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. hydrophila were determined as the sensitive bacteria.

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

Algal Ekstraktların Gıda Kaynaklı Patojenlere Karşı Antimikrobiyel Aktivitesi

Öz
Algal biyoteknoloji, ilaclı ve nutrasötkün endüstrilere değerlendirilmek üzere gün geçtikçe daha fazla dikkat çeken bir alt alandır. Algular hücre içinde birikirdikleri protein, karbohidrat, yağ asitleri, vitamin, mineral, pigmentler ve daha pek çok önemli metabolitler ile insanlar tarafından besin desteği ve gıda katkı maddesi olarak değişik amaçlarla kullanılmaktadır. Oleik asit, linoelik asit, palmitoleik asit, E vitamini, β-karoten, lutein ve zeaksantin gibi algal biyoaktif bileşikler antimikrobiyel, antioksidan, antifungal ve antiviral özelliklere sahip olup, gıda kaynaklı hastalıkların azaltılması ve önlenmesinde önemli rol oynarlar. Yeni farmasötik maddeler geliştirilerek ve kınıyasal ve farmakolojik yenilik sağlamak için mikroalgal kaynaklı biyoaktif bileşikler araştırılmaktadır. Çeşitli mikroalg ekstraktlarının patojen mikroorganizmalarına karşı in-vitro antimikrobiyel aktiviteye sahip olduğu bilinmektedir. Bu çalışmaın amacı, U. lactuca makroalg ve C. vulgaris, C. minutissima ve C. protothecoides mikroalg ekstraktlarının Fusarium oxysporum fungal mikroorganizmalarına karşı antimikrobiyel aktiviteye sahip olduğunu bulmaktadır. Bu çalışmının amacı, U. lactuca makroalg ve C. vulgaris, C. minutissima ve C. protothecoides mikroalg ekstraktlarının Fusarium oxysporum fungal mikroorganizmalarına karşı antimikrobiyel aktiviteye sahip olduğunu bulmaktadır.
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 Özka 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 hydrophila is commonly found in salt water. It is isolated from seafood, chicken meat, dairy products and many other foods (Durlu Özka 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 Özka 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 (Gökşan 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 (Şimat 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. hydrophila 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 BC6624 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. Continuous 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 - microalgal 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 - microalgal 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 analyzers 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 - microalgal samples (Lowry et al., 1951). The phenol-sulfuric acid method was used to determine the total carbohydrate content in the macro - microalgal sample (Dubois et al., 1965). Soxhlet extraction method was used to determine the lipid content in macro - microalgal 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 (Hauoujar 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 1×10^6 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 - microalgal 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 °C 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):

\[
growth\ \text{inhibition}\ % = \frac{(\text{Control} – \text{Treated})/\text{Control}}{100}
\]

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

**2.9. Determination of Antibacterial Effect**

Antibacterial effects of macro - microalgal 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 °C 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 (Baltacioglu 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 - microalgal 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 (Krzemieńska 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                           |

Eicosapentaenoic acid contained in macro - microalgae has antiinflammatory 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 (C\(_{18}\) = 1) and linoleic acid (C\(_{18}\) = 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 algic acid and alginates, carrageenan and agar, laminaran, fucoidan, ulvan and derivatives (Gökpınar et al., 2006).

Table 2. Biochemical and phenolic contents of algae samples

| Algae Extracts | 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 microalgal extract against *Fusarium oxysporum* did not have a significant effect in fumigation of *C. protothecoides*.

Macro and microalgal 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. oxysporum 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      | 41.25±0.35          | 41.25±0.35             | 41.25±0.35      |
|               | 20 µL/petri      | 38.50±1.06      | 40.00±0.00          | 34.50±0.70             | 34.50±2.12      |
|               | 40 µL/petri      | 35.50±3.53      | 38.50±2.12          | 33.25±0.35             | 36.00±1.41      |
| 4 day         | Control          | 49.50±0.70      | 49.50±0.70          | 49.50±0.70             | 49.50±0.70      |
|               | 20 µL/petri      | 44.00±4.24      | 49.50±2.12          | 42.25±1.76             | 40.50±6.36      |
|               | 40 µL/petri      | 37.00±1.41      | 44.00±7.07          | 35.75±0.35             | 37.50±0.00      |
| 5 day         | Control          | 64.50±2.12      | 64.50±2.12          | 64.50±2.12             | 64.50±2.12      |
|               | 20 µL/petri      | 51.00±4.59      | 54.00±4.24          | 48.75±6.71             | 44.50±6.36      |
|               | 40 µL/petri      | 43.00±1.41      | 49.00±5.65          | 39.50±0.70             | 42.00±1.41      |
| 6 day         | Control          | 76.50±2.12      | 76.50±2.12          | 76.50±2.12             | 76.50±2.12      |
|               | 20 µL/petri      | 53.00±7.07      | 59.00±2.82          | 54.50±10.6             | 47.00±5.65      |
|               | 40 µL/petri      | 44.00±1.41      | 53.00±2.82          | 43.75±1.76             | 46.50±1.41      |

