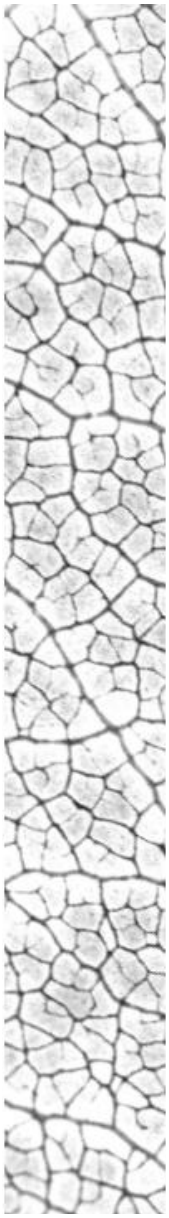

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Antibacterial activity of ethanol extracts of two algae species against some pathogenic bacteria isolated from hospital patients

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Abstract

Microalgae play a significant role in the development of new products for medical and pharmaceutical research due to their ability to generate different biologically active metabolites. They are target organisms in the search for new antibiotic molecules to deal with antibiotic resistance. In addition, the use of natural antibiotics could satisfy consumer demand to avoid the side effects of chemicals. Our results showed antimicrobial activity of two algal species *Spirulina platensis* and *Chlorella vulgaris* against nine human pathogenic bacteria by agar well diffusion method. Seven concentrations of algal extract (10, 50, 100, 150, 200, 250, and 300 mg/ml) were used. It was observed that ethanolic extract of *Spirulina platensis* was most effective against *Streptococcus agalactiae* with maximum inhibition zone of 21.6 mm, while the minimum inhibition zone (8.5mm) was found in case of *Pseudomonas aeruginosa* at concentration 300 mg/ml. In the case of ethanolic extract of *Chlorella vulgaris*, the inhibition zone was the highest (31.6 mm) against *Staphylococcus lentus*, while the lowest inhibition zone (20.6 mm) was in case of *Staphylococcus aureus* at concentration 300 mg/ml. While the concentrations less than 300 mg/ml showed varying inhibition of pathogenic bacteria, some bacterial isolates showed resistance to low concentrations of algal extracts. The results of gas chromatography–mass spectrometry (GC-MS) analysis of the two algal extracts showed that chemical composition analysis consisted of terpenes (monoterpenes and sesquiterpenes)

Keywords: *Spirulina platensis*, *Chlorella vulgaris*, Bioactive compounds, Pathogenic bacteria, Antibacterial activity, Gas chromatography–mass spectrometry (GC-MS)

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INTRODUCTION

Microorganisms that are capable of causing disease are called pathogens. Pathogenic bacteria can cause disease through a number of mechanisms in human hosts. The term “disease” refers to conditions that impair normal tissue function. The harm that pathogens cause to hosts during infection is called virulence, which varies between species ranging from minimal to immediate deaths (Leggett *et al*. 2017). Bacteria causing infection are considered pathogenic bacteria, creating toxic substances called endotoxins and exotoxins. These substances are responsible for the symptoms of diseases related to bacteria. The symptoms can vary from mild to severe and even lethal (Wilson *et al*. 2002). Bacteria infect human body causing many diseases, including lung, skin, and urinary tract infections. For example, the most popular causative agent for both uncomplicated and complicated urinary tract infections (UTIs) is uropathogenic *Escherichia coli*, followed by *Enterococcus faecalis*, group B *Streptococcus* (GBS), *Proteus mirabilis*, *Pseudomonas aeruginosa* and *Staphylococcus aureus* (Foxman 2014).

Antibiotics, also identified as antibacterials, are medicines that kill or delay bacterial growth. These include a number of potent drugs used to treat bacteria-induced diseases. Antibiotics are powerful medicines that fight certain infections and can save lives when used properly; they prevent the bacteria from reproducing or eliminate them (Fair and Tor 2014). Roca *et al*. (2015) observed a dramatic increase in the proportion and an absolute number of resistant bacterial pathogens to multiple antibacterial agents over the past decade. Currently, multidrug-resistant (MDR) bacteria are considered an emerging global disease and a major public health issue. Bacterial strains resistant to the inhibitory effect of antibiotics pose a global threat to the potential for chemotherapy. The problem was

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compounded by bacteria's ability to transfer resistance genes to antibiotic-sensitive bacteria using different mechanisms leading to clinically significant antibiotic resistance. Unwise and excessive use of antibiotics has undoubtedly contributed to the problem's complexity. It was found that an increase in the rate of resistance to certain antibiotics was associated with an increase in their use (Wright 2010).

Over the past several decades, dangerous antibiotic-resistant bacteria have been observed with increasing frequency. Bacterial antibiotic resistance has been a recognized actuality almost since the dawn of the antibiotic age; but it has only occurred with disturbing regularity in the past twenty years when dangerous resistant strains emerged (Appelbaum 2012). Microbial resistance to currently available antibiotics is a public health problem in the fight against infectious diseases. Also, most antibiotics are characterized by numerous side effects that may be harmful to normal body cells. (Mgbeahuruike *et al*. 2019).

The use of microalgae compounds as promising sources of natural antibiotics against human pathogens is mean used different alternatives of natural compounds are available for control of pathogenic bacteria, mainly microalgae-derived. They have the advantages of reducing the side effects of synthetic antibiotics as well as being less expensive (Falaise *et al*. 2016).

