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EVALUATION OF ANTIBACTERIAL ACTIVITY ON BIOACTIVE COMPOUNDS FROM *DUNALIELLA SALINA* BY GC – MS ANALYSIS

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ABSTRACT

Marine microalgae, *Dunaliella salina* (green algae) was selected for the present secondary metabolite investigation. In the present work, the production of bioactive compounds by *Dunaliella salina* and their antibacterial activities were investigated against different bacterial pathogens. The antibacterial effect of the methanol extract of *Dunaliella salina* exhibited maximum zone of inhibition (16.18±0.23 mm) against *S. epidermidis.* The methanol extracts were characterized by FTIR and GC–MS. Dimethyl sulfoxide and 2-Pyridinecarboxylic acid from polar compounds were determined with the highest amount in methanol extracts of the microalga were detected. However, pigments such as chlorophyll a, b and total carotenoid with antibacterial activity were identified, Dimethyl sulfoxide and 2-Pyridinecarboxylic acid responsible for the antibacterial activity were analyzed. The GC-MS analysis revealed that the presence of unique chemical compounds like Dimethyl sulfoxide (M.W. 78), 2-Pyridinecarboxylic acid (M.W. 137), Dimethyl Sulfone (M.W. 94), and Benzenepropanoic acid, alpha., 4-dihydroxy-, methyl ester (M.W. 196) respectively from the crude extract of *Dunaliella salina*.

Keywords: *Dunaliella salina,* methanol solvent, antibacterial activity, bioactive compounds, FTIR and GC–MS

INTRODUCTION

Ocean covers nearly 70% of the earth's surface and possesses nearly three lakhs described species of plants and animal from marine sources, representing 34-36 phyla and some of them are exclusive of the marine ecosystem.In marine and aquatic environment, the microalgae are the most important and basic component, and they well known as a primary producer at the base of the aquatic food chain **[1]**. Microalgae are eucaryotic photosynthetic organisms that play a key role in aquatic ecosystems and account for approximately 40 % of global photosynthesis. They produce several active metabolites with variety of biologically active metabolites such as, carotenoids, phycobilin's, polyunsaturated fatty acids, proteins, polysaccharides, vitamins and sterols among other chemicals. Most algae are autotrophs and they can vary from small unicellular microalgae, such as green algae, blue-green algae and diatoms, to large multicellular macroalgae, such as giant kelp **[2]**. Bioactive compounds of microalgal origin can be sourced directly from primary metabolisms, many of the bioactive compounds have antimicrobial antioxidant, and anti-inflammatory capacities, with the potential for the reduction and prevention of diseases **[3-6]**.

Extracts of *Dunaliella* species have been investigated as having antimicrobial activity against several pathogenic microorganisms. Chang *et al*., **[7]** reported that the crude extracts of *D. primolecta* had antimicrobial activity against *Staphylococcus aureus, Bacillus cereus, Bacillus subtilis*and *Enterobacter aerogenes* and these extracts contained substances having antibiotic properties.In a previous research done by Krishnakumar *et al.,* **[8]** proposed that the effect of the environmental conditions like pH, temperature, and salinity on growth of*D. salina* and its antimicrobial activity. Several of the bioactive compounds found their application in human diseases and others as structural models for the development of new drugs. The antimicrobial activity of microalgae extracts is generally assayed using various organic solvents **[9]**. An organic solvent always provides higher efficiency in extracting compounds for antimicrobial activity as compared to aqueous extract **[10, 11]**. Therefore, the present study aims to describe the antibacterial activity of *D. salina* against human pathogenic bacteria andbioactive compounds analysis in FTIR and GC-MS examination.

MATERIALS AND METHODS

Isolation of *Dunaliella salina*

The microalgae *D. salina* were obtained from southwest part of the Parangipettai estuarine region, South east coast of India. *D. salina* were cultivated in 250 mL Erlenmeyer flasks containing 1000 mL of Johnson's medium containing $NaNO_3$ -0.15g, $MgCl_2$ '$6H_2O$ - 1.5g, $MgSO_4 \cdot 7H_2O$ - 0.5g, KCl - 0.2g, KNO_3 - 1.0g, K_2HPO_3 - 0.045g, NaH_2PO_4 'H_2O - 0.010g, TRIS - 2.45g, CaCl2 - 0.2g, NaCl - 33g, $ZnCl_2$ - 04041mg, H_2BO_3 - 0.61mg, N – 1.0 g/L, EDTA - 2mL, Vitamins B_1 - 0.1mg, B_{12} - 0.5µg in 1000 mL of distilled water (pH: 7.5) (12) at 30 °C, under continuous light intensity as 2400 lx at a growth chamber (BINDER, KBW 400 (E5.1)) for 14 days.

