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Research Article

Antimicrobial Activity of Algal Extracts Against Foodborne Pathogens

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Abstract

Algal biotechnology has been gaining increased attention to be evaluated in pharmaceutical and nutraceutical industries. Since proteins, carbohydrates, fatty acids, vitamins, minerals, pigments and many other important metabolites accumulate in their cells, algae are used by humans as the main nutritional support and food additive for various purposes. Algal bioactive compounds such as oleic acid, linoleic acid, palmitoleic acid, vitamin E, β-carotene, lutein and zeaxanthin have antimicrobial, antioxidant, antifungal and antiviral properties and play an important role in the reduction and prevention of foodborne diseases. Bioactive compounds of microalgae should be investigated in order to develop new pharmaceuticals and to provide chemical and pharmacological innovation. Various microalgae extracts are known to have in-vitro antimicrobial activity against pathogenic microorganisms. The aim of this study was to investigate the antifungal and antibacterial effects of the extracts of *U. lactuca* macroalgae and *C. vulgaris, C. minutissima* and *C. protothecoides* microalgae against *Fusarium oxysporum* fungal microorganisms and *Mycobacterium smegmatis* RUT, *Proteus mirabilis* BC6624 and *Aeromonas hydrophila* ATCC7965 bacterial microorganisms. The antimicrobial effects of the extracts were tested on fungal and bacterial microorganisms by using agar disk diffusion method. As a result of this study, the inhibition zone diameter of algae against *F. oxysporum* was found to be 53.00 mm for *C. vulgaris*; 59.00 mm for *C. minutissima*; 54.50 mm for *C. protothecoides* and 47.00 mm for *U. lactuca* at the dose of 20µL/petri on the 6th day of incubation. While *P. mirabilis* and *M. smegmatis* were resistant to the extracts of all macro - microalgae species used in the study, *A. hydrofila* were determined as the sensitive bacteria.

Keywords: Antimicrobial activity, *Chlorella* sp., *Ulva lactuca*, Foodborne pathogens.

Algal Ekstraktların Gıda Kaynaklı Patojenlere Karşı Antimikrobiyal Aktivitesi

Öz

Algal biyoteknoloji, ilaç ve nutrasötik endüstrilerde değerlendirilmek üzere gün geçtikçe daha fazla dikkat çekmektedir. Algler hücre içinde biriktirdikleri protein, karbonhidrat, yağ asitleri, vitamin, mineral, pigmentler ve daha pek çok önemli metabolitler ile insanlar tarafından besin desteği ve gıda katkı maddesi olarak değişik amaçlarla kullanılmaktadırlar. Oleik asit, linoleik asit, palmitoleik asit, E vitamini, β-karoten, lutein ve zeaksantin gibi algal biyoaktif bileşikler antimikrobiyal, antioksidan, antifungal ve antiviral özelliklere sahip olup, gıda kaynaklı hastalıkların azaltılması ve önlenmesinde önemli rol oynarlar. Yeni farmasötik maddeler geliştirmek ve kimyasal ve farmakolojik yenilik sağlamak için mikroalgal kaynaklı biyoaktif bileşikler araştırılmalıdır. Çeşitli mikroalg ekstraktlarının patojen mikroorganizmalara karşı in-vitro antimikrobiyal aktiviteye sahip olduğu bilinmektedir. Bu çalışmanın amacı, *U. lactuca* makroalg ve *C. vulgaris, C. minutissima* ve *C. protothecoides* mikroalg ekstraktlarının *Fusarium oxysporum* fungal mikroorganizmaya

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karşı antifungal ve *Mycobacterium smegmatis* RUT, *Proteus mirabilis* BC6624 ve *Aeromonas hydrophila* ATCC7965 bakteriyel mikroorganizmalara karşı antibakteriyal etkilerini araştırmaktır. Elde edilen ekstraktların antimikrobiyal etkileri agar disk difüzyon yöntemi kullanılarak fungal ve bakteriyel mikroorganizmalar üzerinde denenmiştir. Bu çalışmanın sonucu olarak, *F. oxysporum*'a karşı 6. inkübasyon gününde 20 µL / petri dozunda *C. vulgaris* ekstraktı için 53.00 mm*; C. minutissima* için 59.00 mm; *C. protothecoides* için 54.50 mm ve *U. lactuca* için 47.00 mm inhibisyon zon çapı gözlenmiştir. *P. mirabilis* ve *M. smegmatis* çalışmada kullanılan tüm makro - mikroalg türlerinin ekstraktlarına karşı dirençli bakteriler iken, *A. hydrofila* duyarlı bakteri olarak belirlenmiştir.

