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RESEARCH ARTICLE

EVALUATION AND QUANTITATIVE ANALYSIS OF BIOACTIVE COMPOUNDS FROM CHAETOCEROUS CALCITRANS AGAINST HUMAN PATHOGEN'S

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Abstract

The distribution of secondary metabolites and pharmacologically dynamic complexes from marine microalgae has increased in the previous decade. The antibacterial metabolites lessons in the current search were taught by Chaetoceroscalcitrans.

Methods :Chaetoceroscalcitrans(green algae) was chosen as the subject of the existing secondary metabolites study. The properties of pH, temperature, and salinity were tested to see if they aided in the growth of microalgae. Dissimilar solvent extracts of Chaetoceroscalcitrans were tested for antibacterial activity against *Vibrio cholerae ,Klebsiella pneumoniae, Escherichia coli, Pseudomonas aeruginosa, Salmonella sp.,Proteus sp., Streptococcus pyrogens, Staphylococcus aureus, Bacillus megaterium,* and *Bacillus subtilis*.

Result : The uppermost cell growth was detected during the 9th day of incubation when the medium was adjusted with pH of 9.0 in 30 ppt of salinity at 25C. Butanol + Isopropanol(1:1) crude extract of Chaetoceroscalcitrans had the largest zone of inhibition (13.4 mm) against Salmonella of all the solvents tested. . For the crude extract of Chaetoceroscalcitrans, GC-MS analysis revealed the presence of unique chemical compounds such as 3, 3, 5-Trimethylheptane (M.W. 142.2) and n-Hexadecane (M.W.226.2), respectively.

Conclusion: These results show that the Butanol + Isopropanol (1:1) extract of Chaetoceroscalcitranshas significant antimicrobial activity and thus has great potential as a solvent for extracting bioactive compounds from natural sources for biomedical and pharmaceutical applications.

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Introduction:-

Marine microalgae could be used as suitable leads in the development of new pharmacological medicines [7]. Biologically active compounds derived from natural resources have always piqued the curiosity of disease researchers [8]. Algae has been used in medicine for a long time, and it has bacteriostatic, antibacterial, antifungal, antiviral, and anticancer action [9]. As a rich source of physically unique and physiologically active metabolites, microalgae have been studied as a possible bioactive compound of interest in the pharmaceutical industry [10,11]. This is a diverse variety that is also high in bioactive components such vitamins [12],pigments, fatty acids, sterols, and polysaccharides [13,14]. Resistance has arisen as a result of frequent use of chemotherapeutic medications and a

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delay in effective pathogen treatment, resulting in undesired side effects and perhaps increased mortality [15]. Due to these constraints, researchers discovered a constant demand for new antimicrobial chemicals from previously unknown habitat for the development of innovative treatments for already existing diseases [16].

As a result, for the treatment of human pathogenic microorganisms, the current study focused on the prospective applications of the marine microalgae Chaetoceroscalcitrans, which can be utilised as a substitute for commonly employed dormant chemotherapeutic occulta Chaetoceroscalcitrans, a motile unicellular halotolerant green alga belonging to the Eustigmatophyte and Monodopsidaceae families, is most typically found in salt marshes [17]. Chaetoceroscalcitrans produces a biomolecule of -carotene, which is employed as a colouring agent, antioxidant [19], anti-tumour agent [20], and cardiac disease preventive [21]in the food, cosmetic, and pharmaceutical industries. With various organic solvent extracts of microalgae, a wide range of pharmacologically active substances have been detected. Many bioactive compounds find use as structural models for the development of new drugs in human pathogens. Antimicrobial activity of microalgae extracts is frequently assessed using a variety of organic solvents [22]. In comparison to aqueous extract, organic solvents extract antimicrobial activity more efficiently. Detecting biomedical compounds by screening organic solvent extracts from microalgae and other marine organisms is a common method. To assess the efficacy of a variety of organic solvents in this context, researchers used GC-MS analysis of crude marine microalgae extracts to determine antimicrobial activity and identify chemical constituents and structure against the most common human diseases.

Materials And Method:-

Microalgae Culture Collection

Chaetoceroscalcitrans

Phylum:
Bacillariophyceae

Order:
Incertae Sedis

Family:
Chaetocerotaceae

Genus:
Chaetocerous was collected in a sterile screw cap tube from the Centre for Marine Fisheries Research Institute (CMFRI) Tuticorin, Tamilnadu, India, and transported to our laboratory in an ice chest box. The microalgae were sub-cultured and kept as a pure culture for the current study.