Determination of *D. salina* growth

The growth activity of *D. salina* was determined by taking UV spectral readings using UV-vis spectroscopy (Shimadzu UV-1800) every two days throughout the growth period (13). In brief, 2 mL of culture was taken in the cuvette and placed in a sample holder. The spectral readings were recorded at a 620 nm wavelength. The growth rate was calculated using the following formula of OECD.

$$\mu = \frac{N\times - N_\circ}{T\times - T_\circ}$$

where, $N_\times$ - Number of cells in time x, $N_\circ$ - Number of cells in time zero, T× - Time x (in days), $T_\circ$- starting time (0).

Optical Density Measurements and Manual Cell Counting

Exponential phase *D. salina* culture was serially diluted to obtain 5 tubes in duplicates where the dilution factor was ½ in each step. Two hundred µL from each tube was used to obtain optical density values at 750 nm measured on a Biotek Epoch microplate reader (BioTek, Winooski, Vermont, USA). From each dilution, 3 ml sub-samples were fixed with 60 µL acidic Lugol's iodine solution **[14]**. Two mL of the fixed samples were transferred to Utermöhl settling chambers (Aquatic Research Instruments, ID, USA) and cells were allowed to settle for 24 hours **[15]**. The number of cells per mL was calculated according to the formula below **[14]**;

$$\text{Field count } \left(\frac{\text{No.}}{\text{mL}}\right) = \frac{\text{C x At}}{\text{Af x F}_\circ} \text{x } V$$

Where, C= number of cells counted, At= total area of settling chamber, mm^2, Af= area of a field, mm^2,F= number of fields counted, V= volume of sample settled, mL.

Pigment estimation (Chlorophylls & Carotenoids)

The algal cells of *D. salina* were estimated to chlorophyll and total carotenoid content in microalgal cells, the spectrophotometric technique was used. An algal cell was extracted with 100% acetone. An algal sample was extracted with 100% acetone. The absorbance of light green supernatant was measured at three wavelengths, 661.6(A661.6), 644.8(A644.8), and 470 (A470), using the

UV-1800 UV spectrophotometer (Shimadzu). The chlorophyll and total carotenoid content of the algal sample were calculated using the following methods Lichtenthaler and Buschmann **[16]**.

Extraction of bioactive compounds

The algae were cultured in 1000 mL of Johnson's medium and incubated at 30 °C at a constant light intensity of 2400 lux lens in a growth chamber for 14 days. At the end of the incubation period, biomass was collected by centrifugation (MPW-351R) at 10,000 rpm for 5 minutes. The resulting biomass was dried (Millrock Technology, Inc., Kingston, NY 12401, USA) overnight and 100 g of dried biomass was exposed to 300 mL of methanol solvent with a purity of 96%. After incubation for 72 h, the mixture was centrifuged at 10,000 rpm for 5 min. and the supernatant was used as a *D. salina* extract. These solutions were stored at 4 ° C and used for 2 days **[17, 18]**.

Antibacterial activity of Bioactive Compounds

Microalgal bioactive compounds'effectiveness was designated with antimicrobial activity. Antibacterial activity was determined by the disc diffusion method **[19]**. For this purpose, standard bacterial strains like *Escherichia coli* (MTCC- 1258), *Klebsiella pneumoniae* (MTCC- 109), *Staphylococcus aureus* (MTCC- 6908), *Streptococcus pneumoniae* (MTCC – 5542), and *Staphylococcus epidermidis* (MTCC- 2639) were used. These bacteria were cultivated in Nutrient Broth for 24 h and were inoculated uniformly using sterile cotton swab onto Muller Hinton Agar (MHA) to test the antibacterial activities of *D. salina* extract. Extracts were dissolved in DMSO and then loaded on sterile discs at different concentrations (50, 100, 150, and 200 µg/mL) then impregnated disks were placed on the plates using sterile forceps properly spaced at equal distance. In this experiment, the standard antibiotic (Chloramphenicol (20 µg) was used as control. The plates were stored for 2 h to allow the extracts into the agar. Then, these plates were incubated for 24 h at 30 ℃ for the growth of bacterial strains. The zone of inhibition was measured and expressed in mm in diameter.

FTIR spectroscopy and quantification

Fourier Transform Infrared Spectroscopy (FTIR) was conducted using the Shimadzu IRPrestige-21 Fourier Transformation Infrared Spectrophotometer (Shimadzu, U.K.) to characterize the various functional groups present on the whole cell surface and also the extracellular polymeric substances (EPS). At least 64 scans with a resolution of 4 cm^{-1}were collected for all samples. *D. salina* extract 1.5 mL sample was taken from each replicate flask for each treatment and

centrifuged before the supernatant was removed and the cells were re-suspended in 0.9% NaCl. They were washed twice to remove EPS. A portion of the sample was then applied to the FTIR plate and dried at room temperature for up to 30 min before being scanned by FTIR. To demonstrate the band corresponding to lipids in living cells, samples were treated with methanol to extract lipids from the live cells (20). The lipid-containing organic phase was then removed and the pellet was washed twice with 0.9% NaCI before measurement by FTIR. Dried lipids previously isolated from *D. salina* extract samples by methanol extraction were resuspended in methanol and measured by FTIR in the same way. The spectra for each sample were measured twice and then collected over the wavenumber range 4000–600 cm^{-1}.

