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Determination of Antibacterial Activities of St. John's Wort (*Hypericum perforatum L.*) Oil, *Nigella Sativa* Oil, Clove (*Eugenia caryophyllata*) oil, Orange Peel (*Citrus sinensis*) and Garlic (*Allium sativa*) Oil Against Microorganisms Isolated from Clinical Samples

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Abstract: The aim of this study is to detect St. John's Wort, *Nigella sativa*, Clove, Orange Peel and Garlic oil on bacteria isolated from blood culture to determine its antibacterial effect. One hundred blood samples were sent to Atatürk University Medical Microbiology Laboratory between 1 December 2021 and 1 January 2022 and analyzed with a blood culture system. Bacteria isolated from blood culture were passaged into blood agar. The bacterial suspension was prepared from the bacterial colonies at 0.5 Mc Farland turbidity. To determine the antibacterial activity of plant extract oils, Minimum Inhibition Concentration and Minimal Bactericidal Concentration values were determined by the liquid microdilution method. Also, the zone diameters of the disc diffusion method were measured. The antibacterial effect of plant extract oils were detected on only 10 of the 100 clinical samples included in the study. St. John's Wort oil used in these ten samples showed the most effective antibacterial effect of 7.81 µg/mL against *Staphylococcus haemolyticus* and *Enterobacter aerogenes*. Garlic oil showed the most effective antibacterial effect against *Escherichia coli* and *Staphylococcus haemolyticus* at 7.81 µg/mL. *Nigella sativa* oil showed the most effective antibacterial effect against *Staphylococcus haemolyticus* at 3.9 µg/mL. Orange Peel oil showed the most effective antibacterial effect against Enterococcus faecalis at 1.95 µg/mL. Garlic oil on *Escherichia coli*, *Staphylococcus haemolyticus* and *Enterobacter aerogenes*, St. John's wort oil on *Staphylococcus haemolyticus* and Enterobacter aerogenes, *Nigella sativa* oil on *Staphylococcus haemolyticus* has been found to be effective. © 2023 NTMS.

Keywords: **Keywords:** Antibacterial Activity; Plant Extract; Blood Culture; Microdilution; Zone Diameter.

1. Introduction

Nosocomial bloodstream infections are a serious and increasing problem that causes complications parallel to bacteremia and fungemia. These infections show high morbidity and mortality due to the delay in

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managing the infectious agent with hospitalization [1-3]. In addition, the long-term hospitalization of the patient and this hospitalization increase the hospital costs. In this respect, appropriate diagnosis and treatment are very important. The determination of the treatment for the causative agent, the choice of antibiotic in the treatment and the limitation of its negative effects on the host are provided by blood culture results [4]. Blood culture is the best approach in routine laboratories to identify microorganisms and determine antimicrobial therapy when suspected of bloodstream infections [5]. After blood culture results, preliminary treatments are determined based on clinical and epidemiological data, but the response to treatment in drug-resistant organisms is uncertain [6, 7]. At this point, researchers are working on alternative treatment methods. The antibacterial activity on the studied substance/component and microorganisms has been demonstrated in vivo and in vitro. In this context, hyperforin is the active ingredient and responsible for the antibacterial effect of St. John's Wort, which has been studied extensively on microorganisms. It has been determined that the active ingredient hypericin also acts together with antibacterial activity. *Nigella sativa* is an antioxidant and has many activities. It is used as a therapeutic in infectious diseases, and it is reported that this positive effect is due to the radical-scavenging effect of essential oil [8]. Seeds of *Nigella sativa* have been used in traditional medicine for their antioxidant properties, and the oil and its active ingredients seem to reduce toxicity mediated by oxidative stress triggered by environmental or infection-related factors or anti-cancer drugs [9]. Piras et al. (2013) observed the best antimicrobial activity in *Nigella sativa* [10]. Orange peel (*Citrus sinensis*) oil contains 20.2 % linalool, 18.0 % decanol, 14.1 % citral, 5.8 % terpineol, 5.2 % valene, 4.1 % dodecanol, 3.9 % citronellol and 0.3 % limonene [11]. Limonene is one of the important active ingredients in the oil. It is available in various studies showing antimicrobial and antiseptic activity [12]. Clove (*Eugenia caryophyllata*) oil shows many pharmacological activities such as, antimicrobial and anti-cancer, based on its bioactive components such as eugenol, eugenol acetate, α-humulene, 2-heptanone, and β-caryophyllene [13]. The antimicrobial effects of garlic (*Allium sativa*) extracts are well known, however the effects of *Allium sativa* oil are little known. Many studies have been carried out to determine the antimicrobial activity of *Allium sativa*. Antimicrobial activity is greater in media without tryptone or cysteine, suggesting that, as for allicin, the effects of *Allium sativa* oil may include sulfhydryl reactivity. All tested bacteria are susceptible to garlic ingredients, including gram-negative and positive bacteria and pathogenic forms [14]. In the light of this information, we investigated the antibacterial activity of St. John's Wort (*Hypericum perforatum L.*) oil, *Nigella sativa* oil, Clove (*Eugenia* caryophyllata) oil, Orange Peel (*Citrus sinensis*), and Garlic (*Allium sativa*) oil against microorganisms isolated from blood culture samples. We aim to contribute to the treatment of nosocomial bloodstream infections.