Stock Culture Maintenance

A required nutrient of Miquell's medium (solution-A: Potassium nitrate: 20.2 g;distilled water: 100 ml; solution-B: Sodium orthophosphate: 4g; Calcium chloride: 2g; Ferricchloride: 2g; Hydrochloric acid: 2 ml; distilled water: 100 ml) was dissolved in 100 ml of sea water (filtered). One liter of filtered sterilized seawater was added to solution A (0.55 ml) and solution B (0.5 ml) and meticulously mixed to enrich the water before autoclaving. After sterilization, 10% of the actively increasing mid phase inoculum was transported aseptically into the culture flask. The inoculated flask was incubated for 8 days at 282°C with 1000 lux fluorescent light. The light was reduced for further growth when the extreme exponential growth phase was touched.

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

Most microalgal species grew best at neutral or slightly alkaline pH, with minimum growth at pH 5 and maximum growth at pH 9. The optimum growth was achieved at 25 0 C, which was higher than the other temperatures employed in this study. By raising the medium's temperature, the cell density declined. In most cultures, the optimal

salinity for optimum microalgal development was 30 ppt. By increasing the salinity of the media, the cell density was reduced.

Determination Of Cell Density

James and Al-Khars's approach [25] was used to determine cell density. Cell counts were evaluated using a Neubauer enhanced Hemocytometer (DHC-N01). To kill the cells, the microalgae were treated with formalin, and one drop of the culture was removed using a sterile Pasteur pipette. After placing the cover slip on the hemocytometer, the pipetted culture samples were placed on the counting grid and left for a few minutes. The cells were counted under a 40X magnification using a compound microscope (ADELTA OPTEC –DN10), and the total cell count was computed using the formula below.

Total cell count Equals counted cells multiplied by the total number of squares in a group and the total number of squares tallied

Microalgae Extract Preparation Using Different Organic Solvents

Microalgae cells were centrifuged for 10 minutes at 200 rpm (REMI- R24). The pellet was collected at room temperature and air dried to produce a fine powder. 100 ml of dried microalgae cells weighing 10g were extracted separately in different organic solvents, including Acetone+n-butanol (1:1), Acetone + Isopropanol (1:1), Acetone +chloroform(1:1), Butanol + Isopropanol (1:1), Chloroform + Methanol (1:1), Chloroform + Methanol(1:1), Chloroform + Methanol (1 Whatman The solution filtered No. 1 filter paper. The filtrate was desiccated for 24 hours at 40°C. The dried powder's respective solvents were suspended to yield 50 mg/ml crude extract. The crude extract was preserved in a sealed container and kept in the refrigerator for additional antibacterial and GC-MS tests.

Pathogenic Bacteria Used As Test Organism

Due to their common occurrence in most clinical cases, antimicrobial activity of these extracts was investigated against ten human pathogens: Vibrio cholerae, Klebsiella pneumoniae, Escherichia coli, Staphylococcus aureus, Bacillus megaterium, Pseudomonas aeruginosa, Bacillus subtilis, Salmonella sp., Proteus sp., and Streptococcus

Antibacterial Assay

El Maseru et al. described a paper disc assay method for determining antibacterial activity against selected human pathogens. The 6mm diameter Whatman No. 1 filter paper disc was incised and disinfected by autoclaving. The sterile disks were saturated using several solvent extracts. The control disc was also maintained by impregnating each extract with its own organic solvent. Overnight broth cultures of test pathogens were inoculated uniformly using sterile cotton swabs, and Muller Hinton Agar plates were made. The plates were positioned by the impregnated discs using sterile forceps. Equal distances are suitably spaced. Triplicates of each test pathogen were kept on hand.The plates were incubated at 37°C for 24 hours before being used. The inhibitory zone was measured in millimeters and expressed in diameter.

GC-MS Analysis Of Microalgae Extract

Even with trace amounts of ingredients with high sensitivity, gas chromatography paired with mass spectrometry detection technology may detect qualitative and quantitative analysis of crude extracts with high sensitivity. Antibacterial activity against various human illnesses were also tested using the chemical moiety of crude extracts of Chaetoceroscalcitrans. Dissolving 10 mg of crude extracts in one milliliter of ethyl acetate is the typical procedure for GC-MS analysis. The standard phase is a 0.11 aliquot injected automatically into a 0.25 mm 25 mm column of 5 percent phenyl poly siloxane in a GC-MS model (GC 17A, Japan). Helium was employed as the carrier gas, with a pressure of 17.69 psi and a flow rate of 3 ml/min at a flow rate of 0.4m/min.

Compound concentration percentage=$[P1/P2] \times 100$, P1 being the peak area of the compound and P2, whole peak areas in the fractionated extracts.

Data Analysis

The data were statistically analyzed using Two-way ANOVA with MINITAB software, and the means for individual parameters were separated using the least significant difference (LSD) test at the 0.05 percent level of probability to determine their significance status [27].

