QUALITATIVE AND MICROBIAL ASSESMENT OF *NANNOCHOLOROPSIS OCCULATA* AGAINST HUMAN PATHOGENS THROUGH GC-MS ANALYSIS

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ABSTRACT

Objective: From marine microalgae the broadcast of secondary metabolites and pharmacologically dynamic complexes has enhanced in the ancient decade. Nannochloropsis occulta has been choice for the antibacterial metabolites lessons in the present search.

Methods: For the existing secondary metabolites study Marine microalgae Nannochloropsis occulta (green algae) was designated. for the development of microalgae the properties of pH, temperature and salinity were tested. The antibacterial effect of dissimilar solvent extracts of Nannochloropsis occulta against selected human pathogens such as Vibrio cholerae, Klebsiella pneumoniae, Escherichia coli, Pseudomonas aeruginosa, Salmonella sp, Proteus sp., Streptococcus pyrogens, Staphylococcus aureus, Bacillus megaterium and Bacillus subtilis were examined.

Results: when the medium adjusted with pH of 9.0 in 30 ppt of salinity at 250C the uppermost cell growth was detected during 9th day of incubation. Among the solvents used, Butanol + Isopropanol(1:1) crude extract of Nannochloropsis occulta exhibited maximum zone of inhibition (13.4 mm) against Salmonella.. GC-MS analysis revealed that, the presence of unique chemical compounds like 3, 3, 5-Trimethylheptane (M.W. 142.2) and n-Hexadecane (M.W. 226.2) respectively for the crude extract of Nannochloropsis occulta

Conclusion: These findings demonstrate that, the Butanol + Isopropanol (1:1) extract of Nannochloropsis occulta displayed appreciable antimicrobial activity and thus have great potential solvent to extract bioactive compounds from the natural sources for current biomedical and pharmaceutical importance.

Keywords: Microalgae, *Nannochloropsis occulta* ,Secondary metabolites, GC-MS analysis.

INTRODUCTION

Water encloses more than 70% of the world surface. The most leading group of living organism in water is the algae. Microalgae only makes the major productivity of the oceans. Pharmacology significant miscellaneous collections of natural products [1,2,3,4] is yielded by marine organisms which also yields new and uncharted bases of potentially valuable bioactive compounds[5,6] that might characterize suitable leads in the growth of new pharmaceutical agents [7]. Biologically active compounds from natural resources have always been a major interest for scientists working on different pathogens [8]. Ancient medicine used Algae for long time and also bacteriostatic, bactericidal, antifungal, antiviral and antitumor activity are present in [9]. Microalgae serves as rich source of physically novel and biologically active metabolites ,therefore it has been studied in pharmaceutical industry as potential bioactive compounds of interest[10,11]. This is a rich varied collection and also constitutes a rich source of bioactive ingredients such as vitamins[12], pigments, fatty acids, sterols and polysaccharides [13,14]. Because of frequent use of chemotherapeutic drugs and delay in adequate therpay of pathogens and causing some unwanted side effects and potentially enlarged mortality, resistance has been developed [15]. These limitations caused demand to improve pharmacokinetics properties, while researches found continuous demand for new antimicrobial compounds from unexplored habitat for the growth of novel drugs for already existing diseases [16]. Hence for the treatment of human pathogenic microorganisms, the present study has particularly paid attention on the potential applications of marine microalgae Nannochloropsis occulata ,which can be used as an alternative source for the usually used dormant chemotherapeutic agents

Nannochloropsis occulata belonging to the class Eustigmatophyceae and family Monodopsidaceae is a motile unicellular halotolerant green algae most commonly found naturally in habitats like salt marshes [17]. A biomolecule of β-carotene is produced by Nannocholoropsis occulata which is used in the food [18] , cosmetic, pharmaceutical industries as a coloring agent, antioxidant [19], anti-tumor agent [20], and cardiac disease preventive [21].

Detection of a wide range of pharmacologically active substances have been done with various organic solvent extracts of microalgae. Majority of bioactive compounds originate their application as human pathogens and other bioactive compounds as structural models for new drugs development.. Using many organic solvents the antimicrobial activity of microalgae extracts is commonly evaluated [22A higher efficiency in extracting antimicrobial activity is offered by organic solvent as related to aqueous extract [23,24]. A common approach to detect the compounds of biomedical importance is by screening of organic solvent extracts from microalgae and other marine organisms. To evaluate the efficiency of numerous organic solvents in this context an effort has been originated, antimicrobial activity and identify the chemical constituents and structure by GC-MS analysis of crude marine microalgae extracts towards the most common human diseases.

