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Antibacterial Effect of Microalgae (*Chlorella vulgaris*, *Scenedesmus obliquus*, *and Spirulina platensis*) Extracts Against Gram-Negative (*Escherichia coli*, *Yersinia ruckeri* and Gram-Positive *Staphylococcus aureus*, *Enterococcus faecalis*) Bacteria

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Abstract: Algae possess antibacterial, antifungal, antiviral, antiparasitic, antioxidant, anticancer properties, serving as a rich source of various bioactive compounds. In this context, the antibacterial activities of the essential oil components of *Chlorella vulgaris, Scenedesmus obliquus, Spirulina platensis* were investigated in the study. For this purpose, 10% ethanol-methanol was extracted for 48 hours for antibacterial analyses, the solvents were evaporated in a rotary evaporator at 92°C. 10% stock solutions of the obtained algal extracts were prepared with dimethyl sulfoxide. Antibacterial tests were carried out with 10, 5, 1, 0.1% dilutions of stock solutions. The effects of methanol-ethanol dilutions of algae on Gram-negative (*Escherichia coli, Yersinia ruckeri*), Gram-positive (*Staphylococcus aureus, Enterococcus faecalis*) have been investigated. For this purpose, disk diffusion, minimal inhibitory concentration, minimum bactericidal medium tests were performed. In addition, 25% methanol extracts were prepared for the analysis of oil components found in algae by gas chromatography-mass spectrometry. As a result, it was determined that only 10% methanol extract of *S. platensis* had antibacterial activity against *S. aureus* (zone 15-16 mm), the minimum inhibitory concentration was 8 mg L^{-1}, the minimum bactericidal concentration was 16 mg L^{-1}. It was observed that ethanol-methanol extracts of the other two algae, ethanol extract of S. platensis, did not have antibacterial activity. A total of 22 essential oil components were found in the algae. The highest oleic acid levels were found in S. platensis at 69.31%, while it showed the highest antibacterial activity only against *S. aureus* bacterium.

Keywords: Antibacterial activity, Chlorella vulgaris, disk diffusion, Scenedesmus obliguus, Spirulina platensis.

INTRODUCTION

Microalgae are microscopic organisms that can be prokaryotic, such as cyanobacteria, or eukaryotic, such as green algae [1]. Microalgae can be found in various aquatic environments, can survive even in adverse conditions [2]. Intensive research is needed to identify algae that can grow in adverse conditions, show promising antibacterial activity [3]. Microalgae are used as live feed for various stages of crustaceans, mollusks, and marine fish larvae in aquaculture [4]. They are natural resources that people have begun to use for their

benefit [5]. They help maintain the ecological balance of the world by producing various metabolic substances, including carbohydrates, enzymes, lipids, and antimicrobial compounds [6, 7]. They are also used as food raw materials, additives in many industrial areas [8, 9, 10]. The antimicrobial activities of microorganisms such as *Chlorella* sp., *Spirulina* sp. have been studied; it has been reported that they have inhibitory effects on various pathogens. The antibacterial activity of cyanobacteria has been associated with some compounds, such as alkaloids, fatty acids, eicosapentaenoic acid (EPA), hexadecatrienoic acid, unsaturated fatty acids such as palmitoleic acid, indoles, macrolides, peptides, phenols, pigments, and terpenes [11, 12, 13]. In addition, microalgae can produce both polar, non-polar oligopeptides, proteins in bacterial cytoplasmic membranes [14]. Antimicrobial peptides are considered as potential alternatives to antibiotics due to their broad antibacterial spectrum [15]. Polyunsaturated fatty acids (PUFAs) play an important role in the defense of microalgae. Antimicrobial activity depends on the chain length and, degree of unsaturation. The composition, concentration of free lipids should be taken into account [16].

