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Research Article

An In-Vitro Investigation of the Antibacterial Effects of the Acetone and Ethanol Extracts and the Supernatant of the Algae *Chlorella vulgaris* CCATM-210-1 on Some of the Gram-Negative Bacterial Foodborne Pathogens

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ABSTRACT:
Algae are rich sources of amino acids, terpenoids, florentines, alkanes, halogenated ketones, steroidal compounds, fatty acids, and phenols that can be used in the pharmaceutical industry. The acetone and ethanol extracts, and the supernatant of the algae *Chlorella vulgaris* CCATM- 210-1, were used in this study. After culturing the Algae, and preparing the supernatant and extracts, the antibacterial effects of the extracts and supernatant of this algae against some of the gram-negative bacterial foodborne pathogens were determined using the well plate and agar disk diffusion methods. According to the study conducted, the acetone and ethanol extracts and the supernatant of the algae *Chlorella vulgaris* CCATM- 210-1 showed acceptable antibacterial properties against some of the bacteria being studied, at concentrations of 1.25, 2.5, 5, and 10 mg/ml in the well plate and agar disk diffusion methods. The maximum inhibition zone diameters in the well plate and agar disk diffusion methods, were 36.8 and 35.4 mm, respectively, which were related to the bacterium *Proteus mirabilis* PTCC 1776 and were observed at a 10 mg/ml concentration of the acetone extract. By comparing the mean inhibition zone diameters among the acetone and ethanol extracts and a mixture of the two extracts using the well plate method, it can be concluded that the acetone and ethanol extracts of *Chlorella vulgaris* CCATM- 210-1 are indifferent to each other. This analysis was also true of the agar disk diffusion method.

Keywords: *Chlorella vulgaris*, antibacterial effect, well plate, agar disk diffusion

INTRODUCTION

Algae have many active metabolites, which can be used in the pharmaceutical industry. Different materials have been obtained from Algae, such as: amino acids, terpenoids, florentines, alkanes, halogenated ketones, steroidal compounds, annular polysulfides, fatty acids, phenols, etc., some of which have antibacterial effects (Taskin et al., 2007). The antibiotic secreted by the algae *Chlorella* (Chlorellin) has an inhibitory effect on bacteria, and the free fatty acids of this algae can damage bacterial cell membrane (DellaGreca et al., 2010). The unicellular algae *Chlorella vulgaris* CCATM- 210-1 with a diameter of 2 to 10 microns, is one of the most famous micro-Algae living in fresh water. *Chlorella*, similar to plants, is one of the most active photosynthetic organisms and has a high density of chlorophyll. Part of the healing properties of *Chlorella* in the

body, is related to the large amount of chlorophyll and the structure of its cell wall, especially the constituents of this cell wall. This algae increases the health and defensive power of the body skin (Safari et al., 2011).

Food consumption makes it possible for many pathogens (bacteria, viruses, and parasites) to be transmitted to the human body (Newell et al., 2010).A large variety of micro-organisms or their toxins, with different mechanisms, have a role in foodborne illnesses (Mozafari et al., 2002). According to national health agencies, the average incidence of foodborne illnesses in the European countries and the third world countries, has been reported to be 38.3 and 915.8 cases per 100,000 population, respectively. The incidence rate of foodborne illnesses has had a growing trend in developing countries. Although there is no statistics on the incidence of food infections and food poisonings in developing countries such as Iran, but with no doubt, due to the poor conditions of food production, storage, distribution, and consumption, which are often without appropriate control by responsible organizations, and due to the low level of public health education, the prevalence of food infections has been far more in these countries than in the developed countries (Tavakoli et al., 2007). Increased use of antibiotics, and undue and inappropriate use, such as patients' excessive intake and/or non-compliance with recommended doses have led to the development of bacterial resistance to antibiotics (Kotze and Eloff., 2002). This study was conducted with the aim of identifying the inhibition properties of the acetone and ethanol extracts, and the supernatant of the algae *Chlorella vulgaris* CCATM- 210-1 on some of the gram-negative bacterial foodborne pathogens.

MATERIALS AND METHODS

Materials and devices used in this study

Consuming materials:

The Mueller-Hinton agar (MHA) medium and the Trypticase soy broth (TSB) medium were all prepared from Qlab Company in Canada. Tamiya medium, ethanol 96%, acetone 99%, dimethyl sulfoxide (DMSO) 99.9%, and distilled water (all made by Merck Company in Germany), the physiological serum, Gentamicin Sigma commercial powder, Gentamicin disk made by Padtan Teb Company in Iran.