2. Material and Methods

2.1. Clinical Isolates

One hundred blood samples sent to Atatürk University Medical Microbiology Laboratory between 1 December 2021 and 1 January 2022 were analyzed with the BacTAlert (Biomerieux, France) blood culture system. Identification and antibiotic susceptibility of the growing isolates were studied with automated Vitek version 2.0 (Biomerieux, France). One hundred bacterial species identified in line with the cultivation of selective agars from the bottles were included in the study. Control of these bacteria was done with standard strains. Bacteria isolated from blood culture were passed. In accordance with Clinical Laboratory Standards Institute (CLSI) recommendations, a bacterial suspension was prepared from 24-hour bacterial colonies, equal to 0.5 Mc Farland turbidity [15, 16].

2.2. Preparation of the Medium

Microorganisms were inoculated on Eozyne Methylene Blue and blood agar to obtain new cultures from the stock culture to be used in antibacterial activity tests. A commercially purchased Tryptic Soy Broth medium to be used to determine the Minimum Inhibition Concentration (MIC) was prepared at 21 g/l in distilled water.

2.3. Preparation of Plant Extract Oils

In studies on surfactants, plant extract oils dissolve easily. Substances such as Tween 20, Tween 80 and Sodium dodecyl sulfate (SDS) have positively affected antibacterial activity [17-19]. Kuang et al. (2018) In their study, it was determined that the essential oil dissolved in Tween 20 has the strongest antibacterial activity [17]. In line with the studies, Tween 20 was preferred as the solvent. In this study, plant extract oils for antibacterial tests were prepared by filtering through 0.22 μm filters with a solution concentration of 10 % (v/v)[17].

2.4. Antibacterial Activity Tests

2.4.1. Determination of the Minimum Inhibition Concentration (MIC)

CLSI was applied for the identification and valuation of MIC [16]. St. John's Wort oil, *Nigella sativa* oil, Clove oil, Orange Peel, and Garlic oil were dissolved with Tween 20 to determine the final concentration of 500 μg/ml. In the Minimum Inhibition Concentration Determination method, ten microorganisms taken from Eosin Methylene Blue and blood agar were taken to the study and 24-hour fresh culture. The colonies taken from the prepared cultures prepared a bacterial suspension in sterile 0.9 % saline with a turbidity of 0.5 Mc Farland $5x10^5$ CFU/ml. Then, 100 μl of Tryptic Soy Broth medium was added to all wells 1 to 12 of the sterile 96-well microplate. First, 100 μl of the relevant commercial plant oil (500-50 μg/ml) was added to the

1st well, and 100 µl dilution was made up to the 10th well in a 1:1 ratio. Then, 100 µl of bacterial suspension was added to the 10th well. Bacteria control only bacterial suspension was added to the well, and only commercial plant extract oils were added to the 12th well. Then, the microplate was covered with parafilm and incubated for 24 hours at 37 °C. The last well without growth was determined as the Minimum Inhibition Concentration (MIC) value. The antibacterial effect of plant extracts was tested by minimum inhibitor concentrations (MIC) against ten control bacterial strains Gentamicin was used to control the study [20-24].

2.4.2. Determination of Minimal Bactericidal Concentration (MBC)

The lowest antibiotic concentration without turbidity was accepted as the MIC value. To determine the MBC values, ten µl was taken twice from all wells that did not show growth at the end of the incubation period. Colonies formed as a result of incubation were counted, and the lowest antibiotic concentration that killed 99.9 % of the initial inoculum was determined as the MBC value.