In recent years, the use of microalgae as a source of antimicrobial compounds has attracted attention due to the emergence of antibiotic-resistant bacteria. The production of antimicrobial compounds by microalgae provides a natural defense mechanism against microorganisms in their environment [10]. Microalgae have promising potential for biotechnological applications, and can even be used to create products with antibacterial properties alone [10,17]. Bacterial infections are a major problem in aquaculture as they can lead to high mortality rates in cultured fish, invertebrate populations [18]. However, the use of antibiotics in areas such as medicine, agriculture, and aquaculture still has serious negative effects on the environment [19]. Excessive use of antibiotics transforms beneficial bacteria in the body into harmful ones; it promotes the development of pathogens resistant to standard antimicrobial practices [30]. The increase in antibiotic resistance indicates an urgent need for new antibacterial sources [3]. Therefore, it is well known that many biotechnological products rich in active compounds, such as microalgae, have the potential to be developed [17, 20]. Under the influence of environmental stress, microalgae change their internal mechanisms, and the possibility of synthesizing valuable metabolites with different properties arises [21, 22].

In this study, the antibacterial activities of the essential oil components of microalgae (*S. platensis*, *S. obliquus*, *C. vulgaris*) extracts produced using different solvents (methanol, ethanol) were investigated. Antibacterial activities of the extracts have been obtained from algae were determined against Gram (-) *Escherichia coli* (ATCC 43895), *Yersinia ruckeri* (Wiklund, T., Finland), Gram (+) *Staphylococcus aureus* (ATCC 25923), and *Enterococcus faecalis* (ATCC 29212) bacteria using the disk diffusion method.

MATERIAL AND METHODS

Microalgae Material

Chlorella vulgaris, Scenedesmus obliguus, and *Spirulina platensis* were supplied by Çukurova University, Faculty of Fisheries. Convay nutrient medium Walne [23] was used for *C. vulgaris, Scenedesmus obliguus,* and Schlösser [24] nutrient medium for *S. platensis.*

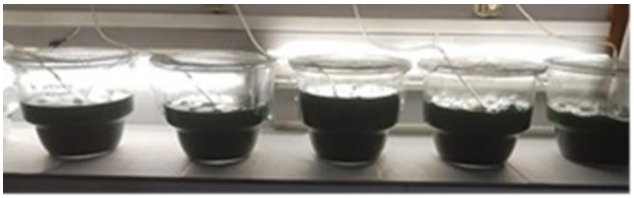

Figure 1: Microalgae grown in a 10 L glass cylinder container

Microalgae were cultured in cylindrical 10 L glass containers using daylight fluorescent lamps under continuous illumination at 22±3˚C (Figure 1). Microalgae were harvested at maximum density, algae were centrifuged for wet biomass. Algae were washed with distilled water, placed in pre-weighed 50 mL Falcon tubes. Centrifuged at 3000 rpm for 5 min, weighed wet.

Reference Bacteria

Two Gram (-) bacilli, Escherichia coli, Yersinia ruckeri two Gram (+), Staphylococcus aureus, and Enterococcus faecalis were used.

Extraction of Microalgae

For each algae, 10% dilutions were prepared by adding methanol to two 15 mL Falcon tubes, ethanol to the other. Extract samples were shaken under UV light for 48 h. Samples were filtered through sterile filter paper containing methanol-ethanol in a tarred, ground glass bottle, and evaporated at 92 °C with a rotary evaporator. Samples were weighed, and 10% stock solutions were prepared with Dimethyl sulfoxide. Stock solutions were stored at +4 °C [25].

Determination of Volatile Oil Components of Algae Extracts by GC-MS

Analyses were performed at Mersin University Advanced Technology Education, Research, and Application Center. Microalgae were extracted with 25% methanol for 48 hours.

Preparation of Reference Strains

Reference strains were revived in Nutrient Broth at 35ºC for 18-24 hours, isolated on Tryptic Soy Agar at the same temperature, time. Fresh colonies were suspended in salinity McFarland 0.5 (1% $BaCl_2$, 9.5 mL, sulfuric acid 0.5 mL) (1.5x108 cfu mL^{-1}).