GC-MS Analysis for Bioactive Compounds

The methanolic crude extract was subjected to identify the bioactive compounds by GC-MS (Shimadzu 2010 plus comprising an AOC-20i) analysis. GC-MS analysis was carried out by following conditions: Column RTX 5Ms (Column diameter was 0..32 mm, column length was 30 m, column thickness 0.50 µm), operating in electron impact mode at 70eV; Helium gas (99.999 %) was used as carrier gas at a constant flow of 1.73 mL /min and an injection volume of 0.5 µL was employed (split ratio of 10:1) injector temperature 270 ºC; ion-source temperature 200 ºC. The oven temperature was programmed from 40 ºC (isothermal for 2 min), with an increase of 8 ºC/min, to 150 ºC, then 8 ºC/min to 250 ºC, ending with a 20 min isothermal at 280 ºC. Mass spectra were taken at 70eV; a scan-interval of 0.5 seconds and fragments from 40 to 450 Da. Total GC running time was 51.25 min. The relative percentage amount of each component was calculated by comparing its average peak area to the total areas. Software adapted to handle mass spectra and chromatograms was a Turbo Mass Ver 5.2.0 (21). Interpretation on GC-MS was conducted using the database of National Institute Standard and Technology (NIST) having more than 62,000 patterns. The spectrum of the unknown component was compared with the spectrum of the known components stored in the NIST library. The name, molecular weight, and structure of the components of the test materials were ascertained **[22]**.

RESULT

Cultivation of *D. salina*

The *D. salina* used in the study was tested to determine the presence of the most effective bioactive compound. For these experiments, *D. salina* was inoculated into Johnson's medium and at the end of the incubation period, the efficacy of the bioactive compounds was determined.

From these tests, *D. salina* the production of the most effective bioactive compound has been found and further tests have been carried out with this *D. salina*.

Determination of optical density andcell count on *D. salina*

D. salina experimented with growth rate and cell count on incubation periods. The OD value (0.115) was observed on the 13th day and the highest cells observed cell counting 2.732x10^{6} (cells/ mL). The OD value and cell counting values are shown in **Table 1**.

Pigmentation (Chlorophylls & Carotenoids)

The pigments of *D. salina* were estimated to chlorophyll (a and b) and total carotenoid content analyzed on spectrophotometric technique. In this study chlorophyll a was observed value of 4.623 mg/mL, and chlorophyll b estimated value has observed as 0.6566 mg/mL. The total chlorophyll (a and b) was observed values of 5.2796 mg/mL. The carotenoid pigment analyzed in this study was observed at a total carotenoid value of 0.916 mg/mL.

Antibacterial activity of Effectiveness of Bioactive Compounds

The examination on antibacterial activity at different solvent extracts against the pathogenic bacteria showed different levels of the zone of inhibition. The *D. salina* extract was performed in antibacterial activity was shown a growth-inhibitory zone of 16.18±0.23 mm against as observed *S. epidermidis,* which was followed by *S.aureus* (15.08±0.78 mm), and *E. coli* (12.09±0.84 mm). The lowest zone of growth inhibition (10.42±0.62 mm) was observed against *K. pneumoniae* and *S. pneumoniae* (09.12±0.42 mm) was recorded around the disc.The standard antibiotic Chloramphenicol (20 µg) showed a zone of growth inhibition ranged from 17 to 19 mm. The zone of inhibition of antibacterial activity data showed in **Table 2 and Figure 1**.

FTIR analysis of methanolic extract of *D.salina*

The FTIR spectra of the *D. salina* methanol extract were recorded to identify functional groups. Their phytochemical profile revealed the presence of alkyl halides, proteins, phenolic and aromatic compounds with transmission peaks at 3449.48 cm^{-1} is corresponding to the strong broad O-H stretching of alcohol. The band decreased at 1635.29 cm^{-1} is corresponding to the medium N - H stretching of amine. Peak raised at 1520.05 cm^{-1} is corresponding to the strong N - O stretching of the nitro compound. The increased peak at 1385.80 cm^{-1} is corresponding to the medium C - H stretching of alkane. They peak at 1147.59 cm^{-1} is corresponding to the strong S = O stretching of sulfonic acid. The absorbed band at 997.50 cm^{-1} is corresponding to the

strong C - C bending of the alkene. Peak range at 832.80 cm^{-1} is corresponding to the medium C - C bending of an alkene. Then followed peak at 661.48 and 608.58 cm^{-1} is corresponding to the strong C - Br stretching of halo compound respectively **(Figure 2)**.