2.4.3. Kirby Bauer Disc Diffusion Method

Microorganism colonies taken from 24-hour cultures were adjusted in sterile saline to Mc Farland 0.5 turbidity and inoculated into Mueller Hinton medium with a swab stick. Plant extract oils were dissolved in Tween 20. Then, 6 mm diameter Sterile Paper Discs (Oxoid, Oxoid Antibacterial Susceptibility Blank Test Disc, Hampshire, UK) were placed on a Muller Hinton Medium containing an inoculated culture medium, then a 20 µg/mL sample was impregnated into the Oxford plate; 10 % Tween 20 solvent was used as a blank control. Gentamicin (10 µg/mL) was used as a positive. Zone diameters were measured after 24 hours of incubation [20-24].

3. Results

The antibiotic susceptibility results obtained from the automated system VITEK 2 (bioMerieux/ France) device were interpreted according to the European Committee on Antimicrobial Susceptibility Testing (EUCAST) criteria [25].

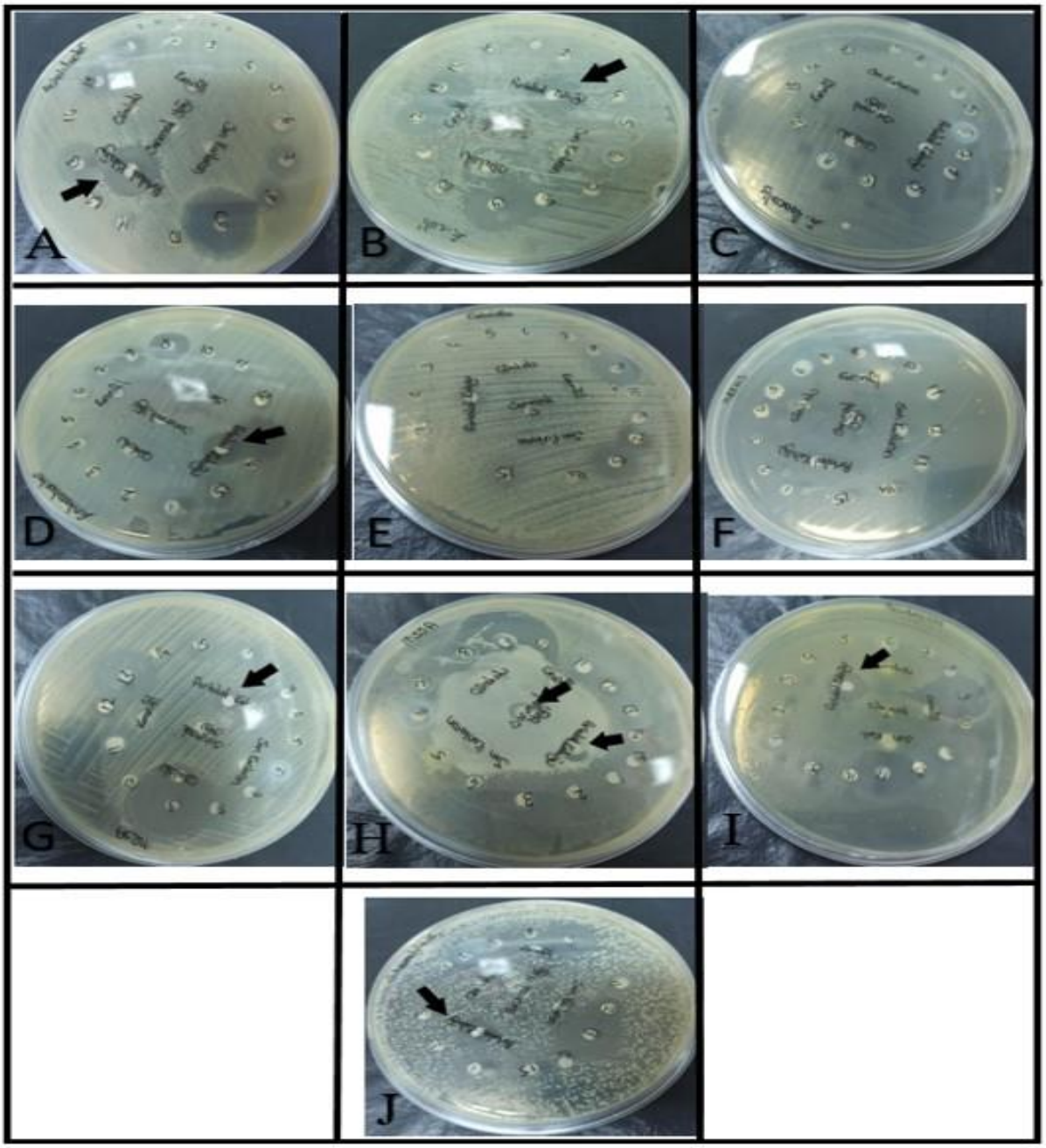
In our study, antimicrobial susceptibility testing of bacteria isolated from blood cultures using plant extract oil was performed by liquid microdilution method. The antibacterial effect of plant extract oils was detected on only 10 of the 100 clinical samples included in the study. No antibacterial activity was detected on the other 90 samples. The antibacterial effect of 10 microorganisms was determined, and MIC and MBC values are shown in Table 1. The lowest antibiotic concentration without turbidity was accepted as the MIC value. Colonies formed as a result of incubation were counted, and the lowest antibiotic concentration that killed 99.9 % of the initial inoculum was determined as the MBC value. The effect of plant extract oils on standard bacterial strains used in the study was evaluated by MIC, MBC and Kirby Bauer Disc Diffusion method. Kirby Bauer Disc Diffusion method determined all bacterial strains resistant to plant extract oils. In addition, the MIC value was determined as 31.25 µg/mL only in *Escherichia coli* ATCC 25922, *Klebsiella pneumoniae* ATCC700603 strains.