In the last decade, there has been increasing interest in microalgae screening for antibiotics and pharmacologically active compounds. A large number of antibiotic compounds have been isolated and characterized, many with new structures. Microalgae are especially attractive as natural sources of bioactive molecules because these algae have the potential to produce such compounds in a culture that makes it possible to produce structurally complex molecules that are difficult or impossible to produce through chemical synthesis (Borowitzka 1995). Due to their extensive application potential in the renewable energy, biopharmaceutical, and nutraceutical industries microalgae have recently attracted significant interest worldwide. Microalgae are sources of biofuels, bioactive medicinal products, and food ingredients that are renewable, economical, and sustainable (Khan *et al*. 2018). A variety of studies have investigated the therapeutic potential of algal extracts and extracellular compounds from a wide range of microalgae; and they have reported antibacterial, antiprotozoal, antiviral, antifungal, and anti-plasmodial activity. Chemical groups such as phenols, fatty acids, indoles, terpenes, acetogenins, and some volatile halogenated hydrocarbons extracted from microalgae have shown antimicrobial activity (Jena and Subudhi 2019). Mgbeahuruike *et al*. (2019) indicated that the dosage combinations of these bioactive compounds with the antibiotics used may be a better option for the treatment of bacterial infections aimed at minimizing the adverse effects associated with the use of these conventional antibacterial drugs.

Cyanobacteria and algae with complex photosynthetic processes can turn absorbed solar energy into other forms of energy for nutrition; metabolites such as phenolics, phytoene/terpenoids, phytols, sterols, free fatty acids, photoprotective compounds (MAAs, scytonemines, carotenoids, polysaccharides, halogenated compounds, etc.), phytohormones, cyanotoxins, biocides, phytohormones, and cyanotoxins biocide the importance of these metabolites as antibiotics, immunosuppressants, anticancer, antivirals, anti-inflammatory agents. Metabolites derived from cyanobacteria and algae have several biotechnological, agricultural, medicinal and cosmetic applications (Singh *et al*. 2017).

Terpenoids are the generally distributed class of organic compounds in cyanobacteria and algae (Keeling and Bohlmann 2012). They have been categorized into seven groups according to their five-carbon isoprene structure, i.e. hemiterpenes (C5), monoterpenes (C10), sesquiterpenes (C15), diterpenes (C20), triterpenes (C30), tetraterpenes (C40), and polyterpenes (>C40) (Singh and Sharma 2015). Terpenoids have gained more interest in recent years at the commercial level due to their active roles in therapeutics (Pattanaik and Lindberg 2015).

Naturally, cyanobacteria produce many diterpenes with specific functions such as tolypodiol, a compound of diterpenoids isolated from Tolypothrix nodosa that has been shown to have anti-inflammatory properties (Prinsep *et al*. 1996). Two isolated abietane diterpenes from the cyanobacterium Microcoleous lacustris showed antibacterial activity against a few specific bacteria (Gutiérrez *et al*. 2008). The diterpenoid, anthraquinone, and indane derivative, first reported as a natural product, were isolated from the cells of the cultivated cyanobacterium Nostoc commune (EAWAG 122b); these natural products have antibacterial activity for all bacterial isolates used in the study (Jaki *et al*. 2000).

Five microalgae cultures (*Chlorella minutissima, Tetraselmis chui, Nannochloropsis sp., Arthrospira platensis, and Isochrysis sp*.) have been tested for their ability to inhibit the growth of six Vibrio bacterial strains (*V. parahaemolyticus, V. anguillarum, V. splendidus, V. scophthalmi, V. alginolyticus, and V. lentus*). Compared to the control treatments, all microalgae cultures inhibited bacterial growth (Kokou *et al*. 2012). The dried biomass of *Chlorella vulgaris* showed high antibacterial activity against gram-negative and gram-positive human pathogenic bacteria, like *Klebsiella pneumoniae, Proteus mirabilis, Vibrio cholerae, Salmonella typhi, Escherichia coli, Staphylococcus aureus, Bacillus subtilis, Enterococcus sp., Clostridium botulini,* and *Nocardia sp*. because it has phytochemicals such as

Table 1. Pathogenic bacterial identification according to the biochemical tests

Pathogenic bacterial isolates	Clinical cases isolation	Biochemical tests											
		Gram stain	Catalase	Oxidase	Urease production	Hemolysis	Coagulase	Mannitol	Novobiocin Susceptibility	Indole	Citrate Utilization	Methyl Red	Voges–Proskauer
Enterococcus faecalis	Stool	+	-	-	-	*	-	*	*	-	*	*	-
Escherichia coli	Urine	-	+	-	-	+	*	*	*	+	-	+	-
Proteus mirabilis	Urine	-	+	-	+	-	*	*	*	-	+	+	-
Pseudomonas aeruginosa	Sputum	-	+	+	+-	β	-	*	*	-	+	-	-
Staphylococcus aureus	Urine	+	+	-	+-	+	+	+	S	*	*	*	*
Staphylococcus lentus	Urine	+	+	-	+-	+-	-	-	R	*	*	*	*
Staphylococcus xylosus	Urine	+	+	-	+-	+-	-	-	R	*	*	*	*
Streptococcus agalactiae	Vaginal	+	-	-	+	β	-	*	*	*	*	*	*
Streptococcus pyogenes	Skin	+	-	-	+	β	-	*	*	*	*	*	*

+ Positive, - negative, * test not achieve, β blood beta analysis, S sensitive, R resist

phenol, tannins, flavonoids, terpenes, terpenoids, alkaloids, and saponins (Dineshkumar *et al*. 2017).

The objective of this study is to estimate the antibacterial activity of two algae species, *Spirulina platensis* and *Chlorella vulgaris* against pathogenic bacteria isolated from different clinical cases.

MATERIALS & METHODS

Algae Species

Two identified and pure microalgae *Spirulina platensis* and *Chlorella vulgaris* powders were purchased from Golden Horizon (Chengdu, Technology Co., Ltd, China).

Soxhlet Extraction

Solvent extraction method was performed using modified Soxhlet extraction (SE). SE was implemented with 25 g of *Spirulina platensis* and *Chlorella vulgaris* biomass powder with extraction solvent 250 ml of ethanol on a Soxhlet system. The extraction process was performed at 78°C until the extract was clear, followed by solvent evaporation. The extracts were transferred to a hot air oven, where they were dried at 40°C and stored at 4°C. Portions of the extracts were used for phytochemical analysis carried out by gas chromatography–mass spectrometry (GC-MS) method while the rest were used for bacterial susceptibility test. Algal crude extracts were dissolved in dimethyl sulfoxide (DMSO) to a final concentration 300 mg/mL, sterilized by filtration and stored at 4°C (Li *et al*. 2014).

