Journal Homepage: - www.journalijar.com

INTERNATIONAL JOURNAL OF ADVANCED RESEARCH (IJAR)

Article DOI: 10.21474/IJAR01/18483
DOI URL: http://dx.doi.org/10.21474/IJAR01/18483

RESEARCH ARTICLE

STANDARDISATION OF BIOACTIVE MATERIALS FROM CHROMULINA FREIBERGENSISAGAINST HUMAN PATHOGENS USING GC-MS ANALYSIS

Dr. V. Dooslin Mercy Bai, S. Kousik Saravana, S. Sabarish, S. Nabha Sindu, Shanmugapriya, Godwinjose and A.D Harshini

Department of Biomedical Engineering, Sri Shakthi Institute of Engineering and Technology, Coimbatore - 641062.

Manuscript Info

Manuscript History
Received: 28 January 2024
Final Accepted: 29 February 2024
Published: March 2024

Key words:-
Microalgae, Secondary Metabolites, Pharmaceutical, GC-MS Analysis

Abstract

In the preceding ten years, there has been a surge in the distribution of secondary metabolites and pharmacologically dynamic compounds from marine microalgae. Chromulinafreibergensis provided the antibacterial metabolites lessons for the current research .The current secondary metabolites study was chosen to focus on the green algal Chromulinafreibergensis. To see whether the properties of pH, temperature, and salinity on the development of microalgae aided in the growth of microalgae, they were tested. Antibacterial activity against Vibrio cholerae, Klebsiella pneumonia, Escherichia coli, Pseudomonas aeruginosa, Salmonella sp., Proteus sp., Streptococcus pyrogens, Staphylococcus aureus, Bacillus megaterium, and Bacillus subtilis was tested for in different solvent extracts of Chromulinafreibergensis.On the ninth day of incubation, when the medium was adjusted to a pH of 9.0 in 30 ppt of salinity at 250C, the uppermost cell growth was noticed. Of all the investigated solvents, the butanol + isopropanol (1:1) crude extract of Chromulinafreibergensis tested the highest zone of inhibition (13.4 mm) against Salmonella. . The presence of rare chemical substances like 3, 3, 5-Trimethylheptane (M.W. 142.2) and n-Hexadecane (M.W. 226.2) in the crude extract of Chromulinafreibergensis was discovered using GC-MS analysis.These results demonstrated the strong antibacterial activity of the Chromulinafreibergensis Butanol + Isopropanol (1:1) extract, demonstrating its enormous potential as a solvent for the extraction of bioactive chemicals from natural sources for biomedical and pharmaceutical applications.

Introduction:-

Marine microalgae might make good leads for the development of novel pharmalogical medications [7]. Researchers who study diseases have always been intrigued by biologically active substances that are obtained from natural resources [8]. Long used in medicine, algae has been shown to have bacteriostatic, antibacterial, antifungal, antiviral, and anticancer properties [9]. Microalgae have been studied as a potential bioactive chemical of interest in the pharmaceutical industry because they are a rich source of physically distinctive and physiologically active metabolites [10,11]. This variety is varied and rich in bioactive substances, including vitamins [12], pigments, fatty acids, sterols, and polysaccharides [13,14]. Due to repeated use of chemotherapeutic drugs and a delay in the

Corresponding Author:- Dr. V. Dooslin Mercy Bai
Address:- Department of Biomedical Engineering, Sri Shakthi Institute of Engineering and Technology, Coimbatore - 641062.

successful treatment of the disease, resistance has developed, possibly leading to increased mortality [15]. Due to these limitations, researchers found a continuing need for fresh antimicrobial chemicals from the previously undiscovered environment for the creation of cutting-edge cures for diseases that already exist [16].

Therefore, the current study concentrated on the potential applications of the marine microalgae Chromulinafreibergensis for the treatment of human pathogenic microorganisms, which can be used as a replacement for commonly employed dormant chemotherapeutic .Chromulinafreibergensis is a motile unicellular halotolerant green algae belonging to the Eustigmatophyte and Monodopsidaceae families, which is found mostly in salt marshes[17]. A biomolecule of -carotene is produced by Chromulinafreibergensis, and it is used in the culinary, cosmetic, and pharmaceutical industries as a coloring agent, antioxidant [19], anti-tumor agent [20], and preventative for heart disease [21]. Several organic solvent extracts of microalgae have been found to include a variety of pharmacologically active compounds. Numerous bioactive substances are used as structural models for the creation of novel medications against human infections. Various organic solvents are often used to test the antimicrobial properties of microalgae extracts [22]. Organic solvents extract antibacterial activity more effectively than aqueous extract. Organic solvent extracts from microalgae and other marine organisms are frequently screened for biological chemicals. Researchers employed GC-MS analysis of crude marine microalgae extracts to evaluate the effectiveness of various organic solvents in this context, identifying chemical components and structures against the most prevalent human diseases.

Materials and Method:-

Microalgae Culture Collection

Chromulinafreibergensis

CLADE: SAR
PHYLUM: OCHROPHYTA
CLASS:CHRYSOPHYCEA
ORDER: CHROMULINALES
FAMILY: CHROMULINACEAE
GENUS: CHROMULINA

was brought to our lab in an ice chest box after being collected in a sterile screw cap tube from the Centre for Marine Fisheries Research Institute (CMFRI) Tuticorin, Tamilnadu, India. For the current study, the microalgae were sub-cultured and maintained as a pure culture.

