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RESEARCH PAPER

ANTIMICROBIAL ACTIVITY OF THREE ALGAL SPECIES AGAINST TWO PATHOGENIC BACTERIA AND FUNGI

Chiyai M. Shareef[1], Farhad H. Aziz[2]

[1,2]Department of Environmental science and health, College of Science, Salahaddin University-Erbil, Kurdistan Region, Iraq

ABSTRACT

Three different species of algae, namely *Scenedesmus quadricauda* 1 (OQ408126), *Scenedesmus quadricauda* 2 (OQ408127) and *Dunaliella primolecta* (OQ408128) were extracted by methanol, ethanol and ethyl acetate against one type of gram positive, one type of gram negative bacteria and one type of fungi by using 96-well microliter plate method. There were used eleven various concentrations of the algal activity extract (2, 5, 10, 15, 20, 25, 30, 35, 40, 45, and 50 mg/ml). *Scenedesmus quadricauda* 1 was shown to be more effectiveness for both pathogenic bacteria. While, extract of all three species of algae by methanol was more effective against pathogenic fungi, *Candida albicans* (ATCC:10231) followed by ethanol then by ethyl acetate. Additionally, extraction of *Scenedesmus quadricauda* 1 by ethanol was more effective than methanol and ethyl acetate against studied pathogenic bacteria. Extract of all three algal species by ethyl acetate demonstrated low effects on two used bacteria and fungi in comparison with the other types of solvent. In addition, extraction of three algal species by methanol was more effective than ethanol and ethyl acetate against *Candida albicans*. Through examining the algae extraction by gas chromatography-mass spectrometry (GC-MS), many chemical components with antibacterial and antifungal properties were discovered.

KEYWORDS: Algae, Activity, Antimicrobial, Genera, Pathogenic
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1. INTRODUCTION

Depending on their size, the varied group of photosynthetic eukaryotic and prokaryotic microorganisms known as algae are categorized as either macroalgae or microalgae. Numerous microalgae species have been shown to possess various biochemical traits linked to advantages for human nutrition and health (Martínez-Ruiz et al., 2022). Variety of chemicals from numerous metabolic pathways, including amino acids, fatty acids, polysaccharides, carotenoids, lectins, polypeptides, and toxins are produced by microalgae (Martínez-Ruiz et al., 2022).

* **Corresponding Author:**
Chiyai M. Shareef
E-mail:
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An important global health issue is microbial infectious diseases. One of the most common fungal infections is candidiasis, especially in critically patients, such as immune compromised people, people taking broad-spectrum antibiotics for an extended period of time, people with HIV, cancer, and even the elderly (Alves et al., 2022). Among the reported pharmacological effects of bioactive substances are their anti-inflammatory, anti-bacterial, antioxidant, antiviral, and anthelmintic properties (El-Sheekh et al., 2020), (El-Sheekh et al., 2021). Antimicrobial resistance, which affects the efficient prevention and treatment of an expanding array of diseases brought on by harmful bacteria, is one of the biggest public health challenges of the twenty-first century (Potocki et al., 2021). These compounds' antibacterial action is

influenced by a range of parameters, sulfate content, charge density, molecular weight, and distribution, structural and conformational properties, and molecular weight (Vera et al., 2011). Chemical medicines are universally threatened by bacterial strains that are resistant to the antibiotics' inhibitory effects. Additionally, the majority of antibiotics differ from one another by having a variety of adverse effects that could affect healthy human body cells (Mgbeahuruike et al., 2019). The use of microalgal compounds as positive sources to antibiotics derived in nature and devoid of synthetic chemicals that could harm human diseases entails using various natural compounds that are readily available to control pathogenic bacteria, particularly microalgal-derived compounds. They have the benefit of decreasing the adverse effects or side effects of synthetic antibiotics and are also thought to be inexpensive (Falaise et al., 2016).Many chemical compounds derived from microalgae, including phenols, fatty acids, and some volatile halogenated hydrocarbons, have demonstrated antimicrobial activity (Jena and Subudhi, 2019). Numerous investigations found that the extract of *Scenedesmus* species has highly effective antimicrobial action against a variety of harmful microorganisms (Marrez et al., 2019). Numerous ecologists and scientists have studied the importance of naturally occurring algae in various ecosystems, including freshwater, because they may have biological effects on microorganisms like bacteria and fungi (Thomas et al., 2021). Infective endocarditis, bacteremia, skin and soft tissue infections, and other human pathogens can all be brought on by *Staphylococcus aureus*, a Gram-positive bacteria (Karakonstantis and Kalemaki, 2019). Comparing with other species, which are unable to survive in harsh settings, *Dunaliella* microalgae is relatively simple (Bombo et al., 2023). Recently, increased the focus has been devoted to the idea of cultivating these microscopic microalgae since they can produce a range of bioactive chemicals that can be used for commercial applications, at a huge scale (Anwer et al., 2022) and (Hassan et al., 2022). In this study, three different species of algae were extracted with the primary goal of evaluating the activity of active chemicals (*Scenedesmus quadricauda* 1, *Scenedesmus quadricauda* 2 and *Dunaliella primolecta*) against pathogenic bacteria and fungi were obtained from Medya diagnostic Center Erbil.

2. Materials and Methods:

2.1. Collection of algae

Three types of algae from some springs and ponds along the Greater Zab River the path from Bekhma to Gwer District, *Scenedesmus quadricauda* 1, *Scenedesmus quadricauda* 2, and *Dunaliella primolecta* were collected or obtained within Erbil governorate.

2.2. Identification of algal species

Algae books were used to identify and diagnose algal species, (Pentecost, 1975 and (Matthews, 2016).

2.3. Isolation of algal species

Necrotic parts from algal Sample were removed. A glass container was then used to incubate the algae sample. Blue Green-11 medium was applied for algal growth. Algae were isolated and made pure using staining process plate techniques. Following that, they were incubated for 14 days at 25 ±2 °C with light intensity distribution of 3000-5000 lux for 16 hours of light and 8 hours of darkness and pH 8.2. Repeating this process several times allowed us to obtain purified algal species, which we then transferred to tube containing 25 ml of BG-11 media and incubated for 14 days below the same situations as above to produce algal inoculum. According to the Streak Plating Technique, this alga was isolated (Hussein et al., 2018).