Measurement of Antibacterial Activity

Antibacterial activities were performed by the disk diffusion method. After inoculation of reference strains in suspension onto Müller-Hilton Agar with sterile swabs, antibacterial disks, and blank disks appropriate for the bacterial species were placed. 20 µL of (10, 5, 1, 0.1)% algae dilutions, 10 µL of ethanol, methanol, and DMSO for controls were placed on the blank disks. Inhibition zones were measured after 24 h of incubation at 35 °C. Pozitif kontrol

amacıyla *S. aureus* için de gentamisin ve eritromisin, *E. faecalis* için siprofloksasin ve vankomisin, *E. coli* için siprofloksasin (CIP, 5 µg) ve trimetoprim-sulfametoksazole (SXT, 1,25-23,75 µg) ve *Y. ruckeri* için siprofloksasin (CIP, 5 µg) ve enrofloksasin diskleri (Becton Dickenson BBL, ABD); negatif kontrol amacıyla da steril DMSO ve distile su kullanılmıştır. Studies were conducted in a double-blind parallel design (EUCAST [26]; Table 1). The evaluation was made according to the reference values of antibacterial drugs. Accordingly, no zones were evaluated as ineffective, zone diameters <7-9.9 mm as weakly effective, 10-11.9 mm as moderately effective, and≥12 mm above were evaluated as highly effective [27].

Minimal Inhibitory Concentration Measurement

The MIC test was performed in 96-well microplates using the microtiter broth dilution method EUCAST [26] with a 10% main stock solution of *S. platensis*, which was found to be effective against *S. aureus* based on disk diffusion results. Stock solutions of erythromycin, thiocyanate, and ciprofloxacin were prepared as antibacterial drugs, used at a concentration of 512 µg mL^{-1}. Appropriate dilutions of 0.1 N HCl were used to dissolve ciprofloxacin, 95% ethyl alcohol for erythromycin, and DMSO used to dilute algae were prepared with distilled water. Reference bacteria solutions ($1x10^8$ cfu mL^{-1}) prepared according to McFarland 0.5 were used by diluting 1:20 with salinity ($5x10^6$ cfu mL^{-1}). The minimum bactericidal concentration of Müller-Hinton Broth was used as medium. Tests were repeated twice for reference bacteria. After 24 h of incubation at 35°C, turbidity in the wells was considered bacterial growth. The first well without turbidity was considered the minimum inhibitory concentration value.

Minimum Bactericidal Concentration Measurement

10 µL of liquid was taken from the first well without growth in the microplate, from all previous wells without turbidity, and incubated at 35°C for 24 hours. The first dilution without growth was considered MBC [28].

RESULTS AND DISCUSSIONS

Antibacterial Effects of Microalgae (*S. platensis*, *S. obliquus*, *C. vulgaris*) Extracts Obtained Using Different Solvents (methanol-ethanol) on Bacteria (*E. coli*, *Y. ruckeri*, *S. aureus and E. faecalis*)

This study investigated the antibacterial effects of extracts obtained from algae (*S. platensis, S. obliquus, C. vulgaris*) produced under suitable culture conditions using different solvents (methanol-ethanol). The antibacterial effects of algal extracts on the bacteria used in the study (*Escherichia coli, Yersinia ruckeri, Staphylococcus aureus*, and *Enterococcus faecalis*) were examined using the disk diffusion method. According to the data obtained, it was determined that the methanol extract (SP) of *S. platensis* was effective against *S. aureus* in a 10% dilution stock solution; disk diffusion, minimum inhibitory concentration (MIC) test, and minimum bactericidal concentration (MBC) tests were performed for antibacterial activity measurements. The antibacterial activities of the algal samples by the disk diffusion method are given in Tables 1, 2; Figures 2, 3, and 4.