MATERIALS AND METHODS

Microalgae culture collection

Marine microalgae Nannocholoropsis occulata (Kingdom: Chromista Phylum: Ochrophyta ; Class: Eustigmatophyceae ; Order: Eustigmatales ; Family: Monodopsidaceae ; Genus: Nannochloropsis Species: Collection of Nannochloropsis occulata was done in a sterile screw cap tube which was kept in an ice chest box and brought to our laboratory from Centre for Marine Fisheries Research Institute(CMFRI) Tuticorin,Tamil Nadu.The microalgae which were subcultured and maintained as pure culture was chosen for the existing investigation...

Stock culture maintenance

Sea water (100 ml) was taken into 250 ml of conical flask filtered and a required nutrient of Miquell's medium (solution-A: Potassium nitrate: 20.2 g; distilled water: 100ml; solution-B: Sodium orthophosphate: 4g; Calcium chloride: 2g; Ferric chloride: 2g; Hydrochloric acid: 2 ml; distilled water: 100 ml) was dissolved. Solution A (0.55 ml) and solution B (0.5 ml) were added to one liter of filtered sterilized seawater and mixed meticulously to enrich the water and autoclaved. 10% of actively increasing

mid phase inoculum was transported into culture flask aseptically after sterilization. The inoculated flask was incubated at 28±2°C for underneath the fluorescent light of 1000 lux 8 days. When the extreme exponential growth phase was touched, for further growth the light was reduced.

Chemicals

All chemicals and media components were procured from Hi media Laboratories Private Limited, (Mumbai, India) used to perform the current investigation.

Growth optimization of marine microalgae

Miquell' medium (100ml) was prepared in 250 ml of Erlenmeyer flask. Independent optimization of different growth parameters including pH(3,5,7,9 and11), temperature (20,35,30 and 35°C) and salinity (20,30 and 40 ppt) was performed. Authentication of salinity was done with the help of 300 PX- Refractometer (300×225-traditional hand land). Then to the culture flask , 10 ml of actively growing log phase inoculum was transferred aseptically and for 14 days it was reserved under the fluorescent light of 1000 lux.

Determination of cell density

the cell density was determined by the method given by James and Al-Khars [25]. Using a Neubauer improved Haemocytometer (DHC-N01) Cell counts were examined. The microalgae were treated with formalin to kill the cells and one drop of the culture was taken with the help sterile Pasteur pipette. The pipetted culture samples were poured on the counting grid of the haemocytometer after placing the cover slip on the haemocytometer, and left for a few minutes. The cells were counted with the aid of compound microscope (ADELTA OPTEC – DN10) under the magnification of 40X and the total cell count was calculated by the following formula.

Total cell count = Number of cells counted X Number of square in a group Number of square counted

Microalgae extract preparation using different organic solvents

At 200 rpm the Microalgae cells were centrifuged (REMI- R24) for 10 minutes. Under room temperature the pellet was collected and air dried to get a fine powder.In different organic solvents 100 ml of dried microalgae cells of 10g were extracted specifically Acetone, n-butanol,Isopropanol. ,Acetone+n-butanol(1:1),Acetone + Isopropanol(1:1),Acetone +chloroform(1:1), Butanol + Isopropanol (1:1), Chloroform + Methanol (1:1) separately under continuous stirring of 50rpm for 7 days at room temperature. Whatman No.1 filter paper was filtered by the solution. For 24 hr the filtrate was dried at 40oc by desiccator. The respective solvents of the dried powder was suspended to give 50mg/ml of crude extract.For further antimicrobial and GC-MS studies the crude extract was kept in sealed container and stored in a refrigerator

Human pathogens

The human gram negative pathogens such as Vibrio cholerae, Klebsiella pneumoniae, Escherichia coli, Pseudomonas aeruginosa, Salmonella sp, Proteus sp., and gram positive pathogens namely Streptococcus pyogens, Staphylococcus aureus, Bacillus megaterium and Bacillus subtilis collected at Kanyakumari medical college and Hospital (KMCH), Kanyakumari District, Tamilnadu, India and for the present antibacterial susceptibility study our laboratory was chosen maintained.

Antibacterial assay

Against the chosen human pathogens antibacterial activity was determined with paper disk assay method was described by El Maseru etal. By Autoclaving the whatman No.1 filter paper disk of 6mm diameter was incised and sterilized. Using different solvent extract the sterile disk was saturated. Control disk was also sustained by impregnating respective organic solvents alone for each extract. Using sterile cotton swab the overnight broth culture of test pathogens were inoculated uniformly and Muller Hinton Agar plates were prepared. Using sterile forceps the plates were placed by the impregnated disks Equal distances are spaced properly. For each test pathogen there was maintenance of triplicates. The plates were incubated at 37°C for 24 hours.The zone of inhibition been measured in mm and expressed in diameter.