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

### 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<sup>b</sup> | 42.48<sup>a</sup> |
| C. minutissima | 22.88<sup>b</sup> | 30.72<sup>a</sup> |
| C. protothecoides | 28.76<sup>b</sup> | 42.81<sup>a</sup> |
| U. lactuca | 38.56<sup>b</sup> | 39.22<sup>a</sup> |

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 | 5 | 8.76 | 151.80 | <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 |
|        | 7 | 33.43 | 4 | 8.35 | 108.56 | 00000 |
| C. minutissima | 4 | 141.30 | 6 | 23.35 | 424.00 | <0.0001 |
|        | 5 | 379.90 | 5 | 75.99 | 115.80 | <0.0001 |
|        | 6 | 644.50 | 5 | 128.90 | 1031.2 | <0.0001 |
|        | 3 | 56.48 | 5 | 11.29 | 217.80 | <0.0001 |
| C. protothecoides | 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 |
|        | 3 | 66.32 | 5 | 13.26 | - | - |
| U. lactuca | 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.
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 13.16 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. protothecoides* macroalgae. 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. hidrofila,*
*M. smegmatis* and *P. mirabilis*

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

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

(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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References

Ak, I. & Cirik, S. (2017). Blue-green algae (Cyanobacteria) and thermalism. Ege Journal of Fisheries and Aquatic Sciences, 34(2), 227-233.

Al-Reza, S.M., Rahman, A., Ahmed, Y. & Kang, S.C. (2010). Inhibition of plant pathogens in vitro and in vivo with essential oil and organic extracts of *Cestrum nocturnum* L. Pesticide Biochemistry and Physiology, 96, 86–92.

Al-Ghanayem, A.A., Al-Sobeai, M.S., Alhussaini, S.M., Joseph, B. & Saadabi, A.M. (2017). Antifungal activity of *Anastatica hierochuntica* L. extracts against different groups of fungal pathogens: An in-vitro test. Romanian Biotechnological Letters, 23(6), 14135. doi: 10.26327/RBL2018.147.

Amaro, H.M., Guedes, A.C. & Malcata, F.X. (2011). Antimicrobial activities of macro - microalgae: an invited review. In: Méndez-Vilas A (ed). Science against microbial pathogens: communicating current research and technological advances. Formatex Resresearch Center Spain, 3, 1272-1280.

Baltacıoğlu, H., Baltacıoğlu, C., Okur, I., Tanrivermiş, A., & Yalıç, M. (2021). Optimization of microalgae-assisted extraction of phenolic compounds from tomato: Characterization by FTIR and HPLC and comparison with conventional solvent extraction. Vibrational Spectroscopy, 113, 103204.

Castillo, E., Hernández, D., Gallegos, G., Rodriguez, R. & Aguilar, C.N. (2004). Antifungal properties of bioactive compounds from plants. Fungicides for Plant and Animal Diseases, 82-98.

Ceylan, S., & Goldfarb, J. L. (2015). Green tide to green fuels: TG–FTIR analysis and kinetic study of *Ulva prolifera* pyrolysis. Energy Conversion and Management, 101, 263-270.

Chinnasamy, S., Ramakrishnan, B., Bhatnagar, A. & Das, K.C. (2009). Biomass production potential of a wastewater alga *Chlorella vulgaris* ARC 1 under elevated levels of CO2 and temperature. International Journal of Molecular Sciences, 10(2), 518-532.

de Morais, M.G., Da Silva Vaz, B., de Morais, E.G. & Vieira Costa, J.A. (2014). Biologically active metabolites synthesized by macro - microalgae. BioMed Research International, 1, 15.

Du, Z., Li, Y., Wang, X., Wan, Y. & Chen, Q. et al. (2011). Microwave-assisted pyrolysis of macro - microalgae for biofuel production. Bioresource Technology, 102(7), 4890-4896.

Dubois, M., Gilles, K.A. & Hamilton, J.K. (1956). Colorimetric method for determination of sugars and related substances. Analytical Chemistry, 28(3), 350-356.

Duru ÖzKayaya, F. & Cómez, M. (2008). Efficient factors for food poisoning. Turkish Bulletin of Hygiene and Experimental Biology, 65(3), 149-158.

Gowda, C.T., Purama, S. N. S., & Kammarra, R. (2020). TLPdb: A Resource for Thaumatin-Like Proteins. The Protein Journal, 39(4), 301-307.

Gökşan, Ş. & Torun, M. (2009). Biomass production potential of a wastewater alga *Chlorella vulgaris* ARC 1 under elevated temperature. Avrupa Bilim ve Teknoloji Dergisi, 76, 270.

Güler, M., Gölyurt, M.Ö., Özçimen, D. & İnan, B. (2016). Biodiesel production from *Chlorella protothecoides* oil by microwave-assisted transesterification. International Journal of Molecular Sciences, 17(4), 579. doi:10.3390/ijms17040579.

Gupta, A.K., Baran, R. & Summerbell, R.C. (2000). Fusarium infections of the skin. Current Opinion in Infectious Diseases, 13(2), 121-128.