Table 1: Antibacterial Activity Zones of 10% Methanol Extracts on Algae, Antibacterial Drugs

Bacterıa Species	Medicines Treatment	Antibacterial Activity Zones (mm)					
		Chlorella vulgaris		*Scenedesmus obliquus*		*Spirulina platensis*	
E. coli	CIP	38	40	40	40	20	20
	SXT	27	27	28	28	30	30
	%10 ekstract	0	0	0	0	0	0
Y. ruckeri	CIP	36	30	40	38	22	22
	ENR	30	30	36	36	38	38
	%10 ekstract	0	0	0	0	0	0
S. aureus	GN	27	23	23	24	25	25
	E	26	26	26	28	26	26
	%10 ekstract	0	0	0	0	16	15
E. faecalis	CIP	20	20	20	20	24	24
	VA	20	20	20	20	18	18
	%10 ekstract	0	0	0	0	0	0

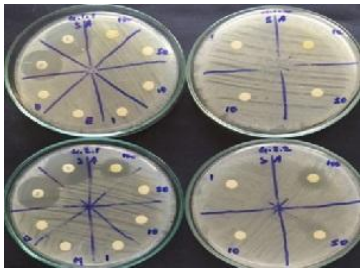

Figure 2: Antimicrobial activity of *S. platensis on S. aureus* bacteria in methanol solution (See 100)

It was determined that the 10% dilution of the methanol extract of S. platensis, whose antibacterial activity was investigated by disk diffusion method, was effective against *S. aureus*, while other dilutions and all dilutions of the ethanol extract were ineffective. Both extracts of the other two algae were found to be ineffective It was observed that ethanol, DMSO, HCl used as positive controls did not show any antibacterial effect.

Table 2: Minimal inhibitory concentration of S. platensis against S. aureus

Bacteria Control (SA)	1	2	3	4	5	6	7	8	9	10	11	12
Dilations (µL mL^{-1})	256	128	64	32	16	8	4	2	1	0,5	0,25	0,125
Ciprofloxasin	-	-	-	-	-	-	-	-	-	-	-	+
Eritromisin	-	-	-	-	-	-	-	-	-	-	-	+
Spirulina	-	-	-	-	-	-	+	+	+	+	+	+
Metil Alkol	+	+	+	+	+	+	+	+	+	+	+	+
DMSO	+	+	+	+	+	+	+	+	+	+	+	+
Etil Alkol	+	+	+	+	+	+	+	+	+	+	+	+

C: Ciprofloxacin, E: Erythromycin, SP: S. *platensis*, M: Methanol, D: Dimetil sülfoksit (DMSO), Et: Ethanol, H: HCl, MHB: Müller Hinton Broth, SA: Bacteria control

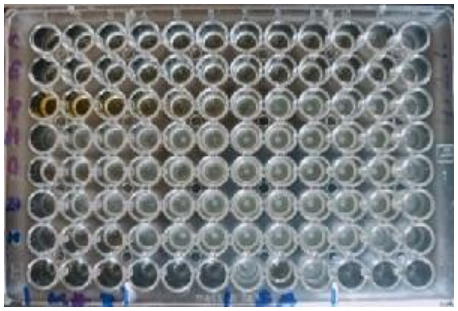

Figure 3: *S. aureus* bakterisinin Minimal inhibitory concentrations (MIC) test results

According to the MIC test results, the MIC test was performed on the 10% dilution of methanol extract of *S. platensis*, which had an antibacterial effect only against *S. aureus*; the minimal inhibitory concentration was determined as 8 mg L^{-1}. As a result of the MIC test, starting from the first well where there was no growth/turbidity in the microplate, including the first well where turbidity/growth was observed, growth was observed in the samples taken from the 6^{th}, 7^{th} wells as a result of the inoculation on TSA from 7 wells. Thus, the minimum bactericidal concentration of the 10% dilution of the methanol extract of *S. platensis* against *S. aureus* was determined as 16 mg L^{-1} (Table 2; Figures 3, 4).