Anahtar Kelimeler: Antimikrobiyal aktivite, *Chlorella* sp., *Ulva lactuca*, Gıda patojenleri.

1. Introduction

Food-borne diseases account for significant economic losses and serious health problems all over the world. During food transport and storage, foodborne pathogens can reach dangerous numbers and cause food poisoning in humans. Individuals with the highest risk of food-borne disease are pregnant women, children, the elderly and those with weakened immune systems (Durlu Özkaya and Cömert, 2008). Live microorganisms that cause food poisoning cause disease by multiplying in the digestive system or by mixing with blood (Lisete et al., 2016). *M. smegmatis* is defined as a new opportunistic agent that may be responsible for the disease spreading in immune compromised individuals (Pierre-Audigier et al., 1997). *Aeromonas hydrophilia* is commonly found in salt water. It is isolated from seafood, chicken meat, dairy products and many other foods (Durlu Özkaya and Cömert, 2008). *A. hydrophila* is considered as a human pathogen that produces infection primarily in immune compromised patients (Morgan et al., 1985). *Proteus* species are the causative agent of various opportunistic hospital infections including respiratory tract, eye, ear, nose, skin, burns, throat and wounds. *Proteus* bacilli are associated with urinary tract infections in individuals with structural or functional abnormalities (Jacobsen et al., 2008).

Mycotoxin is one of the serious dangers produced by fungi that is present in food and threatens human and animal health (Lisete et al., 2016). Fumonisins, trichothecenes and zearalenone mycotoxins are produced by various food-borne fungi belonging to the *Fusarim* species (Durlu Özkaya and Cömert, 2008). Fusarium species may cause mycotoxicosis in humans following food intake colonized by the fungal organism. This pathogen usually affects individuals with poor immune system and immune compromised individuals (Gupta et al., 2000).

The majority of foodborne diseases occur as a result of microbial contamination. These microorganisms lead to poisoning of the person taking the food orally. To prevent this, fungicides and synthetic chemicals are frequently applied on vegetables and fruits today (Göksan et al., 2003). The most common concerns are pesticide residues, chemical contaminants and the possibility of food additives resulting in unexpected health consequences. As a result of treatment of foods with high amounts of synthetic chemicals, they cause negative effects on food safety and human health. For all these reasons, food safety and different methods of combating against pests have become an increasingly important public health issue (Amaro et al., 2011). In last decade, functional and bioactive compounds from marine plants, animals and microorganisms have become sustainable solution that offers new compounds with high biological activity (Šimat et al., 2020). In recent years, the need to develop environmentally friendly biological preservatives as an alternative to chemicals has become a priority (Gowda et al., 2020; Vehapi et al., 2020).

Macro - microalgae contain a large number of bioactive molecules which are pharmaceutically important such as proteins, lipids, vitamins, enzymes, sterols, pigments (Ak and Cirik, 2017). Proteins and peptides with antifungal activity have potential value in protecting crops and food as well as preventing fungal infections in humans (Gowda et al., 2020). Bioactive compounds such as oleic acid, linoleic acid, palmitoleic acid, vitamins A, C, E, D, B12, β carotene, phycocyanin, lutein and zeaxanthin exhibit antioxidant, antifungal, antiviral or antibiotic properties (Ak and Cirik, 2017). The aim of this study was to investigate the antimicrobial effects of *U. lactuca* macroalgae, *C. vulgaris, C. minutissima* and *C. protothecoides* microalgae against *F. oxysporum* fungal and *M. smegmatis, P. mirabilis* and *A. hydrophilav* bacterial microorganisms.

2. Material and Method

2.1. Materials

The microalgae species used in the study were obtained from Algal Biotechnology and Bioprocess Laboratory in Bioengineering Department of Yıldız Technical University. *Ulva lactuca* macroalgae was collected from the coastal areas of Marmara Sea. Methanol and DMSO were purchased from Merck. *Fusarium oxysporum, Mycobacterium smegmatis* RUT, *Proteus mirabilis* BC 6624 and *Aeromonas hydrophila* ATCC 7965 were obtained from the Microbiology Laboratory of Food Engineering Department of Yıldız Technical University. Potato Dextrose Agar (PDA, Merck), Nutrient Broth and Nutrient Agar (NA, Merck) medium were used to determine the antifungal effect.

