 mM Fe (III) and 0.83 mM TPTZ [31-32]. *R.ribes* extract (10 µg/mL) and standarts (BHT and Trolox) (10 µg/mL) were added to final solution (1.67 mM Fe (III) and 0.83 mM TPTZ) and incubated in dark for 10 minutes at room temperature. Standarts and *R.ribes* extract were compared against the absorbance of blank at 595 nm (Figure-2.C).

2.5. Total phenolic content (Toplam Fenolik içerik)

The determination of the total amount of phenolic substance is generally carried out by measuring the absorbance of the blue color formed by the reduction of the Folin–Ciocalteu reagent [33]. The color intensity formed is directly proportional to the phenolic substance concentration, and the total amount of phenolic substance can be calculated. Using this method, 0.5 N Folin–Ciocalteu reagent and 10% concentration Na_2CO_3 were prepared. Then, 30 min after pipetting, the absorbance was read at 760 nm. Gallic acid, a phenolic compound, was used in the preparation of the standard graph. Different concentrations of gallic acid, 1–0.031 mg mL^{-1} (50% percent to each dilution), were prepared with methanol, and their absorbances were read. A graph of absorbance versus concentration was constructed, and the total phenolic contents of the sample were determined as gallic acid equivalent.

2.6. Total flavonoid (Toplam Flavonoid)

The determination of the total flavonoids was achieved by observing the pink color formation, which is directly proportional to the flavonoid concentration. In this method, 10% $AlCl_3$ was prepared in a fume hood. $NaNO_2$ and 1 N NaOH were prepared at 5% concentrations. Absorbance was read at 510 nm, 15 min after pipetting.

Rutin was used in the preparation of the standard chart. Different concentrations of the rutin standard were prepared with methanol, and their absorbances were read. A graph of absorbance versus concentration was constructed, and the total flavonoid amounts of the sample were determined as rutin equivalent [34].

Table 1. Acineto-bacter baumannii Antibiotic resistance/susceptibility testing (*Acinetobacter baumannii* Antibiyotik direnci/duyarlılık testi)

Antibiotic	**Resistant abundance**	**Intermediate abundance**	**Sensitive abundance**
Amikacin	20	9	8
Cefepime	15		3
Ceftazidime	15		4
Ceftriaxone			
Cefuroxime			
Cefuroxime axetil			
Ciprofloxacin	33		4
Colistin	5		25
Ertapenem			
Gentamicin	35		3
Imipenem	33	1	
Levofloxacin	31		3
Meropenem	32	1	4
Netilmicin	29		6
Piperacillin	15	1	1
Piperacillin/Tazobactam	19		1
Tetracycline	8	1	7
Tigecycline		13	22
Tobramycin	22		11
Trimethoprim/sulfamet	29		7
Amoksisilin/Klavulonat			

Resistant abundance, n (%). Intermediate abundance, n (%). Sensitive abundance, n (%)

Tablo 2. *Klebsiella pneumoniae* Antibiotic resistance/susceptibility testing (*Klebsiella pneumoniae* Antibiyotik direnci/duyarlılık testi)

Antibiotic	**Resistant abundance**	**Intermediate abundance**	**Sensitive abundance**
Amikacin	5	11	4
Aztreonam	4		
Ampicillin	16		
Cefepime	13		
Ceftazidime	20		
Ceftriaxone	16		
Cefuroxime	16		
Cefuroxime axetil	12		
Ciprofloxacin	19	1	
Colistin	1		9
Ertapenem	10		6
Gentamicin	11		9
Imipenem	3	4	
Levofloxacin	4		
Meropenem	15		5
Netilmicin	4		
Piperacillin	4		
Piperacillin/Tazobactam	16	3	1
Tetracycline	1		
Tigecycline	3	6	4
Tobramycin	4		
Trimethoprim/sulfamet	20		1
Amoksisilin/Klavulonat	7		
Fosfomycin			2

Resistant abundance, n (%). Intermediate abundance, n (%). Sensitive abundance, n (%)

Tablo 3. Pseudomonas aeruginosa Antibiotic resistance/susceptibility testing (Pseudomonas aeruginosa Antibiyotik direnci/duyarlılık testi)