Stock Culture Maintenance

Miquell'smedium's essential nutrient (solution-A: 100 ml of seawater was mixed with 20.2 g of potassium nitrate, 100 ml of distilled water, 4 g each of sodium orthophosphate, calcium chloride, ferric chloride, and hydrochloric acid, and 2 ml each of hydrochloric acid and hydrochloric acid (filtered). Solution A (0.55 ml) and Solution B (0.5 ml) were properly combined to enrich the water before being poured into one literof filtered, sterilized saltwater. 10% of the actively growing mid-phase inoculum was transferred aseptically into the culture flask after sterilization. The inoculation flask was kept at 282°C under 1000 lux of fluorescent light for 8 days of incubation. When the extreme exponential growth phase was touched, the light was dimmed to prevent further expansion.

Chemicals

Hi Media Laboratories Private Limited (Mumbai, India) provided all of the chemicals and media components for all microbiological and analytical prodecures

Growth Optimization Of Marine Microalgae

Maximum and minimum growth rates for most microalgal species were at pH 5 and pH 9 and they grow best at neutral pH or slightly alkaline pH. At 25°C, which was higher than the other temperatures used in this study, the best growth was accomplished. The cell density decreased as the medium's temperature increased. The majority of cultures required a salinity of 30 ppt for the best possible development of microalgae. The media's salinity was increased, which decreased the cell density.

Determination Of Cell Density

To calculate cell density, The James and Al-Kharsapproach[25] was applied. With the help of a Neubauer-enhanced hemocytometer, cell counts were assessed (DHC-N01). The microalgae were formalin-treated to kill the cells, and a drop of the culture was taken out using a sterile Pasteur pipette. The pipetted culture samples were placed on the

counting grid and left for a short period after the coverslip was secured to the hemocytometer. A compound microscope (ADELTA OPTEC -DN10) was used to count the cells, and the formula below was used to calculate the total number of cells.

The count of all cells equals the sum of the squares in each group multiplied by the number of squares that were counted in each cell.

Microalgae Extract Preparation Using Different Organic Solvents

Microalgae cells were centrifuged for 10 minutes at 200 rpm (REMI- R24) (REMI- R24). The pellet was collected at room temperature and ground into a fine powder by air drying. The solution was filtered through No. 1 filter paper, the filtrate was desiccated for 24 hours at 40°C, and the solvents from the dried powder were suspended to produce 50 ml. That is, 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 powders respective solvents were suspended to yield 50 mg/ml crude extract. To conduct additional bactericidal and GC-MS testing, the crude extract was maintained in a sealed container and kept in the refrigerator.

Pathogenic Bacteria Used As Test Organism

The antimicrobial activity of these extracts was examined against ten human pathogens due to their frequent occurrence in the majority of clinical cases: Vibrio cholerae, Klebsiella pneumoniae, Escherichia coli, Staphylococcus aureus, Bacillus megaterium, Pseudomonas aeruginosa, Bacillus subtilis, Salmonella sp., Proteus sp., and Streptococcus

Antibacterial Assay

A paper disc assay method was described by El Maseru et al. for assessing antibacterial efficacy against particular human infections. The 6mm Whatman No. 1 filter paper disc was cut, then sterilized in an autoclave. Several solvent extracts were used to fully saturate the sterile discs. Additionally, the control disc was kept in working order by soaking each extract in its respective organic solvent. Using sterile cotton brushes, test pathogen overnight broth cultures were uniformly infected, and Muller Hinton Agar plates were created. Using sterile forceps, the impregnated discs were used to position the plates. Suitably spaced equal intervals are used. Each test pathogen was maintained on hand in three copies. Before usage, the plates were incubated for 24 hours at 37°C. The diameter of the inhibitory zone was given in millimeters.

GC-MS Analysis Of Microalgae Extract

Gas chromatography combined with mass spectrometry detection technology can detect qualitative and quantitative analyses of crude extracts with great sensitivity, even with tiny levels of components. The chemical component of crude extracts of Chromulinafreibergensis was also investigated for its antibacterial effectiveness against a variety of human ailments. The standard method for GC-MS analysis involves dissolving 10 mg of crude extracts in 1 milliliter of ethyl acetate. In a GC-MS setup, a 0.1l aliquot of the standard phase is automatically injected into a 0.25 mm by 25 mm column of 5 percent phenyl poly siloxane (GC 17A, Japan). As the carrier gas, helium was used at a pressure of 17.69 psi and a flow rate of 3 ml/min at 0.4 m/min.

Compound concentration percentage=[P1/P2] ×100, P1 is the compound peak area, and P2 is the total peak area in the fractionated extracts.

Data Analysis

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

Result:-

Microalgae Culture Conditions

Microalgal cultures were grown in a variety of pH, temperature, and salinity conditions using Miquell's medium. To enhance pH, the microalgal inoculum was grown at pH 5, 7, 9, and 11. The ideal temperature was determined to be

between 20 and 35 degrees Celsius. Using 20, 30, and 40 ppt, the ideal salinity was identified. Every time, the cell density was used as the standard (James and Al-Khars, 1990).

Table. 1:- Antimicrobial activity of Chromulinafreibergensisin different Solvents.