2.2. Microalgae cultivation

The microalgae species were allowed to grow in an agitated incubator operating at 28 ± 2°C, 150 rpm using BBM medium prepared with distilled water in a closed semi-batch culture system. Continous illumination was provided with 18W fluorescent tubes. Optical density analysis was carried out with UV Visible Spectrophotometer (PG Intruments T-60) at 540 nm for two weeks. When the growth curve was determined and the microalgae reached the stationary phase, the cells were harvested by centrifugation. The microalgae were centrifuged for 5 min at 8000 rpm and algal pellets were dried overnight in the oven at 65°C (Vehapi et al., 2018a).

2.3. Preparation of algae extracts

The collected macroalgae was washed with distilled water and dried for 24 h at the temperature of 65 °C, then it was stored in an air-tight container. Dried macro - microalgae samples were extracted in soxhlet extraction with methanol. Excess methanol was evaporated using a rotary evaporator. Extracted macro - microalgae samples were prepared at concentrations of 10 mg / mL with DMSO for evaluation of antimicrobial activity (Vehapi et al., 2018b; Al-Ghanayem et al., 2017). DMSO was used instead of methanol for preparing the extract samples because DMSO is

considered non-toxic to cells. DMSO is placed in the safest category, class 3 solvents, with low toxic potential (Vehapi et al., 2019).

2.4. FT-IR Measurements

Functional groups in the structure of organic compounds, whether the two compounds are the same, the state of the bonds in the structure can be determined by FT-IR spectrometer. In addition, biochemically; the structures of carbohydrates, phospholipids, amino acids and proteins can be determined (Koçer and Özçimen, 2018). FT-IR measurements of macro - microalgae samples were determined by Bruker Alpha FT-IR spectrometer.

2.5.Chemical Identification by GC Analysis

YL Instruments 6100 gas chromatography (GC) was used to determine fatty acid methyl ester (FAME) content of macro - microalgae species. The temperature program of the column was started at 50 °C and increased to 175 °C at 15 °C / min and then 230 °C at 5 °C / min. Hydrogen gas was used as the carrier gas. The injector temperature was set to 230 °C and the flow rate to 1.8 mL / min. The analyzes were performed using the flame ionization detector (FID) and the ZB-FFAP column. The detector temperature was kept constant at 280 °C. The injection volume was adjusted to 1 µL. Methyl margarate was used as an internal standard and the samples were prepared by mixing methyl margarte and n-heptane for GC analysis (Gülyurt et al., 2016).

2.6.Determination of biochemical and total phenolic content

Lowry method was used to determine the protein content of macro - microalgae samples (Lowry et al., 1951). The phenol-sulfuric acid method was used to determine the total carbohydrate content in the macro - microalgae sample (Dubois et al., 1965). Soxhlet extraction method was used to determine the lipid content in macro - microalgae samples (Soxhlet, 1879; Koçer and Özçimen, 2018).

The total phenolic content of the samples was determined by the Folin–Ciocalteu method. Briefly, 200 µL of the diluted extract was mixed with 1 mL of Folin–Ciocalteu reagent in test tubes, and then 800 µL (75 g/L) of sodium carbonate was added. The samples were incubated in darkness for 30 min at room temperature, and then absorbance at 765 nm was measured by spectrophotometer. The total phenol content of the extracts is expressed in milligrams of Gallic acid equivalent (Haoujar et al. 2019).

2.7.Pathogenic Isolations

Fusarium oxysporum was isolated from tomato seedlings. Sport suspensions were cultured on potato PDA with 50 mg / L streptomycin at 25 ± 2 °C for 7 days. The spores were collected by washing the surface with distilled water and gently shaking the plate to remove spores. The spores were counted and 1×10^5 spore / mL was adjusted to the inoculum concentration by hemocytometer. Prior to inoculation, the resulting suspensions were shaken for 30 seconds using vortexing (Yilmaz et al., 2016a, 2016b).

2.8.Determination of Antifungal Effect

Fungal discs taken from fungal cultures of 7 days of fungal cultures developed in PDA medium were placed in the middle of petri dishes. Macro - microalgae oils were prepared by dissolving at 10 mg / mL concentration in DMSO. Discs impregnated with 20 and 40 µL / petri algae extracts were placed on the top lids of prepared petri dishes. Plates were incubated for 6 days at 25 ± 2 ºC for fungal strains. Negative controls were prepared using DMSO. The colony diameters of the fungi growing in petri dishes were measured on the 3rd, 4th, 5th and 6th days (Yilmaz et al., 2016a). The relative growth inhibition % of treated plates compared to the control plates were calculated using the following formula (Al-Reza et al, 2010; Vehapi et al., 2019):

$$\text{growth inhibition \%} = ([\text{Control}–\text{Treated}]/\text{Control}) \times 100 \quad (1)$$

where Control and Treated correspond to mean diameter of growth (mm) of fungi colonies.