Antibiotic	Resistant abundance	Intermediate abundance	Sensitive abundance
Amikacin	10		7
Aztreonam	2		
Ampicillin			
Cefepime	10	5	
Ceftazidime	11	4	5
Ceftriaxone			
Cefuroxime			
Cefuroxime axetil			
Ciprofloxacin	12	1	4
Colistin			11
Ertapenem			
Gentamicin	10		10
Imipenem	5	6	5
Levofloxacin	2		
Meropenem	10	2	4
Netilmicin	2		1
Piperacillin	2		
Piperacillin/Tazobactam	15	2	4
Tetracycline			
Tigecycline			
Tobramycin	3		
Trimethoprim/sulfamet			
Amoksisilin/Klavulonat			
Fosfomycin			

Resistant abundance, n (%). Intermediate abundance, n (%). Sensitive abundance, n (%)Tablo

3.RESULTS (BULGULAR)

In this study, the highest amount of *R.ribes* extract on pneumococcal bacteria was detected in *K.pneumonia*-2 isolate and *K.pneumonia*-21 and (19.32 and 18.45 mm zone diameter, respectively) as shown in Table-4. Antimicrobial effect was detected in the other two klebsiella isolates (15.14 and 14.56 mm zone diameter, respectively). It was observed that it showed a strong antimicrobial effect in *S.aureus* isolate (18.32 mm zone diameter). Antimicrobial effect was detected in *P.aeruginosa* isolate (13.54 mm zone diameter).

Table-4. Antimicrobial zone diameters of *Rheum ribes* extract on Pneumococ microorganisms (*Rheum ribes* ekstraktının Pnömokok mikroorganizmaları üzerindeki antimikrobiyal bölge çapları)

Microorganism	**Diameter region (mm)**
Staph aureus	18.32
Pseudomonas aeruginosa	13.54
K. pneumonia -20	15.14
K. pneumonia -21	18.45
K. pneumonia -16	14.56
K. pneumonia -2	19.32

The graph of the growth concentrations of *R. ribes* extract applied to 4 isolates of *K. pneumonia* bacteria at 600 nm compared to the control group is given in Figure 2. For all *K.pneumonia* isolates of *R. ribes*, at 0.5 ppm, 100% inhibitory effects of *K.pneumonia* 21 and 2 isolates were determined, while the highest 16 and 20 isolates were found to have 98.3% and 94.88%, respectively. It was observed that showed a strong inhibition effect at the rate of 50.36% at 0.25 ppm concentration in *S. aureus* (Figure 3A), and 82.82% at 0.5 ppm concentration in *P.aeruginosa* isolate (Figure 3B.).