Bacterial Strain Used

Pathogenic bacterial species were isolated from Al-Diwaniyah Teaching Hospital in Diwaniyah Governorate, Iraq. Nine bacterial species were isolated from different clinical cases including samples of urine, feces, sputum, skin, and vaginal smears. Isolates were diagnosed based on phenotypic characteristics, cultural characteristics, biochemical tests, API 20E Test System, API 20 Staph, and API 20 Strep (CLSI 2016). Bacterial isolates were diagnosed after cultivation on the MacConkey agar nutrient agar and Mannitol Salt Agar (MSA).

Pure cultures of pathogenic bacteria, including *Enterococcus faecalis, Escherichia coli, Proteus mirabilis, Pseudomonas aeruginosa, Staphylococcus aureus, Staphylococcus lentus, Staphylococcus xylosus, Streptococcus agalactiae,* and *Streptococcus pyogenes* were isolated from different clinical cases (**Table 1**). The cultures were used as the test microorganism for antibacterial testing. The organism was suspended in 2 ml of sterile saline water to prepare the inoculum. Then, turbidity was adjusted to 0.5 McFarland standard before the Mueller-Hinton agar plate was inoculated.

Determination of Antimicrobial Activity

Antibacterial activity was tested using the agar well diffusion method as described in CLSI (2016). Muller Hinton Agar Medium was prepared, the pH kept at 7.4, then autoclaved for 15 min at 121°C and 15 lbs pressure. In the sterilized Petri dish, 20 ml of the sterilized media was poured and allowed to solidify at room temperature. A sterile cotton swab was used equally on the Muller Hinton Agar Medium plates to spread the test microorganism from the 24-hour inoculated broth. Likewise, for each test microorganism, swabbing was done separately on the Muller Hinton Agar plates and left for a few minutes to allow the inoculum to be fully absorbed. Using an appropriate size sterilized cork borer, wells with a diameter of 6 mm were made at the center of each of these plates. 50 µl from different concentrations of each algal extract on the Muller Hinton Agar plates was transferred to the wells (10, 50, 100, 150, 200, 250, and 300 mg/ml at room temperature). Loaded plates of the extract were kept for incubation at 37°C for 24 hours. A clear zone around the well was observed after incubation, which was evidence of the presence of active antibacterial compounds in algal extracts. Diameters of the inhibition zone (including well diameter) were measured in millimeters by Vernier

Table 2. Inhibition zones of *Spirulina platenis* and *Chlorella vulgaris* extraction against pathogenic bacteria isolated from different cases were measured in millimeters

Pathogenic bacteria	*Enterococcus faecalis*		*Escherichia coli*		*Proteus mirabilis*		*pseudomonas aeruginosa*		*Staphylococcus aureus*		*Staphylococcus lentus*		*Staphylococcus xylosus*		*Streptococcus agalactiae*		*Proteus mirabilis*	
Algal Extracts	S. platensis	C. vulgaris	S. platensis	C. vulgaris	S. platensis	C. vulgaris	S. platensis	C. vulgaris	S. platensis	C. vulgaris	S. platensis	C. vulgaris	S. platensis	C. vulgaris	S. platensis	C. vulgaris	S. platensis	C. vulgaris
	Mean±SE	Mean±SE	Mean±SE	Mean±SE	Mean±SE	Mean±SE	Mean±SE	Mean±SE	Mean±SE	Mean±SE	Mean±SE	Mean±SE	Mean±SE	Mean±SE	Mean±SE	Mean±SE	Mean±SE	Mean±SE
10	0.0±0.0	3.6±0.33*	0.0±0.0	0.0±0.0	0.0±0.0	4.3±0.33*	0.0±0.0	0.0±0.0	0.0±0.0	10.6±0.33*	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	4.3±0.33*	2.1±0.16	0.0±0.0*
	d A	e C	f A	e D	c A	g B	e A	g D	e A	e A	c A	f D	e A	f D	f A	g B	e A	f D
50	0.0±0.0	16.6±0.88*	3.3±0.33	12±0.57*	0.0±0.0	13.6±0.66*	2.6±0.33	7.1±0.01*	0.0±0.0	13±0.57*	0.0±0.0	11.3±0.33*	3.6±0.66	10.6±0.33*	0.0±0.0	10.6±0.33*	2.6±0.33	4.3±0.33*
	d C	d A	e A	d CD	e C	f BC	d B	f C	e C	d B	c C	e D	d A	e E	f C	f E	e B	e F
100	4.5±0.28	18.3±0.33*	8±0.57	15.1±0.01*	0.0±0.0	15±0.57*	5.3±0.33	12.6±0.33*	0.0±0.0	17.3±0.66*	0.0±0.0	14.3±0.33*	3.3±0.33	12.6±0.33*	3.1±0.01	11.3±0.66*	5±0.57	10.6±0.33*
	c C	d A	d A	d C	e E	e C	c B	e D	e E	c B	c C	d C	d A	d D	e D	e D	d B	e E
150	10.6±0.33	20.3±0.88*	7.6±0.66	16.6±0.33*	5.3±0.33	16.67±2.4*	5.3±0.33	14±0.57*	3.3±0.66	20.3±0.66*	5.6±0.66	16.6±0.66*	8.6±0.33	15.6±0.66*	4.3±0.33	13±0.57*	7.3±0.33	13.6±0.66*
	b A	c A	d C	c B	d D	d B	c D	d C	d F	a A	b D	d B	c B	b BC	d E	d D	c C	d DC
200	10.3±0.33	21.3±0.66*	10.3±0.33	18.6±0.33*	7.6±0.33	20.3±0.88*	7.6±0.66	15±0.57*	8.10±0.01	17.3±0.88*	6.6±0.66	18.6±0.33*	9.5±0.26	13.6±0.66*	10.3±0.66	22.3±0.33*	10.6±0.33	21.3±0.66*
	b A	b B	c A	b D	c C	c C	b C	c F	c C	c E	b D	c D	bc B	c G	c A	c A	b A	c B
250	11.3±0.33	21.6±0.88*	17.6±0.66	18.3±0.33	11±0.57	24.3±0.66*	7.4±0.29	18.6±0.33*	10.6±0.33	19.1±0.01*	13.83±2.83	20±0.57*	10.3±0.33	15.6±0.66*	12±0.57	23.3±0.33*	11.3±0.66	22.3±1.2*
	b BC	b C	a A	b F	b C	b A	b E	b E	b C	b E	a E	b D	b C	b F	b B	b B	b C	b C
300	14.6±0.33	22.3±0.33*	12.6±0.33	21.6±0.33*	13.6±0.66	28.3±0.66*	8.5±0.46	21±0.5*	13.6±0.33	20.6±0.66*	13±0.57	31.6±0.88*	13.3±0.33	21±0.57*	21.6±0.33	29.6±0.33*	17.3±0.33	23.6±0.33*
	a C	a ED	b E	a E	a D	a B	a F	a FE	a D	a F	a D	a A	a D	a FE	a A	a B	a B	a D