GC-MS analysis of microalgae extract

Qualitative and quantitative analysis of the crude extracts with high sensitivity can be detected by the gas chromatography combined with mass spectrometry detection technique even with trace amount of constituents with high sensitivity.By the chemical moiety of crude extracts of Nannochlotopsis occulata , valuable antibacterial activities against the selected human diseases was also determined.The standard specification of doing GC-MS analysis is by dissolving 10 mg of crude extracts in one milliliter of ethyl acetate.The aliquot of 0.1µl injected automatically into 0.25 mm × 25 mm column of GC-MS model (GC 17A,Japan) 5% phenyl poly siloxane serves as standard phase.Helium, at 17.69 psi pressure and flow of 3 ml/ min at flow rate of 0.4m/min was used as carrier gas.To identify the chemical constituent, the temperature gradient program was implemented for the evoperation of organic solvent. The initial temperature being 70° C ,been gradually accelerated to 250° C at a rate of 10°C per minute.After 18 minutes the sample was injected at 250°C .Comparision was done between the maximum peaks representing mass to charge ratio characteristics of antimicrobial fractions with those in the mass spectrum library of the corresponding organic compounds[26].The concentration of such compund was calculated by the following formula :

Compund concentration percentage=[P1/P2]×100

P1 being the peak area of the compound and P2, whole peak areas in the fractionated extracts.

Data analysis

Through TWO way ANOVA using MINITAB software the data were statistically analyzed and by applying least significant difference (LSD) test at 0.05% level of probability to know their significance status means for different parameters were separated [27].

RESULTS

Microalgae Culture conditions

The flourishing source of bioactive compounds is possessed by the Marine halophilic microalgae to compete the harmful pathogens. The important aspect to be considered in the development of fermentation technology is the culture media optimization. In large scale production of algal metabolites a wide range of search for optimization of culture conditions is also involved,which was achieved by altering the diverse culture conditions to the microalgae through a systematic study.For marine microalgae Nannochlotopsis occulata , optimum culture conditions relative to temperatures,pH and salinities levels were adopted. The growth characteristic of microalgae is shown in the following figure at various temperatures. Maximum cell growth of microalgae recorded at 25°C and minimum growth 35°C on 9 th day of incubation is shown in Fig.1. Fig.2 depicts the cell growth of microalgae at different pH. On the 9 th day of incubation maximum cell growth was observed at the pH of 9.0 and minimum growth was recorded at the pH of 5.0. Different salinity rates were used for the study of the microalgae cell growth rate such as 20,30 and 40 ppt concentration. Fig.3 presents algal cell growth at various salinity.On the 9 th day of incubation minimum growth was recorded at 20 ppt and maximum cell growth at 40 ppt.The confirmation that the microalgae Nannochloropsis occulata belongs to halophytes was confirmed by these results.Logarithmic increase in cell count of microalgae was observed from the first day to the 8 th day ,the maximum value was observed on the 9 th day of incubation ,after which a gradual decline in almost all the culture conditions was observed.

Antibacterial assay

Microalgae extracts were prepared by paper disk assay method by using different organic solvents for antibacterial assay.Table 1 represents antibacterial activity of crude extract . Fig.4 shows maximum zone of inhibition (10.4 mm) against Vibrio cholerae is exhibited by chloroform + methanol (1:1) extract of Nannochloropsis occulata among the other solvents used

Minimum zone of inhibition (2.0 mm) against Proteus species was shown by Isopropanol solvent extract.In chloroform + methanol (1:1) extract of Nannochloropsis occulata ,the highest inhibition zone was observed against gram negative bacteria Vibrio cholerae (10.4 mm) and gram positive bacteria Staphylococcus aureus (10.0 mm) and Streptococcus pyogens (10.0 mm) and Acetone + chloroform (1:1) extract against Streptococcus pyogens(10.0 mm) respectively Table 2 depicts the execution of two way ANOVA on the data of antibacterial activity of bioactive substance extracted from Nannochloropsis occulata by using different organic solvents and their combinations against selected human pathogens. Variation due to bacterial pathogens P- value was > 0.05 is statistically non- significant while variation due to organic solvent based extracts P- value was<0.05 is statistically significant