Result:-

Microalgae Culture Conditions

The pH, temperature, and salinity of microalgal cultures were varied in Miquell's medium. The microalgal inoculum was cultivated at pH 5, 7, 9, and 11 to maximize the pH. Similarly, the optimum temperature was identified at 20, 25, 30, and 35 degrees Celsius. The optimum salinity was determined using 20, 30, and 40 ppt. The cell density was employed as the criterion in every case (James and Al-Khars, 1990).

Figure 1:- Growth characterization of Chaetoceroscalcitrans at various Ph.

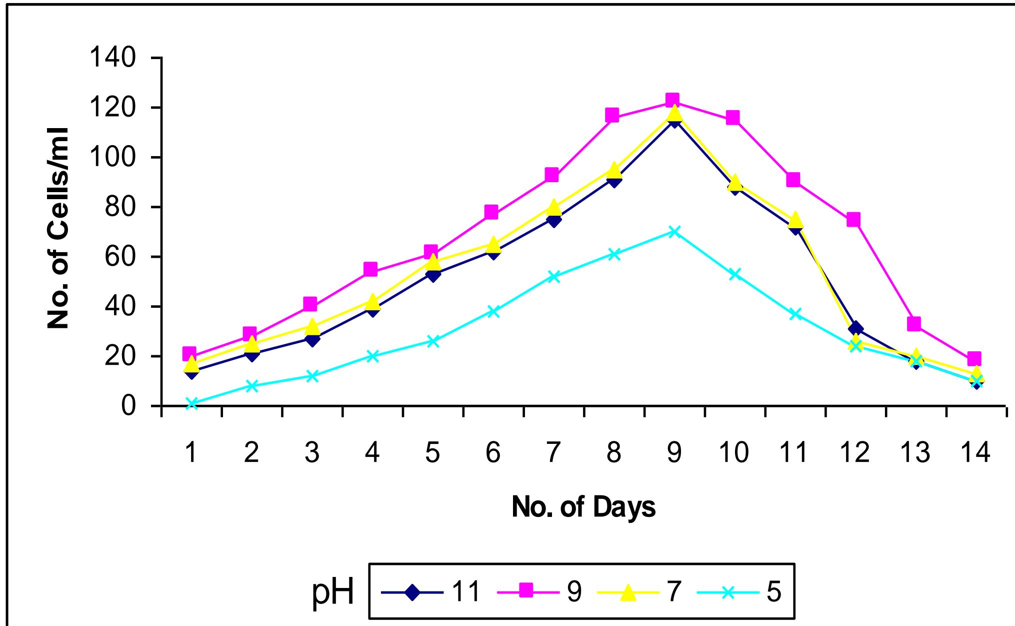


Figure 2:- Growth characterization of Chaetoceroscalcitrans at various temperature (°C).