Mean±SE: mean±standard error
*indicated to differences between the concentration of two algal extracts (LSD= Spirulina platensis: 1.043; Chlorella vulgaris: 0.568)
small letters indicated to differences between concentration for every bacterium (LSD= Spirulina platensis: 0.924; Chlorella vulgaris: 0.831)
capital letters indicated to differences between bacteria for all concentration (LSD= Spirulina platensis: 1.594; Chlorella vulgaris: 1.44)

scale. Antibiotic Disc Diffusion (Kirby–Bauer) technique was conducted according to CLSI (2016) to compare the antibacterial activity of the two algae extracts with the therapeutic action of a number of known broad spectrum antibiotics including Amikacin 30 µg/disc, Augmentin 30 µg/disc, Cefoperazone 30 µg/disc, Ceftriaxone 30 µg/disc, Chloramphenicol 30 µg/disc, Levofloxacin 5 µg/disc, Meropenem 10 µg/disc, Netilmicin 30 µg/disc, Nitrofurantoin 300 µg/disc, Tetracycline 30 µg/disc, and Trimethoprim 5 µg/disc. Muller Hinton Agar was prepared and sterilized. 20 ml of the sterilized media was poured into the sterile Petri dishes after sterilization and allowed for solidification at room temperature. The test pathogenic bacteria from the 24-hour liquid inoculated nutrient broth was evenly spread on each Muller Hinton Agar plate using a sterile cotton swab. Each of the antibiotic disks was put on the Muller Hinton Agar plates using sterile forceps and then kept for incubation at 37°C for 24 hours.

Chemical Composition of Algae Extracts

An analysis of chemical composition of the two algae extracts was performed by GC-MS analytical method (Agilent technologies, USA) equipped with a single quadrupole detector with an HP-5 capillary column (30 m×0.25 mm I.D., 1 µm film thickness). The oven temperature was set at 40°C (hold 2 min) to 150°C at 5°C min, then to 300°C at 15°C min. The temperature of the injector port was kept at 280°C. Helium was used as a carrier gas and 1 µl of the sample was injected into the system (dissolved in 100% dimethyl sulfoxide).

Statistical Analysis

All experiments were carried out in triplicates. The findings were expressed as the mean value ± standard error and evaluated by multiple comparisons using variance analysis (two-way ANOVA) with the least significant differences (LSD) test. All data were processed by SPSS V.26 (P<0.05).

RESULTS

In the present study, antimicrobial activity of two algal species *Spirulina platensis* and *Chlorella vulgaris* were tested against nine human pathogenic bacteria by agar well diffusion method. Seven concentrations of algal extracts were used (10, 50, 100, 150, 200, 250, and 300 mg/ml). The inhibition zones formed by the extracts at different concentrations against the specific test bacteria were measured (**Table 2**). It was observed that ethanolic extract of *Spirulina platensis* was the most effective one against *Streptococcus agalactiae* with maximum inhibition zone of 21.6 mm, while the minimum inhibition zone (8.5 mm) was found in *pseudomonas aeruginosa* at 300 mg/ml concentration of algal extract. While the concentrations less than 300 mg/ml showed varying inhibition of pathogenic bacteria, some bacterial isolates showed resistance to low concentrations of algal extracts, especially 10, 50, and 100 mg/ml.

Table 3. Inhibition zones of antibiotic against pathogenic bacteria isolated from different clinical cases were measured in millimeters

Pathogenic bacteria / Antibiotics	*Enterococcus faecalis*	*Escherichia coli*	*Proteus mirabilis*	*pseudomonas aeruginosa*	*Staphylococcus aureus*	*Staphylococcus lentus*	*Staphylococcus xylosus*	*Streptococcus agalactiae*	*Streptococcus pyogenes*
Amikacin 30 µg	12 R	18 S	20 S	19 S	21 S	20 S	19 R	11 R	17 R
Augmentin 30 µg	0 R	12 R	12 R	0 R	0 R	0 R	0 R	27 S	0 R
Cefoperazone 30 µg	18 S	12 R	15 R	28 S	26 S	28 S	30 S	0 R	4 R
Ceftriaxone 30 µg	0 R	7 R	16 R	28 S	30 S	27 S	30 S	14 R	0 R
Chloramphenicol 30 µg	3 R	11 R	0 R	0 R	20 S	0 R	21 S	8 R	0 R
Levofloxacin 5 µg	0 R	0 R	23 S	27 S	0 R	28 S	25 S	0 R	0 R
Meropenem 10 µg	30 S	30 S	29 S	25 S	26 S	26 S	25 S	13 R	30 S
Netilmicin 30 µg	0 R	15 S	13 R	15 S	18 S	17 S	15 S	0 R	10 R
Nitrofurantoin 300	30 S	26 S	14 R	16 R	12 R	8 R	0 R	21 S	17 S
Tetracycline 30 µg	0 R	0 R	0 R	0 R	0 R	0 R	24 S	0 R	0 R
Trimethoprim 5 µg	4 R	0 R	0 R	0 R	24 S	0 R	20 S	0 R	0 R

R: Resist, S: Sensitive According to CLSI (2018)

In case of ethanolic extract of *Chlorella vulgaris*, the inhibition zone was highest (31.6 mm) against *Staphylococcus lentus*, while the lowest inhibition zone was 20.6 mm in case of *Staphylococcus aureus* at concentration 300 mg/ml of algal extract. Also, the concentrations less than 300 mg/ml showed varying inhibition of pathogenic bacteria. However, some bacterial isolates showed resistance to low concentrations of algal extracts, especially 10 mg/ml (**Table 2**). Comparing the results of **Table 2**, it is noted that the concentrations of *Spirulina* extract had less inhibitory effect than the concentrations of *Chlorella* extract against the pathogenic bacteria used in the study. This is confirmed by the results of statistical analysis at $p<0.05$.