2.4. Biomass preparation and harvesting

Twenty five ml of isolated algae was put into a flask with 100 ml of Blue Green-11 media, where they were cultured for 14 days with the same circumstances as before. Following then, this cultural media was put to 500 ml glass flask which contained 100 ml of Blue Green-11. Additionally, the BG11 media was incubated for 14 days under controlled circumstances in an incubator, after the growth of algae (2 weeks) the culture centrifuged with 3500rpm for 10 min to separate the algae from the culture (Elnabris et al., 2013). Then algal

sample were evaporated at a temperature of around 40 ° C. These samples of algae were weighed and kept in the fridge (Hassan et al., 2020).

2.5. Antimicrobial activity

Staphylococcus aureus (ATCC:25923), *Escherichia coli* (ATCC:35218), and fungi *Candida albicans* (ATCC:10231) were obtained from the Media Diagnostic Center Erbil, and used in the current investigation. On Muller Hinton agar, the bacterial culture was incubated (24 hr. at 37 °C), and fungal culture incubated on Sabouraud Dextrose Broth (24-72 hr. at 37 °C).

2.6. Determining minimum inhibitory concentration (MIC) of algal extraction

96-well microliter plates were used for test various concentrations of algal extracts for anti-microbial activity and the insulate of anti-microbial substances (0, 2, 5, 10, 15, 20, 25, 30, 35, 40, 45 and 50 mg/ml) by mixing them with nutrient broth for bactria and Sabouraud Dextrose Broth for fungi. This method was useful in testing algae for anti-microbial activity and the separate of anti-microbial substances. All wells were inoculated with 10µl of the activated culture of *Staphylococcus aureus*, *Escherichia coli*, and *Candida albicans* over night at 37 °C. Wave length 490 nm was used to measure the absorbency both before and after incubation, and the MIC was then determined using the ELIZA equipment (EL 800 Biotek, Epison LQ-300+II) (Ismaeil and Saleh, 2019).

2.7. Phytochemical examination of the algal species

According to (Alghanmi and Omran, 2020), 10 gram for each powder of *Scenedesmus quadricauda*1, *Scenedesmus quadricauda* 2 and *Dunaliella primolecta* were extracted with 100 ml of 97% ethanol, methanol and ethyl acetate each discretely, these solvents are extracted by Soxhlet at 76°C for 3–4 hours until they are insoluble. Algal extract was dried at 40°C using a rotary evaporator. The extracts were used for gas chromatography-mass spectrometry (GC-MS)-based phytochemical screening. Algal crude extracts was sterilized by filtration, dissolved in dimethyl sulfoxide to a final concentration of 300 mg/mL, then stored at 4°C (Li et al., 2014).

2.8. Chemical Composition of Algae Extracts

The algal extract's biochemical components were identified using the Gas Chromatography-Mass Spectrometry analytical technique (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 three degrees including 40°C for two minute and 150°C for five minutes, then 300°C for fifteen minutes. The injector port's temperature was maintained at 280°C. Used as a carrier gas was helium and 1 µl of the sample was injected into the system (dissolved in 100% dimethyl sulfoxide) (Alghanmi and Omran, 2020).

2.9. DNA extraction and PCR amplification for Microalgae Molecular Identification Using the ITS Region

Total genomic DNA form microalgae cells was extracted using Genomic DNA purification kit: thermo scientific/USA) depending on manufacturer's instructions. The target sequence 750bp of the Microalgae in the rDNA fragments were successfully amplified using universal primers designed by(White et al., 1999). The total of 25 µl PCR master mix reaction volume was performed containing 3µl of genomic DNA, 12.5 µl of 2X GoTaqGreen Master Mix (Promega/USA) and 1µl was added for each of the forward and reverse primer for both ITS1 and ITS4, forward (ITS1, F´5-TCC GTA GGT GAA CCT GCG G-´3), reverse (ITS4, R-5´ TCC TCC GCT TAT TGA TAT GC-´3) then the mixture was completed by adding 7.5 µl of nuclease free water. The PCR amplification process was carried out with a Techne/UK thermocycler under the following conditions: an initial denaturation cycles at 95°C for five minutes, followed by 35 cycles at 94°C for one minute, 55°C for one minute, 72°C for one minute and a last extension at 72°C for seven minutes. The size of PCR products was confirmed by using 2% agarose gel electrophoresis in 1XTBE buffer and PCR products of algae isolates were sent to (Macrogen/South Korea) for sequencing (White et al., 1990).

2.10. The data sequence analysis of the target region:

MEGA5 was used to analyze the DNA target sequence and align it with NCBI BLAST (http://www.ncbi.nlm.nih.gov/BLAST/). The PCR results were sequenced on an Applied Biosystems 3500 Genetic Analyzer (Applied Biosystems).

3. RESULT AND DISCUSSION

In this study, antimicrobial activity of three species of algae *Scenedesmus quadricauda* 1, *Scenedesmus quadricauda* 2 and *Dunaliella primolecta* was tested against bacteria *Staphylococcus aureus* (ATCC:25923), *Escherichia coli* (ATCC:35218) and fungi *Candida albicans* (ATCC:10231) by 96-microlitre plate technique . There were utilized eleven distinct concentrations of algal extract, containing (2, 5, 10, 15, 20, 25, 30, 35, 40, 45, and 50 mg/ml). For testing, the extracts' minimum inhibitory concentrations (MIC) against particular bacteria and fungus at varied concentrations were calculated (Tables 1, 2 and 3). *Scenedesmus quadricauda* 1 purified antimicrobial compound was more effective against *Staphylococcus aureus*, *Escherichia coli* and fungi *Candida albicans*. But the highest activity of algal extracts by ethanol and diethyl acetate higher than methanol against *Staphylococcus aureus* while higher activity of algal extract by diethyl acetate against *Eschershia coli*. *Scenedesmus quadricauda* 1 was more effective against bacteria with antibacterial action starting at concentration 5mg/ml for *Candida albicans*, 10 mg/ml for *Staphylococcus aureus* and 15 mg/ml for Escherichia coli. The methanol extracts of algal genera indicated the minimum inhibition to fungi under study specifically at 5 mg/ml concentration. Extracts of *Dunaliella primolecta* by methanol showed that the minimum inhibitory concentration was (10 mg/ml) against *Staphylococcus aureus* bacteria, the minimum inhibitory concentration of *Dunaliella primolecta* extract by ethanol against *Escherichia coli* bacteria was (25 mg/ml).