2.9.Determination of Antibacterial Effect

Antibacterial effects of macro - microalgae extracts were determined against Gram-positive and Gram-negative bacteria by using disk diffusion method. Algae extracts prepared at concentrations of 10 mg / mL in discs with a diameter of 6 mm were absorbed in disc papers with an automatic pipette at 20 and 40 µL / petri dose. The disc of algae extracts were placed in the suspension of bacteria spreading onto the NA medium by incubation and allowed to incubate at 37 ºC for 24 hours (Vehapi et al., 2018b).

2.10.Statistical Analysis

Variance analysis was performed using JMP (release 6.0.0, SAS) package program. The significance levels between the means were determined by Student's t comparison test. Data were presented as mean ± standard deviation ($p < 0.05$ was considered significant).

3. Results and Discussion

3.1. Characterization of Algal Species and Their Extracts

The functional groups identified from the FTIR spectra were presented in Table 1. It was seen that there are similar peaks in the range of 4000–2000 cm^{-1}. FTIR functional groups have shown the presence of alkanes, amines, carboxylic acids, esters, ketones and phenols (Du et al., 2011).

Phenols are known as membrane toxins that destroy cell walls. It is known that microalgae, especially *C. vulgaris*, contain phenolic compounds. Antimicrobial activity of phenolic compounds; alteration of the permeability of the microbial cell results from loss of internal macromolecules, loss of membrane function and loss of cellular integrity and results in cell death (Chinnasamy et al., 2009). Evaluation of the fingerprint region in FT-IR spectrum which was found between 1800 and 700 cm^{-1} is the best way to identify phenolic compounds (Baltacıoğlu et al. 2021). According to the literature, the peak at the wave number of 1618 cm^{-1} is assigned to ring C-C stretch of phenyl and the band at 813 cm^{-1} which is caused by ring CH deformation can indicate polyphenols (Lu et al. 2011). In addition to that, band between 1300 cm^{-1} and 1200 cm^{-1} which is C-O stretching shows the presence of phenols, and the peak at 1200 cm^{-1} in the fingerprint region indicates phenols (Ceylan and Goldfarb 2015). In the present study, it was considered that the, peak at 1238 cm^{-1} shows the presence of phenol. Peaks in the range of 1500-1700 cm^{-1} seen in all macro - microalgae samples are thought to be caused by

protein content and a large peak in the range of 900-1000 cm^{-1} is thought to be caused by high carbohydrate content as shown in Figure 1 (Krzemińska et al., 2015).

Table 1. Wave number and functional groups of macro - microalgae samples

Wave number (cm^{-1})	Functional groups
3250	Stretching vibration of the OH group
2900 - 2950	C-H stretching vibrations of CH_2
1625 - 1730	Amide C = O originated from protein
1530	Amide N-H originated from protein
1420	Stretching of CH_3 and CH_2 groups
1300-1200	C-O stretching
1210	P = O stretch associated with phosphorus compounds
1012 - 1030	C-O Ester and C-N stretching

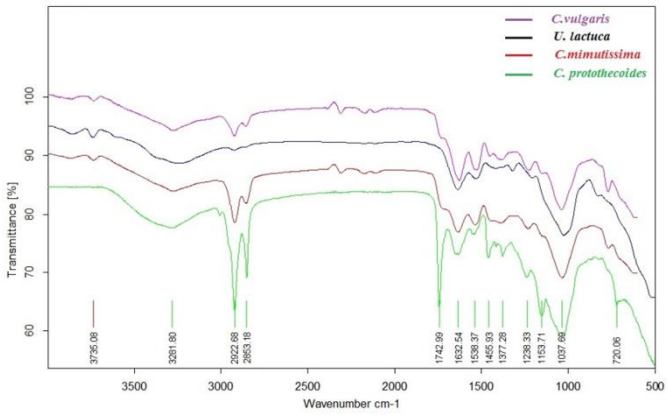

Figure 1. Fourier transform infrared spectroscopy (FTIR) spectrum of algae samples.

Eicosapentaenoic acid contained in macro - microalgae has antimicrobial activity against pathogens. *C. minutissima* is rich in amino acids and polyunsaturated fatty acids. The action mechanism of fatty acids affects various structures of microorganisms; which cell membranes are most affected. Membrane damage probably leads to the loss of internal substances of cell and the introduction of harmful components, in addition to reducing nutrient absorption and inhibiting cellular respiration. The biological activity of fatty acids depends on the ability to inhibit bacterial growth, chain length and degree of unsaturation (de Morais et al., 2014).