Devices:

Autoclave model D121 manufactured by Iran Tolid Company, Digital Scale model SBA 32 manufactured by SCALTEC Company in Germany, Spectrophotometer model M259 manufactured by Sherwood Company in the U.K., Freeze-Dryer model CHRIST-21165 made in Germany, Tube Shaker LS-100 manufactured by Labtron Company in Iran, Plate Shaker (PARS Teb Novin Iranian International Company), Incubator and Hot Air Oven (both manufactured by Behdad Company in Iran).

The conditions of culturing and preparing the supernatant of the algae *Chlorella vulgaris*:

To conduct this research, the green algae *Chlorella vulgaris* CCATM-210-1 was prepared from the culture collection of Algae of Department of Marine Biology, Tarbiat Modarres University in Noor City.

Sterilization: A UV lamp was used for 20 minutes to sterilize the culture room. All the glass tools were first washed by distilled water, then were dried and sterilized at a temperature of 180 °C in the oven for one hour. The Erlenmeyer flasks containing liquid media, were covered by cotton and aluminum foil, and were placed in the autoclave at a temperature of 121 °c and pressure of 15 Pounds/Inch^2 for 20 minutes, to be sterilized.

Light setting: In this study, fluorescent lamps with an intensity of 60 $\mu M.m^{-2}.s^{-1}$ and a light/dark cycle of 9:15 were used.

Temperature setting: The cultivation temperature for the algae *Chlorella vulgaris* CCATM 210-1, was set in the range of 27 ± 1 °C.

Cultivation: Cultivation of the Algae *Chlorella vulgaris* CCATM 210-1 was done in a specific cultivation room (Ficolab) with fluorescent lamps on its walls. First, one liter of Tamiya medium

was added to the 2-liter Erlenmeyer flask, and the algael samples were added. Then during the logarithmic phase and near the flame, the sterile culture media were inoculated to the extent that the optical density was set between 0.03-0.04. The optical density was read by UV-VIS spectrophotometer on a daily basis, and when the optical density reached 1.2 (stationary phase), the suspensions were centrifuged and finally the Algae and supernatant were separated. To conduct the research, the supernatant was poured into a large glass plate and put at the ambient temperature (25-28 °C) away from sunlight, to be dried (Ghasemi et al., 2007).

Preparing the powder of the algae *Chlorella vulgaris* CCATM 210-1

To prepare the algae powder, first, using a centrifuge machine, the algae paste was obtained from the obtained suspension during a 21-day period. Then, the algae paste was moved to the freeze dryer model CHRIST-21165, and was dried using the freeze drying method (Ghasemi et al., 2007).

The method for extracting the Acetone and Ethanol extracts of *Chlorella vulgaris* CCATM 210-1

First, the powder of the algae *Chlorella vulgaris* CCATM 210-1 was solved to the ratio of 5 gr per 100 ml of Acetone and Ethanol solvents, and then they were placed on a shaker for 72 hours. After 72 hours, the extract was strained and poured into a large glass plate and then put at the ambient temperature (25-28 °C) away from sunlight, so that the solvent is removed and the extract is dried (Vishnu and Sumathi., 2014).

Preparing a suspension from the extracts and the supernatant

For this purpose, amounts of 1 gr from the powders of the dried extracts (Acetone and Ethanol) and supernatant were weighed separately and precisely, and inserted into a sterile container, which contained 100 ml of pure dimethyl sulfoxide, and then dilutions of 10 mg/ml were prepared from each extract and the supernatant to conduct the experiment (Annamalai et al., 2012).

The bacteria being tested

The bacteria that were used in this study, are as follows:

Proteus mirabilis PTCC1776, *Pseudomonas aeruginosa* PTCC 1074, *Escherichia coli* PTCC 1399, *Shigella dysentriae* PTCC 1188, and *Salmonella enterica* PTCC 1709.

These bacteria were purchased in the form of lyophilized vials from the Iranian center for the collection of industrial bacteria and fungi, were opened in aseptic conditions, were transported to the culture medium; Tryptic soy broth (TSB), and were incubated at a temperature of 37 ° C for 18 hours. In order to investigate the antimicrobial effects, every time, a new 24-hour culture was prepared.