In all type of extracts of *Scenedesmus quadricauda* 1, *Scenedesmus quadricauda* 2 and *Dunaliella primolecta* they were considered. Extraction of *Scenedesmus quadricauda* 2 by ethanol against *Staphylococcus aureus* was seen no inhibitory concentration. This may be as a result of microalgae capacity to create highly desired chemicals with broad-spectrum activity for the creation of antibiotic (Alghanmi and Omran, 2020). In this study, the ethyl acetate extract also helped to explain why bacteria that because diseases grew with the least amount of resistance. This may be caused by the kind of extraction agent used for algae, which is crucial for isolating active chemicals from algae, as well as the quality and amount of chemical compounds that were isolated by the extract of the algae (Toma and Aziz, 2023). *Scenedesmus quadricauda* 1 shown the best level of growth inhibition of all pathogenic bacteria in the current study when utilizing ethanol and ethyl acetate rather than methanol. This might be as a result of the algal sample's phytochemical makeup, which includes used active ingredients. These bioactive components in *Scenedesmus quadricauda* 1 are crucial for producing a range of bioactive substances that can be used as precursors. Worrisome human infections have been linked to fungi, especially in underdeveloped and rural areas where access to diagnosis and treatment is limited (Cochrane and Lohans, 2020). The findings of the present investigation demonstrated that the three extracted algal methanol extracts had the maximum antifungal activity against the chosen humans infectious *Candida albicans*. The minimum inhibitory concentration of *Scenedesmus quadricauda* 1, *Scenedesmus quadricauda* 2 and *Dunaliella primolecta* extracts by methanol against fungi *Candida albicans* were 5 mg/ml, 20 mg/ml and 20 mg/ml respectively. These outcomes were consistent with those attained by (Musbah et al., 2019). Several investigations have indicated the possible antibiotic activity of a particular type of algae, which is consistent with the present findings, also the timing of sample collection and dose dependency were the main factors influencing the antibacterial efficacy, Additionally, the considerable presence of the bioactive phenolics could be responsible for the ethanol extract's antibacterial activities (Thomas et al., 2021). The difference of extracted compounds may due to the efficiency of the extraction method (Fayyad et al., 2022). The inhibitory activity is influenced by the type of algae under investigation, the effectiveness of the extraction technique, the active ingredients, and the type of host employed (Mohammed et al., 2021). Phenolic compounds have reportedly been found to offer a variety of desirable qualities. These compounds have a wide range of possible health benefits; among of the most researched bioactivities include their antioxidant, antibacterial, anti-inflammatory, and cytotoxic actions (Vuolo et al., 2019).

In the current investigation, several chemical components related to human health were found in the extract of three algae taxa (Tables 4, 5, 6, 7, 8, 9, 10, 11 and 12). In the current investigation using organic solvents that were discovered to prepare *Scenedesmus quadricauda* 1 (OQ408126), *Scenedesmus quadricauda* 2 (OQ408127) and *Dunaliella primolecta* (OQ408128) extract and diagnosis many compounds by GC-MS. During GC-MS screening of solvent extract of *Scenedesmus quadricauda* 1, it showed that ethanol, methanol and ethyl acetate extract showed eighteen, fifteen and twelve compounds respectively (Tables 4, 5 and 6) and for *Scenedesmus quadricauda* 2 showed twenty four, twenty three and sixteen compounds through extract by ethanol, methanol and ethyl acetate (Tables 7, 8 and 9) respectively, finally extraction of *Dunaliella primolecta* by ethanol, methanol and ethyl acetate were including thirty, seventeen and nineteen compounds extract (Tables 10, 11 and 12). The majority of these chemicals have a variety of antibiotic effects, and Tables (4–12) list chemical compounds that may be antioxidant and anticancer. Three algae genera' extracts by ethanol, methanol, and ethyl acetate contained some or all of these chemicals, including Alkaloids, Phenolic, Alcohol, Carboxylic acid, Flavonoids, Ketone, Phenyl, Amine, Nitrile, Phenolic acid, Fatty acid, Dicarboxylic acid, Biphenyls, Heterocycle and other natural compounds as their surface area was described in (Tables 4-12). Algae have been found to contain a variety of chemically unique substances that have antimicrobial activity; many of these substances, New medications are currently being created using substances such polysaccharides, phenols, and heterocyclic carbons (Saeed et al., 2020). The composition of phytochemicals in the crude extract depends on the polarity of the extraction solvent (Awotedu et al., 2020). Phenotypic screening was used in conjunction with comprehensive or specific initial gene magnification to identify the algal species. Data for the molecular sample from the ITS nucleotide sequencing provided the smallest characteristics and isolation diagnostic. *Scenedesmus quadricauda* 1, *Scenedesmus quadricauda* 2, and *Dunaliella primolecta* were the three taxa of algae whose data sequences were provided by supplied Gene bank accession number nucleotide sequencing (Table 13).

Depending on the result of the current studies some bacterial and fungal species have resistance to antibiotic , therefore it is important to increase the request new antimicrobials for the therapy of infectious diseases, microalgae made up - depending on the kind and the family to which related to it, and the factors in which it has growth in a wealthy source of bioactive compounds, with a broad range of using and may be in the future a valuable therapeutic provided opening new fields for the utilization of new and still unexploited sources of drugs.Over the years, several screening experiments have been conducted to find new cytotoxic or antibiotic metabolic components of microalgae, particularly cyanobacteria and green algae (Toma and Aziz, 2023) (Fayyad et al., 2022, Thomas et al., 2021).