GC-MS analysis of selected Fractions

The gas chromatogram shows that the relative concentration of various compounds getting fractionated at their specific retention time. Several peaks were obtained from the mass spectrum of *D. salina* extract. All the major compounds and their molecular weights present at respective peaks were analyzed using the NIST database to locate the probable compound as well as their molecular weight (**Figure 3**). The heights and percentage of peak area indicate the relative concentrations of the components present in the *D. salina* extract. The peaks at RT 78.05, 79.05, 63.00, 79.00, and 107.00 were identified as Dimethyl Sulfoxide, 2-Pyridinecarboxylic acid, Methyl ester, Methane, Sulfinylbis, Dimethyl Sulfone, and Benzenepropanoic acid, alpha., 4-dihydroxy-, methyl ester, respectively **(Table 3)**.

Table 1: Optical Density andCell count on *D. salina*

Days	OD values	Cell counting (cells/ mL)
1st	0.001	$6.5x10^4$
2nd	0.019	$2.02x10^5$
3rd	0.022	$3.2x10^5$
4th	0.019	$5.6x10^5$
5th	0.022	$7.77x10^5$
6th	0.025	$1.11x10^6$
7th	0.030	$1.337x10^6$
8th	0.046	$1.715x10^6$
9th	0.058	$1.985x10^6$
10th	0.071	$2.212x10^6$
11th	0.085	$2.612x10^6$
12th	0.118	$2.67x10^6$
13th	0.115	$2.732x10^6$
14th	0.091	$2.36x10^6$

Table 2: Antibacterial activity of *D. salina* extract against pathogenic bacteria

Bacteria Name	Zone of inhibition (mm in diameter)				
	50 µg/mL	100 µg/mL	150 µg/mL	200 µg/mL	Chloramphenicol
Escherichia coli	_	09.16±0.54	11.24±0.34	12.09±0.84	18.12±0.41
Klebsiella pneumoniae	_	_	09.06±0.24	10.42±0.62	19.24±0.67
Staphylococcus aureus	08.24±0.42	10.12±0.06	12.06±1.65	15.08±0.78	18.65±0.84
Streptococcus pneumoniae	_	_	_	09.12±0.42	17.34±1.06
Staphylococcus epidermidis	08.06±064	11.34±0.28	13.05±0.84	16.18±0.23	18.72±0.36

-: No zone of inhibition. Values expressed in mean ± standard deviation

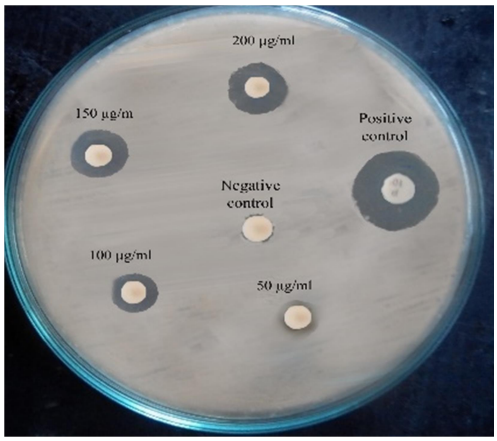

Figure 1: Antibacterial activity of *D. salina* extracts against pathogenic bacteria *Staphylococcus aureus*

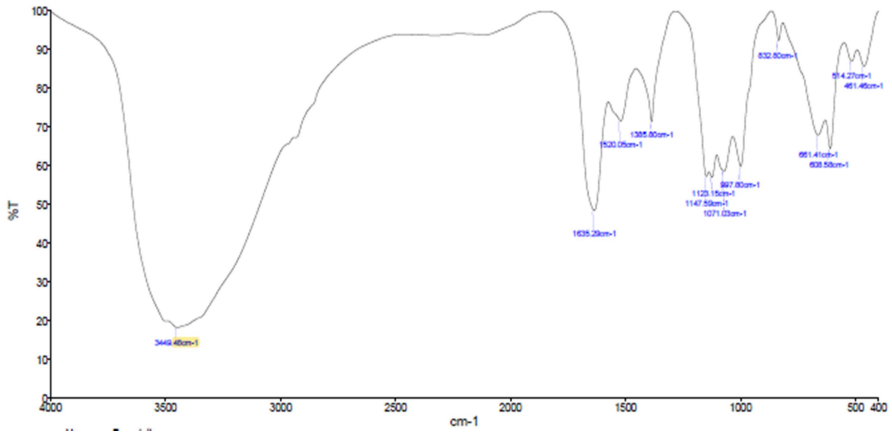

Figure 2: FTIR analysis of *D. salina* extract

Table 3: Major compounds identified in the mass spectrum of *D. salina* extract

Identified compound	Retention Time	Base peak	Molecular formula
Dimethyl Sulfoxide	4.160	78.05	C2H6OS
2-Pyridinecarboxylic acid, Methyl ester	4.720	79.05	C7H7NO2
Methane, Sulfinylbis	5.415	63.00	C2H6OS
Dimethyl Sulfone	6.215	79.00	C2H6O2S
Benzenepropanoic acid, alpha., 4-dihydroxy-, methyl ester	9.060	107.00	C10H12O4