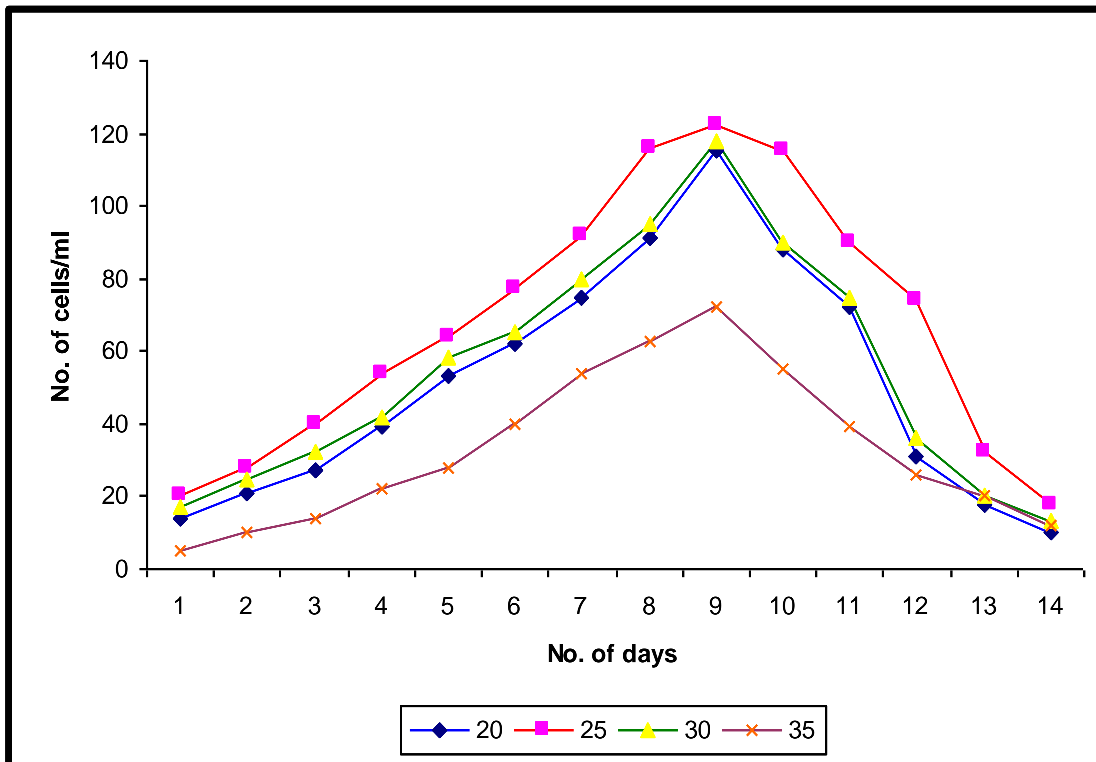
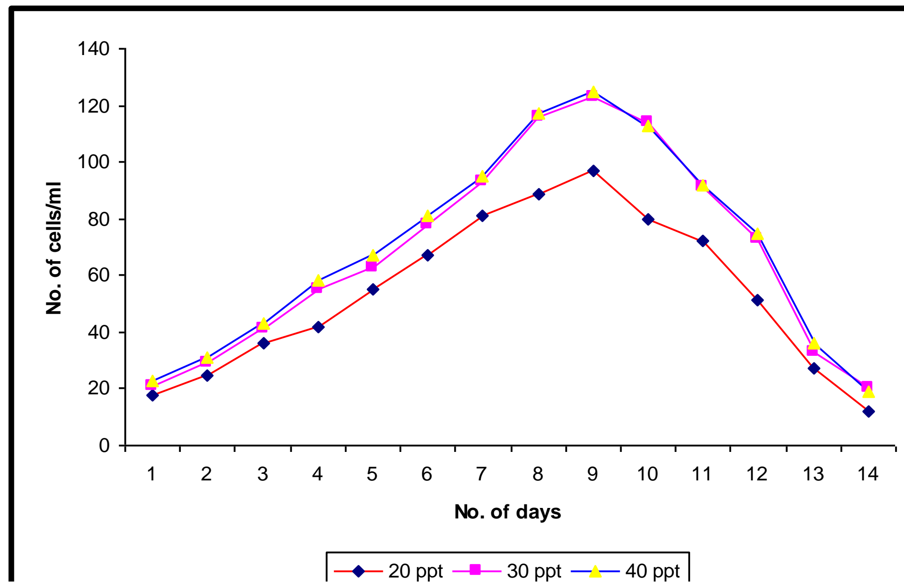


Figure 3:- Growth characterization of Chaetoceroscalcitrans at various salinity(ppt).



Antibacterial Assay

In 1986, Rao et al used a sterile Whatman No. 1 filter paper disc with a diameter of 5 mm that was impregnated with 10 different algal extracts. To make a lawn, cotton swabs were infected on Muller Hinton agar plates with individual test pathogens. Filter paper discs coated with algal extract were placed at a regular distance on the agar surface. In order to generate an antibacterial zone, all plates were incubated in an incubator for 24 hours at 37 degrees Celsius (Gonalaz et al., 2001).

Antimicrobial Activity Of Algal Cultures

Presence of the acetone is thus higher in proteus sp , Presence of the n-butanol alage is thus higher in pseudomonas aeruginosa ,Presence of the isopropanol is thus higher in E-coli, Presence of the acetone + n-butanol (1:1) is thus higher in bacillus subtilis , Presence of the acetone+isopropanol (1:1) is thus higher in vibriocholrae,presence of acetone+ chloroform(1:) is higher in pseudomonas aeruginosa, Presence of the butanol + isopropanol is thus higher in salmonella sp , Presence of the choloroform + methanol (1:1) is thus higher in vibrio cholerae,

It shows the highest results in presence of chloroform + methanol (1:1) is thus higher in vibrio cholerae against the human pathogens and the leastest results shows in the isopropanol is present in the bacillus megaterium shown in Figure 3

Antimicrobial activity of different solvent extracts of Chaetoceros calcitrans against Vibriocholerae

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

Figure 4:-

Table 1:- Two-way ANOVA for the data on antibacterial activity of bioactive substance extracted from Chaetoceroscalcitrans using different organic solvents and their combinations against selected human pathogens

Source of Variation	SS	df	MS	F	P-value
Total variance	69.9849	79			
Variation due to bacteria	7.01612	9	0.77957	1.08896	> 0.05*
Variation due to solvent based extracts	17.8679	7	2.55255	3.56558	< 0.05**
Error variance	45.1009	63	0.71589		

* Statistically non-significant ; ** Statistically significant

Table 2:- Two-way ANOVA test carried out for the data on antimicrobial activities of algal associates against human pathogen at pH 5.