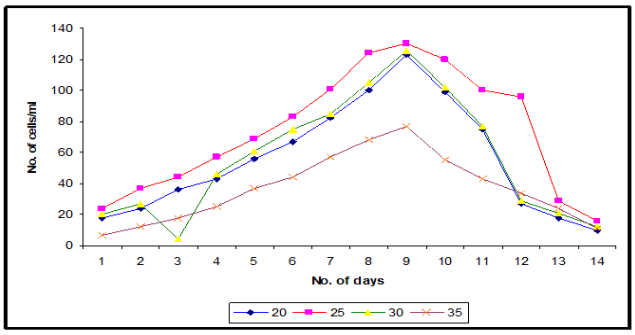


Fig. 1: Growth characterization of *nannochloropsis occulata* at various temperature (°C)

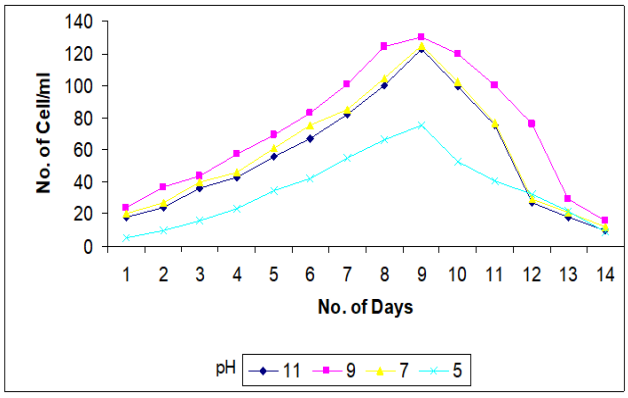


Fig. 2: Growth characterization of *Nannocholoropsis occulata* at various pH

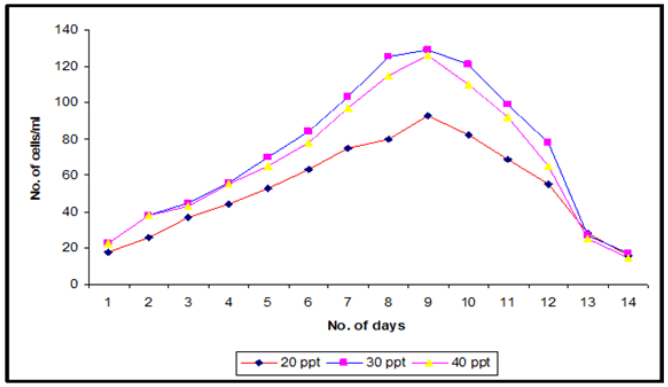


Fig. 3: Growth characterization of Nannocholoropsis occulata at various salinity

Fig. 4: Antimicrobial activity of different solvent extracts of Nannocholoropsis occulata against *Vibrio cholerae*

A- acetone + n-butanol (1:1); B – isopropanol; W - chloroform + methanol; C - control

Table 1: Antimicrobial activity of bioactive substance extracted from N.occulata

Solvent used	Zone of inhibition (mm)									
	Control	*Vibrio cholerae*	*Klebsiella pneumonia*	*Escherichia coli*	*Staphylococcus aureus*	*Bacillus megaterium*	*Pseudomonas aeruginosa*	*Bacillus subtilis*	*Salmonella sp*	*Proteus sp.*	*Streptococcus pyogens*
Acetone	-	5.8 + 0.79	10.5 ± 1.75	10.1 ± 1.70	5.6 + 0.92	4.7 + 0.95	4.8 + 0.85	5.5 + 0.99	11.9± 0.95	11.5 ± 0.80	10.3 ± 0.80
n-butanol	-	11.4 ± 2.19	3.9 + 1.53	9.6 ± 0.74	10.9 ± 2.54	8.9 ± 0.93	10.4 ± 1.44	9.8 ± 2.83	10.8 ± 0.93	11.4 ± 2.24	12.1 ± 1.70
Isopropanol	-	3.6 + 2.64	10.6 ± 2.14	4.9 + 1.83	6.4 + 2.85	10.9 ± 1.73	7.1 + 0.91	10 ± 1.37	12 ± 3.12	4.0 + 0.85	4.6 + 1.73
Acetone + n-butanol (1:1)	-	11.5 ± 0.44	5.4 + 1.72	9.4 ± 1.54	8.8 ± 2.93	10.8 ± 1.63	10.6 ± 1.54	9.9 ± 2.34	8.8 ± 1.45	8.2 ± 1.44	9.8 ± 1.43
Acetone + Isopropanol (1:1)	-	4.85 + 1.81	4.72 + 3.85	10.36 ± 2.70	10.6 ± 4.89	10.4 ± 1.24	6.7 + 2.5	6.5 + 1.43	8.8 + 0.93	11.47 ± 2.86	6.92 + 3.02
Acetone + Chloroform (1:1)	-	6.3 + 1.56	5.4 + 3.54	5.76 + 10+	10.56 ± 1.86	11.6 ± 1.83	10.21 ± 2.48	10 ± 2.34	6.61 + 2.22	7.8 + 3.01	11 ± .70
Butanol + Isopropanol (1:1)	-	10 ±3.7	9.44 ± 2.24	7.8 ± 1.34	11 ± 2.2	6.1 + 1.82	12.2 ± 1.83	10.6 ± 2.2	**13.4 ±** 2.24	9.99 ± 0.94	9.65 ± 1.38
Chloroform + Methanol (1:1)	-	12.14 ± 2.54	11.61 ± 1.4	11.4 ± 1.5	12 ± 2.7	10.6 ± 2.9	12 ± 2.42	9.5 ± 2.44	10.8 ± 2.43	12 ± 1.72	11.0 ± 2.42