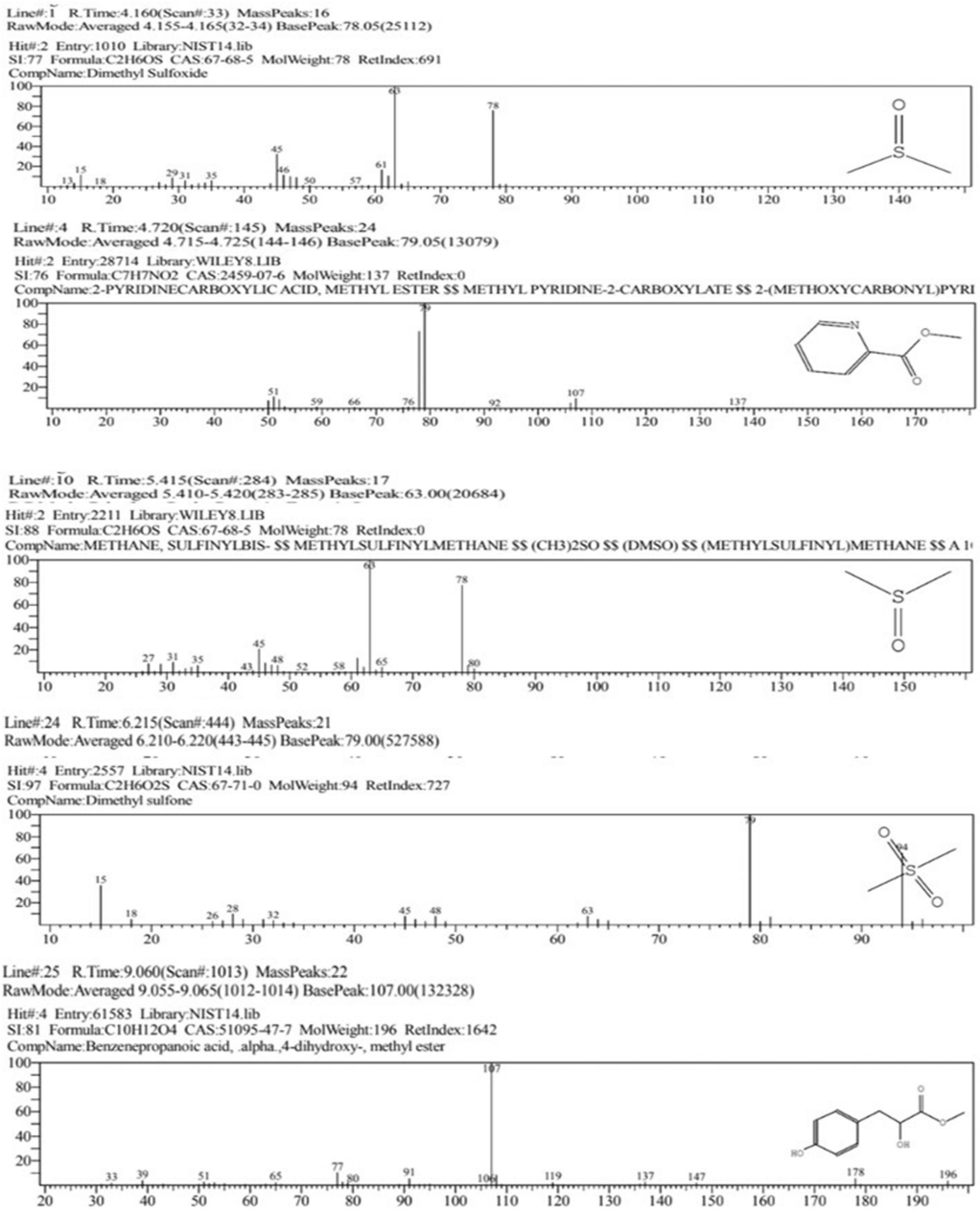

Figure 3: GC-MS analysis of *D. salina* extract

DISCUSSION

In the current study, the *D. salina*was isolated and cultivated in Johnson's medium containing 1.0 g/L of nitrogen concentration at 30 °C. Previous studies on nitrogen limitation generally related to the increment of certain fatty acids accumulation when nitrogen concentration decreased **[23]**. On the other hand, in another previous study, *D. salina* was cultivated in media with nitrogen-free and 250 mM nitrogen; the biomass extracts obtained from nitrogen-containing medium were more effective against the tested

cancer cells than extracts obtained from nitrogen-free medium **[24]**. The present study was observed that the optical density and cell counting determine the highest values on the 13th day, later the growth was reduced.