Source of Variation	SS	df	MS	F	P-value
Total variance	331.954	59			
Variation due to organisms	108.962	11	9.90564	2.89957	> 0.05*
Variation due to algal associates	72.6773	4	18.1693	5.31851	< 0.05**
Error variance	150.315	44	3.41624		

* Statistically non-significant ; ** Statistically significant

Table 3:- Two-way ANOVA test carried out for the data on antimicrobial activities of algal associates against human pathogen at pH 7.

Source of Variation	SS	df	MS	F	P-value
Total variance	248.919	59			
Variation due to organisms	35.2873	11	3.20794	3.10564	< 0.05**
Variation due to algal associates	168.183	4	42.0457	40.7049	< 0.01**
Error variance	45.4493	44	1.03294		

** Statistically significant

Table 4:-Two-way ANOVA test carried out for the data on antimicrobial activities of algal associates against human pathogen at pH 9.

Source of Variation	SS	df	MS	F	P-value
Total variance	237.65	59			
Variation due to organisms	34.1698	11	3.10635	2.57258	> 0.05*
Variation due to algal associates	150.351	4	37.5877	31.1289	< 0.01**
Error variance	53.1293	44	1.20748		

* Statistically non-significant ; ** Statistically significant

Table 5:- Two-way ANOVA test carried out for the data on antimicrobial activities of algal associates against human pathogen at temperature 25^0C.

Source of Variation	SS	df	MS	F	P-value
Total variance	143.3458	49			
Variation due to organisms	12.7738	9	1.419311	0.462579	> 0.05*
Variation due to algal associates	20.1148	4	5.0287	1.638944	> 0.05*
Error variance	110.4572	36	3.068256		

* Statistically non-significant

Table 6:- Two-way ANOVA test carried out for the data on antimicrobial activities of algal associates against human pathogen at temperature 30^0C.

Source of Variation	SS	df	MS	F	P-value
Total variance	144.895	49			
Variation due to organisms	11.3272	9	1.25858	0.4095	> 0.05*
Variation due to algal associates	22.9232	4	5.7308	1.8646	> 0.05*
Error variance	110.645	36	3.07347		

* Statistically non-significant

Table 7:- Two-way ANOVA test carried out for the data on antimicrobial activities of algal associates against human pathogen at temperature 35^{0}C.

Source of Variation	SS	df	MS	F	P-value
Total variance	137.493	49			
Variation due to organisms	15.0528	9	1.67253	0.59879	> 0.05*
Variation due to algal associates	21.8848	4	5.4712	1.95876	> 0.05*
Error variance	100.555	36	2.7932		

* Statistically non-significant

Table 8:- Antimicrobial Activity Of Bioactive Substances Extracted From Chaetocerous Calcitrans.