4. CONCLUSION

It was found that *Scenedesmus quadricauda* 1 ethanolic extraction was superior to methanol and ethyl acetate against all pathogenic bacteria, including *Staphylococcus aureus* and *Escherichia coli*. The extract of Scenedesmus quadricauda 1 by ethanol was more effective against *Candida albicans*. This study shows different chemical compounds which have antimicrobial activities were attained from analyzing the extraction of three algal genera by gas chromatography–mass spectrometry (GC-MS) that prevents or causes them to kill. Three algae species extracts by ethanol, methanol, and ethyl acetate contained some or all of these chemicals, including Alkaloids, Phenolic, Alcohol, Carboxylic acid, Flavonoids, Ketone, Phenyl, Amine, Nitrile, Phenolic acid, Fatty acid, Di carboxylic acid, Biphenyls, Heterocycle and other natural compounds. In this study, screening for algae species using ethanol, methanol, and ethyl acetate revealed that several algal genera have strong antibacterial activity against pathogenic bacteria as well as fungi.

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Conflict of Interest

-Conflicts of Interest: None.

-We hereby emphasize that every Figures and Tables in the manuscript are mine.

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Table 1. Minimum Inhibitory Concentration (MIC) of *Scenedesmus quadricauda* 1 against pathogenic bacteria and fungi.

Concentration of extract	Absorbency of bacterial culture (extract concentration mg/ml)											
Name of bacteria with solvent	Control	2	5	10	15	20	25	30	35	40	45	50
Staphylococcus aureus (Methanol)	0.586	0.509	0.414	0.378	0.324	0.19	0.145	0.118	0	0	0	0
Staphylococcus aureus (Ethanol)	0.775	0.538	0.008	0	0	0	0	0	0	0	0	0
Staphylococcus aureus (Ethyl acetate)	0.41	0.415	0.465	0	0	0	0	0	0	0	0	0
Escherichia coli (Methanol)	0.374	0.465	0.621	0.406	0.323	0.194	0.11	0.104	0	0	0	0
Escherichia coli (Ethanol)	0.523	0.381	0.358	0.323	0.315	0.197	0.311	0.274	0.263	0.123	0	0
Escherichia coli (Ethyl acetate)	0.611	0.475	0.325	0.258	0	0	0	0	0	0	0	0
Candida albicans (Methanol)	0.324	0.17	0.155	0.02	0	0	0	0	0	0	0	0
Candida albicans (Ethanol)	0.616	0.321	0	0	0	0	0	0	0	0	0	0
Candida albicans (Ethyl acetate)	0.435	0.264	0.277	0.303	0.101	0.213	0	0	0	0	0	0

0 means no growth observed

Table 2. Minimum Inhibitory Concentration (MIC) of *Scenedesmus quadricauda* 2 against pathogenic bacteria and fungi.

Concentration of extract	Absorbency of bacterial culture (extract concentration mg/ml)											
Name of bacteria with solvent	Control	2	5	10	15	20	25	30	35	40	45	50
Staphylococcus aureus (Methanol)	0.461	0.428	0.458	0.239	0.233	0.26	0.226	0	0	0	0	0
Staphylococcus aureus (Ethanol)	1.038	0.671	0.456	0.483	0.379	0.463	0.350	0.313	0.309	0.152	0.168	0.197
Staphylococcus aureus (Ethyl acetate)	1.113	0.823	0.742	0.575	0.682	0.376	0.545	0	0	0	0	0
Escherichia coli (Methanol)	0.559	0.468	0.476	0.487	0	0	0	0	0	0	0	0
Escherichia coli (Ethanol)	0.547	0.468	0.358	0.439	0.148	0.443	0.121	0.279	0.261	0.085	0	0
Escherichia coli (Ethyl acetate)	0.604	0.514	0.400	0.333	0.301	0.283	0.166	0	0	0	0	0
Candida albicans (Methanol)	0.372	0.218	0.155	0.036	0.048	0	0	0	0	0	0	0
Candida albicans (Ethanol)	0.632	0.317	0.381	0.333	0.25	0	0	0	0	0	0	0
Candida albicans (Ethyl acetate)	0.907	0.272	0.149	0.193	0.002	0.152	0	0	0	0	0	0

0 means no growth observed

Table 3. Minimum Inhibitory Concentration (MIC) of *Dunaliella primolecta* against pathogenic bacteria and fungi.

Concentration of extract	Absorbency of bacterial culture (extract concentration mg/ml)											
Name of bacteria with solvent	Control	2	5	10	15	20	25	30	35	40	45	50
Staphylococcus aureus (Methanol)	0.843	0.678	0.631	0.351	0.456	0.278	0.227	0.11	0.079	0	0	0
Staphylococcus aureus (Ethanol)	1.129	0.872	0.64	0.403	0.471	0.243	0.167	0.079	0.077	0.027	0	0
Staphylococcus aureus (Ethyl acetate)	0.346	0.325	0.376	0	0	0	0	0	0	0	0	0
Escherichia coli (Methanol)	0.573	0.675	0.424	0.395	0.509	0.356	0.231	0.211	0	0	0	0
Escherichia coli (Ethanol)	0.584	0.505	0.557	0.44	0.418	0.33	0.239	0.126	0	0	0	0
Escherichia coli (Ethyl acetate)	0.353	0.094	0.023	0.385	0.19	0.299	0	0	0	0	0	0
Candida albicans (Methanol)	0.402	0.128	0.097	0.017	0	0	0	0	0	0	0	0
Candida albicans (Ethanol)	0.275	0.132	0.107	0.154	0.061	0.021	0	0	0	0	0	0
Candida albicans (Ethyl acetate)	0.48	0.145	0.142	0.062	0.035	0.057	0.018	0.029	0.008	0	0	0

0 means no growth observed

Table 4. GC-MS screening of various substances in ethanol extract of *Scenedesmus quadricauda* 1.