Chlorophyll contents of the microalga were found adaptable with antimicrobial activities determined. *D. salina* had the highest chlorophyll (a and b) content, carotenoids, and bioactive property when it was cultivated in media with 1.0 g/L nitrogen. The amount of total chlorophyll (a and b) was observed with the values of 5.2796 mg/mL and carotenoid value of 0.916 mg/mL respectively. BenMoussa-Dahmen*et al.,* **[25]** showed that with an increase in salt concentration up to 3 M, *Dunaliella* sp. had more chlorophyll like in the current study. In that study, it was also found that because of salt osmotic stress, photosynthesis decreased and at the salinity, reduction of chlorophyll content has occurred. A similar effect was shown by Kirrolia *et al.,* **[26]**. It was known that carotene had antioxidant properties, while phenolic compounds as a photoprotective response can be useful as antimicrobial **[27]**. *D. salina* most probably produced more phenolic compounds under high light and therefore antimicrobial activity was found more efficient under these conditions.

In recent years, antibacterial metabolites extraction from algae has attracted the most attention among other possible sources. The antimicrobial activity of microalgae has been attributed to compounds belonging to several chemical classes – including indoles, terpenes, acetogenins, phenols, fatty acids, and volatile halogenated hydrocarbons **[28, 29]**. Antimicrobial activity depends on both algal species and the solvents used for their extraction **[30]**. The antimicrobial activity of algae extracts is generally assayed using various organic solvents, such as acetone, ether and chloroform, methanol **[9]**. An organic solvent always provides higher efficiency in extracting compounds for antimicrobial activity **[31]**.

Several factors affect the yield of pigments extraction; of these, the solvent to be used comes first. In the current study, methanol was found the best organic solvent to obtain bioactive compounds from *D. salina*. In a previous study, methanol extracts of *D. salina* had the most efficient antimicrobial activity as and need in the current study. On the other hand, Srinivasakumar & Rajashekhar **[32]** showed butanol extracts of *D. salina* had the most efficient bioactive compound. Herrero*et al.,* **[33]** showed that methanol extracts of *D. salina* had lower bioactive properties than obtained from organic solvents as it is found in the current study

also. In the present study, *D. salina* extract was inhibiting the growth of tested pathogenic bacteria, according to the results the most zone of inhibition against *S. epidermidis* and *S.aureus* with a nitrogen concentration from 1.0 g/L, antibacterial effect increased for the tested bacteria. Based on these results, further experiments were done in media with 1.0 g/L nitrogen related to extracts having the highest bioactive property.

The FTIR analysis of crude extract allows to verify the metabolic compound present in the extract. In this study, for example, the lipid has several bands including 3449.48 cm^{-1}, 1635.29 $cm^{-1,}$ and 1147.59 cm^{-1} **[34]**. The previous study strongly suggests that FTIR is an effective means of determining genuine lipid changes in cells, i.e. the increase of lipid/protein ratio was not caused solely by a decrease of protein.GC-MS analysis of a crude extract of *D. salina* demonstrated interesting compounds with significant antibacterial activity. In the present investigation, different chemical constituents such as Dimethyl Sulfoxide, 2-Pyridinecarboxylic acid, Methyl ester, Methane, Sulfinylbis, Dimethyl Sulfone and Benzenepropanoic acid, alpha., 4-dihydroxy-, methyl ester with antibacterial activity and pharmaceutical importance were identified. *D. salina* extract analysis of the described species using gas chromatography-mass spectrometry (GC-MS) had revealed several important organic volatile compounds and its derivatives. The microalgae produce active extracts in terms of both antioxidant and antimicrobial activity. In the earlier research, different fatty acids and volatile compounds such as phytol, fucosterol, neophytadiene or palmitic, palmitoleic and oleic acids, with antimicrobial activity were identified, which were obtained by the organic solvent extracts of *Synechocystis* sp. Chemically characterized by GC-MS analysis **[35]**. Al-Wathnani **[36]** identified that cyanobacteria and green algae have been found potential for the production of several compounds including biomedically important organic metabolites such as 3-Methyl-2-(2-Oxopropyl) Furan, ethane1,1-diethoxy butanal, heptanal, and octanal by GC-MS analysis. Dooslin Mercy Bai and Krishnakumar **[37]** noticed that GC-MS analysis of *Tetraselmiss uecica* crude extract contained 1-ethyl butyl 3-hexyl hydroperoxide and methyl heptanoate which is known to demonstrate valuable therapeutic uses including anti-inflammatory**,** antipsychotics, antiseptic, antineoplastic, anti-allergic, antipyretic and analgesic effects.

CONCLUSION

The current study has shown the antibacterial activity of bioactive compounds by *D. salina*. The extract of *D.*

salina had the highest capacity in producing effective bioactive compounds against different bacteria. Further, antimicrobial activity increased in the zone of inhibition was found the most effective. Pigments of chlorophyll (a and b) and total carotenoid from *D. salina* and a Dimethyl Sulfoxide and 2-Pyridinecarboxylic acid from polar compounds were determined with the highest amount in methanol extracts of the microalga. It can be concluded that polarity compounds were responsible for higher antibacterial activity and *D. salina* is a safe beneficial biomaterial to be utilized in the field of pharmacology according to its bioactive properties.