The fatty acid profile of macro - microalgae species as; *U. lactuca, C. vulgaris, C. minutissima* and *C. protothecoides* was determined using GC analysis. GC analysis showed four main fatty acids: palmitic, oleic, linoleic and linolenic acid. The highest fatty acid methyl ester oleic acid (C18 = 1) and linoleic acid (C18 = 2) determined in all samples were not determined by these results because other fatty acids were found in trace amounts (Gülyurt et al., 2016).

In Table 2, the biochemical and total phenolic contents of algal species were given. It was seen that, *C. minutissima* has the highest total phenolic content in comparison with the other algal species in this study. Algal-derived peptides show antimicrobial properties by inhibiting bacterial spread and micelle development of fungal pathogens (Ak and Cirik, 2017; Gowda et al., 2020). Also alkaloids in *C. vulgaris* are bioactive compounds with antibacterial activity. It can be seen in Table 2, the protein content of microalgal sample was higher than macroalgal sample. Algae are composed of a variety of polysaccharides, including alginic acid and alginates, carrageenan and agar, laminaran, fucoidan, ulvan and derivatives (Gökpınar et al., 2006).

Table 2. Biochemical and phenolic contents of algae samples

	Protein (%)	Carbohydrate (%)	Lipid (%)	Total Phenolics (mg/g GAE)
C. minutissima	35.6	23.1	24.8	188.54
C. vulgaris	28.6	24.5	28.3	75.81
C.protothecoides	30.3	22.2	32.5	78.82
U. lactuca	28.8	44.1	5.3	33.27

3.2. In Vitro Fumigation of Algae Extracts

In Table 3, the extracts *of U. lactuca, C. minutissima, C. vulgaris* and *C. protothecoides* were given at 20 and 40 µL /petri in the fumigation application on the 3rd, 4th, 5th and 6th incubation day. According to Table 3; in the fumigation of *U. lactuca* extract on the 6th day of incubation, the micellar growth of *Fusarium oxysporum* was obtained as 47.00 – 46.50 mm. Additionally the micellar growth of *Fusarium oxysporum* in fumigation of *C. vulgaris* extract was obtained as 44.00 - 53.00 mm. Furthermore the micellar growth of *Fusarium oxysporum* was obtained as 53.00 and 59.00 mm in fumigation of *C. minutissima* extract on the 6th day of incubation. Finally the antifungal activity of *C. protothecoides* extract on the 6th day of incubation against the micellar growth of *Fusarium oxysporum* was obtained as 43.75 - 54.50 mm. The micellar development of the *Fusarium oxysporum* as a control was 76.50 mm. It was observed that the increase of the dose of microalgae extract against *Fusarium oxysporum* did not have a significant effect in fumigation of *C. protothecoides*.

Macro and microalgae extracts with antifungal activity act by inhibiting of micellar growth, by preventing germination of *Fusarium oxysporum* from 22.88 % to 42.81 % (Table 4). The lowest inhibiting rate (22.88 %) was observed at 20 µL /petri *C. minutissima* application and the highest inhibiting rate (42.81 %) was seen at 40 µL /petri *C. protothecoides* application. As a result, the highest inhibition rates of microalgal extracts against *Fusarium oxysporum* were determined using the JMP package program for variance analysis. *C. protothecoides* and *C. vulgaris* were similar and highly effective, however, *C. minutissima* showed the lowest inhibition rate against *Fusarium oxysporum* as shown in Table 5 and Figure 2.

In the study of Vehapi et al. (2018a); *C. vulgaris* and *C. minutissima* microalgae samples were grown in ISKI municipal wastewater, Bold Basal medium and Iroko tree water, and they

examined the antifungal effect of the microalgal extracts at 40 µL / petri and 60 µL / petri. The ratio of *C. vulgaris* extract grown in Bold Basal medium to *Fusarium oxysporum* mycelial growth rate was obtained as 49.00 mm at 60 µL / petri dish and 63.00 mm at 40 µL / petri and *C. minutissima* extract was obtained as 59.00 mm in 40 µL / petri dose and 57.00 mm in 60 µL / petri on the 6th day of incubation. In present study, the inhibition rate was found to be 53.00 mm for *C. vulgaris* and 59.00 mm for *C. minutissima* at dose 20 µL / petri on the 6th day of incubation. As a result of this study, it has been proven that even at lower doses, high effect can be observed.