"-" No activity ; Each value is the mean ± SD of three individual estimates

Table 2: Two-way ANOVA for the data on antibacterial activity of bioactive substance extracted from *N.OCCULATA* using different organic solvents and their combinations against selected human pathogens

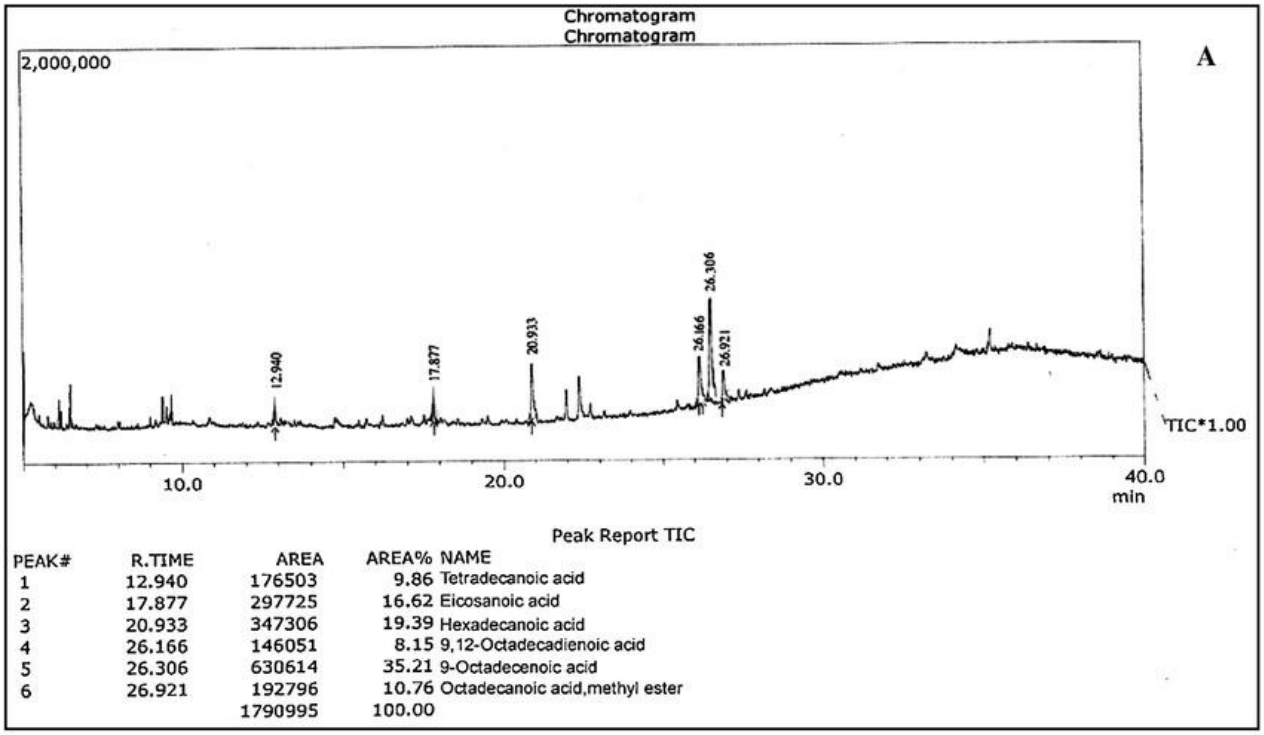
Source of Variation	SS	df	MS	F	P-value
Total difference	504.191	78			
Variation due to bacteria	31.2642	8	4.38262	1.76384	> 0.06*
Variation due to solvent based extracts	142.884	6	19.9706	4.72359	< 0.06**
Error variance	311.058	68	6.09645		