Peak	R.Time	No.	Compounds	Chemical formula	%
1	3.776	1	2-Chloro-7-methoxy-4-methylquinoline, Quinoline	$C_{11}H_{10}ClNO$	9.34
		2	1H-Pyrazol-5-amine,1-(3-Chlorophenyl)-3-methyl-1H-pyrazol-5amine	$C_{10}H_{10}ClN_3$	
		3	6-(4-Chlorophenyl)-5-nitro-2-piperidinone	$C_{11}H_{11}ClN_2O_3$	
2	3.848	4	1H-imidazole-2-methanol, 1-decyl-	$C_{14}H_{26}N_2O$	6.10
3	3.905	5	2,4,4,6,6,8,8-heptamethyl-1,3,5,7,2,4,6,8-tetraoxatetrasilocan-2-ol	$C_7H_{22}O_5Si_4$	2.05
4	4.876	6	1,2,3,6-Tetrahydrophthalimide	$C_8H_9NO_2$	3.00
5	5.074	7	benzoic acid	$C_7H_6O_2$	1.57
6	5.117	8	4-Bromo-3-chloroacetanilide	$C_8H_7BrClNO$	2.45
7	6.173	9	Thiazolo[3,2-b][1,2,4]triazol-2-one	$C_{11}H_6ClN_3O_2S$	1.23

8	6.487	10	Pyridazin-3(2H)-one, 6-chloro-2-phenyl-	$C_{10}H_7FN_2O_2$	1.16
9	7.890	11	Hexadecanoic acid	$C_{16}H_{32}O_2$	34.50
10	8.267	12	Sophoridine	$C_{15}H_{24}N_2O$	1.74
11	8.510	13	Pyrimethamine	$C_{12}H_{13}ClN_4$	1.30
12	8.755	14	6-Isopropyl-9-methyl-4-oxo-3,4-dihydroazuleno(2,1-d)pyrimidine-10-carboxamide	$C_{17}H_{17}N_3O_2$	2.63
13	9.029	15	1,2,3,6,7,8,9,10,11,12-Decahydrobenzo[e]pyrene	$C_{20}H_{22}$	14.46
14	9.306	16	3-methoxy-5,5-dimethyl-6,6a-dihydroisoindolo[2,1-a]quinolin-11(5H)-one	$C_{19}H_{19}NO_2$	2.11
15	9.701	17	1-Naphthalenamine, N-phenyl-	$C_{16}H_{13}N$	4.06
16	9.944	18	2-Propylthiazolo[4,5-c]quinolin-4-amine	$C_{13}H_{13}N_3S$	12.31

Table 5. GC-MS screening of various substances in methanol extract of *Scenedesmus quadricauda* 1.

Peak	R.Time	No.	Compounds	Chemical formula	%
1	6.915	1	malononitrile	$C_{12}H_2F_4N_4$	0.85
		2	4-Thiazoleacetic acid, 2-(p-chlorophenyl)-, hydrazide	$C_{11}H_{10}ClN_3OS$	
2	7.630	3	6-Amino-4-phenyl-1H-quinazolin-2-one	$C_{14}H_{11}N_3O$	0.39
3	7.713	4	Benzo[a]pyren-7(8H)-one	$C_{20}H_{14}O$	91.50
4	7.916	5	6-Isopropyl-3-phenoxytropolone	$C_{16}H_{16}O_3$	1.21
		6	5-Methyl-7-phenyl-1,3-diazaadamantan-6-one Hydrazone	$C_{15}H_{20}N_4$	
		7	Benzo[g]pteridine-2,4(3H,10H)-dione	$C_{13}H_{12}N_4O_3$	
		8	Hexadecanoic acid	$C_{16}H_{32}O_2$	
5	8.503	9	Levo-3.alpha.,5.beta.-tetrahydronorgestrel	$C_{21}H_{28}O_2$	0.85
6	8.541	10	2,4-Hexanediol	$C_6H_{14}O_2$	0.92
7	8.670	11	Succinic acid	$C_4H_6O_4$	0.47
8	9.270	12	1,2,3,6,7,8,9,10,11,12-Decahydrobenzo[e]pyrene	$C_{20}H_{22}$	0.53
9	9.321	13	6-Isopropyl-9-methyl-4-oxo-3,4-dihydroazuleno(2,1-d)pyrimidine-	$C_{17}H_{17}N_3O_2$	1.44

			10-carboxamide		
10	9.602	14	2-isopropyl-3-phenyl-, 4-oxide	$C_{17}H_{16}N_2O$	0.98
11	9.732	15	Benzoic acid, pentachloro-	$C_7HC_{l5}O_2$	0.88

Table 6. GC-MS screening of various substances in ethyl acetate extract of *Scenedesmus quadricauda* 1.

Peak	R.Time	No.	Compounds	Chemical formula	%
1	3.765	1	2-Chloro-7-methoxy-4-methylquinoline	$C_{11}H_{10}ClNO$	9.92
		2	1-(3-Chlorophenyl)-3-methyl-1H-pyrazol-5-amine	$C_{10}H_{10}ClN_3$	
		3	6-(4-Chlorophenyl)-5-nitro-2-piperidinone	$C_{11}H_{11}ClN_2O_3$	
2	3.827	4	1H-imidazole-2-methanol, 1-decyl-	$C_{14}H_{26}N_2O$	1.63
3	3.894	5	2,4,4,6,6,8,8-heptamethyl-1,3,5,7,2,4,6,8-tetraoxatetrasilocan-2-ol	$C_7H_{22}O_5Si_4$	1.99
4	6.115	6	[1,2,4]triazolo[1,5-a]pyrimidin-7-ol, 2-methyl-5-phenyl-	$C_{12}H_{10}N_4O$	0.66
5	7.794	7	[1,3,4]thiadiazol-2-yl-amine	$C_2H_3N_3S$	6.55
6	7.900	8	Hexadecanoic acid	$C_{16}H_{32}O_2$	67.60
7	8.326	9	2-Phenyl-2,5-dihydro-4H-pyrazolo[3,4-c]quinolin-4-one	$C_{16}H_{11}N_3O$	2.53
		10	1,2,4-1H-Triazole	$C_2H_3N_3$	
8	9.697	11	1-Naphthalenamine, N-phenyl-	$C_{16}H_{13}N$	6.09
9	9.903	12	Vittatine	$C_{16}H_{17}NO_3$	3.02

Table 7. GC-MS screening of various substances in ethanol extract of *Scenedesmus quadricauda* 2.