In the study of Özçimen (2018), the antifungal effect of *Chlorella protothecoides* microalgae prepared at concentrations of 50 and 100 mg / mL using DMSO, ethanol and methanol solvents on *Botrytis cinerea* and *Aspergillus niger* fungal pathogens was investigated by impregnating the discs at 50 µL / petri dose. As a result, *C. protothecoides* extracts prepared using DMSO, was reported as the highest with 44.20 mm antifungal activity against *Aspergillus niger* on the 6th day of incubation. In our present study, macro - microalgae extracts were prepared at lower concentrations of 10 mg / mL with DMSO and the micelle growth at lower doses such as 20 and 40 µL / petri were investigated against bacterial microorganisms *Mycobacterium smegmatis* RUT, *Proteus mirabilis* BC6624 and *Aeromonas hydrophila* ATCC7965 and fungal microorganisms *Fusarium oxysporum.*

Terpenes, alkaloids and polypeptides found in *C. vulgaris* are the main groups with antifungal activity (Castillo et al., 2004). Antifungal proteins of plant origin are the basic focus of biotechnology owing to its antifungal activity (Gowda et al., 2020). Eicosapentaenoic acid and phenolic compounds present in *C. minutissima* microalgae have antimicrobial activity against pathogens (Castillo et al., 2004). *U. lactuca, C.minutissima, C. vulgaris* and *C.protothecoides* macro - microalgae species have different effects against *F. oxysporium* because it is thought to be related to the presence of secondary metabolites with different ratios and antifungal activity in all algae species.

Table 3. Antifungal activity of algae extracts at 20 and 40 µL/petri doses against Fusarium oxysporum micellar growth

Incubation day	Impregnated Dose	*C.vulgaris* mm	*C.minutissima* mm	*C.protothecoides* mm	*U.lactuca* mm
3 day	Control	41.25±0.35^{a}	41.25±0.35^{a}	41.25±0.35^{a}	41.25±0.35^{a}
	20 µl/petri	38.50±1.06^{b}	40.00±0.00^{a}	34.50±0.70^{c}	34.50±2.12^{c}
	40 µl/petri	35.50±3.53^{b}	38.50±2.12^{a}	33.25±0.35^{c}	36.00±1.41^{b}
4 day	Control	49.50±0.70^{a}	49.50±0.70^{a}	49.50±0.70^{a}	49.50±0.70^{a}
	20 µl/petri	44.00±4.24^{b}	49.50±2.12^{a}	42.25±1.76^{c}	40.50±6.36^{d}
	40 µl/petri	37.00±1.41^{b}	44.00±7.07^{a}	35.75±0.35^{c}	37.50±0.00^{b}
5 day	Control	64.50±2.12^{a}	64.50±2.12^{a}	64.50±2.12^{a}	64.50±2.12^{a}
	20 µl/petri	51.00±4.59^{b}	54.00±4.24^{a}	48.75±6.71^{c}	44.50±6.36^{d}
	40 µl/petri	43.00±1.41^{b}	49.00±5.65^{a}	39.50±0.70^{c}	42.00±1.41^{b}
6 day	Control	76.50±2.12^{a}	76.50±2.12^{a}	76.50±2.12^{a}	76.50±2.12^{a}
	20 µl/petri	53.00±7.07^{b}	59.00±2.82^{a}	54.50±10.6^{b}	47.00±5.65^{c}
	40 µl/petri	44.00±1.41^{b}	53.00±2.82^{a}	43.75±1.76^{b}	46.50±1.41^{c}

Numbers; mean colony diameter ± SD (mm) represents standard deviation values (n = 6).

a-d: in each row, the lower case superscripts shows the differences between each types of algae on the day of incubation. $p < 0.05$ was considered to be statistically significant

Table 4. The growth inhibition rates (%) of algae extracts at 20 and 40 µL/petri doses against F. oxysporum at 6. incubation day

Algae	20 µL	40 µL
C. vulgaris	30.72^{B}	42.48^{A}
C. minutissima	22.88^{B}	30.72^{A}
C. protothecoides	28.76^{B}	42.81^{A}
U. lactuca	38.56^{B}	39.22^{A}

A-B: in each row, the upper case superscripts shows the differences between 20 and 40 µL / petri concentration. $p < 0.05$ was considered to be statistically significant.