The test of determining the antibacterial effects of the acetone and ethanol extracts, and the supernatant of the algae *Chlorella vulgaris* CCATM- 210-1 using the well plate method

In order to implement the well plate method, a suspension equivalent to 0.5 McFarland standard (at wavelength of 630 nm and optical density of 0.08-0.13) was prepared from the 24-hour microbial culture of the bacteria. Then 20 µl of the obtained bacterial suspension was inoculated on the Mueller-Hinton agar culture medium, and was cultured uniformly on the surface of the culture medium using a sterile swab. And using a sterile Pasteur pipette, some wells with a diameter of 6 mm were created on the culture medium at distances of 2.5 cm from each other. Ninety µl of the dilutions (1.25, 2.5, 5, and 10 mg/ml) of these extracts and the supernatant was separately added to each well (10 µl of the culture medium was previously used to close the bottom of the wells). Dimethyl sulfoxide was used as a negative control, and the antibiotic Gentamicin 10 mg/ml as a positive control. In order to investigate the interaction between these two extracts using the well plate method, an amount of 45 µl of the acetone extract and 45 µl of the ethanol extract were added to each well. After 24 hours of incubation at a temperature of 37 ° C, the diameter of the inhibition zone around each well, was

measured using a millimeter ruler. In order to verify the obtained results, the experiments were carried out in 5 replications (Salem et al., 2014).

The test of determining the antibacterial effects of the acetone and ethanol extracts, and the supernatant of the algae *Chlorella vulgaris* CCATM- 210-1 using the agar disk diffusion method

In order to implement the disk diffusion method, about 90 µl of the dilutions (1.25, 2.5, 5, and 10 mg/ml) of these extracts, and the supernatant of the algae *Chlorella vulgaris* CCATM- 210-1 were separately added to the standard disks, and they were put in the hot air oven at 45-50 °C to dry. A suspension equivalent to 0.5 McFarland standard was prepared from the 24-hour microbial culture of the bacteria. Then 20 µl of the obtained bacterial suspension was inoculated on the Mueller-Hinton agar culture medium, and then was cultured uniformly on the surface of the culture medium using a sterile swab. And the disks were placed on the culture medium at specified distances from each other. Dimethyl sulfoxide was used as a negative control, and a 10-µg antibiotic disk of Gentamicin as a positive control. In order to investigate the interaction between these two extracts using the agar disk diffusion method, 45 µl of the acetone extract and 45 µl of the ethanol extract were applied to each disk. After 24 hours of incubation at a temperature of 37 ° C, the diameter of the inhibition zone around each disk, was measured using a millimeter ruler. In order to verify the obtained results, the experiments were carried out in 5 replications (Ghasemi et al., 2004).

Statistical analysis:

Using SPSS software version 23, a statistical analysis was carried out on the results investigated in this research, and comparison of means was performed by ANOVA test.

RESULTS

According to the study conducted, the acetone and ethanol extracts and the supernatant of the algae *Chlorella vulgaris* CCATM- 210-1 showed acceptable antibacterial properties against some of the gram-negative bacterial foodborne pathogens, at concentrations of 1.25, 2.5, 5, and 10 mg/ml in the well plate and agar disk diffusion methods. According to tables 4 and 8, the maximum inhibition zone diameters in the well plate and agar disk diffusion methods, were 36.8 and 35.4 mm, respectively, which were related to the bacterium *Proteus mirabilis* PTCC 1776 and were observed at a 10 mg/ml concentration of the acetone extract. By comparing the mean inhibition zone diameters among the acetone and ethanol extracts and a mixture of the two extracts using the well plate method, it can be concluded that the acetone and ethanol extracts of *Chlorella vulgaris* CCATM- 210-1 are indifferent to each other. This analysis was also true of the agar disk diffusion method. The mortality due to microbial agents and increased bacterial resistance to antibiotics, forced human beings to think about ways to cope with these microorganisms. One of these solutions is the substances extracted from plants and Algae, which are considered as antimicrobial compounds and an alternative to synthetic medicines.

Table 1. The mean inhibition zone diameters of a number of standard gram-negative bacterial foodborne pathogens against a 1.25 mg/ml concentration of the extracts and supernatant of *Chlorella vulgaris* in mm, using the well plate method

1.25 mg/ml / Bacteria	Acetone extract	Ethanol extract	Mixture of acetone and ethanol extracts	Supernatant of *Chlorella vulgaris*	*Positive control
Proteus mirabilis PTCC 1776	16.20	18.40	16.20	20.40	27.80
Pseudomonas aeruginosa PTCC 1074	17.80	17.60	17.60	18.40	31.40

Escherichia coli PTCC 1399	18.60	12.40	16.20	8.60	35.60
Shigella dysentriae PTCC 1188	8	8.25	8	18	30.80
Salmonella enterica PTCC 1709	11.40	18.20	16.60	8.20	32.4