The results of antibiotic susceptibility of the isolated bacterial species against 11 different antibiotics were tested based on the disc diffusion (Kirby–Bauer) technique. The results showed that the highest inhibitory effect of all pathogenic bacterial isolates occurred in the meropenem antibiotic; all isolates were susceptible except *Streptococcus agalactiae* which resisted to this antibiotic isolated from the vaginal area. Meanwhile, the antibiotics Augmentin and Tetracycline showed the least inhibitory effect on all pathogenic bacterial isolates except *Streptococcus agalactiae* which was susceptible to Augmentin and *Staphylococcus xylosus* susceptible to Tetracycline (**Table 3**). Significant difference was confirmed at $p<0.05$.

The results of the instrumental analysis by GC/MS (**Figs. 1** and **2**) of the two algal extracts showed that chemical composition analysis consists of terpenes compounds (monoterpenes and sesquiterpenes) as mentioned in **Table 4**.

DISCUSSION

Algae have a significant attraction as a natural source of bioactive molecules with a wide range of biological activities, including antibiotics, antivirals, anti-tumors, antioxidants, and anti-inflammatory evidence from phytochemical and pharmacological studies. They produce a large majority of the surrounding chemical metabolites like amino acids, terpenoids, phlorotannins, hormones, phenolic compounds, halogenated ketones, alkenes, and cyclic polysulphides, which are some of the bioactive constituents derived from algae. The use of various organic solvents of increasing order of polarity has identified many lipid compounds with antimicrobial properties (Prarthana and Maruthi 2019).

The antibacterial activity of *Spirulina platensis* and *Chlorella vulgaris* was carried out to determine inhibition against some of the pathogenic bacteria such as E*nterococcus faecalis, Escherichia coli, Proteus mirabilis, Pseudomonas aeruginosa, Staphylococcus aureus, Staphylococcus lentus, Staphylococcus xylosus, Streptococcus agalactiae,* and *Streptococcus pyogenes* which are pathogenic to humans and vector diseases causing severe impacts. According to the results obtained from **Table 2**, the effect of ethanolic extracts of two algal species showed the highest inhibition to pathogenic bacteria under study especially at 300 mg/ml concentration of extract compared to the lesser concentration of extract; however, some pathogenic bacteria showed resistance to low concentrations of algal extract (no zone inhibition was observed). This may be due to the ability of microalgae to produce compounds with wide-spectrum activity that are highly desired for the production of antibiotics. Most compounds derived from these species are likely to be