Peak	R.Time	No.	Compounds	Chemical formula	%
1	3.275	1	Acetic acid	$C_{13}H_{12}ClNO_2S$	0.99
2	3.784	2	2-Chloro-7-methoxy-4-methylquinoline	$C_{11}H_{10}ClNO$	2.09
		3	1H-Pyrazol-5-amine,1-(3-Chlorophenyl)-3-methyl-1H-pyrazol-5-amine	$C_{10}H_{10}ClN_3$	
3	3.850	4	(1-decyl-1H-imidazol-2-yl)methanol	$C_{14}H_{26}N_2O$	2.68
		5	6-(4-Chlorophenyl)-5-nitro-2-piperidinone	$C_{11}H_{11}ClN_2O_3$	
4	3.907	6	2,4,4,6,6,8,8-heptamethyl-1,3,5,7,2,4,6,8-tetraoxatetrasilocan-2-ol	$C_7H_{22}O_5Si_4$	1.30
5	4.396	7	Succinic acid	$C_{13}H_{13}BrF_2O_4$	0.73
6	4.556	8	2,6-Dihydroxyacetophenone	$C_{14}H_{24}O_3Si_2$	0.86
7	4.766	9	2-Chloro-4,6-di-piperidin-1-yl-[1,3,5]triazine	$C_{13}H_{20}ClN_5$	0.35
8	4.874	10	2-Hydroxychalcone	$C_{15}H_{12}O_2$	2.60
9	5.390	11	3,6-Bis(N-dimethylamino)-9-ethylcarbazole	$C_{18}H_{23}N_3$	0.46
10	5.745	12	Quinoxaline, 2-phenyl-	$C_{14}H_{10}N_2$	3.10
11	5.885	13	benzoic acid	$C_{13}H_{22}O_3Si_2$	0.62
12	6.491	14	4H-1,2,4-Triazol-4-amine	$C_9H_7ClN_4$	0.86
13	6.573	15	Naphtho[2,1-b:7,8-b']difuran,1,2,9,10-tetrahydro-2,9-dimethyl-	$C_{16}H_{16}O_2$	24.96
14	7.382	16	malononitrile	$C_{12}H_2F_4N_4$	0.31
15	7.707	17	Benz[a]anthracene, 8-propyl-	$C_{21}H_{18}$	3.45
		18	Benzo[a]pyren-7(8H)-one	$C_{20}H_{14}O$	
16	7.797	19	2-Methyl-2-phenyl-1H-indene-1,3(2H)-dione	$C_{16}H_{12}O_2$	1.83
17	7.896	20	1-(3-Hydroxyphenyl)-3-(1-propylpyrazol-4-yl)prop-2-en-1-one	$C_{15}H_{16}N_2O_2$	37.28
		21	Benzo[g]pteridine-2,4(3H,10H)-dione	$C_{13}H_{12}N_4O_2$	

18	7.970	22	Quinazolin-4(3H)-one	$C_{15}H_{12}N_4O_2$	0.33

Table 7. Continued

19	8.604	23	Glyceryl monostearate	$C_{21}H_{42}O_4$	10.49
20	8.756	24	6-Isopropyl-9-methyl-4-oxo-3,4-dihydroazuleno(2,1-d)pyrimidine-10-carboxamide	$C_{17}H_{17}N_3O_2$	4.73

Table 8. GC-MS screening of various substances in methanol extract of *Scenedesmus quadricauda* 2.

Peak	R.Time	No.	Compounds	Chemical formula	%
1	3.349	1	2,6-Dihydroxyacetophenone	$C_{14}H_{24}O_3Si_2$	0.22
2	3.853	2	2,4,4,6,6,8,8-heptamethyl-1,3,5,7,2,4,6,8-tetraoxatetrasilocan-2-ol	$C_7H_{22}O_5Si_4$	0.43
3	4.779	3	benzoic acid	$C_{17}H_{14}O_3$	0.57
4	5.745	4	Quinoxaline, 2-phenyl-	$C_{14}H_{10}N_2$	1.90
5	6.073	5	Chloroacetic acid	$C_2H_3ClO_2$	0.16
		6	Acetic acid	$C_8H_4C_{l4}O_2$	
6	6.406	7	Benzene, 1,4-dibromo-2-nitro-	$C_6H_3Br_2NO_2$	0.16
7	6.583	8	Naphtho[2,1-b:7,8-b']difuran,1,2,9,10-tetrahydro-2,9-dimethyl-	$C_{16}H_{16}O_2$	11.61
8	6.824	9	Benzene, hexachloro-	C_6C_{l6}	0.41
9	6.928	10	Dowicide	$C_6HC_{l5}NaO$	0.53
10	7.383	11	Benz[a]anthracene-7,12-dicarbonitrile	$C_{20}H_{10}N_2$	0.29
11	7.610	12	6-Amino-4-phenyl-1H-quinazolin-2-one	$C_{14}H_{11}N_3O$	2.14
		13	2-Methyl-2-phenyl-1H-indene-1,3(2H)dione	$C_{16}H_{12}O_2$	
12	7.708	14	Benzo[a]pyren-7(8H)-one	$C_{20}H_{14}O$	17.53
13	7.795	15	Fensulfothion oxon	$C_{11}H_{17}O_5PS$	2.98
14	7.898	16	Benzo[g]pteridine-2,4(3H,10H)-dione	$C_{13}H_{12}N_4O_2$	10.68
		17	1-(3-Hydroxyphenyl)-3-(1-propylpyrazol-4-yl)prop-2-en-1-one	$C_{15}H_{16}N_2O_2$	
15	8.137	18	2-Propylthiazolo[4,5-c]quinolin-4-amine	$C_{15}H_{17}N_3S$	2.38
16	8.509	19	Quinoline	$C_{17}H_{10}FN$	0.47

17	8.655	20	Glyceryl monostearate	$C_{21}H_{42}O_4$	38.60
18	8.759	21	6-Isopropyl-9-methyl-4-oxo-3,4 dihydroazuleno(2,1-d)pyrimidine-10-carboxamide	$C_{17}H_{17}N_3O_2$	5.41
19	9.602	22	Flunixin	$C_{14}H_{11}F_3N_2O_2$	2.34
20	9.703	23	1-Naphthalenamine, N-phenyl-	$C_{16}H_{13}N$	1.19

Table 9. GC-MS screening of various substances in ethyl acetate extract of *Scenedesmus quadricauda* 2.