In our study, Kirby Bauer disc diffusion zone diameters of 10 microorganisms showing antibacterial activity are shown in Figure 1, and their sensitivity to oils is shown in Table 2.

4. Discussion

In the present study, antibacterial activity of plant extract oils (St. John's Wort (*Hypericum perforatum L.*) oil, Nigella sativa (*Nigella sativa*) oil, Clove (*Eugenia caryophyllata*) oil, Orange Peel and Garlic (*Allium sativa*) oil were investigated by determining the MIC, MBC values and inhibition zones on agar plates. All the tests determined that Orange Peel (Citrus sinensis) had inhibitory properties.

Orange Peel oil could not show antibacterial activity against *Klebsiella pneumoniae*, MRSA, and *Staphylococcus haemolyticus.* Orange Peel (*Citrus sinensis*) oil showed the most effective antibacterial effect against *Enterococcus faecalis* at 1.95 µg/mL. In a study on the in vitro antibacterial effect of Orange Peel essential oil against bacterial fish pathogens, the effect of the oil on microorganisms was examined, respectively, *L. anguillarum, Y. ruckeri, V. alginolyticus, V. salmoninarum, A. hydrophilave L. garvieae* has also been determined. It was determined that the pathogen in which the obtained essential oil showed the strongest antibacterial activity was *L. anguillarum* at 10% concentration. When the microdilution results were evaluated, the highest MIC value was found in *L. anguillarum* (31.25 µg/ml), followed by *Y. Ruckerive, V. salmoninarum* (62.5 µg/ml), *A. hydrophilave, V. alginolyticus* (125 µL/mL) respectively, followed by *L. garvieae* (250 µg/ml). It has been determined that orange oil is effective against *L. anguillarum*, which is the most effective bacteria, at a dose of 31.25 µg/ml [26].



*A. *Acinetobacter spp. Citrus sinensis* oil (25 mm) zone diameter, B. *Escherichia coli Citrus sinensis* oil (20 mm) zone diameter, D. *Enterobacter aerogenes Citrus sinensis* oil (14 mm) zone diameter, G. MRSA *Citrus sinensis* oil (10mm) zone diameter, H. MSSA *Citrus sinensis* oil (10 mm) and *Allium sativa* oil (10 mm) zone diameter, I. *Pseudomonas aureginosa Citrus sinensis* oil (12 mm) zone diameter, J. *Staphylococcus heamolyticus Citrus sinensis* oil (16 mm) zone diameter

Figure 1: Antibacterial zone diameters of plant extract oils by disk diffusion method.