Solvent used	Zone of inhibition (mm)										
	Control	Vibrio	Klebsiella	Escherichia	Staphylococcus	Bacillus	Pseudomonas	Bacillus	Salmonella	Proteus	Streptococcus
Acetone	*	6.7 ± 0.72	9.2 ± 0.83	9 ± 1.22	6.8+ 0.85	6.1 ± 0.68	9.8 ± 1.30	7.8 ± 0.83	6.1 ± 0.98	10.2 ± 0.83	8.6 ± 0.54
n-butanol	*	5.2 ± 0.86	4.1 ± 0.56	8.4 ± 0.89	4.2 ± 0.62	10.8 ± 0.44	8.6 ± 0.54	4.3 ± 0.85	10.8 ± 1.30	9.2 ± 1.09	9.6 ± 0.89
Isopropanol	*	6.3 ± 0.95	6.7 ± 0.82	6.5 ± 0.72	3.2 ± 0.85	2.7 ± 0.55	3.8 ± 0.76	8.4 ± 0.89	8.4 ± 0.54	8.4 ± 0.54	10.6 ± 0.54
Acetone + n-butanol (1:1)	*	11 ± 1.22	9.6 ± 0.89	15.1 ± 0.43	9 ± 0.70	11.2 ± 2.04	8.8 ± 1.48	9.8 ± 1.92	4.3 ± 0.30	8.6 ± 1.51	8 ± 1
Acetone + Isopropanol (1:1)	*	8.8 ± 0.44	8.4 ± 0.89	7.6 ± 0.54	9.4 ± 1.14	10.2 ± 0.83	10.6 ± 0.89	9.4 ± 1.51	11 ± 1.58	10.2 ± 1.30	10.6 ± 1.51
Acetone + Chloroform (1:1)	*	11.2 ± 1.78	10.8 ± 0.44	9.4 ± 0.54	6.1 ± 0.33	10.6 ± 1.14	11 ± 1.22	10 ± 1	8.8 ± 1.48	10 ± 1.58	10.6 ± 0.54
Butanol + Isopropanol (1:1)	*	5.1 ± 0.22	12 ± 1.73	10.2 ± 1.30	9.2 ± 1.64	8.8 ± 0.83	8.2 ± 0.83	9 ± 1.22	8.6 ± 0.54	11 ± 1.58	11.2 ± 1.09
Chloroform + Methanol (1:1)	*	11 ± 0.70	11.2 ± 0.44	9.6 ± 1.67	8 ± 1.22	9.8 ± 2.38	10.6 ± 2.40	10.8 ± 2.04	9.8 ± 1.30	10.4 ± 1.51	9 ± 0.70

* No growth was observed ; Each value is the mean ± SD of three individual estimates

Fig. 1:- Growth characterization of Chromulinafreibergensis at various pH.

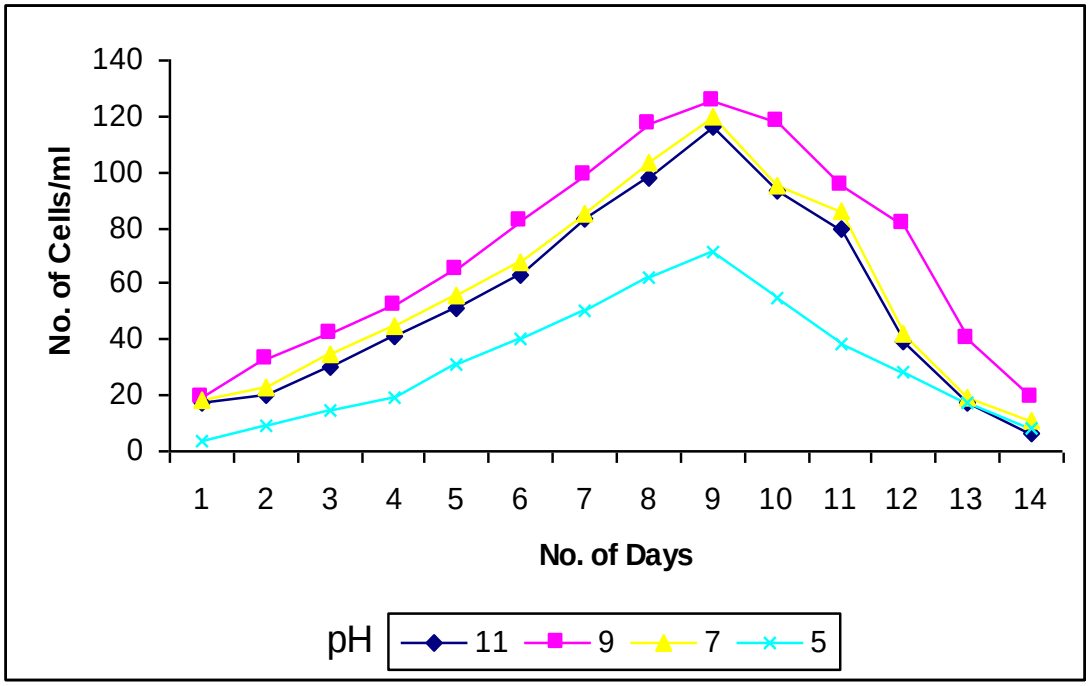


Fig. 2:- Growth characterization of Chromulinafreibergensis at various temperature (°C).