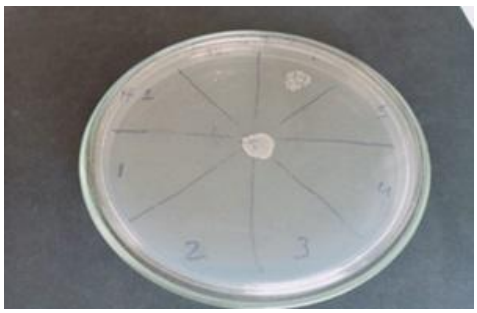

Figure 4: Minimum bactericidal concentrations (MBK) test result

Volatile Fatty Acid Chemical Components of Algal Extracts Determined by GC-MS

Volatile chemical components determined from the 25% methanol extract of algae; a total of 22 different components were determined, 11 in *Chlorella vulgaris*, 9 in *Scenedesmus obliquus*, 8 in *Spirulina platensis* (Table 3). Of these components, oleic acid was the highest, determined as 51.67%, 27.42%, 69.31%, respectively. It was observed that there were two chemicals common to all three algae: 2-Hexadecene-1-ol, oleic acid.

Table 3: Volatile fatty acid Component Rates of Chlorella vulgaris, Scenedesmus obliquus, Spirulina platensis

Number	Volatile fatty acid Chemical Components	CV (%)	RT (dk)	SC (%)	RT (dk)	SP (%)	RT (dk)
1	Dodecanol Trimethyl	0,91	20,65	2,42	20,8		
2	Bicyclo Heptane	8,76	20,73				
3	2-Hexadecene-1-ol	2,95	20,80	12,6	23,9	8,42	23,9

4	9-Octadecen-1-ol	3,26	21,03				
5	Eicosyn	4,50	21,26				
6	Hexadecanoic acid	2,83	21,78				
7	Tetrahydroxycyclopentadienone	0,94	22,32				
8	Octadecadienoic acid	3,97	23,67	3,57	15,4		
9	Linoneic Acid	2,84	23,75				
10	Benzyl Methyl Eter	6,39	23,92				
11	Oleic Acid	51,7	32.81	27,4	32,3	69,31	33,2
12	Neophytadine			4,22	20,7		
13	2 Methyl Octadecyne			1,06	21,0		
14	Neophytadine			1,66	21,3		
15	Octadecadienoyl Chloride			2,42	21,5		
16	Pentadecanoic Acid			6,97	21,8		
17	Methylnonadecane					1,86	18,9
18	Neophytadine					1,50	20,7
19	Octadecenoic acid					3,43	21,6
20	Tetradecanoic acid					2,45	21,8
21	Octadecadienoyl chloride					3,99	23,5
22	Octadecenoic acid					3,84	33,5
	Total fatty acid ratio	61,3		37,96		79,0	
	Total (%)	89,2		62,34		94,8	
	Number of fatty acid components	11		9		8	

CV(%): Chlorella vulgaris, SC(%): Scenedesmus obliguus, SP(%): Spirulina platensis, RT: Rete

Microalgae are used as a sustainable resource for the production of high-value compounds. A significant portion of microalgal biomass has the potential for producing various high-value compounds, including polyunsaturated fatty acids (PUFAs), carotenoids, phycobiliproteins, polysaccharides, and phytotoxins. Many studies have shown that fatty acids, proteins, polysaccharides, lipids, vitamins, enzymes, and active compounds in the structure of some microalgal species are effective against bacteria, viruses, fungi, and cancer [29]. Furthermore, studies have indicated that continuous antibiotic use can cause beneficial bacteria in the body to become harmful, leading to hormonal imbalances, digestive problems, and a negative impact on the immune system [30]. To solve this problem, it is necessary to discover alternative antibiotics and compounds with antibacterial properties [31]. Additionally, species such as *Chlorella* sp., *Scenedesmus* sp., *Spirulina* sp., *Oscillatoria* sp., *Phormidium* sp., and *Microcystis* sp. are also studied. The antimicrobial activities of microalgae such as those mentioned have been investigated, and their inhibitory effects on various pathogens have been noted [32, 33, 34]. The antibacterial activity of microalgal extracts has been investigated by various researchers [35], and green algae such as *Chlorella* sp. and *Scenedesmus* sp. have been reported to be a valuable source of a wide variety of bioactive compounds with antimicrobial activity. The first antibacterial compound discovered in algae is a substance called "chlorelin" in the *Chlorella* algae. This substance has an inhibitory effect on both gram-positive and gram-negative bacteria. These include *Bacillus subtilis*, *Pseudomonas aeruginosa*, *Streptococcus pyogenes*, and *Staphylococcus aureus* bacteria [36].