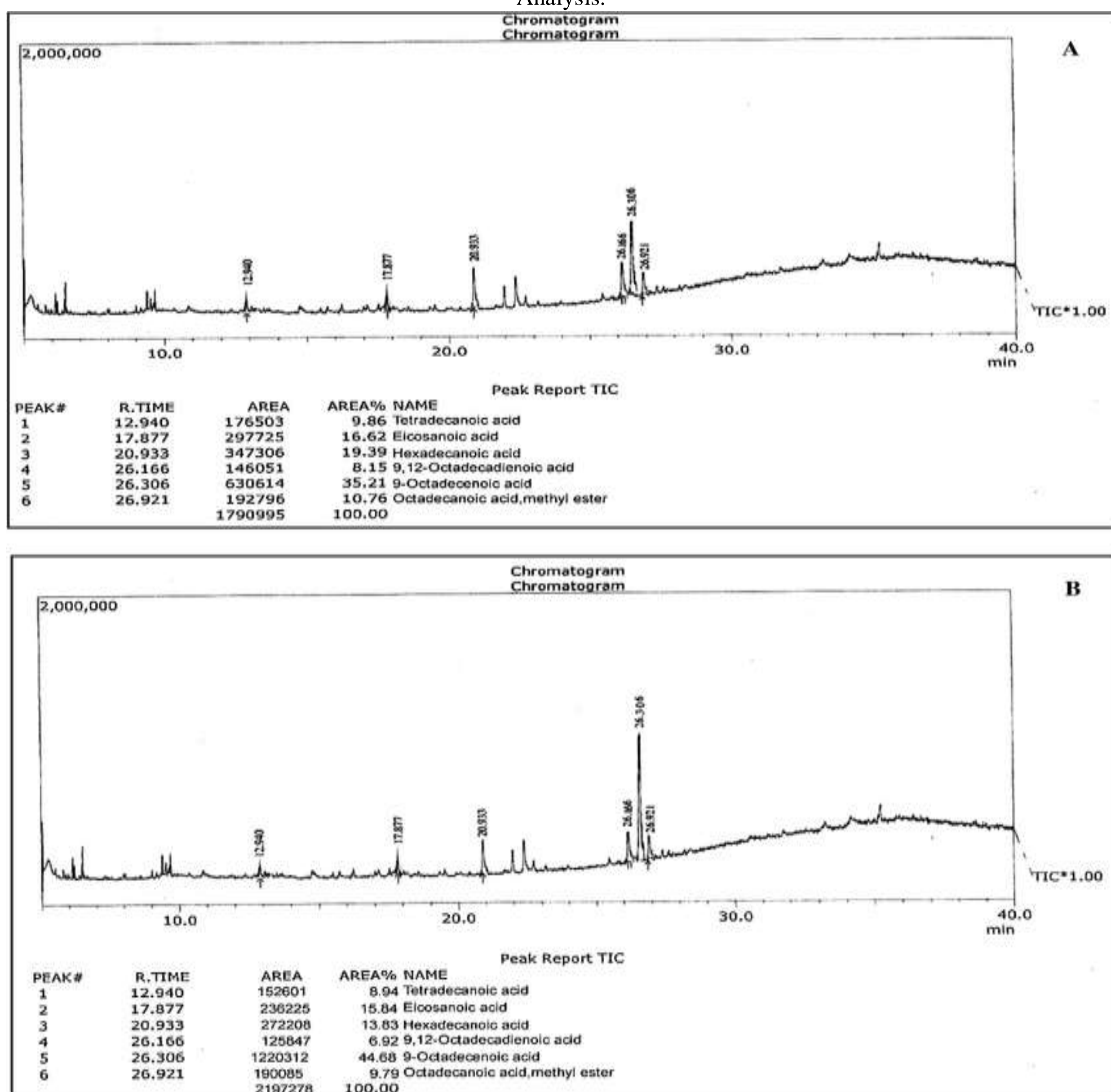
Solvent used	Zone of inhibition (mm)										
	Control	Vibrio cholerae	Klebsiella pneumoniae	Escherichia coli	Staphylococcus aureus	Bacillus megaterium	Pseudomonas aeruginosa	Bacillus subtilis	Salmonella sp.	Proteus sp.	Streptococcus pyogens
Acetone	*	7.4 ± 0.54	9 ± 0.70	8.8 ± 0.83	7.2 ± 0.44	6.8 + 0.66	9 ± 0.70	7.5 + 0.88	9.6 ± 0.54	10.6 ± 0.89	7.7 + 0.79
n-butanol	*	8.8 ± 0.83	8.8 ± 0.83	9.2 ± 0.83	9.8 ± 0.83	10 ± 0.70	10 ± 0.70	8.2 ± 1.30	8.2 ± 0.83	8.6 ± 1.14	8.6 ± 0.54
Isopropanol	*	8.8 ± 1.09	8.2 ± 0.44	9.8 ± 0.83	7.4 ± 0.54	5.8 + 0.95	9.6 ± 1.14	9.4 ± 1.14	7.8 ± 0.83	8.4 ± 0.54	7.6 ± 0.54
Acetone + n-butanol (1:1)	*	10.4 ± 0.54	8.8 ± 0.44	9.6 ± 0.54	8.8 ± 0.44	9 ± 1.58	8.8 ± 0.83	10 ± 0.70	9 ± 0.70	8.6 ± 1.51	9 ± 0.70
Acetone + Isopropanol (1:1)	*	10.6 ± 1.14	10.4 ± 0.54	8.6 ± 0.54	10.2 ± 0.83	9.6 ± 1.14	8.8 ± 1.09	9.2 ± 0.83	9.6 ± 0.54	8.8 ± 0.83	9 ± 0.70
Acetone + Chloroform (1:1)	*	9.8 ± 1.30	8.6 ± 0.54	8.4 ± 0.54	7.6 ± 0.54	9 ± 0.70	10 ± 0.70	8.6 ± 1.14	9 ± 0	8.4 ± 0.54	7.5 + 0.81
Butanol + Isopropanol (1:1)	*	9.2 ± 0.83	8.8 ± 0.83	9.2 ± 0.83	8.2 ± 0.83	8.6 ± 0.54	9 ± 0.70	8.4 ± 0.54	10.2 ± 0.44	9.8 ± 0.83	9.4 ± 0.54
Chloroform + Methanol (1:1)	*	10.8 ± 1.30	9.8 ± 0.83	10 ± 1.22	10 ± 0.70	9.4 ± 0.89	8.2 ± 0.83	10 ± 0.70	9.6 ± 0.89	9.6 ± 0.54	9.6 ± 1.14

GC – MS Analysis

Identification of biochemical markers in the biomedicines is very important. Therefore, it is essential to find out the various constituents present and their chemical structure of these substances from marine source. The terrestrial counterpart is not able to produce variety of natural products but it can be produced with Marine microalgae by its 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.