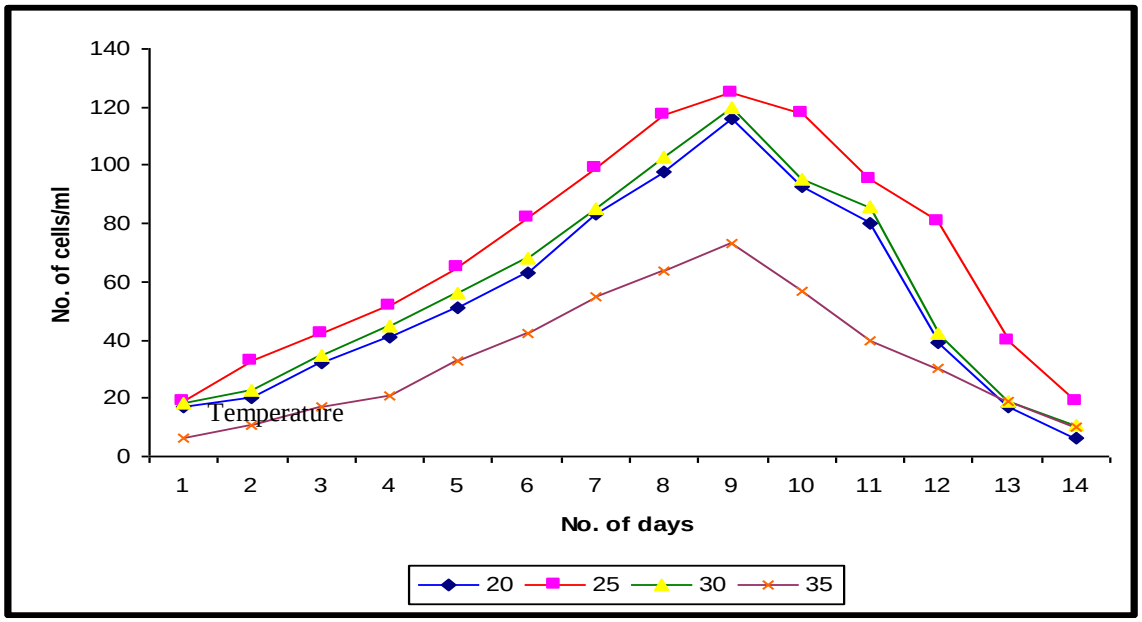
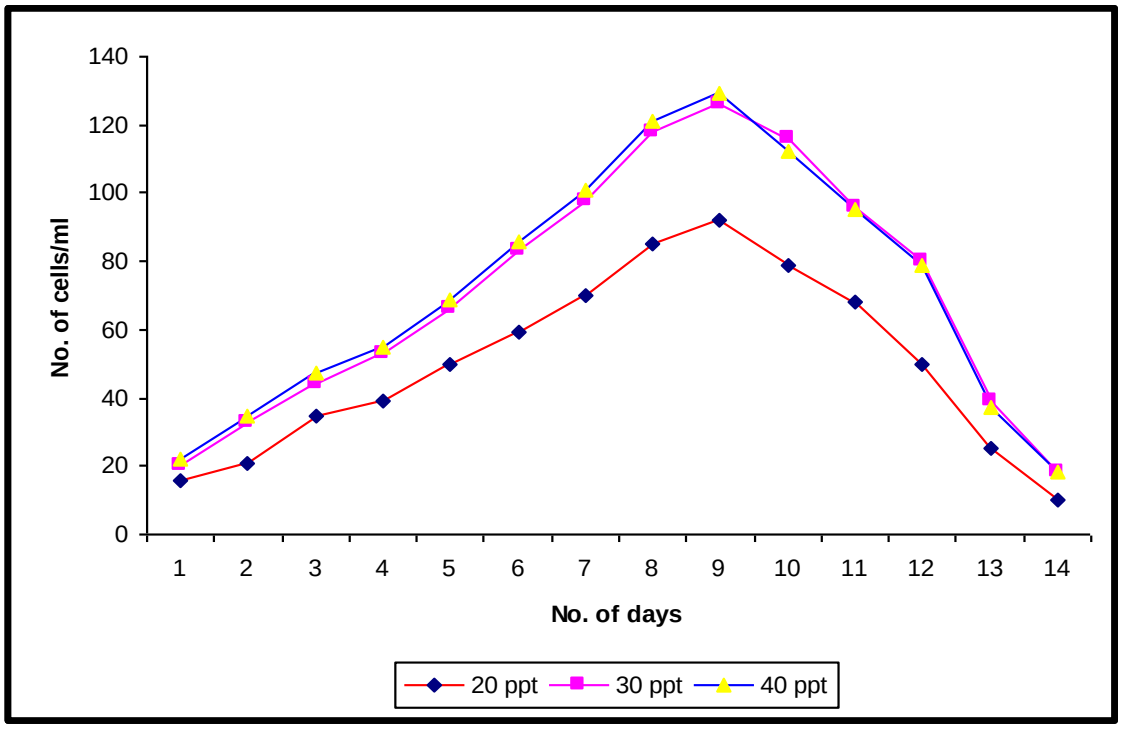


Fig. 3:- Growth characterization of Chromulinafreibergensis at various salinity.