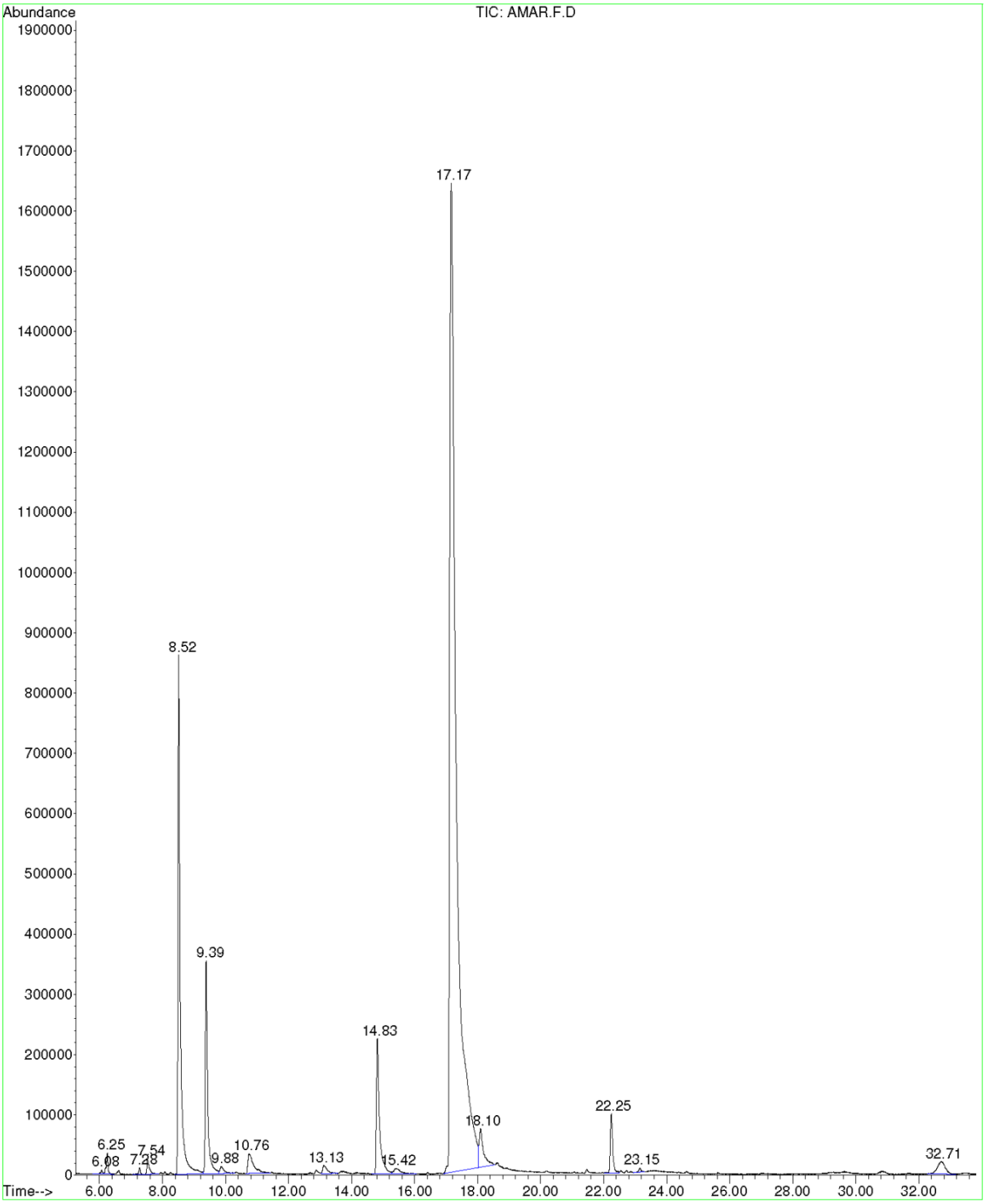


Fig. 1. GC/MS analysis of Spirulina platensis extract

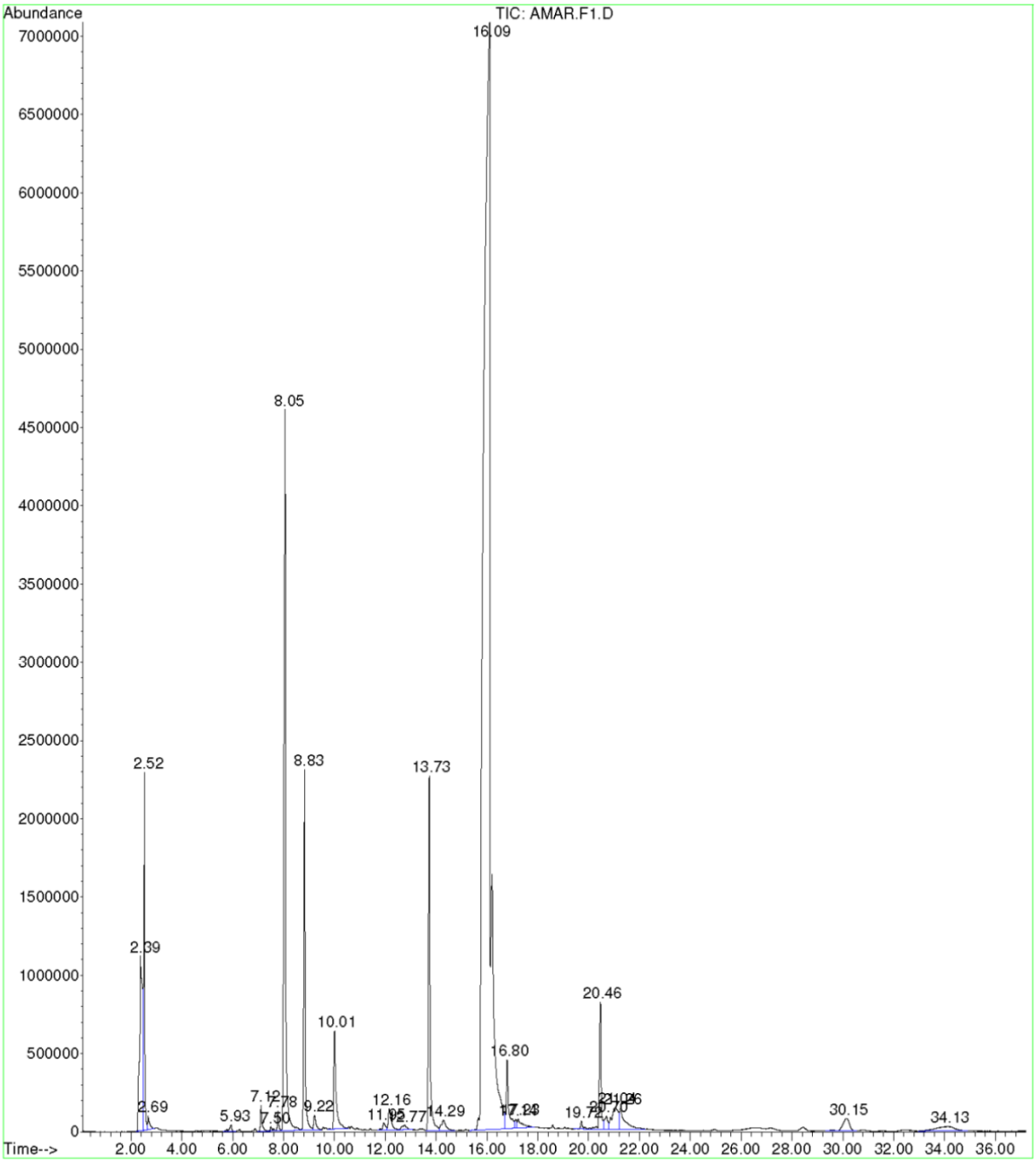
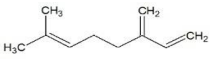
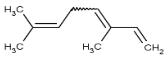
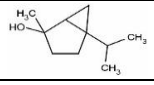
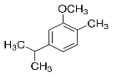
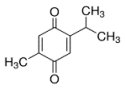
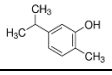
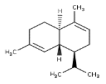
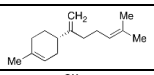
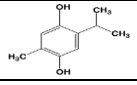


Fig. 2. GC/MS analysis of Chlorella vulgaris extract

Table 4. Terpenes compound (monoterpenes and sesquiterpenes) detection by GC/MS of the two algal extract