Blue-green algae support the body's protection against radiation thanks to 31 chlorophyll compounds and maintain the balance of minerals, vitamins, and nutrients [37].

Compounds such as polyunsaturated fatty acids (PUFAs) and cholesterol play a significant role in the defense of microalgae [34]. Furthermore, antimicrobial activity depends on chain length, degree of unsaturation of the fatty acid [38], composition, and free fat concentration [39]. Compounds synthesized by *Scenedesmus costatum* have been found to possess antibacterial activity against aquatic bacteria. High levels of linoleic acid found in *Spirulina* stimulate the synthesis of prostaglandin (PGE1), a hormone that affects blood cholesterol levels [40]. Additionally, *Spirulina* is used in the treatment of AIDS, skin, and stomach cancer due to its known antitumor properties [41]. Extracts obtained from *Nostoc, Phormidium, Anabaena, Oscillatoria, Pseudoanabaena, Synechocystis, Oscillatoria anguistisima*, and *Calothrix parietina* have been reported to have antimicrobial effects on some pathogenic organisms. Additionally, algal extracts obtained from different solvents have been reported to be effective against both Gram (+) and Gram (-) organisms [42]. Bioactive compounds such as lutein, astaxanthin, and zeaxanthin found in *Chlorella protothecoides* possess antioxidant activity; their effectiveness against bacterial and fungal microorganisms is particularly emphasized [43, 44]. According to Maadane et al. [45], the antioxidant activity of algae, especially *Chlorella* species, increased over time and showed the highest antioxidant effect at 120 minutes.

According to Özdemir et al. [32], the antibacterial activities of *S. platensis* against *S. aureus* and *E. coli* bacteria were determined in methanol, ethanol, chloroform, acetone, and hexane solvents; it was found that the methanol extract of *S. platensis* showed greater antimicrobial activity against *S. faecalis* and *S. epidermidis* compared to other solvents. The antibacterial activity of *S. platensis* extracts was determined as 7 mg mL^{-1} in the methanol extract of *S. aureus*. Kreitlow et al. [42] investigated the antimicrobial activities of various isolates of *Anabaena, Oscillatoria, Pseudoanabaena*, and *Synechocystis* and found them to have antimicrobial activity against various pathogenic microorganisms. Scheuer [46], in his study on algae belonging to the *Chlorophyta, Rhodophyta,* and *Phodophyta* groups, reported that methanol extracts showed greater antimicrobial activity than n-hexane and ethyl acetate extracts. On the other hand, chloroform extracts have been reported to have better antimicrobial activity than methanol and benzene extracts [47].