Antibacterial Assay

Rao et al. impregnated a sterile Whatman No. 1 filter paper disc with 10 distinct algal extracts in 1986, and it had a diameter of 5 mm. Cotton swabs were contaminated with individual test pathogens and placed on Muller Hinton agar plates to create a lawn. On the agar surface, filter paper discs were spaced uniformly apart and covered with the algal extract. All plates were incubated in an incubator for 24 hours at 37 degrees Celsius to create an antibacterial zone (Gonalaz et al., 2001).

Antimicrobial Activity Of Algal Cultures

A higher amount of Acetone is present in proteus sp, Presence of the n-butanol alage is thus higher in pseudomonas aeruginosa, the 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 chloroform + methanol (1:1) is thus higher in vibrio cholerae,

Vibrio cholerae has the strongest results against human pathogens when chloroform + methanol (1:1) is present, while the bacillus megaterium in Figure 3 exhibits the lowest results when isopropanol is present.

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

Source of Variation	SS	df	MS	F	P-value
Total variance	402.347	79			
Variation due to bacteria	45.5206	9	5.05785	1.33703	> 0.05*
Variation due to solvent based extracts	118.504	7	16.9291	4.47518	< 0.05**
Error variance	238.322	63	3.78289		

* Statistically non-significant ; ** Statistically significant

Figure 04;- Antimicrobial activity (Zone formation).

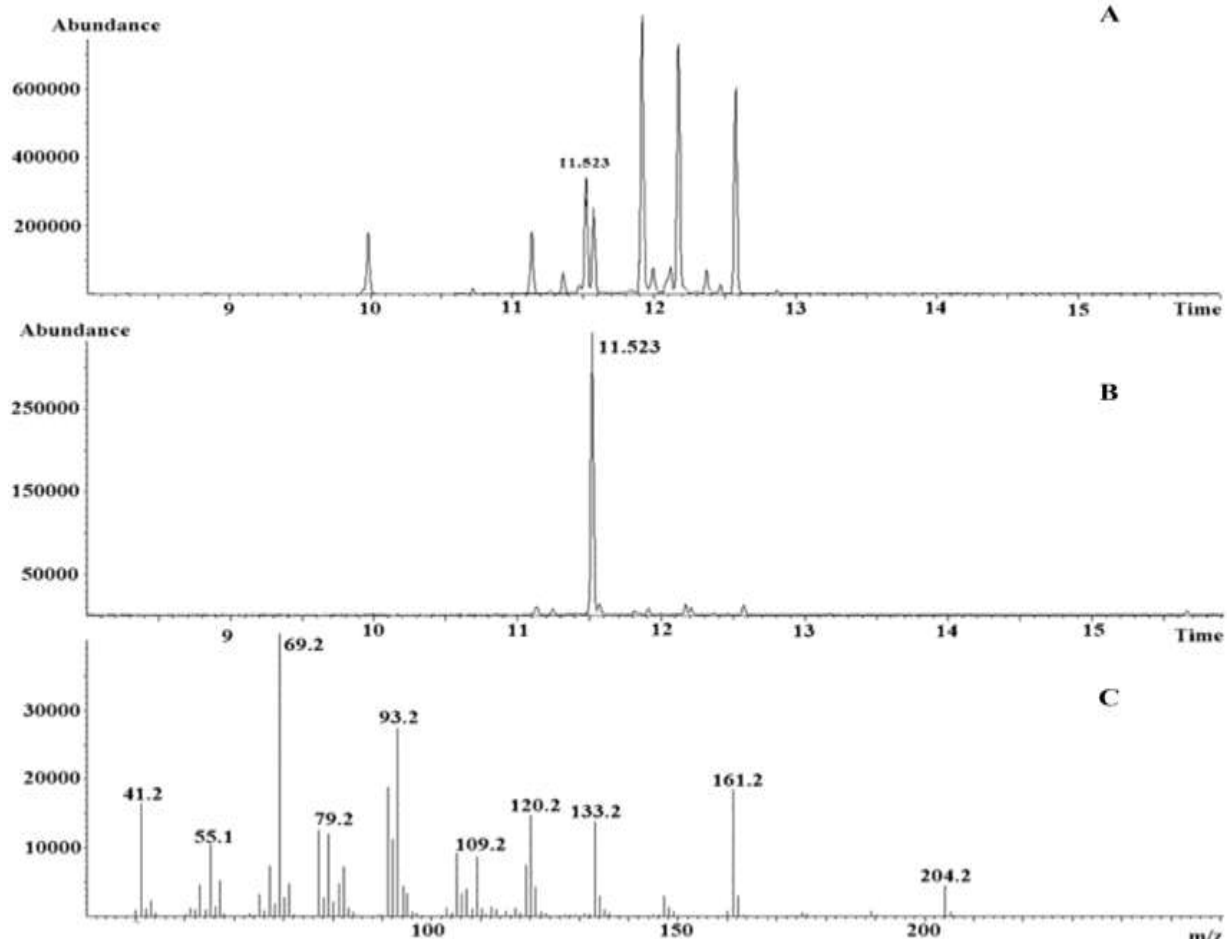
GC – MS Analysis

Finding out the many ingredients present and the chemical structures of these compounds from marine sources is crucial because identifying biochemical markers in biomedicines is crucial. The terrestrial equivalent cannot create a wide range of natural goods, however, due to its unfavorable environmental home, marine microalgae may. The goal of current research in the pharmaceutical and medicinal fields is to create distinctive compounds for identifying the chemistry of marine natural products. This study offers a rare opportunity for the examination of a novel chemical derived from halophilic microalgae for the treatment of human ailments