* Positive control: Antibiotic Gentamicin 10 mg/ml

Table 2. The mean inhibition zone diameters of a number of standard gram-negative bacterial foodborne pathogens against a 2.5 mg/ml concentration of the extracts and supernatant of *Chlorella vulgaris* in mm, using the well plate method

2.5 mg/ml / Bacteria	Acetone extract	Ethanol extract	Mixture of acetone and ethanol extracts	Supernatant of *Chlorella vulgaris*	*Positive control
Proteus mirabilis PTCC 1776	28	28.20	28	28.40	27.80
Pseudomonas aeruginosa PTCC 1074	22.60	24	22.60	25.20	31.40
Escherichia coli PTCC 1399	23.40	17.20	23	9.40	35.60
Shigella dysentriae PTCC 1188	8.50	10.75	10	24.40	30.80
Salmonella enterica PTCC 1709	23	24	23	9.20	32.4

* Positive control: Antibiotic Gentamicin 10 mg/ml

Table 3. The mean inhibition zone diameters of a number of standard gram-negative bacterial foodborne pathogens against a 5 mg/ml concentration of the extracts and supernatant of *Chlorella vulgaris* in mm, using the well plate method

5 mg/ml / Bacteria	Acetone extract	Ethanol extract	Mixture of acetone and ethanol extracts	Supernatant of *Chlorella vulgaris*	*Positive control
Proteus mirabilis PTCC 1776	35.80	34.60	35	33.40	27.80
Pseudomonas aeruginosa PTCC 1074	29.60	29.20	29.20	34.20	31.40
Escherichia coli PTCC 1399	29.40	22.60	28.80	12.20	35.60
Shigella dysentriae PTCC 1188	12.50	12.25	12.25	32	30.80
Salmonella enterica PTCC 1709	32	32.40	32	17.80	32.4

* Positive control: Antibiotic Gentamicin 10 mg/ml

Table 4. The mean inhibition zone diameters of a number of standard gram-negative bacterial foodborne pathogens against a 10 mg/ml concentration of the extracts and supernatant of *Chlorella vulgaris* in mm, using the well plate method

10 mg/ml	Acetone	Ethanol	Mixture of	Supernatant of	*Positive

Bacteria	extract	extract	acetone and ethanol extracts	*Chlorella vulgaris*	control
Proteus mirabilis PTCC 1776	36.80	36.60	36.60	34.60	27.80
Pseudomonas aeruginosa PTCC 1074	33	32.80	32.80	35.60	31.40
Escherichia coli PTCC 1399	33.40	31	33	21.60	35.60
Shigella dysentriae PTCC 1188	13.75	18.20	16	36.60	30.80
Salmonella enterica PTCC 1709	33.60	33.60	33.60	20.40	32.4

* Positive control: Antibiotic Gentamicin 10 mg/ml

Table 5. The mean inhibition zone diameters of a number of standard gram-negative bacterial foodborne pathogens against a 1.25 mg/ml concentration of the extracts and supernatant of *Chlorella vulgaris* in mm, using the agar disk diffusion method

1.25 mg/ml / Bacteria	Acetone extract	Ethanol extract	Mixture of acetone and ethanol extracts	Supernatant of *Chlorella vulgaris*	*Positive control
Proteus mirabilis PTCC 1776	22.40	19.60	21	20.20	27.40
Pseudomonas aeruginosa PTCC 1074	14	14.20	14.20	19	30.40
Escherichia coli PTCC 1399	14.40	10.80	14	8.40	35.20
Shigella dysentriae PTCC 1188	8	8.25	8.25	17	30.60
Salmonella enterica PTCC 1709	14.80	19	15.80	8.60	32

* Positive control: 10-µg Gentamicin disk

Table 6. The mean inhibition zone diameters of a number of standard gram-negative bacterial foodborne pathogens against a 2.5 mg/ml concentration of the extracts and supernatant of *Chlorella vulgaris* in mm, using the agar disk diffusion method

2.5 mg/ml / Bacteria	Acetone extract	Ethanol extract	Mixture of acetone and ethanol extracts	Supernatant of *Chlorella vulgaris*	*Positive control
Proteus mirabilis PTCC 1776	26.20	26.20	26.20	27.20	27.40
Pseudomonas aeruginosa PTCC 1074	19.60	19.60	19.60	26.80	30.40
Escherichia coli PTCC 1399	19	15.20	16	8.60	35.20
Shigella dysentriae PTCC 1188	8.75	8.75	8.75	23.80	30.60
Salmonella enterica	24.20	24.20	24.20	11.40	32