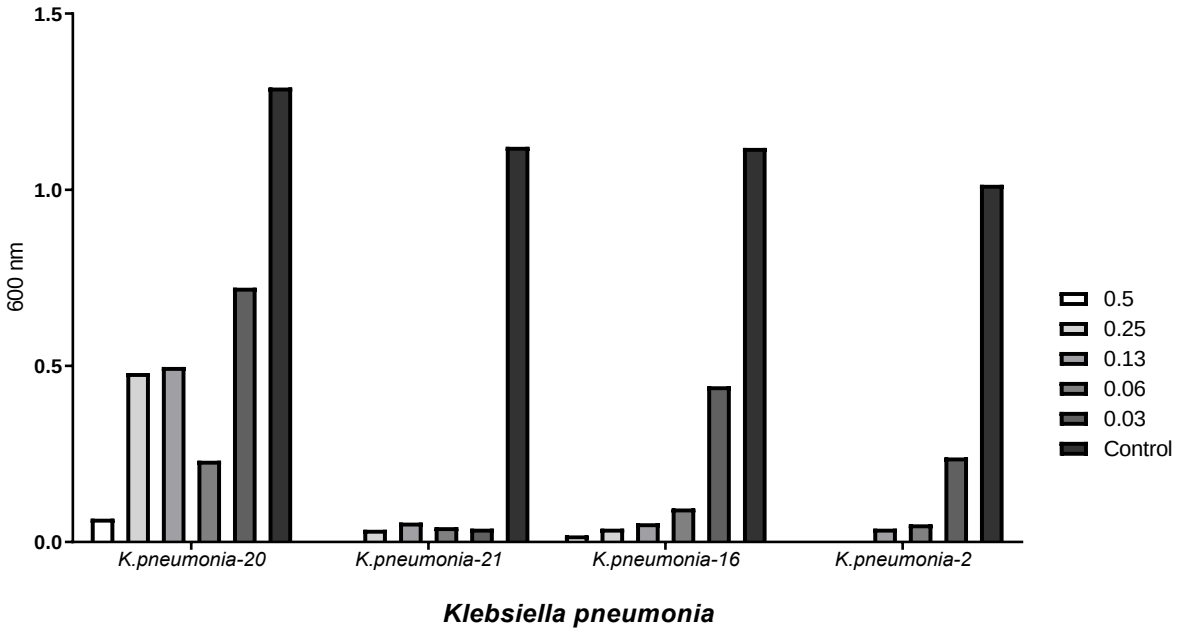

Figure 2. Growth concentrations *of R.ribes extract* doses (14.17-0.89 mg/L) applied to *K.pneumonia* isolates by spectrophotometric (at 600 nm) method compared to the control group. *(K.pneumonia* izolatlarına spektrofotometrik (600 nm'de) yöntemle uygulanan *R.ribes* ekstraktı dozlarının (14,17-0,89 mg/L) büyüme konsantrasyonlarının kontrol grubuyla karşılaştırılması)