Biomedicines require the identification of biochemical markers. Determining the different components and their chemical structures in marine sources is crucial because of this. The marine counterpart can create a large variety of natural goods due to its hard environment, however, its terrestrial counterpart cannot. The goal of current research in the pharmaceutical and biomedical fields is to create a distinctive chemical for recognizing the chemistry of marine natural product. This study offers a rare chance to look into potential new medicines made from halophilic microalgae to treat human illnesses. Fig. 5a depicts the results of the current investigation, which used GC-MS analysis to identify antibacterial chemicals in an organic solvent extract of Chromulinafreibergensis. Table 3

displays the number of chemicals (peak) identified in the crude extract. Some chemical components are stated to have known biomedical relevance in pharmaceutical disciplines, and the mass spectra of compounds are compared to those of related compounds in the PubChem database (data not shown). The two chemical elements that make up the crude extract of Chromulinafreibergensis in Figures 5a and 5b,5c respectively, are tetradeconic acid and 9,12 octadecadienoic acid. These secondary metabolites open up a brand-new field of inquiry for future study of the chemical elements with antibacterial activity.

Figure 5a,5b,5c:- Graphical Representation Of Gc Ms Analysis.



Discussion:-

A significant amount of algal biomass is required for the synthesis of microalgal bioactive metabolites. The final step of the optimization process involves testing the top strains and most successful tactics under optimum circumstances. Numerous cultivation techniques have been created by academics and industry for high microalgal biomass output. The goal of the current study is to optimize the growing conditions to meet our need for significant volumes of microalgae biomass. The culture conditions, such as temperature, pH, salinity, and other micronutrients and macronutrients, have an impact on the chemical makeup of some microalgae. The earlier researchers [28, 29, 30, 31] supported the pattern of Chromulinafreibergensis (Australian and Kuwaiti) cultivated at various temperatures, attaining growth rates of up to 2.90x10 6 and 2.40x10 6 cell ml-1, respectively. [31] and has been thoroughly examined by Abu-Rezq et al. Both samples' development patterns changed less as the temperature increased. As a result, it may be concluded that Chromulinafreibergensis prefers low temperatures (20oC) to high temperatures (32oC). The maximum production range of cells in a closed tube system in outdoor culture was attained by Garcia-Gonzalez et al. [32], ranging from 2 to 4x10 6 cells ml-1. They determined that Chromulinafreibergensis exhibits its best culture performance at a temperature of 25°C and a pH of 7.50.5 and that the addition of CO2 gas regulates it. Chromulinafreibergensis strains' development patterns and carotenogenesis are examined by Cifuentes et al. [33] at varied experimental temperatures. They discovered that a photoperiod of 12:12

(light and dark phases) at a temperature of 20°C resulted in the maximum growth rate and carotene production. These results support our ongoing research on Chromulinafreibergensis and the generation of secondary metabolites on the ninth day of incubation under experimental circumstances at 20° C. On the other hand, Singh et al. [34] propose that water temperature be set at 30oC because they found that the greatest cell growth induction occurs under experimental conditions of restricted nutrients. At various pH values, the growth pattern of microalgae culture media is examined. As the pH rises, algae growth accelerates. Microalgae Chromulinafreibergensis showed that the highest cell growth was seen on the ninth day of the experiment when the pH was 9.0 rather than 5.0. Maximum growth for the marine microalga Chlorella sp. is seen on the seventh day of incubation at a pH of 9 to 9.5, according to Zhao et al. [35]. The present Chromulinafreibergensis culture optimization study that we are conducting has been approved and is supported by earlier studies.

In contrast to low salinity, Chromulinafreibergensis' development rate rose under experimental conditions with increasing salinity (40 ppt) (20 ppt). Dolapsakis et al. [36] found that areas with higher salt concentrations, such as those near salt lagoons, salt-producing factories, and the seashore, are where Chromulinafreibergensis can develop at their best during the natural season. Oren [37], whose findings concur with those of earlier scientists. Farahat et al. [38] observed the growth of Chromulinafreibergensis. According to Hadi et al. [39], Chromulinafreibergensis can tolerate NaCl concentrations between 0.2 percent and roughly 35 percent. The microalgae can thrive in conditions containing salt concentrations as low as 0.17 M and as high as 4.0 M NaCl. The greatest number of cells was seen on the 18th day of incubation, when the media was updated with 4.0M NaCl, according to Raja et al. [39]. Increased -carotene accumulation, however, supports 3.5M NaCl. Leach et al. [42] reported that maintaining the culture at an 8.5 pH and 18 percent NaCl w/w salinity allowed for a cell concentration of 0.8x10 6 cells ml-1. A hyper-halotolerant creature called Chromulinafreibergensis can be found in salt lakes at great densities.