Peak	R.Time	No.	Compounds	Chemical formula	%
1	3.187	1	benzoic acid	$C_{20}H_{30}O_7$	0.70
2	3.349	2	2,6-Dihydroxyacetophenone	$C_{14}H_{24}O_3Si_2$	3.32
3	3.766	3	2-Chloro-7-methoxy-4-methylquinoline	$C_{11}H_{10}ClNO$	7.73
		4	1H-Pyrazol-5-amine,1-(3-Chlorophenyl)-3-methyl-1H-pyrazol-5-amine	$C_{10}H_{10}ClN_3$	
		5	6-(4-Chlorophenyl)-5-nitro-2-piperidinone	$C_{11}H_{11}ClN_2O_3$	
4	3.894	6	2,4,4,6,6,8,8-heptamethyl-1,3,5,7,2,4,6,8-tetraoxatetrasilocan-2-ol	$C_7H_{22}O_5Si_4$	1.42
5	6.116	7	[1,2,4]triazolo[1,5-a]pyrimidin-7-ol, 2-methyl-5-phenyl-	$C_{12}H_{10}N_4O$	0.70
6	6.581	8	Naphtho[2,1-b:7,8-b']difuran, 1,2,9,10-tetrahydro-2,9-dimethyl-	$C_{16}H_{16}O_2$	24.03
7	7.133	9	1,4-Thiazine,perhydro-1-benzoylimino-4-(3-chloro-5-trifluoromethyl-2-pyridyl)-	$C_{17}H_{15}ClF_3N_3OS$	0.49
8	7.287	10	1,3,5-triazine-2,4-diamine	$C_{10}H_{14}N_6S$	2.50
9	7.490	11	Succinic acid	$C_{13}H_{13}BrF_2O_4$	0.79
10	7.706	12	s-Triazine, 2,4-dichloro-6-piperidino-	$C_8H_{10}C_{l2}N_4$	9.20
11	7.897	13	Benzo[g]pteridine-2,4(3H,10H)-dione	$C_{13}H_{12}N_4O_2$	33.57
12	8.267	14	Acetic acid	$C_{14}H_{32}O_3Si_2$	0.72
13	8.621	15	Glyceryl monostearate	$C_{21}H_{42}O_4$	8.41
14	8.754	16	6-Isopropyl-9-methyl-4-oxo-3,4-dihydroazuleno(2,1-d)pyrimidine-10-carboxamide	$C_{17}H_{17}N_3O_2$	6.42

Table 10. GC-MS screening of various substances in ethanol extract of *Dunaliella primolecta*.

Peak	R.Time	No.	Compounds	Chemical formula	%
1	3.775	1	2-Chloro-7-methoxy-4-methylquinoline Quinoline	$C_{11}H_{10}ClNO$	0.85
		2	1-(3-Chlorophenyl)-3-methyl-1H-pyrazol-5-amine	$C_{10}H_{10}ClN_3$	
2	3.849	3	(1-decyl-1H-imidazol-2-yl)methanol	$C_{14}H_{26}N_2O$	0.97
		4	1,3,5-triazine-2,4-diamine	$C_3H_5N_5$	
3	4.029	5	7,7,9,9,11,11-Hexamethyl-3,6,8,10,12,15-hexaoxa-7,9,11-trisilaheptadecane	$C_{14}H_{36}O_6Si_3$	0.28
4	4.425	6	benzoic acid	$C_{14}H_{24}O_3Si_2$	0.28
5	4.876	7	1,2,3,6-Tetrahydrophthalimide 1H-Isoindole-1,3(2H)-dione	$C_8H_9NO_2$	0.92
6	5.183	8	Quinazolin-4(3H)-one	$C_{15}H_{13}N_3O$	0.59
		9	3-Amino-2-benzyl-4(3H)-quinazolinone	$C_{15}H_{13}N_3O$	
7	5.482	10	2,6-Dihydroxyacetophenone, 2TMS derivative	$C_{14}H_{24}O_3Si_2$	1.66
8	5.743	11	9,10-Dimethylphenanthrene	$C_{16}H_{14}$	7.45
9	5.777	12	1-(3-Chlorophenyl)-3-methyl-1H-pyrazol-5-amine	$C_{10}H_{10}ClN_3$	1.05
10	6.174	13	3,6-Bis(N-dimethylamino)-9-ethylcarbazole	$C_{18}H_{23}N_3$	0.36
11	6.571	14	1-Phenyl-3-(4-fluorophenyl)-2-pyrazoline	$C_{15}H_{13}FN_2$	1.83
		15	Ferrocene	$C_{14}H_{16}Fe$	
		16	Naphtho[2,1-b:7,8-b']difuran, 1,2,9,10-tetrahydro-2,9-dimethyl-	$C_{16}H_{16}O_2$	
		17	1-Phenyl-3-(4-fluorophenyl)-2-pyrazoline	$C_{15}H_{13}FN_2$	
12	7.014	18	1,3-Oxathiolan-4-one	$C_3H_4O_2S$	0.79
13	7.383	19	Malononitrile	$C_{12}H_2F_4N_4$	0.64
14	7.485	20	8H-Pyrazolo[5,1-a]isoindol-8-one	$C_{17}H_{14}N_2O_2$	0.48
		21	Benz[a]anthracene-7,12-dicarbonitrile	$C_{20}H_{10}N_2$	
15	7.707	22	Benz[a]anthracene, 8-propyl-	$C_{21}H_{18}$	1.90
16	7.889	23	6-Isopropyl-3-phenoxytropolone	$C_{16}H_{16}O_3$	7.62