*Statistically non-significant; ** Statistically significant

GC – MS analysis

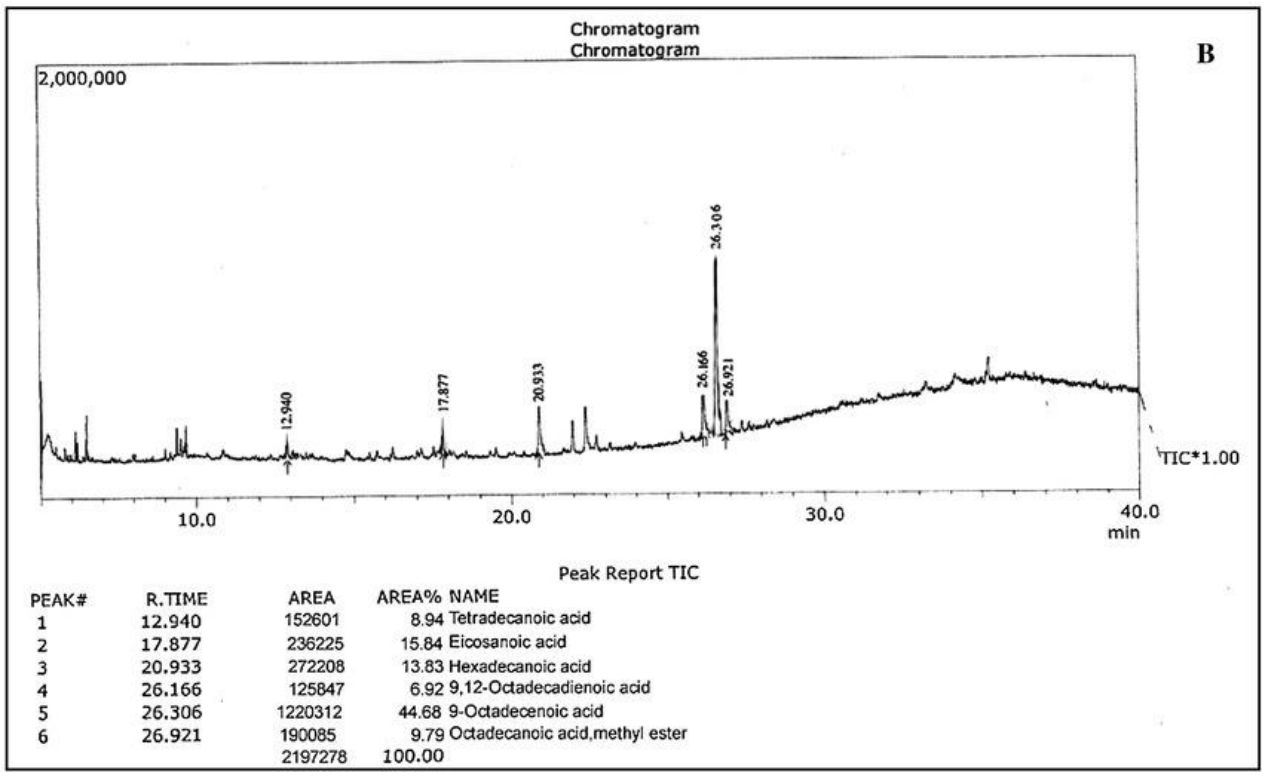
The terrestrial counterpart are not able to produce variety of natural products but it can be produced with Marine microalgae by it's adverse environmental habitat. In field of pharmaceutical and biomedical industries, the current scenario of research is to develop unique compound for identification of marine natural product chemistry. In this research, exceptional opening for the investigation of novel compound for treatment of human diseases from halophilic microalgae is provided. In Fig 5a, present investigation undertaken to discover antibacterial compound from organic solvent extract of Nannochloropsis occulata using GC-MS analysis is illustrated. Table 3 portrays number of compounds (peak) reported in crude extract. In PubChem database, the mass spectra of the compounds are investigated with similar compounds and in pharmacological fields, some of the chemical components reports to have known biomedical value (data not shown). In Fig 5a , Tetradeconic acid and in Fig 5b, 9,12 octadecadienoic acid are the unique chemical compound which is the chief constituent of the crude extract of nannochloropsis occulata. For future research, these secondary metabolites pay for a new avenue to pinpoint the chemical constituents that possess antimicrobial activity.