The extraction of antimicrobial metabolites from algae has received the greatest attention recently compared to other potential sources. A wide range of chemical classes, including indoles, terpenes, acetogenins, phenols, fatty acids, and volatile halogenated hydrocarbons, are produced by the antimicrobial activity of microalgae [43, 44]. Solvents are employed to extract the antibacterial properties of both algae species [46]. Several organic solvents, including methanol, acetone, ether, and chloroform, as well as other microorganisms, were used to investigate the antibacterial efficacy of algal extracts [45]. Organic solvents are always more effective at extracting substances with antibacterial action [49]. The antibacterial action observed in numerous pressurized extracts from Chromulinafreibergensis is attributed to some fatty acids as well as substances including cyclocitral, neophytadiene, and phytol [47]. Despite Spirulina platensis extracts having antibacterial activity in dichloromethane, petroleum ether, and ethyl acetate [48], the methanol extract was shown to be more effective. The marine alga Jania rubens demonstrated strong antibacterial action against both gramme negative and gramme positive bacteria, according to KarabayYavasoglu et al. [50]. In line with our current discovery, Table 1 demonstrates that a methanol and chloroform (1:1) extract exhibits promising antibacterial activity against both gram-negative and gram-positive bacteria. According to Mhadhebi et al. [50], extracts made from the marine algae Cystoseiracrinita and Cystoseirasedoides have greater antifungal activity. When Chromulinafreibergensis crude extract is subjected to GC-MS analysis, intriguing chemicals with notable antibacterial activity are discovered. In this investigation, various chemical components were shown to have antibacterial action and medicinal significance, including Tetradecanoic acid, Eicosanoic acid, hexadecenoic acid, 9,12-octadecadienoic acid, 9-octadecenoic acid, and methyl ester. Crude extract examination of the indicated species using gas chromatography-mass spectrometry reveals numerous significant organic volatile chemicals and their derivatives (GC-MS)

Organic solvent extracts from Synechocystis sp. that have undergone GC-MS chemical characterization [51] produce a variety of fatty acids and volatile chemicals with antibacterial activity, including phytol, fucosterol, neophytadiene, or palmitic, palmitoleic, and oleic acids. According to Al-research Wathnani's [53], cyanobacteria and green algae create many substances, including biomedically significant organic metabolites such as heptanal, ethane1,1-diethoxy butanal, 3- Methyl-2-(2-Oxopropyl) Furan, and octanal.

According to Dooslin Mercy Bai ,S.kousik Saravanaresearch [54,55], GC-MS analysis of Tetraselmissuecica crude extract revealed the presence of 1-ethyl butyl 3hexyl hydroperoxide and methyl heptanate, which have anti-inflammatory, antipsychotic, antiseptic, antineoplastic, anti-allergic, antipyretic, and analgesic effects. Leading chemical compounds with medicinal relevance, such as 3, 3, 5-Trimethylheptane, and n-Hexadecane, are present in the fractionated matrices of the extract of Chromulinafreibergensis. Interestingly, some of the chemicals in our final chromatograms showed prominent biological characteristics. Based on the findings of this study, it is recommended

that chloroform + methanol (1:1) be used as the best organic solvent for removing bioactive substances from marine microalgae that are currently important for biomedical and pharmaceutical use.

Conclusion:-

To achieve maximum algal biomass of 56, the following ideal cultivation conditions must be maintained: salinity of 40 ppt, a temperature of 20 oC, and a high pH of 9.0 on the ninth day of incubation. Additional investigation into the precise chemical component responsible is needed for clinical trials, biocidal action, and medicinal applications in humans. Researchers may have the chance to study an exciting new area of microalgal biotechnology in the future. Future research on Chromulinafreibergensis will involve the creation of biofuels and novel antibiotics, among other microalgal projects.

Acknowledgement:-

The authors are grateful to Sri Shakthi Institute of Engineering and Technology , Department of Biomedical Engineering, Coimbatore, Tamil Nadu, India for providing all the needed facilities complete this work successfully. Our exceptional thanks to (RTD) Dr.A.Kumaresan, Professor and Head, ICAR centre, Sri Parasakthi College for women, Courtallam, Tamilnadu, India for the valuable guidance and constant support.