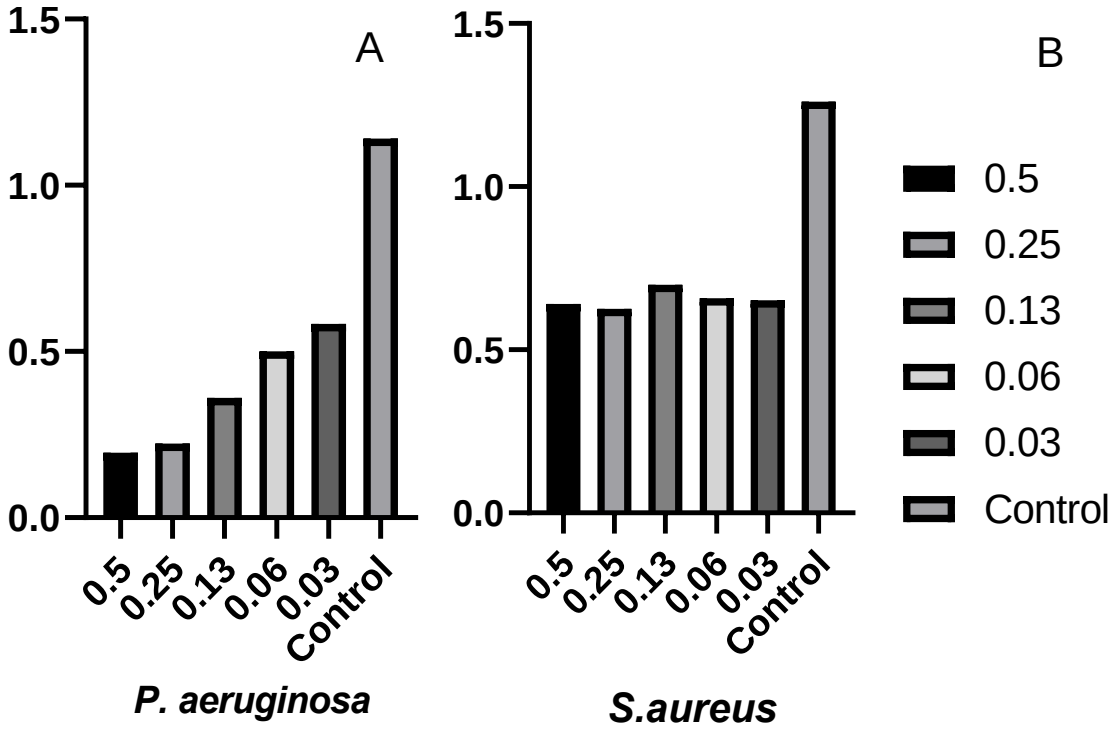

Figure 3. Growth concentrations of *R.ribes* extract doses (14.17-0.89 mg/L) applied to *P.aeruginosa* (PANEL A) and S. aureus (PANEL B) isolates by spectrophotometric (at 600 nm) method compared to the control group (*P.aeruginosa* (PANEL A) ve S. aureus (PANEL B) izolatlarına spektrofotometrik (600 nm'de) yöntemle uygulanan *R.ribes* ekstraktı dozlarının (14,17-0,89 mg/L) büyüme konsantrasyonlarının kontrol grubuyla karşılaştırılması)

The results are given graphically in Figure 4. analysis of the data revealed that all of the values of *R.ribes* were lower than the values of the standards (Throlox and BHT), but close to the standard. DPPH inhibition effect (PANEL A), FRAP analysis (PANEL B) and chelating activity values of iron ions (Fe^{2+}) (PANEL C) for the antioxidant effects of *R.ribes* extract were determined as 17.22%, 1.18 and 2.14%, respectively.

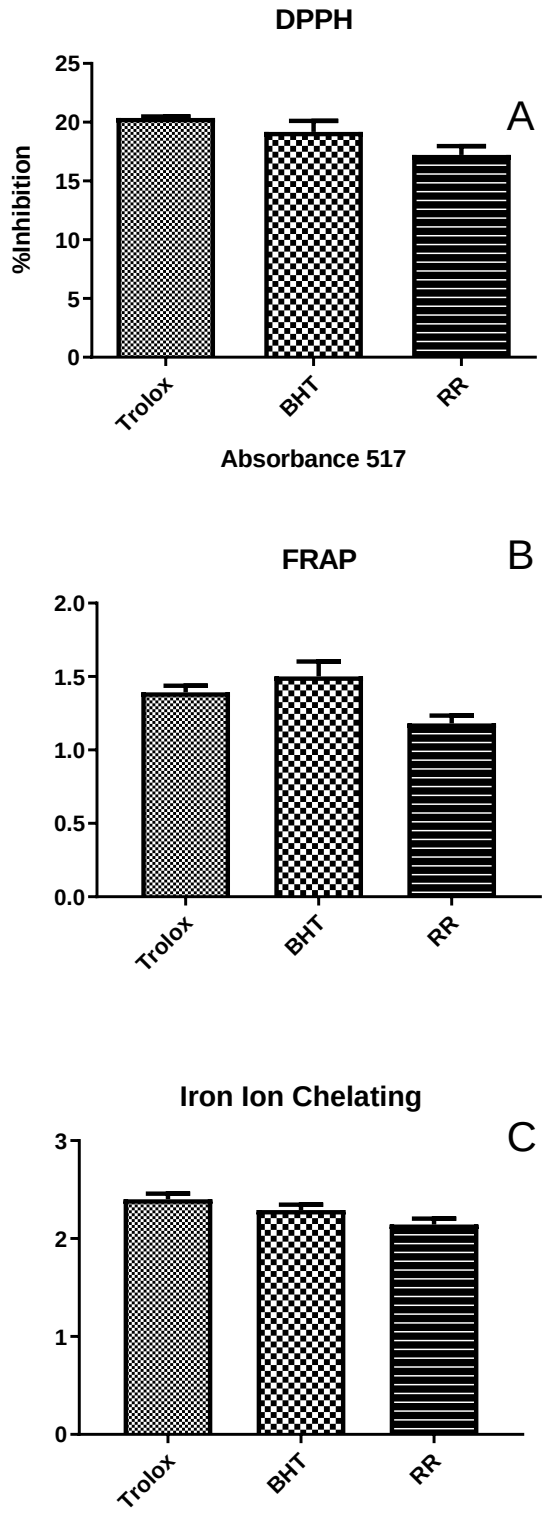

Figure 4. Antioxidant capacity (mg mL^{-1}) of *R.ribes* for the DPPH (PANEL A), FRAP (PANEL B), and Iron Ion Chelating (PANEL C) determinations (Absorbance). BHT and Trolox was used as a standard for DPPH, FRAP and Iron Ion Chelating (DPPH (PANEL A), FRAP (PANEL B) ve Demir İyonu Şelatlama (PANEL C) tayinleri için)

In addition, the total flavonoid and phenolic contents made are given in Table 5. According to the analyzes performed, the total flavonoid value was determined as 155.53 mg routine equivalent / mL extract, and the total phenolic value was determined as 69.57 mg gallic acid equivalent / mL.