Table 1: Minimum Inhibition Concentration and Minimal Bactericidal Concentration Values.

Clinical Isolates /*Plant Extracts*	**St. John's Wort oil**		**Nigella sativa oil**		**Clove oil**		**Orange Peel oil**		**Garlic Oil**	
	MIC (µg/mL)	MBC (µg/mL)	MIC (µg/mL)	MBC (µg/mL)	MIC (µg/mL)	MBC (µg/mL)	MIC (µg/mL)	MBC (µg/mL)	MIC (µg/mL)	MBC (µg/mL)
E. coli	125	93.75	62.5	46.87	125	93.75	125	93.75	7.81	5.855
K. pneumoniae	1.95	1,46	500 ≤	500 ≤	500 ≤	500 ≤	500 ≤	500 ≤	125	93.75
P. aeruginosa	31.25	23.42	62.5	46.87	62.5	46.87	62.5	46.87	62.5	46.87
E. faecalis	125	93.75	500 ≤	500 ≤	250	187.5	1.95	1.46	15.62	11.71
MSSA	250	187.5	250	187.5	250	187.5	125	93.75	125	93.75
MRSA	500 ≤	500 ≤	500 ≤	500 ≤	500 ≤	500 ≤	500 ≤	500 ≤	250 ≤	500 ≤
MRCNS	500 ≤	500 ≤	500 ≤	500 ≤	125	93.75	250	187.5	31.25	23.42
S. haemolyticus	7.81	5.85	3.9	2.44	500 ≤	500 ≤	500 ≤	500 ≤	7.81	5.855
E. aerogenes	7.81	5.85	500 ≤	500 ≤	250	187.5	250	187,5	3,9	2.44
A. baumannii	125	93.75	125	93.75	125	93.75	62.5	46.87	500 ≤	500 ≤

*Ten clinical samples were included in the study, St. John's Wort oil used in our study showed the most effective antibacterial effect of 7.81 µg/mL against *Staphylococcus haemolyticus* and *Enterobacter aerogenes*. *Nigella sativa* oil showed the most effective antibacterial effect against *Staphylococcus haemolyticus* at 3.9 µg/mL. *Orange Peel* oil showed the most effective antibacterial effect against *Enterococcus faecalis* at 1.95 µg/mL.

Table 2: Plant Extract Oil Sensitivity.

	P. aeruginosa			***K. pneumoniae***			***E. faecalis***			**MSSA**			**MRSA**			***E. coli***			**MRCNS**			**S. *haemolyticus***			***E. aerogenes***			***A. baumanii***		
	S	I	R	S	I	R	S	I	R	S	I	R	S	I	R	S	I	R	S	I	R	S	I	R	S	I	R	S	I	R
1			x			x			x			x			x			x			x			x			x			x
2			x			x			x			x			x			x			x			x			x			x
3			x			x			x			x			x			x			x			x			x			x
4			x			x			x			x			x			x			x			x			x			x
5	x					x			x			x		x			x			x		x			x			x		

1- St. John's Wort (*Hyperic um perforatum L.*) oil, 2- *Nigella sativa* oil, 3- Clove (*Eugenia caryophyllata*) oil, 4- Garlic (*Allium Sativa*) Oil, 5- Orange Peel (*Citrus sinensis*) oil.

*S: Sensitive, I: Intermediate, R: Resistance.

In our study, clove oil showed a MIC value of 62.5 µg/mL as effective on *Pseudomonas aureginosa*. It did not show any inhibitory properties on any microorganisms in the Kirby Bauer Disc diffusion method.

Emeka et al. (2015) examined black cumin oil against *Staphylococcus aureus* isolated from the wounds of 34 diabetic patients at varying concentrations by pit diffusion method. While 8 of 19 isolates were sensitive to undiluted oil samples, 4 were sensitive to 200 mg/mL, 400 mg/mL, and 800 mg/mL, and 11 were resistant to all oil concentrations. They found that more than half of the isolates were sensitive to black cumin oil at different concentrations [28]. Disc diffusion method, which we used to determine the antibacterial activity of black cumin oil against ten microorganisms, we were unable to detect any inhibitory effect against any microorganisms. We believe this is not the effective amount of the stock solution (500 µg/ml) we used compared to the studies.