According to Demiriz [48], the antibacterial activities of algal extracts (*Chlorella vulgaris, Oscillatoria limosa, Oscillatoria limnetica, Phormidium tenue, Spirulina major*) produced using different solvents (acetone, ethanol, hexane, methanol, n-butanol, 0.5 M Tris-HCl, pH: 8.00) were investigated using the disk diffusion method. The antibacterial effects of algal extracts were tested against *Bacillus subtilis, Escherichia coli, Staphylococcus aureus*, and *Salmonella enteritidis* bacteria. *Spirulina major* was determined to have the highest antibacterial activity. Other researchers have reported that the antimicrobial activity found in *S. platensis* extracts may be due to the synergistic effect of γ-linolenic acid, active fatty acids, lauric, and palmitoleic acids [34]. The antimicrobial activity of microalgae can be explained by the presence of cyclic peptides, alkaloids, and lipopolysaccharides [33]. Many previous studies have found methanol, acetone, and hexane solvents to be effective [49]. The antimicrobial activity of *S. platensis* extracts prepared using three different solvents was investigated by the disk diffusion method. The best antimicrobial activity was found in the *Spirulina* extract obtained from methanol; this extract showed antibacterial activity against five out of seven bacteria (*B. cereus, B. subtilis, E. coli, K. pneumoniae, P. aeruginosa*). The extract obtained from acetone showed antibacterial activity only against *P. aeruginosa*, while the extract obtained from hexane

showed antibacterial activity against *B. cereus* and *B. subtilis*. The inhibitory zone of *S. platensis* extracts against bacteria ranged from 12 mm to 10 mm at 30 mg mL^{-1}. The methanol extract was reported to form an inhibitory zone at 15 mg mL^{-1} (*S. aureus* except *E. faecalis*), but showed no effect at lower concentrations. The acetone extract showed antibacterial activity only against *P. aeruginosa* bacteria, forming an inhibitory zone of 10 mm at 30 mg mL^{-1}. The hexane extract showed antibacterial activity against *B. cereus* (30, 15 mg mL^{-1}) and *B. subtilis* (30, 15, 6 mg mL^{-1}); the inhibition zone diameter was determined to be 9 mm at 15 mg mL^{-1} for *B. cereus* and 9 mm at 6 mg mL^{-1} for *B. subtilis*. When the results of previous studies on the antimicrobial activities of algal extracts were examined, it was reported that the methanolic extract was more effective against bacterial strains [50]. In our study, it was determined that the antibacterial activity of *S. platensis* algal extracts against *Staphylococcus aureus* bacteria was significantly higher in methanol solvent compared to ethanol solvent.

Alsenani et al. [13] did not detect eicosapentaenoic acid in *S. obliquus* extracts, but determined the presence of other long-chain fatty acids with antibacterial activity. Fatty acids with more than 10 carbon atoms have been proven to cause bacterial degradation. Oleic and linoleic acids found in microalgae species have been highlighted as effective against pathogenic bacteria in many organisms [43]. According to many studies, oleic acid lowers cholesterol levels and supports heart health. Oleic acid has anti-inflammatory properties and supports cell health by increasing the flexibility of cell membranes. Oleic acid, along with other free fatty acids, strengthens the immune system and fights infections [51].

Spirulina is rich in gamma-linolenic acid, alpha-linolenic acid, oleic acid, EPA, and DHA [52]. The essential fatty acid oleic acid is a fatty acid that occurs naturally in various animal and plant oils. The antimicrobial or other biological activities of essential oils are related to the presence of bioactive volatile components [53]. The mechanism of action of essential oils is linked to the ability of phenolic monoterpenes to alter microbial cell permeability, disrupt cytoplasmic structure, interfere with the cellular energy system, and impair proton transport. Impaired cell permeability leads to the leakage of molecules and ions out of the cell, and thus to cell death. Lipoteichoic acids present in the cell membrane of Gram-positive bacteria facilitate the penetration of hydrophobic compounds of essential oils into the cell, while the lipopolysaccharide layer in the outer membrane of Gram-negative bacteria limits the diffusion rate of hydrophobic compounds. Therefore, Gram-positive bacteria are more susceptible to essential oils than Gram-negative bacteria [54]. In our study, the highest oleic acid content was found in *S. platensis* algae at 69.31%, indicating the highest level of antibacterial activity against Staphylococcus aureus, a Gram-positive bacterial species. This is thought to be related to the presence of bioactive volatile fatty acid components. According to El-Baz et al. [37], the antibacterial and antiviral effects of ethanol extracts of *S. platensis* were tested, and their antibacterial effects against different bacterial species (*Escherichia coli, Salmonella typhi*, *Enterococcus faecalis*) were determined by the disk diffusion method. While it was observed that it did not form an inhibition zone with *Escherichia coli, Salmonella typhi* (gram-negative bacteria), and *Staphylococcus aureus* (gram-positive bacteria), it was determined that it formed a significant inhibition zone against *Enterococcus faecalis* and *Candida albicans*. According to Heidari-Soureshjani et al. [21], a study conducted found that *Escherichia coli*, a significant bacterial species in infections, had a C18:1 oleic acid ratio of 35-50% in sesame oil and 55-