Table 5. Total flavonoid and phenolic contents of the *R. ribes* methanol-chloroform extract from Elazig/Aricak. Rutin and gallic acid were used as standards for flavonoids and phenols, respectively[a] (Elazığ/Arıcak'tan elde edilen R. ribes metanol-kloroform ekstraktının toplam flavonoid ve fenolik içeriği. Rutin ve gallik asit, sırasıyla flavonoidler ve fenoller için standart olarak kullanılmıştır)

Analysis	***R.ribes* roots extract**
Total Flavonoid (µg RE/mL extract)[b]	155.53 ± 0.36
Total Phenolic (µg GAE/mL extract)[c]	69.57 ± 1.29

[a]Values expressed are means ± SD of three parallel measurements. [b]RE, Rutin equivalent. [c]GA, Gallic acid equivalent.

In the current study, we examined the antimicrobial effects of chloroform-methanol extract of the *R. ribes* plant obtained from Aricak district, Elazig Province, Turkey, on pathogen pneumonia isolates obtained from VAP patients in Adana Çukurova Hospital. As a result of the analyzes, it was determined that it showed strong antimicrobial effect on four isolates of *K. pneumonia, S. aureus* and *P. aeruginosa*. In addition, it was determined that *R. ribes* has very high antioxidant capacity with total flavonoid- phenolic and antioxidant experiments (DPPH, FRAP and Metal ion Chelating). Thus, it was determined that it gave effective results on *R. ribes* pneumonia isolates.

Mostly typical infections in intensive care units is VAP. Depending on the context and diagnostic criteria, reported incidences range greatly, from 5% to 40%. Long-term mechanical ventilation and length of stay in the ICU are linked to VAP [35]. An estimated 10% of deaths are attributed to VAP, and patients in surgical intensive care units have greater fatality rates. Strongly advised is microbiological confirmation of infection [7]. Although *P. aeruginosa* and methicillin-resistant *S.aureus* are among the common etiological agents of VAP, *A. baumannii* and *K. pneumoniae* are known to be the most important causes of VAP in many intensive care units worldwide [36]. In a clinical study, oral

herbal medicine mixture and mouthwash chlorhexidine (as a positive control) were compared in VAP patients. Oral herbal medicine formulation was given as miswak (stem of *Salvadora persica*), chamomile flower extract, listerine, veramin, zufa, *Echinacea angustifolia*, aloe vera green tea, and *Boswellia serrata* extracts. As a result of the study, it was revealed that oral herbal medicines are beneficial in preventing VAP, supporting our current study. It has been determined to play a significant function in maintaining oral health and preventing VAP by lowering the mouth's microbial flora [37].