Biochemical markers need to be identified in biomedicines. As a result, it is critical to determine the various constituents present as well as their chemical structures in marine sources. The terrestrial counterpart is unable to produce a wide range of natural products, whereas marine microalgae can do so due to its harsh environment. The current research scenario in the pharmaceutical and biomedical industries is to develop a unique compound for identifying marine natural product chemistry. This research provides a unique opportunity to investigate novel compounds derived from halophilic microalgae for the treatment of human diseases. The current study, which used GC-MS analysis to discover antibacterial compounds from an organic solvent (chloroform + methanol (1:1))extract of Chaetoceroscalcitrans, is shown in Fig 5 . The number of compounds (peak) reported in crude extract is shown in Table 3. The mass spectra of compounds are compared to those of similar compounds in the PubChem database, and some chemical components are reported to have known biomedical value in pharmacological fields (data not shown). Tetradeconic acid and 9,12 octadecadienoic acid are the two chemical compounds that make up the crude extract of Chaetoceroscalcitrans in Figure 5, respectively. These secondary metabolites provide a new avenue for future research into the chemical constituents that have antimicrobial activity.

Figure 5:- Detection Of Mixed Secondary Metabolities Produced By Chaetoceros Calcitrans Using Gc-Ms Analysis.



Discussion:-

The production of microalgal bioactive metabolites necessitates a large amount of algal biomass. The optimization procedure is completed by testing the best strains and most effective strategies under ideal conditions. Several cultivation technologies for high production of microalgal biomass have been developed by researchers and businesses. To meet our demand for large amounts of microalgae biomass, the current study aims to optimise the culture conditions. The chemical composition of several microalgae is influenced by culture conditions such as temperature, pH, salinity, and other micronutrients and macronutrients. The earlier researcher [28, 29, 30, 31] endorsed the growth pattern of Chaetoceroscalcitrans (Kuwaiti and Australian) cultured at different temperatures, achieving growth rates of up to 2.90x10 6 and 2.40x10 6 cell ml-1, respectively. [31] and is well documented by Abu-Rezqetal. The growth pattern in both samples decreased as the temperature dose. This indicates that Chaetoceroscalcitrans prefers low temperatures (20ºC) over high temperatures (32ºC). Garcia-Gonzalez et al. [32] achieved the highest production range of Chaetoceroscalcitrans cells in a closed tubular system in outdoor culture, ranging from 2 to 4x10 6 cells ml-1.They discovered that the maximum culture performance of Chaetoceroscalcitrans occurs at a temperature of 25oC and a pH of 7.50.5, and that the addition of CO2 gas controls it. Cifuentes et al [33] investigate the growth pattern and carotenogenesis of Chaetoceroscalcitrans strains at various experimental temperatures. They found that a temperature of 20±4oC and a 12:12 (light and dark phases) photoperiod produced the highest growth rate and carotene production. These findings corroborate our current investigation on Chaetoceroscalcitrans and the production of secondary metabolites on the 9th day of incubation under experimental conditions at a temperature of 20o C. On the other hand, maximum cell growth induction is seen under experimental conditions of limiting nutrients, as Singh et al. [34] suggest that water temperature be set at 30oC. The growth pattern of microalgae culture media is investigated at various pH levels. The growth of algae increases as the pH rises. Microalgae Chaetoceroscalcitrans demonstrated that on the 9th day of the experimental period, when the pH was 9.0 rather than 5.0, the highest cell growth was observed. According to Zhao et al. [35], maximum growth is observed for the marine microalgae Chlorella sp. at a pH of 9 to 9.5 on the 7th day of incubation. Our current Chaetoceroscalcitrans culture optimization study has been accepted and is backed up by previous research.