No.	Retention Time		Compounds	CAS Number	Chemical Structures	Chemical class
	Spirulina platensis	Chlorella vulgaris				
1	6.08	7.50	α-Phellandrene	99-83-2		Monocyclic Monoterpenes
2	6.25	5.93	α-pinene	25766-18-1		Bicyclic Monoterpenes
3	8.52	8.05	p-cymene	99-87-6		Monocyclic Monoterpenes
4	7.28	ND	β-pinene	127-91-3		Bicyclic Monoterpenes
5	7.53	7.12	β-Myrcene	123-35-3		Cyclic Monoterpenes
6	9.39	8.82	γ-Terpinene	99-85-4		Monocyclic Monoterpenes
7	9.88	ND	β-Ocimene	13877-91-3		Cyclic Monoterpenes
8	ND	9.22	4-Thujanol	546-79-2		bicyclic Monoterpenes
9	10.76	10.01	Linalool	78-70-6		Cyclic Monoterpenes
10	ND	11.95	Borneol	507-70-0		Bicyclic Monoterpenes
11	13.13	12.16	Terpinen-4-ol	562-74-3		Monocyclic Monoterpenes
12	14.83	13.73	Carvacrol Methyl Ether	6379-73-3		Monocyclic Monoterpenes
13	15.42	14.29	Thymoquinone	490-91-5		Monocyclic Monoterpenes
14	17.17	16.09	Thymol	89-83-8		Monocyclic Monoterpenes
15	18.10	16.80	Thymol acetate	528-79-0		Monocyclic Monoterpenes
16	ND	17.13	Carvacrol	499-75-2		Monocyclic Monoterpenes
17	ND	19.72	α-Cadinene	11044-40-9		Bicylic Sesquiterpenes
18	22.25	20.47	β-bisabolene	495-61-4		Monocyclic Sesquiterpenes
19	ND	21.26	Thymohydroquinone	2217-60-9		Monocyclic Monoterpenes

impractical antibiotics for medical use as a result of their in vivo toxicity or inactivity (Martínez-Francés and Escudero-Oñate 2018).

Spirulina platensis' purified antimicrobial compound was more effective against gram-positive (*Bacillus subtilis*), gram-negative bacteria (Esch*erichia coli* and

Pseudomonas aeruginosa,), *Candida albicans* and unicellular fungi (*Aspergillus niger*) the highest biological activity was reported. The findings of this investigation showed that cyanobacteria could be a good source of antimicrobial agents in comparison with contemporary antimicrobial compounds (El-Sheekh *et al*. 2014). Usharani *et al*. (2015) revealed that the methanol extract of *Spirulina platensis* showed antimicrobial activity with the highest inhibition zone against pathogenic isolates of bacteria and fungi, while the hexane extract showed limited inhibition zone.

Syed *et al*. (2015) found the highest inhibition zone of about 13 mm when using ethanol extracted *Chlorella vulgaris* against pathogenic bacteria *E. coli, Klebsiella sp.,* and *bacillus sp*. This may be due to phytochemical analysis of this dried algal sample containing useful bioactive compounds such as flavonoids, tannins, phenolic compounds, terpenes, cardiac glycosides, saponins, and carbohydrates. Substantial evidence of the existence of these seven bioactive compounds showed that *Chlorella vulgaris* plays a major role in obtaining various bioactive compounds as a useful precursor. The drugs derived from these algae species must find some particular application to suppress bacterial growth, which results in more specific control of vector infections without any side effects.

Dineshkumar *et al*. (2017) mentioned that due to the composition of such phytochemicals as phenols, tannins, flavonoids, terpenes, terpenoids, alkaloids, and saponins in the dried biomass, *Chlorella vulgaris* cells were extracted with different solvents including methanol, ethanol, chloroform, and diethyl ether, which showed antibacterial activity against both gram-negative and gram-positive human pathogenic bacteria. In vitro testing of ten freshwater and marine algae organic solvent extracts (methanol, ethanol, and chloroform) showed antimicrobial activity performed on two grams-positive, four grams-negative bacteria, and one fungus by the process of disc diffusion. Green algae are more potent than red and brown ones. Extracts of ethanol are more active than extracts of methanol and chloroform. Also, *Ulva lactuca* and *Chlorella sp.* revealed the best activity in all solvent types among other algal species. *Spirogyra crassa* demonstrated very low antibacterial activity where it had mild antifungal activity. It was obvious that nearly all extracts of all algal species showed antimicrobial activity against all pathogenic bacteria (Chowdhury *et al*. 2015).

Also, the results of the study revealed that *Chlorella vulgaris* extract showed higher inhibition activity compared to *Spirulina platensis* extract. This may be due to the quantity and quality of the phytochemical composition, which has been confirmed by some previous studies. For example, a study examined the biological activity of two species of freshwater algae, *Spirulina platensis* and *Chlorella vulgaris*, and two marine algae, Sargassum vulgaris and *Sargassum wightii*, in vitro against *Trichophyton rubrum, Microsporum canis* and *Candida albicans*. The results showed inhibitory activity against the studied fungi by all algal extracts. The highest inhibition against the tested microorganisms was shown by 70% of methanol extracts from *Chlorella vulgaris*. The findings of this investigation suggest that *Chlorella vulgaris* methanol extract contains a new antifungal compound (El-Sheekh *et al*. 2015).

In another study, five species (four cyanobacterial and one green algae), namely *Nostoc caeruleum, Spirulina platensis, Cylindrospermum majus, Oscillatoria formosa* and *Chlorella vulgaris* were tested for their antibacterial activity against three gram-positive bacteria (*Staphylococcus aureus, Staphylococcus epidermidis,* and *Streptococcus pyogenes*), three gram-negative bacteria (*Klebsiella pneumoniae, Pseudomonas aeruginosa,* and *Escherichia coli*), as well as for their antifungal activity against *Aspergillus fumigatus, Candida albicans, Geotricum candidumn,* and *Trichophyton mentagrophytes* using the agar well diffusion procedure. The results indicated that *Chlorella vulgaris* extract was more effective against bacteria and fungi strains tested, followed by *Spirulina platensis*. Chemical analysis showed that the highest percentages of total phenolic and total flavonoid content were recorded by *Chlorella vulgaris* (Ahmed 2016).