83% in olive oil in vitro. The synergistic effect of the combination of the two oils was investigated. As a result of the study, it was reported that both oils and their combinations inhibited bacterial growth and that the antibacterial properties of sesame oil were approximately equal to those of olive oil and the sesame oil mixture. In Katırcıoğlu et al. [33], antibacterial activities against *Bacillus subtilis, Bacillus thuringiensis, Bacillus cereus, Bacillus megaterium, Yersinia enterocolitica, Escherichia coli, Staphylococcus aureus, Micrococcus luteus, Micrococcus flavus,* and *Pseudomonas aeruginosa* were determined using microalgae (*Chlorella vulgaris, Anabaena* sp., *Oscillatoria* sp., *Synechocystis aquatilis*, *Chrococcus* sp.) and methanol, chloroform, acetone, ethanol, and ether solvents; it was observed that acetone and ether extracts showed antimicrobial activity against Gram-negative bacteria, methanol extracts against Gram-positive bacteria, and ethanol extracts against both Gram-negative and Gram-positive bacteria. Another study found that methanol extracts had higher antibacterial activity than hexane acetate extracts [47]. Our study also determined that methanol extracts exhibited antibacterial activity against Gram-positive bacteria.

CONCLUSIONS

This study determined that *S. platensis* extracts obtained with methanol and ethanol solvents showed high antibacterial activity against *Staphylococcus aureus*, especially in the methanol solvent. The 10% methanol extract of *S. platensis* was found to have a zone diameter of 15-16 mm, a minimum inhibitory concentration of 8 mg L^{-1}, and a minimum bactericidal concentration of 16 mg L^{-1}. The ethanol and methanol extracts of the other two algins and the ethanol extract of *S. platensis* showed no antibacterial activity. GC-MS analysis of the algae revealed a total of 22 volatile oil components. The highest oleic acid levels were observed in all three algae: 69.31% in *S. platensis*, 51.67% in *C. vulgaris*, and 27.42% in *S. obliquus*. It was determined that S. platensis has the highest level of oleic acid fatty acids at 69.31%. It was concluded that the antibacterial activity of *S. platensis* extract stems from antioxidant compounds such as oleic acid, an essential oil component. *Spirulina* is rich in gamma-linolenic acid, alpha-linolenic acid, oleic acid, EPA, DHA, and linoleic acid. Furthermore, oleic acid and other free fatty acids strengthen the immune system, fight infections, and have been proven effective against pathogenic bacteria in many living organisms. Additionally, an important characteristic of essential oils is that they are fatty acid components with a hydrophobic effect. This property makes the cell more permeable. Cell permeability allows molecules and ions to leak out of the cell, thus leading to cell death. Lipoteichoic acids, present in the cell membrane of Gram-positive bacteria, facilitate the diffusion of hydrophobic compounds like volatile oils into the cell, while the lipopolysaccharide layer on the outer membrane of Gram-negative bacteria limits the diffusion rate of hydrophobic compounds. Therefore, Gram-positive bacteria are more sensitive to volatile oils than Gram-negative bacteria. In the study, *S. platensis* showed the highest antibacterial activity against *S. aureus*, a gram-positive bacterium.

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