PTCC 1709					

* Positive control: 10-µg Gentamicin disk

Table 7. The mean inhibition zone diameters of a number of standard gram-negative bacterial foodborne pathogens against a 5 mg/ml concentration of the extracts and supernatant of *Chlorella vulgaris* in mm, using the agar disk diffusion method

5 mg/ml / Bacteria	Acetone extract	Ethanol extract	Mixture of acetone and ethanol extracts	Supernatant of *Chlorella vulgaris*	*Positive control
Proteus mirabilis PTCC 1776	34.40	33.60	33.80	33	27.40
Pseudomonas aeruginosa PTCC 1074	28.80	28.20	28.20	33.20	30.40
Escherichia coli PTCC 1399	28.60	22	27.80	11.40	35.20
Shigella dysentriae PTCC 1188	11.50	12	11.50	31	30.60
Salmonella enterica PTCC 1709	31	31.60	31	17	32

* Positive control: 10-µg Gentamicin disk

Table 8. The mean inhibition zone diameters of a number of standard gram-negative bacterial foodborne pathogens against a 10 mg/ml concentration of the extracts and supernatant of *Chlorella vulgaris* in mm, using the agar disk diffusion method

10 mg/ml / Bacteria	Acetone extract	Ethanol extract	Mixture of acetone and ethanol extracts	Supernatant of *Chlorella vulgaris*	*Positive control
Proteus mirabilis PTCC 1776	35.40	35.40	35.40	34.20	27.40
Pseudomonas aeruginosa PTCC 1074	32.20	31.80	31.80	34.60	30.40
Escherichia coli PTCC 1399	32.40	30.20	30.20	17.40	35.20
Shigella dysentriae PTCC 1188	13.25	17.40	16.80	35.60	30.60
Salmonella enterica PTCC 1709	32.80	32.60	32.60	19.60	32

* Positive control: 10-µg Gentamicin disk

DISCUSSION

Pratt et al (1944) reported that *Chlorella vulgaris* contains an active compound, known as Chlorellin (consisting of a variety of fatty acids), that inhibits gram-positive and gram-negative bacterial activities. Also in the present study, the acetone and ethanol extracts and the supernatant of this algae could inhibit the growth of the bacteria being tested, which may be due to the presence of the antibiotic Chlorellin in the extracts and supernatant of this algae.

Ghasemi et al (2004) stated that microAlgae such as: *Chlorella* species, *Scenedesmus* species, *Euglena viridis*, *Fischerella ambigua*, *Nostoc* species, *Scytonema hofmanni*, *Hapalosiphon fontinalis*, *Anabaena* species, *Microcystis aeruginosa*, and *Phormidium* species are the main

groups of microAlgae that produce antimicrobial substances. Not only is Algae's ability to produce antimicrobial substances considered a means of defense for Algae, but these substances can also be used in the pharmaceutical industry.

Ghasemi et al (2007) reported that using the agar disk diffusion method, the supernatant of *Chlorella vulgaris* (012) formed inhibition zones equivalent to 16, 15, 15, 14, 13 and 14 mm against the bacteria *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Bacillus subtilis*, *Escherichia coli*, *Salmonella typhi*, and *Pseudomonas aeruginosa*, respectively. Continuing in this investigation, they found that the supernatant of *Chlorella vulgaris* (046) showed inhibition zones equivalent to 20, 10, and 16 mm against the bacteria *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Bacillus subtilis*, respectively, whilst it had no effects against the bacteria *Escherichia coli*, *Salmonella typhi*, and *Pseudomonas aeruginosa*.

In the present study, the supernatant of *Chlorella vulgaris* CCATM- 210-1 showed an acceptable antimicrobial activity against the tested bacteria, such that in the present study, the supernatant of this algae inhibited the growth of standard gram-negative bacterial foodborne pathogens, such as: *Escherichia coli* PTCC 1399 and *Pseudomonas aeruginosa* PTCC 1074, as similarly as the supernatant of *Chlorella vulgaris* (012) did in the study conducted by Ghasemi et al.. In the present study, the supernatant of this algae, unlike the supernatant of *Chlorella vulgaris* (046) in the study conducted by Ghasemi et al., was also able to inhibit the growth of the bacteria *Escherichia coli* and *Pseudomonas aeruginosa*. This difference may be due to the further release of fatty acids and Chlorellin in the supernatant of the algae *Chlorella vulgaris* CCATM- 210-1 being studied.