In their study of the effect of three algae species, Arun *et al*. (2012) found that *Chlorella pyrenoidosa* and *Nostoc muscorum* methanolic extracts were most effective against *Pseudomonas aeruginosa*, while *Spirulina platensis* methanolic extract showed maximum activity against *Staphylococcus aureus*. Another investigation showed that the organic extracts of *Chlorella pyrenoidosa* algal strain had the most prominent effect against the tested gram-positive bacteria and fungi strains, while *Spirulina platensis* extracts were more efficient against the tested gram-negative bacteria (Ali and Doumandji 2017). Volatile algae oils contain a wide range of compounds, including linalool, geraniol, citronellol; monocyclic: limonene, 1-8-cineol, p-cymene; bicyclic: α-and β-pinene, cadinene, aromatic eugenol, and isoeugenol. The others are compounds containing benzaldehyde, phenol, p-cresol, various acids, alcohols, aldehydes, amines, ketones, arsenic, and halogenated compounds. The algae were found to be relatively poor when compared with volatile oil components of algae and terrestrial plants. Importantly, they are halogenated compounds only in algae, but not in terrestrial plants (Güven *et al*. 2013, Rezapour-Nasrabad 2018).

The antimicrobial activity of two algae extracts used in this study results from the phytochemical compositions which were done using GC/MS device. The results showed the extraction of two algae represented by terpenes like α-Phellandrene, α-pinene, p-cymene, β-pinene, β-Myrcene, γ-Terpinene, β-

Ocimene, 4-Thujanol, Linalool, Borneol, Terpinen-4-ol, Carvacrol Methyl Ether, Thymoquinone, Thymol, Thymol acetate, Carvacrol, α-Cadinene, β-bisabolene, and Thymohydroquinone (**Table 4**). Essential oils consist of a complex mixture of compounds, usually between 20 and 60, at different concentrations, Terpenes, the main constituents of essential oils, are derived from the pathway of isoprenoids and are produced and secreted from specialized plant tissues. They consist of isoprene units (C5), which are the basis for their classification, i.e. two isoprene units forming monoterpenes (C10), three units forming sesquiterpenes (C15), four units forming diterpenes (C20), six units forming triterpenes (C30), and eight units forming carotenoid (C40). Terpenes may have various chemical functions, including alcohol (linalool, geraniol, carveol, citronellol, terpineol, menthol, borneol, and bisabolol), aldehyde (citral and citronellal), phenol (thymol and carvacrol), ketone (carvone and camphor), ether (eucalyptol), and hydrocarbon (cymene, pinene, limonene, and phellandrene) (Chouhan *et al*. 2017).

The emergence of pathogens' antimicrobial resistance has driven extensive research into alternative therapies. One of these resources for the exploration of potential resources to mitigate this issue is plants abundant with natural secondary metabolites. Terpenes and their hydrocarbon derivatives are generally found in essential oils. Several terpenes and their derivatives such as(+) -Terpinen-4-ol α-Terpinene Terpinolene α-Pinene 1,8-Cineole π-Cymene (+) -Limonene β-Myrcene (+) -β-Pinene, (±)-Linalool, α-Phellandrene, and α-Terpinoel have been shown to be active antimicrobial agents toward drug-resistant pathogens, often bacteria and fungi (Mahizan *et al*. 2019).

Many studies have reported the antimicrobial activity of essential oils, but a vast majority of these studies attribute the activity to the most prevalent compounds without independent analysis. One of these studies examined the antibacterial activity of 33 free terpenes commonly found in essential oils and assessed the cell ultrastructure to verify possible damage to the cell membrane. At the initial screening, only 16 out of the 33 compounds had antimicrobial activity. Eugenol showed rapid bactericidal action against *Typhimurium serovar* and *Salmonella enterica*. Terpineol had excellent anti-*Staphylococcus aureus* strains bactericidal activity. The rapid bactericidal action of carveol, citronellol, and geraniol against *E. coli* is presented. The increased antimicrobial activity was correlated with hydroxyl groups (phenolic and alcohol compounds), while hydrocarbons resulted in less activity. In contrast to sulfanilamide, the first band such as carvacrol, L-carveol, eugenol, trans-Geraniol, and thymol showed increased activity (Guimarães *et al*. 2019).

Essential plant oils have been documented to have extensive antimicrobial activity against different bacterial and fungal pathogens; the results showed that terpenes α-Phellandrene and Nonanal could significantly inhibit the growth of *Penicillium cyclopium* by severely disrupting the integrity of the fungal cell membrane, leading to the leakage of cell components and potassium ions, and triggering an increase of the total lipid content (Zhang *et al*. 2017).

The antimicrobial effect of basil and thyme essential oil and its major constituents' thymol, p-cymene, estragole, linalool, and carvacrol was determined using the agar well diffusion assay. Thyme essential oil and thymol and carvacrol showed inhibition of *Shigella sp.* in the agar well diffusion method (Bagamboula *et al*. 2004). Another study revealed antibacterial effect of the Persian Gulf harvested brown algae *Cystoseira trinodis*. *Staphylococcus aureus* (ATCC 25923), *Staphylococcus epidermidis* (ATCC 14990), *Escherichia coli* (ATCC 25922), and *Pseudomonas aeruginosa* (ATCC 27853) were examined for the activity of *C*. trinodis extract. The extract was active against both gram-positive and gram-negative species tested in this study, which may result from major components of brown algae Cystoseira trinodis extract that was α-pinene about 15.84% (Adepoju et al 2018, Tajbakhsh *et al*. 2011)

CONCLUSION

Microalgae offer significant opportunities as sources of antimicrobial agents through their phytochemical composition. The ethanolic extracts of two algae *Spirulina platensis* and *Chlorella vulgaris* showed the most prominent effect against the tested gram-positive bacteria and fungi strains, while *Chlorella vulgaris* extracts were more efficient against all tested pathogenic bacteria; this bacterial inhibition may result from the chemical composition of extracts represented by terpenes, However, such extracts can be considered as a good alternative to antibiotics and can act as effective therapeutic agents against pathogenic bacteria without any side effect on human body.

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