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REFERENCES

[1] Sampathkumar, P. and l. Kannan. seasonal variations in physico-chemical characteristics in the tranquebar-nagapattinam region, south-east coast of India. Em international vol.17, issue 04, (1998); page no. (397-402)

[2] Kouzuma, K. Watanabe. Exploring the potential of algae/bacteria interactions, Curr. Opin. Biotechno 33, 125–129. (2015). DOI: 10.1016/j.copbio.2015.02.007

[3] Smee, D. F. Bailey, K. W. Wong, M. H. *et al*. "Treatment of influenza A (H1N1) virus infections in mice and ferrets with cyanovirin-N," Antiviral Research, vol. 80, no. 3, pp. 266–271, (2008).

[4] Ibanez, E. and A. Cifuentes. "Benefits of using algae as natural sources of functional ingredients," Journal of the Science of Food and Agriculture, vol. 93, no. 4, pp. 703–709, (2013).

[5] Markou, G. and E. Nerantzis. "Microalgae for high-value compounds and biofuels production: a review with focus on cultivation under stress conditions," Biotechnology Advances, vol. 31, no. 8, pp. 1532–1542, (2013).

[6] Harun, R. M. Singh, G. M. Forde, andM. K. Danquah. "Bioprocess engineering of microalgae to produce a variety of consumer products," Renewable and Sustainable Energy Reviews, vol. 14, no. 3, pp. 1037–1047, (2010).

[7] Chang, T., Ohta, S., Ikegami, N., Miyata, H., Kashimoto, T., & Kondo, M. Antibiotic substances produced by a marine green alga, *Dunaliella primolecta*. Bioresource

Technology, (1993) 44 (2), 149-153. https://doi.org/10.1016/0960-8524(93)90189-I

[8] Krishnakumar, S., Bai, V.D.M., & Rajan, R.A. Evaluation of Bioactive Metabolites from Halophilic Microalgae *Dunaliella Salina* by GC-MS Analysis. International Journal of Pharmacy and Pharmaceutical Sciences, (2013) 5 (4), 296-303.

[9] Cordeiro RA, Gomes VM, Carvalho AFU, Melo VMM. Effect of Proteins from the Red Seaweed *Hypnea musciformis* (Wulfen) Lamouroux on the Growth of Human Pathogen Yeasts. Brazilian Arch Boil Technol. (2006); 49(6): 915-921.

[10] Masuda M, Abe T, Sato S, Suzuki T, Suzuki M. Diversity of halogenated secondary metabolites in the red alga *Laurencia nipponica* (Rhodomelaceae, Ceramiales). J of Phycology. (1997); 33: 196-208.

[11] Lima-Filho JVM, Carvalho AFFU, Freitas SM, Melo VMM. Antibacterial activity of extracts of six macroalgae from the northeastern Brazilian coast. Brazilian J of Microbiology. (2002); 33: 311-313.

[12] Johnson, M.K., Johnson, E.J., MacElroy, R.D., Speer, H.L., & Bruff, B.S. Effects of salts on the halophilic alga *Dunaliella viridis. Journal of Bacteriology*, (1968) 95, 1461–1468.

[13] Teoh, M.L., Chu, W.L., Harvay, M., & Phang S. M. Influence of culture temperature on the growth, biochemical composition and fatty acid profiles of six Antarctic microalgae. Journal of Applied phycology, (2004): 16421 – 430.

[14] Edler, L., Elbrächter, M. The Utermöhl method for quantitative phytoplankton analysis, in: Karlson, B.; Cusack, C. and Bresnan, E. (Eds.), Microscopic and molecular methods for quantitative phytoplankton analysis. Paris, France, UNESCO, (2010) 110pp.

[15] Ferrando, N. S., Benítez, H. H., Gabellone, N. A., Claps, M. C., Altamirano, P. R. A quick and effective estimation of algal density by turbidimetry developed with *Chlorella vulgaris* cultures. Limnetica, (2011) 29; 397–406.

[16] Lichtenthaler HK, Buschmann C. Chlorophylls and carotenoids: Measurement and characterization by UV-VIS spectroscopy. Current

protocols in food analytical chemistry. (2001).

[17] Rao, A.R., Reddy, A.H., & Aradhya, S.M. Antibacterial properties of *Spirulina platensis, Haematococcus pluvialis, Botryococcus brauniimicro* algal extracts. *Current Trends in Biotechnology and Pharmacy,* (2010) *4*(3), 809-819.

[18] Pradhan, J., Das, B.K., Sahu, S., Marhual, N.P., Swain, A.K., Mishra, B.K., & Eknath, A.E. Traditional antibacterial activity of freshwater microalga Spirulina platensis to aquatic pathogens. Aquaculture Research, (2012) 43, 1287–1295. https://doi.org/10.1111/j.1365-2109.2011.02932.x

[19] Murray, P.R., Baron, E.J., Pfalle, M.A., Tenover, F.C., & Yolke, R.H. Manual of clinical microbiology (6th ed.) Washington, DC, United States, ASM Press, (1995) 1482 pp.