DellaGreca et al (2010) noted that, the substance secreted by the algae *Chlorella* (Chlorellin) has an inhibitory effect on bacteria. They also stated that part of the antimicrobial properties of *Chlorella*, is to release fatty acids in the culture medium, and this mechanism helps this algae survive in its surrounding environment. And the fatty acid of this algae damages the bacterial cell membrane. In the present study, the destruction of the tested bacteria may be due to the presence of the antibiotic Chlorellin and fatty acid in this algae.

Annamalai et al. (2012) reported that the ethanol extract of *Chlorella vulgaris* at a concentration of 100 mg/ml, 100 µl of which was added to the wells, formed inhibition zones of 20, 16, 15, 23 and 24 mm against the clinical isolates of *Escherichia coli*, *Proteus vulgaris*, *Staphylococcus aureus*, *Pseudomonas aeruginosa* and *Bacillus subtilis*, respectively.

In the present study, the antibacterial properties of the ethanol extract of *Chlorella vulgaris* CCATM-210-1 was approved using the well plate method, such that the mean inhibition zone diameters of the bacteria *Escherichia coli* PTCC 1399 and *Pseudomonas aeruginosa* PTCC 1074 against a concentration of 10 mg/ml of the ethanol extract of *Chlorella vulgaris* CCATM- 210-1, 90 µl of which was added to the wells, were 31, 32, and 32.8 mm, respectively. The findings of the present study showed that the ethanol extract of this algae could inhibit the growth of the above mentioned bacteria with a more potent antibacterial activity.

Salem et al. (2014) studied the antibacterial activity of the acetone extracts of some micro-Algae at a concentration of 30 mg/ml, 100 µl of which was added to the wells, against a number of gram-positive and gram-negative bacteria, and reported that the acetone extract of *Chlorella vulgaris* was effective against the clinical isolates of *Bacillus subtilis*, *Klebsiella pneumoniae*, *Staphylococcus aureus*, and *Sarcina lutea*, whilst it had no effects against the bacterium *Bacillus megaterium*. Also in the present study, the aceton extract of *Chlorella vulgaris* CCATM-210-1 could inhibit the growth of a number of gram-negative bacterial foodborne pathogens at concentrations of 10, 5, 2.5 and 1.25 mg/ml.

Syed et al. (2015) examined the antibacterial properties of the ethanol, acetone, and chloroform extract of *Chlorella vulgaris* at a concentration of 100 mg/ml against some bacteria, using the agar

disk diffusion method. In order to implement this method, they applied 150 µl of the extract to the disks. They evaluated the antimicrobial activity of the bacteria *Escherichia coli*, *Klebsiella* species, and *Bacillus* and *Pseudomonas* species positively. This result was also consistent with the results of the present study.

Using the agar disk diffusion method, El-Sheik and Al-Souod (2015) examined the intracellular and extracellular extracts of *Chlorella vulgaris* prepared with chloroform, ethanol, methanol, and ethyl acetate solvents against the bacteria *Escherichia coli* and *Staphylococcus aureus*. In order to implement this method, they applied 100 µl of the extract to the disks. They found out that the extracellular extract of *Chlorella vulgaris* had a less inhibitory effect on the growth of the above mentioned bacteria than the intracellular extract did. In the present study, too, the extracellular extract of *Chlorella vulgaris* CCATM- 210-1 (supernatant) showed a less inhibitory effect on the growth of the bacterium *Escherichia coli* PTCC 1399 than the intracellular extracts (acetone and ethanol) of this algae did.

No significant difference was observed among the acetone and ethanol extracts and a mixture of the acetone and ethanol extracts of *Chlorella vulgaris* CCATM- 210-1 by comparing the mean inhibition zone diameters among the acetone and ethanol extracts and a mixture of the acetone and ethanol extracts of *Chlorella vulgaris* CCATM- 210-1 through the well plate method and using the ANOVA statistical test ($p<0.05$), and it can be concluded that the acetone and ethanol extracts of *Chlorella vulgaris* CCATM- 210-1 are indifferent to each other. This analysis was also true of the agar disk diffusion method.

CONCLUSION

Therefore, the findings from this study showed that the acetone and ethanol extracts and the supernatant of the algae *Chlorella vulgaris* CCATM- 210-1 had a good inhibitory properties against a number of gram-negative bacterial foodborne pathogens, and this may be due to the presence of the antibiotic Chlorellin in this algae.

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