Table 5. Analysis of variance of F. oxysporum micelle development with one-way ANOVA

Algae	Day	SS	df	MS	F	p-value
C. vulgaris	3	35.05	4	8.76	115.80	<0.0001
	4	113.41	5	22.68	290.30	<0.0001
	5	426.90	5	86.38	1613.5	<0.0001
	6	932.01	5	186.40	1513.9	<0.0001
C. minutissima	3	33.43	4	8.35	108.56	00000
	4	141.30	6	23.55	4240	<0.0001
	5	379.90	5	75.99	115.80	<0.0001
	6	644.50	5	128.90	1031.2	<0.0001
C. protothecoides	3	56.48	5	11.29	217.80	<0.0001
	4	137.63	6	22.90	152.36	<0.0008
	5	447.10	7	63.80	766.48	<0.0013
	6	924.10	6	154.00	1700.8	<0.0001
U. lactuca	3	66.32	5	13.26	-	<0.0001
	4	174.70	6	29.11	299.50	<0.0003
	5	551.77	5	110.35	1765.6	<0.0001
	6	931.00	4	232.70	5586	<0.0001

The p values obtained as a result of comparison of the data of samples were considered as statistically significant when p values less than 0.01 were obtained.

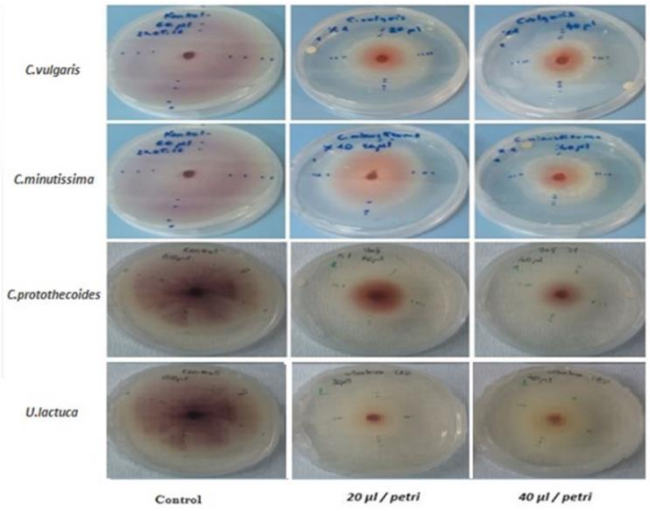

Figure 2. Different doses of algae extracts in-vitro fumigation application of *F. oxysporum* mycelial growth inhibition rates.

3.3.Determination of Antibacterial Activity

The antimicrobial activity of macro - microalgae is dependent on the ability to synthesize fatty acids, terpenoids, sterols, sulfur-containing heterocyclic compounds, carbohydrates and phenolic compounds (Pérez et al., 2016). Antibacterial activities of macro - microalgae extracts were investigated using disk diffusion method against Gram positive; *Mycobacterium smegmatis* and Gram negative; *Proteus mirabilis* and *Aeromonas hydrophila* as shown in Figure 3. According to Table 6, the antibacterial activity of *U. lactuca* macroalgae extract against *P. mirabilis* was determined as microorganism resistant with 12.16 mm at 20 µL / petri dose and 13.00 mm inhibition zone at 40 µL / petri dose. The antibacterial activity against *Mycobacterium smegmatis* was found to be resistant with the inhibition zone diameter of 9.66 mm at 20 µL / petri dose and 11.66 mm inhibition zone diameter at 40 µL / petri dose. The antibacterial activity of *U. lactuca* macroalgae extract against *Aeromonas hydrophila* was determined as microorganism susceptible with 20 µL / petri dose and 19.33 mm and 40 µL / petri dose with inhibition zone diameter of 27.00 mm.

The antibacterial activity of *C. minutissima* extract against *Proteus mirabilis* was determined as microorganism intermediate with a inhibition zone diameter of 17.66 mm at a dose of 40 µL / petri and 14.16 mm inhibition zone diameter at 20 µL /petri dose. The antibacterial activity against *Mycobacterium smegmatis* was found to be resistant with the inhibition zone diameter of 13.16 mm at 20 µL / petri dose and as intermediate with 15.00 mm inhibition zone diameter at 40 µL / petri dose.

The antibacterial activity of *C. vulgaris* extract against *Aeromonas hydrophila* was determined as microorganism susceptibe with 20 µL / petri dose and 18.00 mm and 40 µL / petri dose with inhibition zone diameter of 21.66 mm. Antibacterial activity against *Mycobacterium smegmatis* was determined as microorganism resistant with inhibition zone 8.00 mm diameter of 20 µL / petri dose and with inhibition zone 11.66 mm diameter of 40 mL / petri dose.