Haojui, I., Cacciola, F., Abrini, J., Mangraviti, D., Giuffrida, D., Ouald El Majdoub, Y., Kounnoun, A., Miceli, N., Taviano, M., Mondello, L., Rigano, F. & Skali Senhaji, N. (2019). The contribution of carotenoids, phenolic compounds, and flavonoids to the antioxidative properties of marine microalgae isolated from Mediterranean Morocco. Molecules, 24(22), 4037.

Jacobsen, S.M., Stickler, D.J., Mobley, H.L.T. & Shirliff, M.E. (2008). Complicated catheter-associated urinary tract infections due to *Escherichia coli* and *Proteus mirabilis*. Clinical Microbiology Reviews, 21(1), 26-59.

Koçer, A.T. & Özcimen, D. (2018). Investigation of the biogas production potential from algal wastes. Waste Management & Research, 36(11), 1100-1105.

Krzemińska, I., Nawrocka, A., Piasecka, A., Jagielski, P. & Tys, J. (2015). Cultivation of *Chlorella protothecoides* in photobioreactors: The combined impact of photoperiod and CO2 concentration. Engineering in Life Sciences, 15(5), 533-541.

Lisete, P., Eliseabte, L., Ana, N.I., Massimo, M. & José, B. (2016). Health-promoting ingredients from four selected Azorean macroalgae. Food Research International, 89, 432-438.

Lu, X., Wang, J., Al-Qadiri, H. M., Ross, C. F., Powers, J. R., Tang, J., & Rasco, B. A. (2011). Determination of total phenolic content and antioxidant capacity of onion (*Allium cepa*) and shallot (*Allium oschaninii*) using infrared spectroscopy. Food Chemistry, 129(2), 637-644.

Lowry, O.H., Rosebrough, N.J. & Farr, A.L. (1951). Protein measurement with the folin reagent. Journal of Biological Chemistry, 193, 265-275.

Morgan, D.R., Johnson, P.C., Dupont, H.L., Satterwhite, T.K. & Wood, L.V. (1985). Lack of correlation between known virulence properties of *Aeromonas hydrophila* and *Aeromonas salmonicida*.
Enteropathogenicity for humans. Infection and Immunity, 50(1), 62-65.

Özçimen, D. (2018). Investigation of antifungal effect of Chlorella protothecoides macro - microalgae oil against Botrytis cinerea and Aspergillus niger fungi. Journal of Tekirdag Agricultural Faculty, 15(2), 45-52.

Pérez, M.J., Falqué, E. & Domínguez, H. (2016). Antimicrobial action of compounds from marine seaweed. Marine Drugs, 14, 52.

Pierre-Audigier, C., Jouanguy, E., Lamhamedi, S., Altare, F., Rauzier, J. et al. (1997). Fatal disseminated Mycobacterium smegmatis infection in a child with inherited interferon y receptor deficiency. Clinical infectious diseases, 24(5), 982-984.

Soxhlet, F. (1879). Die gewichtsanalytische bestimmung des milchfettes. Dingler's Polytechnisches Journal, 232, 461-465.

Șimat, V., Elabed, N., Kulawik, P., Ceylan, Z., Jamroz, E., Yazgan, H., ... & Özogul, F. (2020). Recent Advances in Marine-Based Nutraceuticals and Their Health Benefits. Marine Drugs, 18(12), 627.

Vehapi, M., İnan, B., Yılmaz, A., Özçimen, D. (2020). Prevention of foodborne infections with algal biotechnology. II. International Enzyme and Bioprocess Days EBDays 2020, İstanbul, Türkiye, 26.

Vehapi, M., Koçer, A. T., Yılmaz, A., & Özçimen, D. (2019). Investigation of the antifungal effects of algal extracts on apple-infesting fungi. Archives of Microbiology, 1-17.

Vehapi, M., Yılmaz, A. & Özçimen, D. (2018a). Antifungal activities of Chlorella vulgaris and Chlorella minutissima macro - microalgae cultivated in bold basal medium, wastewater and extract water against Aspergillus niger and Fusarium oxysporum. Romanian Biotechnological Letters, 1-8.

Vehapi, M., Yılmaz, A. & Özcimen, D. (2018b). Investigation of antibacterial and antioxidant activities of some algae species. Journal of Biotechnology, 280, 80.

Yılmaz, A., Ermis, E. & Boyraz, N. (2016a). Investigation of in vitro and in vivo anti-fungal activities of different plant essential oils against postharvest apple rot desease Colletotrichum gloesporioides, Botrytis cinerea and Penicillium expansum. Journal of Food Safety and Quality, 67, 113-148.

Yılmaz, A., Bozkurt, F., Cicek, P.K., Dertli, E., Durak, M.Z. et al. (2016b). A novel antifungal surface-coating application to limit postharvest decay on coated apples: molecular, thermal and morphological properties of electrospunzein–nanofiber mats loaded with curcumin. Innovative Food Science Emerging Technology, 37, 74-83.