FIGURE 5a and FIGURE 5b



Peak Report TIC

PEAK#	R.TIME	AREA	AREA%	NAME
1	12.940	176503	9.86	Tetradecanoic acid
2	17.877	297725	16.62	Eicosanoic acid
3	20.933	347306	19.39	Hexadecanoic acid
4	26.166	146051	8.15	9,12-Octadecadienoic acid
5	26.306	630614	35.21	9-Octadecenoic acid
6	26.921	192796	10.76	Octadecanoic acid,methyl ester
		1790995	100.00	



Peak Report TIC

PEAK#	R.TIME	AREA	AREA%	NAME
1	12.940	152601	8.94	Tetradecanoic acid
2	17.877	236225	15.84	Eicosanoic acid
3	20.933	272208	13.83	Hexadecanoic acid
4	26.166	125847	6.92	9,12-Octadecadienoic acid
5	26.306	1220312	44.68	9-Octadecenoic acid
6	26.921	190085	9.79	Octadecanoic acid,methyl ester
		2197278	100.00	

DISCUSSION

Large quantities of algal biomass is required for the production of micro algal bioactive metabolites. By testing the best strains and most effective strategies under optimal conditions, the optimization procedure is done. Researchers and commercial have developed several cultivation technologies that are used for high production of micro algal biomass. In order to get huge quantities of microalgae biomass to meet our demand, in present study an attempt is made to optimize the culture condition. Culture conditions like temperature, pH, salinity and other micronutrients and macronutrients influences the chemical composition of several microalgae. The growth pattern of Nannocholoropsis occulata (Kuwaiti and Australian) cultured at different temperatures achieving growth rates of up to 2.90x10 6 and 2.40x10 6 cell ml-1, respectively was endorsed by the earlier researcher [28, 29, 30, 31]. Abu-Rezq et al. [31] and is well documented. With increase in temperature, the growth pattern in both samples decreased. Rather than high temperatures (32ºC),this indicates that Nannocholoropsis occulata prefers low temperatures (20ºC) .In a closed tubular system, in outdoor culture, Garcia-Gonzalez et al. [32] achieved highest production range of 2 to 4x10 6 cells ml-1 of Nannocholoropsis occulata. At a temperature of 25ºC, with pH of 7.5±0.5 ,they found that the maximum culture performance of Nannocholoropsis occulata, and addition of CO2 gas controls it. Under different experimental temperature, Cifuentes et al [33] studies growth pattern and carotenogenesis in different strains of Nannocholoropsis occulata. They reported that maximum growth rate and carotene production is recorded using a temperature of 20±4oC under a 12:12 (light and dark phases) photoperiod. For highest biomass , at temperature of 20o C, these finding corroborate with our present investigation on Nannocholoropsis occulata and under experimental condition, its secondary metabolites production on 9th day of incubation. On the other hand, under experimental condition on limiting nutrients, maximum cell growth induction is shown as Singh et al. [34] suggest to fix water temperature at 30oC.

At different pH, investigation of growth pattern of microalgae culture media is done. With increase in pH, the algal growth increases. At the pH of 9.0 rather than the pH of 5.0 on 9th day of the experimental period, highest cell growth is observed as Microalgae Nannocholoropsis occulata demonstrated it. For marine microalgae Chlorella sp, maximum growth is observed between a pH of 9 to 9.5 at 7th day incubation as Zhao et al. [35] confirms it. Our present culture optimization study on Nannocholoropsis occulata is accepted and supported on records of earlier researchers.

In present investigation, under experimental condition, with increasing salinity (40 ppt) the growth rate was increased rather than at low salinity (20 ppt) on Nannocholoropsis occulata. During natural season, desired optimal growth of Nannocholoropsis occulata could be achieved along the seashore or close to salt lagoons and salt- producing industries with increased salt concentration, which is found by Dolapsakis et al. [36]. Oren [37] as these results are agreed with earlier researchers. In culture media containing different NaCl concentrations, report of the growth of Nannocholoropsis occulata is observed by Farahat et al. [38]. Hadi et al. [39] that Nannocholoropsis occulata is able to tolerate varying NaCl concentrations, ranging from 0.2% to approximately 35%. In a media containing an extremely wide range of salt concentration from 0.17 M to 4.0 M NaCl, the microalgae is able to grow. When the media amended with 4.0M NaCl on the 18th day of incubation, maximum cell number of Nannocholoropis occulata is recorded as per the report of Raja et al. [39]. However increased accumulation of β-carotene [41] is on favour of 3.5M of NaCl. It was possible to obtain a cell concentration of 0.8x10 6 cells ml-1 when the culture was maintained at a salinity of 18% NaCl w/w with a pH of 8.5 as per conclusion of Leach et al. [42]. In saline lakes, in high densities Nannocholoropsis occulata is a hyper-halotolerant organism is found. By accumulating glycerol to balance osmotic pressure, It adapted to survive in high salinity environments.