[20] Bligh EG and Dyer WJ, A rapid method for total lipid extraction andpurification.CanJBiochemPhysiol: 911-917 (1959).

[21] Reyes-Chilpa R., Rivera J., Oropeza M., Mendoza P., Amekraz B., Jankowsko C. Methanol extracts of Hamelia patens containing oxindole alkaloids relax KCl induced concentration in rat myometrium. Biol Pharm Bull; (2004) 27: 1617-1620.

[22] Donghai Li., Shihong Yan., Peter Proksch., Zhenyi Liang., Qiang Li., Jing Xu. Volatile metabolites profiling of a Chinese mangrove endophytic *Pestalotiopsis* sp. Strain, African j of Biotechnology, (2013) vol 12(24), pp. 3802-3806.

[23] Byrd, S.M., Burkholder. J.M., & Zimba. P.V. Environmental stressors and lipid production by *Dunaliella spp*. I. Salinity. Journal of Experimental Marine Biology and Ecology, (2017) 487, 18–32. https://doi.org/10.1016/j.jembe.2016.11.004

[24] Singh, P., Baranwal, M., & Reddy, S.M. Antioxidant and cytotoxic activity of carotenes produced by *Dunaliella salina* under stress. Pharmaceutical Biology, (2016) 54(10), 2269–2275. https://doi.org/10.3109/13880209.2016.1153660

[25] Ben Moussa-Dahmen, I., Chtourou, H., Rezgui, F., Sayadi, S., &Dhouib, A. Salinity stress increases lipid, secondary metabolites and enzyme activity in *Amphora subtropica* and

Dunaliella sp. for biodiesel production. Bioresource Technology, (2016) 218, 816–825. https://doi.org/10.1016/j.biortech.2016.07.022

[26] Kirrolia, A., Bishnoi, N.R., & Singh, N. Salinity as a factor affecting the physiological and biochemical traits of *Scenedesmus quadricauda*. Journal of Algal Biomass Utilization, (2011) 2, 28–34.

[27] Skjanes, K., Rebours, C., & Lindblad, P. Potential for green microalgae to produce hydrogen, pharmaceuticals and other high value products in a combined process. *Critical Reviews in Biotechnology,* (2013) 33(2), 172–215. https://doi.org/10.3109/07388551.2012.681625

[28] VisoAC, Pesando D, Baby C. Antibacterial and antifungal properties of some marine diatoms in culture. Botanica Marina. (1987); 30:41-45.

[29] Findlay JA, Patil AD. Antibacterial constituents of the diatom *Navicula delognei*. Journal of Natural Products. (1984); 47: 815-818.

[30] Radhika D, Veerabahu C, Priya R. Antibacterial activity of some selected seaweeds from the Gulf of Mannar Coast, South India. Asian journal of pharmaceutical and clinical research. (2012); 5(4): 89-90.

[31] TuneyI, Cadirci BH, Unal D, Sukatar A. Antimicrobial activity of the extracts of marine algae from the coast of Urla (Izmir, Turkey). Turk J Biol. (2006); 30:171-175.

[32] Srinivasakumar, K.P., & Rajashekhar, M. In vitro studies on bactericidal activity and sensitivity pattern of isolated marine microalgae against selective human bacterial pathogens. Indian Journal of Science Technology, (2009) 2(8), 16-23. http://dx.doi.org/10.17485/ijst/2009/v2i8/29503

[33] Herrero M, Ibañez E, Cifuentes A, Reglero G, Santoyo S.*Dunaliella salina* microalga pressurized liquid extracts as potential antimicrobials. Journal of Food Protection. (2006); 69: 2471-2477.

[34] Dean PA, Sigee CD, Estrada B, and Pittman KJ, Using FTIR spectroscopy for rapid determination of lipid accumulation in response to nitrogen limitation in freshwater microalgae. *Bioresource*

Technology **101**: 4499-4507 (2010).

[35] Plaza M, Santoyo S, Jaime L, Garcia-Blairsy Reina G, Herrero M, Senorans FJ, Ibanez E Screening for bioactive compounds from algae. Journal of Pharmaceutical and Biomedical Analysis. (2010); 51: 450–455.

[36] Al-Wathnani H, Ismet Ara RR, Tahmaz TH, Al-Dayel, Bakir MA Bioactivity of natural compounds isolated from cyanobacteria and green algae against human pathogenic bacteria and yeast. Journal of Medicinal Plants Research. (2012); 6(18): 3425-3433.

[37] Dooslin Mercy Bai V, Krishnakumar S Evaluation of antimicrobial metabolites from marine microalgae *Tetraselmis suecica* (Kylin) using gas chromatography – mass spectrometry (GC – MS) analysis. International J Pharmacy and Pharmaceutical Sciences. (2013); 5 (3): 17-23.