The World Health Organization has reported that traditional medicines play an important role in maintaining, preventing chronic diseases, health and treating certain medical conditions. Studies have shown that compounds including, rhein, physcion8-O-glucoside, chrysopanol, emodin, physcion aloe emodin-8-O-glucoside, rhaponticin, aloeemodin, and sennoside A are present in the roots of *R. ribes* [38]. These components can ameliorate many diseases by regulating cellular signaling pathways and oxidative stress and inflammation processes [39]. Several *in vivo* and *in vitro* studies have demonstrated that *R. ribes* exerts its therapeutic effects via a variety of pathways [40]. Numerous studies have demonstrated the many biological properties of *R. ribes* root and leaf extract, such as delaying the progression of Alzheimer's disease, antibacterial and antiviral activity against *Herpes simplex virus*, *Enterobacter aerogenes* and *Bacillus subtilis* [41]. Meydan et al. (2022) stated that in an *in vitro* study in which they applied the aqueous extract and ZnO nanoparticle of *R.ribes*, it showed antimicrobial effect for gram-negative and positive bacteria. They showed that the highest effect was *B.subtilis* (21.5 mm zone), secondly *S.aureus* (14.1 mm zone diameter), which supports our studies [42]. In addition, *R. ribes* extracts from the HajeOmaran mountains in Iraq were subjected to examination, and the results revealed an antibacterial effect that was consistent with our investigation. Although *E. coli* was the most effective bacteria in their study, it was found that *K. pneumonia* were the bacteria that had the greatest antimicrobial effect in our study when compared to the current study [43]. Wijesinghe et al. (2021), in their *in vitro* study, found that cinnamon leaf essential oil showed antibacterial effects against *P. aeruginosa*, *S. aureus* and *K. pneumoniae*, in parallel with our current data [44]. In addition, in a study on some resistant gram-positive/negative bacteria; reported that treatment of drug-sensitive strains with a combination of ciprofloxacin and garlic extract was antimicrobial synergistic on *S. aureus* and *P. aeruginosa* bacteria [45].

Reactive oxidative species, which damages cells, are prevented by natural antioxidant molecules [46]. By preventing the onset or spread of the oxidative chain reaction, which functions as free radical scavengers, singlet oxygen scavengers, and reducing agents, increasing the intake of exogenous antioxidants will lessen the harm caused by oxidative stress. Exogenous antioxidants come primarily from foods and medicinal plants, such fruits, flowers, spices and traditional herbs [47-48]. In an *in vitro* study where anthraquinones extracted from *R.ribes* were applied to bacteria, DPPH, FRAP and ferrous-ions chelating activities were found to be quite high as a result of the analyzes, and it is thought to be related to the secondary metabolites, flavonoids and phenolic substances in its structure [49]. In a study on obese rats, it was reported that *R.ribes* root extract improved DNA damage and MDA levels in brain tissues and showed positive effects on antioxidant parameter activities in different tissues [50]. Another study revealed that the whole plant butanol fraction of *R. ribes* collected from the Pakistan region exhibited significant anticancer (MCF-7) activity [51].

4.CONCLUSIONS (SONUÇLAR)

In conclusion, our research suggests that *R. ribes* extraction may reduce the severity of VAP in *in vitro* studies and that this effect may be mediated by altering bacterial infection mediators and oxidative stress parameters. In addition, it was confirmed that the *R. ribes* formulations applied in the study showed similar effects with antibiotic drugs with known benefits. We believe that herbal therapies, such as *R. ribes*, can provide a safe alternative to conventional treatments for the treatment of clinical VAP, which should be validated by carefully planned research. As a limitation of our study, experimental animal and human studies may compare with antibiotics often used to treat VAP symptoms. Long-term *in vivo* and clinical trials in humans with a focus on the VIP solution are required to elucidate the mechanism by which herbal supplements improve functional status for *in vitro* studies.

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DECLARATION OF ETHICAL STANDARDS (ETİK STANDARTLARIN BEYANI)

This study was approved by the local ethics committee Çukurova University (protocol no: 54/2016).

Bu makalenin yazarları, bu çalışmanın Çukurova Üniversitesi yerel etik kurul tarafından onaylandığını beyan eder (protokol no: 54/2016).

AUTHORS' CONTRIBUTIONS (YAZARLARIN KATKILARI)

***Oğuzhan ÖZDEMİR*:** He conducted the experiments, analyzed the results and performed the writing process.

Deneyleri yapmış, sonuçlarını analiz etmiş ve maklenin yazım işlemini gerçekleştirmiştir.

***Nurten YILMAZ*:** She conducted the analyzed the results and performed the writing process.

Sonuçların analizini ve yazım sürecini yürüttü.

***Mustafa Oğuzhan KAYA*:** He performed the writing process and supervising.

Yazım sürecini ve süpervizörlüğü gerçekleştirdi.

CONFLICT OF INTEREST (ÇIKAR ÇATIŞMASI)

There is no conflict of interest in this study.

Bu çalışmada herhangi bir çıkar çatışması yoktur.

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