Allium sativum L. (Kastamonu and Denizli local) in a study on the comparison of the chemical compound, the antibacterial and antioxidant activity of essential oils of the plant, the inhibition zone of *Escherichia coli* MC 4100 strain was 13 and 12 mm, *Pseudomonas aeruginosa*, in which Garlic essential oil was applied as 30 µl/disc. *Pseudomonas aeruginosa* NRRL-B-2679 strain, 6 and 5 mm, *Enterobacter aerogenes* NRRL-B-3567 strain 9 and 7 mm, *Staphylococcus aureus* ATCC 33862 strain 9 and 11 mm; In the petri dish with 50 µl/disk, the inhibition zone was measured as 15 and 15 mm. *Escherichia coli* MC 4100 strain 15 and 15 mm, Pseudomonas aeruginosa in a petri dish containing 50 µl disc. *Pseudomonas aeruginosa* NRRL-B-2679 strain was 11 and 10 mm, *Enterobacter aerogenes* NRRL-B-3567 strain was 15 and 14 mm, *Staphylococcus aureus* ATCC 33862 strain was 12 and 14 mm [29]. Kim et al. (2004) while they found the antimicrobial activity of the garlic oil obtained to be high, and O'Gara et al. (2000) found that the isolated was low [30, 31]. In our study, while 10 mm zone diameter was detected only on MSSA in the disc diffusion method, it could not show any antibacterial activity on other microorganisms.

In the present study, no inhibition zone was observed on agar plates of Tween 20 extract of *Hypericum perforatum L.* against microorganisms; *Pseudomonas aeruginosa*, *Klebsiella pneumoniae*, *Enterococcus faecalis*, MSSA, MRSA, *Escherichia coli*, MRCNS, *Staphylococcus haemolyticus*, *Enterobacter aerogenes* and *Acinetobacter baumannii*. In this context, the two studies show parallels with each other. The fact that another does not support only the use of the disc diffusion method in vitro study is incomplete in evaluating the results. However, in our study, observing antibacterial activity against many microorganisms can be based on the fact that the strain used is not a reference strain and therefore can be resistant to the extracts used (not tried against comparative antibiotics). Chemicals used as solvents are likely to differ in studies with essential oils. Due to the fact that plant essential oils have different chemical structures, not every oil dissolves to the same degree in the same solvent. On the other hand, since the solvent used is also different, this difference may also be associated with the solvent's ability to decode active compounds.

5. Conclusion

Garlic (*Allium sativa*) oil on *Escherichia coli*, *Staphylococcus haemolyticus* and *Enterobacter aerogenes*, St. John's Wort oil on *Staphylococcus haemolyticus* and Enterobacter aerogenes, *Nigella sativa* oil on *Staphylococcus haemolyticus* has been found to be effective. We think more studies are needed to determine the effect of other doses and time. Studies should shed light on what components might be responsible for the antimicrobial activity of these extracts against target isolates.

Limitations of the Study

The fact that no antibacterial activity was found in 90 out of 100 samples in the study leads to the thought that higher ranges should be selected from these dose ranges in future studies. In addition to Tween 20, other solvents can be preferred as a degreaser. Low dose and solvent type were limitations of the study.

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Conflict of Interests

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Author Contributions

Conceptualization, ÖÇ, SB, MCG, DÇ and S.Ç; methodology, ÖÇ, SB, MCG, DÇ and S.Ç; validation, ÖÇ, SB, MCG, DÇ and S.Ç; formal analysis, ÖÇ, SB, MCG, DÇ and S.Ç; investigation ÖÇ, SB, MCG, DÇ and S.Ç; resources, ÖÇ, SB, MCG, DÇ and S.Ç; data curation ÖÇ, SB, MCG, DÇ and S.Ç; writing-original draft preparation, ÖÇ, SB, MCG, DÇ and S.Ç; writing-review and editing, ÖÇ, SB, MCG, DÇ and S.Ç; visualization ÖÇ, SB, MCG, DÇ and S.Ç; supervision ÖÇ, SB, MCG, DÇ and S.Ç; project administration ÖÇ, SB, MCG, DÇ and S.Ç.

Ethical Approval

Ethical permission was obtained from the Atatürk University Medical Faculty Clinical Research Ethics Committee for this study with date 04.11.2021 and number 07, and Helsinki Declaration rules were followed to conduct this study.

Data sharing statement

Not applicable.

Consent to participate

Not applicable.

Informed Consent
Not applicable.

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