The antibacterial activity of *C. prototthecoides* extract against *P. mirabilis* was determined as microorganism resistant with 9.50 mm at 20 µL / petri dose and 10.00 mm inhibition zone at 40 µL / petri dose. Antibacterial activity against *A. hidrofila* was determined as microorganism resistant with 10.66 mm inhibition zone diameter at 20 µL / petri dose and 13.66 mm inhibition zone diameter at 40 µL / petri dose, anti-bacterial activity against *M. smegmatis* was determined as microorganism resistant with 10.33 mm inhibition zone diameter at 20 µL / petri and 10.66 mm inhibition zone at 40 µL / petri dose. All bacteria were found to be resistant to *C. prototthecoides* microalgae. As a result, it can be reported that secondary metabolites with antifungal activity act by inhibiting or inhibiting the growth of micellar growth, by preventing germination or by reducing the sporulation of fungal pathogens (Table 4).

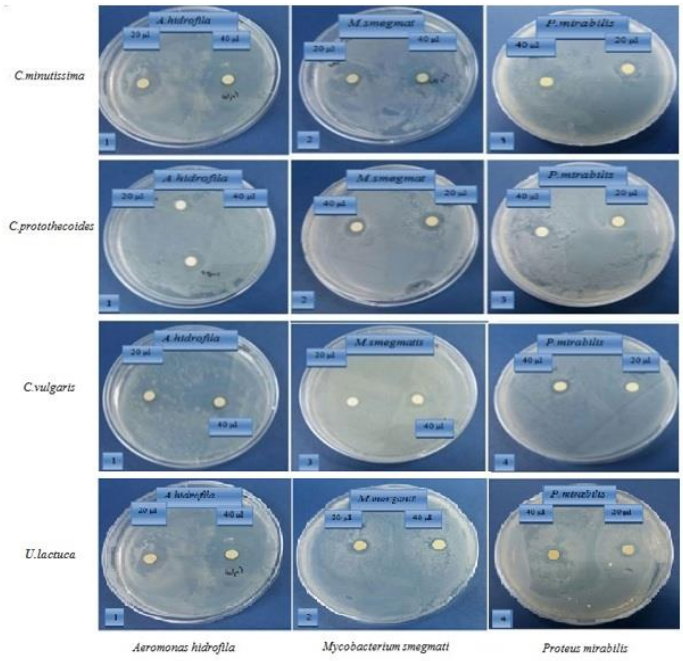

Figure 3. Antibacterial activity of algae extracts on A. hydrofila, M. smegmatis and P. mirabilis

Table 6. Average inhibition zone diameters of the algae extracts against pathogens (mm)

Algae samples	Dose	*Zone of inhibition (mm)*		
	µL	***P.mirabilis***	***A.hidrofila***	***M.smegmatis***
C.minutissima	20	14.16 ± 1.44^{A}	12.66 ± 0.57^{C}	13.16 ± 3.61^{B}
	40	17.66 ± 4.04^{A}	17.33 ± 3.05^{A}	15.00 ± 2.00^{B}
C.vulgaris	20	09.33 ± 1.52^{B}	18.00 ± 4.35^{A}	8.00 ± 1.73^{B}
	40	10.66 ± 1.15^{B}	21.66 ± 5.77^{A}	11.66 ± 3.51^{B}
C.prototthecoides	20	09.50 ± 3.04^{B}	10.66 ± 1.52^{A}	10.33 ± 2.08^{A}
	40	10.00 ± 1.00^{B}	13.66 ± 1.52^{A}	10.66 ± 2.08^{B}
U.lactuca	20	12.16 ± 2.25^{B}	19.33 ± 1.15^{A}	9.66 ± 1.52^{C}
	40	13.00 ± 2.08^{B}	27.00 ± 2.00^{A}	11.66 ± 2.30^{C}

(a) Data are given as mean ± standard deviation (n = 6).

A-C: In each row, the different upper case superscripts of each macro - microalgae extract with the activity of 20 and 40 µl/petri show differences in bacterial strains ($p < 0.05$).

4. Conclusions and Recommendations

Treatment of vegetables and fruits with a high proportion of synthetic chemicals results in environmental pollution, adverse effects on foods, adverse effects on humans and food poisoning. For such reasons, natural fungicides which are obtained from macro - microalgae and which have no side effects, and the natural food additives with antibacterial properties should be produced and used.

In conclusion, the extracts obtained from different algae species have strong antimicrobial effects against *P. mirabilis, M. smegmatis, A. hidrofila* and *F. oxysporium* pathogens. These results are indicative of the presence of antimicrobial compounds in algae species. In this study, it has been proven to be useful as a natural food additive in the treatment of infections, to prevent food poisoning and to prevent food spoilage.

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