Table 10. Continued

		24	5-Methyl-7-phenyl-1, 3-diazaadamantan-6-one Hydrazone	$C_{15}H_{20}N_4$	
17	8.074	25	Acenaphtho[1,2-b]quinoxaline, 9-methoxy-	$C_{19}H_{12}N_2O$	31.45
18	8.136	26	Gallic acid	$C_{16}H_{20}O_8Si$	0.62
19	8.395	27	7-Methoxy-1,2,3-trimethyl-6-nitro-1H-indole	$C_{12}H_{14}N_2O_3$	1.76
20	9.033	28	1,2,3,6,7,8,9,10,11,12-Decahydrobenzo[e]pyrene	$C_{20}H_{22}$	10.90
21	9.193	29	Imidazo[1,2-a]pyridine	$C_{16}H_{16}FN_3$	27.25
22	9.600	30	Flunixin	$C_{14}H_{11}F_3N_2O_2$	0.35

Table 11. GC-MS screening of various substances in methanol extract of *Dunaliella primolecta*.

Peak	R.Time	No.	Compounds	Chemical formula	%
1	3.773	1	2-Chloro-7-methoxy-4-methylquinoline, Quinoline	$C_{11}H_{10}ClNO$	0.61
		2	1-(3-Chlorophenyl)-3-methyl-1H-pyrazol-5-amine	$C_{10}H_{10}ClN_3$	
2	5.447	3	2,6-Dihydroxyacetophenone, 2TMS derivative	$C_{14}H_{24}O_3Si_2$	2.63
3	5.745	4	2-Phenylquinoxaline	$C_{14}H_{10}N_2$	5.46
4	6.241	5	Pyrimidine	$C_4H_4N_2$	0.81
5	6.574	6	Oxazole	C_3H_3NO	2.90
		7	Naphtho[2,1-b:7,8-b']difuran, 1,2,9,10-tetrahydro-2,9-dimethyl-	$C_{16}H_{16}O_2$	
6	6.680	8	Benzoic acid	$C_{15}H_{14}O_3$	1.52
7	6.823	9	Benzene, hexachloro-	C_6Cl_6	7.41
8	6.937	10	Pentachlorophenol, Dowicide	C_6HCl_5O	2.19
9	7.160	11	1-(2-Bromo-5-trifluoromethylphenyl)piperidin-2-one	$C_{12}H_{11}BrF_3NO$	0.94
10	7.348	12	4-Chloro-5-methyl-3-(trifluoromethyl)-1H-pyrazole-1-acetic acid	$C_7H_6ClF_3N_2O_2$	0.71
11	7.483	13	Benz[a]anthracene-7,12-dicarbonitrile	$C_{20}H_{10}N_2$	0.91
12	7.920	14	6-Isopropyl-3-phenoxytropolone	$C_{16}H_{16}O_3$	68.06
13	8.137	15	Ferrocene	$C_{14}H_{18}Fe$	1.89

14	8.396	16	7-Methoxy-1,2,3-trimethyl-6-nitro-1H-indole	$C_{12}H_{14}N_2O_3$	2.83
15	9.601	17	Flunixin	$C_{14}H_{11}F_3N_2O_2$	1.15

Table 12. GC-MS screening of various substances in ethyl acetate extract of *Dunaliella primolecta*.

Peak	R.Time	No.	Compounds	Chemical formula	%
1	3.763	1	2-Chloro-7-methoxy-4-methylquinoline, Quinoline	$C_{11}H_{10}ClNO$	15.02
		2	1-(3-Chlorophenyl)-3-methyl-1H-pyrazol-5-amine	$C_{10}H_{10}ClN_3$	
2	3.827	3	(1-decyl-1H-imidazol-2-yl)methanol	$C_{14}H_{26}N_2O$	1.99
		4	Arsenous acid	$C_9H_{27}AsO_3Si_3$	
3	5.172	5	3-Amino-2-benzyl-4(3H)-quinazolinone	$C_{15}H_{11}N_3O$	0.58
4	5.746	6	9,10-Dimethylphenanthrene	$C_{16}H_{14}$	11.53
5	6.383	7	Thiocyanic acid carbazol-3,6-diyl ester	$C_{14}H_7N_3S_2$	0.81
6	6.575	8	Ferrocene	$C_{14}H_{16}Fe$	3.29
		9	Naphtho[2,1-b:7,8-b']difuran, 1,2,9,10-tetrahydro-2,9-dimethyl-	$C_{16}H_{16}O_2$	
		10	1-Phenyl-3-(4-fluorophenyl)-2-pyrazoline	$C_{15}H_{13}FN_2$	
7	7.287	11	1,3,5-triazine-2,4-diamine	$C_3H_5N_5$	3.00
8	7.443	12	Imidazo[1,2-a]pyridine	$C_{11}H_{17}N_3Si$	1.47
9	7.485	13	Benz[a]anthracene-7,12-dicarbonitrile	$C_{20}H_{10}N_2$	1.07
10	7.898	14	6-Isopropyl-3-phenoxytropolone	$C_{16}H_{16}O_3$	51.83
		15	5-Methyl-7-phenyl-1, 3-diazaadamantan-6-one Hydrazone	$C_{15}H_{20}N_4$	
11	8.131	16	Pyrimidine	$C_4H_4N_2$	2.34
12	8.396	17	7-Methoxy-1,2,3-trimethyl-6-nitro-1H-indole	$C_{12}H_{14}N_2O_3$	3.26
13	8.607	18	2-Bromo-4-chloro-6-nitrophenol	$C_7H_5BrClNO_3$	3.79
		19	Nitrophenol, bromophenol	$C_6H_3BrClNO_3$	

Table 13. Gene Bank Accession Number for algal species

Algal species	Gene Bank Accession Number
Scenedesmus quadricauda 1	OQ408126
Scenedesmus quadricauda 2	OQ408127
Dunaliella primolecta	OQ408128