The growth rate of Chaetoceroscalcitrans was increased under experimental conditions with increasing salinity (40 ppt) rather than at low salinity (20 ppt). Dolapsakis et al [36] discovered that during the natural season, optimal growth of Chaetoceroscalcitrans could be achieved along the seashore or close to salt lagoons and salt-producing industries with increased salt concentration. Oren [37], whose findings are in line with those of previous researchers. Farahat et al. [38] observed the growth of Chaetoceroscalcitrans in culture media containing various NaCl concentrations. Chaetoceroscalcitrans can tolerate NaCl concentrations ranging from 0.2 percent to approximately 35 percent, according to Hadi et al. [39]. The microalgae can grow in a media with a wide range of salt concentrations, ranging from 0.17 M to 4.0 M NaCl. According to Raja et al. [39], the maximum cell number of Nannocholoropisocculata was recorded when the media was amended with 4.0M NaCl on the 18th day of incubation. However, increased -carotene accumulation [41] is in favour of 3.5M NaCl. According to Leach et al. [42], a cell concentration of 0.8x10 6 cells ml-1 could be obtained when the culture was kept at a salinity of 18 percent NaCl w/w and a pH of 8.5. Chaetoceroscalcitrans is a hyper-halotolerant organism found in saline lakes at high densities.

In recent years, antibacterial metabolites extraction from algae has drawn the most attention from other possible sources. The antimicrobial activity of microalgae produces compounds from a variety of chemical classes, including indoles, terpenes, acetogenins, phenols, fatty acids, and volatile halogenated hydrocarbons [43, 44]. Both algal species have antimicrobial activity, and solvents are used to extract them [46]. The antimicrobial activity of algae extracts was tested using various organic solvents such as acetone, ether, and chloroform, as well as methanol [45]. Organic solvents always provide a higher efficiency in extracting compounds for antimicrobial activity [49]. Several fatty acids, as well as compounds like cyclocitral, neophytadiene, and phytol [47], are used to explain the antimicrobial activity found in several pressurised extracts from Chaetoceroscalcitrans. Despite the antimicrobial activity of dichloromethane, petroleum ether, and ethyl acetate extracts of Spirulina platensis [48], the methanol extract was found to be more effective. According to KarabayYavasoglu et al. [50], methanolic and chloroform extracts of the marine algae Janiarubens had significant antimicrobial activity against gram negative and gram positive bacteria. Table 8 shows that the combination of methanol and chloroform (1:1) extract has promising antibacterial activity against gramme negative and gramme positive bacteria, which correlates with our current observation. Mhadhebi et al. [50] show that chloroform and ethyl acetate extracts obtained from the marine algae Cystoseiracrinita and Cystoseirasedoides have higher antifungal activity. GC-MS analysis of crude extract of

Chaetoceroscalcitrans reveals interesting compounds with significant antimicrobial activity.In this study, different chemical constituents such as Tetradecanoic acid, Eicosanoic acid, hexadecenoic acid, 9,12-octadecadienoic acid, 9-octadecenoic acid, octadecanoic acid, methyl ester were identified as having antimicrobial activity and pharmaceutical importance. Using gas chromatography-mass spectrometry, crude extract analysis of the described species reveals several important organic volatile compounds and their derivatives (GC-MS).Synechocystis sp. organic solvent extracts chemically characterised by GC-MS analysis [51] yield different fatty acids and volatile compounds with antimicrobial activity, such as phytol, fucosterol, neophytadiene, or palmitic,palmitoleic, and oleic acids. Several compounds, including biomedically important organic metabolites such as heptanal, ethane1,1-diethoxy butanal, 3- Methyl-2-(2-Oxopropyl) Furan, and octanal, are produced by cyanobacteria and green algae,according to Al-research Wathnani's [53].

The anti-inflammatory, antipsychotic, antiseptic, antineoplastic, anti-allergic, antipyretic, and analgesic effects of 1-ethyl butyl 3hexyl hydroperoxide and methyl heptanate are found in GC-MS analysis of Tetraselmissuecica crude extract, according to Dooslin Mercy Bai and S.Kousiksaravana's research [54,55]. The fractionated matrices of Chaetoceroscalcitrans extract contain leading chemical compounds such as 3, 3, 5-Trimethylheptane and n-Hexadecane, both of which have pharmaceutical significance. Interestingly, some of our resultant chromatogram compounds exhibited significant biomedical features. Based on the results of this study, chloroform + methanol (1:1) is preferred as the most suitable organic solvent for extracting bioactive compounds from marine microalgae for current biomedical and pharmaceutical importance.

Conclusion:-

The following optimum culturing conditions are maintained for bioactive metabolites extraction for possible methods, to get a maximum algal biomass of salinity 40ppt, temperature 20oC, and pH 9.0 on the 9th day of incubation period. For human therapeutic applications, biocidal activity, and clinical trials, more research into the exact chemical constituent responsible is required. In the future, researchers may be able to explore a fascinating new aspect of microalgal biotechnology. Chaetoceroscalcitrans will be used in a variety of future microalgal studies, including the development of new antibiotics and the production of biofuels.

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