Other possible sources had attracted the most attention towards antibacterial metabolites extraction from algae in recent years. Compounds belonging to several chemical classes – including indoles, terpenes, acetogenins, phenols, fatty acids and volatile halogenated hydrocarbons [43, 44] is due to the antimicrobial activity of microalgae. Both algal species is cause for antimicrobial activity and for their extraction, solvents are used[46]. Using various organic solvents, such as acetone, ether and chloroform, methanol [45], the antimicrobial activity of algae extracts assays it. For antimicrobial activity,[49] higher efficiency in extracting compounds is always provided in means of organic solvents. Compounds as cyclocitral, neophytadiene and phytol [47] and several fatty acids are used to explain the antimicrobial activity detected in several pressurized extracts from Nannocholoropsis occulata. Inspite of dichloromethane, petroleum ether and ethyl acetate extracts of Spirulina platensis [48] ,the methanol extract showed more potent antimicrobial activity. Methanolic and chloroform extracts of marine algae Jania rubens had significant antimicrobial activity against gram negative and gram positive bacteria was endorsed on view of Karabay Yavasoglu et al. [50] in present investigation. In Table 1 as shown, promising antibacterial activity against gram negative and gram positive bacteria is shown the combination of methanol with chloroform (1:1) extract where these findings correlated with our present observation. A higher antifungal activity Mhadhebi et al. [50] is shown from chloroform and ethyl acetate extract obtained from marine algae Cystoseira crinita and Cystoseira sedoides,

Interesting compounds with significant antimicrobial activity is demonstrated from GC-MS analysis of crude extract of Nannocholoropsis occulata. The antimicrobial activity and pharmaceutical importance was identified with different chemical constituents such as Tetradecanoic acid ,Eicosanoic acid, hexadecenoic acid,9,12-octadecadienoic acid,9-octadecenoic acid ,octadecanoic acid,methyl ester in present investigation. Several important organic volatile compounds and its derivatives are revealed from crude extract analysis of the described species using gas chromatography-mass spectrometry (GC-MS). Organic solvent extracts of Synechocystis sp. chemically characterized by GC-MS analysis [51] gives different fatty acids and volatile compounds such as phytol, fucosterol, neophytadiene or palmitic, palmitoleic and oleic acids, with antimicrobial activity are identified in earlier research. For the production of several compounds including bio medically important organic metabolites such as heptanal, ethane1,1-diethoxy butanal,3- Methyl-2-(2-Oxopropyl) Furan and octanal, cyanobacteria and green algae is found potentially was identified from the research of Al-Wathnani [53].The valuable therapeutic uses including anti-inflammatory, antipsychotics, antiseptic, antineoplastic, anti-allergic, antipyretic and analgesic effects is demonstrated from 1-ethyl butyl 3hexyl hydroperoxide and methyl heptanate is contained in GC-MS analysis of Tetraselmis suecica crude extract which is noticed from the research of Dooslin Mercy Bai and kousik Saravana [54,55].Leading chemical compounds namely 3, 3, 5-Trimethylheptane and n-Hexadecane has pharmaceutical importance is contained in the fractionated matrices of Nannocholoropsis occulata extract. Significant biomedical features were exhibited from some of our resultant chromatogram compounds interestingly. For current biomedical and pharmaceutical importance, chloroform + methanol (1:1) preferred as the most suitable organic solvent to extract bioactive compounds from marine microalgae, these are obtained based on the result in this study.

CONCLUSION

For bioactive metabolites extraction for possible methods, to get a maximum algal biomass of Nannochloropsis occulata ,the following optimum culturing conditions such as salinity of 40ppt, temperature of 20ºC and a high pH of 9.0 on 9th day of incubation period are maintained. For human therapeutic applications, for the biocidal activity and its clinical trial, regarding exact chemical constituent responsible is required for further research. In future, an interesting new facet of microalgal biotechnology can be opened in the research. For many future microalgal investigations, Nannochloropsis occulata is made for the production of new antibiotic substances and production of biofuels

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