Reference:-

1. Tolpeznikaite e, bartkevics v, ruzauskas m, et al. Characterization of macro- and microalgae extracts bioactive compounds and micro- and macroelements transition from algae to extract. Foods. 2021;10(9):2226. Published 2021 sep 19. Doi:10.3390/foods10092226
2. Wu j, gu x, yang d, et al. Bioactive substances and potentiality of marine microalgae. Food sci nutr. 2021;9(9):5279- 5292. Published 2021 jul 26. Doi:10.1002/fsn3.2471
3. Dooslin mercy bai and s. Kousik saravana (2022); evaluation and quantitative analysis of bioactive compounds from chaetocerouscalcitrans against human pathogens **int. J. Of adv. Res.** 10 (jun). 309-321] (issn 2320-5407).
4. Azizan a, ahamadbustamamms, maulidiani m, et al. Metabolite profiling of the microalgal diatom chaetoceroscalcitrans and correlation with antioxidant and nitric oxide inhibitory activities via ¹h nmr-based metabolomics. Mar drugs. 2018;16(5):154. Published 2018 may 7. Doi:10.3390/md16050154
5. . Shannon e, abu-ghannam n. Antibacterial derivatives of marine algae: an overview of pharmacological mechanisms and applications. Mar drugs. 2016;14(4):81. Published 2016 apr 22. Doi:10.3390/md14040081d
6. krishnakumar s, premkumar j, alexisrajan r, ravikumar s optimization of potential antibiotic production by salt-tolerant actinomycetes streptomyces sp. - msu29 isolated from marine sponge. International j on applied bioengineering. 2011; 5(2):12-17.
7. Iwamoto c, yamadat, ito y, minoura k, numata a cytotoxic cytochalasans from a penicillium species separated from a marine alga. Tetrahedron. 2001; 57: 2904–2997
8. Hemraj upmanyu n, gupta a, jindal a, jalhan s pharmacological activities of stephania glabra, woodfordiafruticosa and cissempelos pareira – a review. International j of pharmacy and pharmaceutical sciences. 2012; 4(3): 16-23.
9. Justo gz, silva mr, queirozmls effects of green algae chlorella vulgaris on the response of the host hematopoietic system to intraperitoneal ehrlich ascites tumour transplantation in mice. Immunopharmimmunotoxico. 2001; l23:199- 131
10. Foo S.C., Yusoff F.M., Ismail M., Basri M., Yau S.K., Khong N.M.H., Chan K.W., Ebrahimi M. Antioxidant capacities of fucoxanthin-producing algae as influenced by their carotenoid and phenolic contents. J. Biotechnol. 2017;**241**:175–183. doi: 10.1016/j.jbiotec.2016.11.026.
11. Foo S.C., Yusoff F.M., Ismail M., Basri M., Chan K.W., Khong N.M.H., Yau S.K. Production of fucoxanthin-rich fraction (FxRF) from a diatom, Chaetoceroscalcitrans (Paulsen) Takano 1968. Algal Res. 2015;**12**:26–32. doi: 10.1016/j.algal.2015.08.004.
12. . Nigjeh S.E., Yusoff F.M., Mohamed Alitheen N.B., Rasoli M., Keong Y.S., Omar A.R. Cytotoxic effect of ethanol extract of microalga, Chaetoceroscalcitrans, and its mechanisms in inducing apoptosis in human breast cancer cell line. Biomed. Res. Int. 2013;**2013**:1–8. doi: 10.1155/2013/783690
13. Goh S.H., Alitheen N.B.M., Yusoff F.M., Yap S.K., Loh S.P. Crude ethyl acetate extract of marine microalga, Chaetoceroscalcitrans, induces Apoptosis in MDA-MB-231 breast cancer cells. Pharmacogn. Mag. 2014;**10**:1–8]
14. Bromke M.A. Amino acid biosynthesis pathways in diatoms. Metabolites. 2013;**3**:294–311. doi: 10.3390/metabo3020294.

15. Loewus F.A., Loewus M.W. Myo-Inositol: Its biosynthesis and metabolism. Annu. Rev. Plant Physiol. 1983;**34**:137–161. doi: 10.1146/annurev.pp.34.060183.001033.
16. Miller M.R., Quek S.Y., Staehler K., Nalder T., Packer M.A. Changes in oil content, lipid class and fatty acid composition of the microalga Chaetoceroscalcitrans over different phases of batch culture. Aquac. Res. 2014;**45**:1634–1647. doi: 10.1111/are.12107.
17. Sirin S., Clavero E., Salvado J. Efficient harvesting of Chaetoceroscalcitrans for biodiesel production. Environ. Technol. 2015;**36**:1902–1912. doi: 10.1080/09593330.2015.1015456.
18. Talero E., García-Mauriño S., Ávila-Román J., Rodríguez-Luna A., Alcaide A., Motilva V. Bioactive compounds isolated from microalgae in chronic inflammation and cancer. Mar. Drugs. 2015;**13**:6152–6209. doi: 10.3390/md13106152
19. 40. Mizoguchi T., Isaji M., Yamano N., Harada J., Fujii R., Tamiaki H. Molecular structures and functions of chlorophylls-a esterified with geranylgeranyl, dihydrogeranylgeranyl, and tetrahydrogeranylgeranyl groups at the 17-propionate residue in a diatom, Chaetoceroscalcitrans. Biochemistry. 2017;**56**:3682–3688. doi: 10.1021/acs.biochem.7b00381.
20. 41. Imaizumi Y., Nagao N., Yusoff F.M., Taguchi S., Toda T. Estimation of optimum specific light intensity per cell on a high-cell-density continuous culture of Chlorella zofingiensis not limited by nutrients or CO_2. Bioresour. Technol. 2014;**162**:53–59. doi: 10.1016/j.biortech.2014.03.123.
21. 42. Lee S.Y., Mediani A., Khatib A., Ismail I.S., Zawawi N., Abas F. Comparison of partial least squares and random forests for evaluating relationship between phenolics and bioactivities of Neptunia oleracea. J. Sci. Food Agric. 2018;**98**:240–252. doi: 10.1002/jsfa.8462.
22. Munro MHG, Blunt JW, Dumdei EJ, et al. The discovery and development of marine compounds with pharmaceutical potential. Journal of Biotechnology. 1999;70(1–3):15–25.
23. Natrah FMI, Yusoff FM, Shariff M, Abas F, Mariana NS. Screening of Malaysian indigenous microalgae for antioxidant properties and nutritional value. Journal of Applied Phycology. 2007;19(6):711–718.
24. Lee JC, Kim HR, Kim J, Jang YS. Antioxidant property of an ethanol extract of the stem of Opuntia ficus-indica var. saboten. Journal of Agricultural and Food Chemistry. 2002;50(22):6490–6496.
25. Mosmann T. Rapid colorimetric assay for cellular growth and survival: application to proliferation and cytotoxicity assays. Journal of Immunological Methods. 1983;65(1-2):55–63.
26. Cochrane CB, Nair PKR, Melnick SJ, Resek AP, Ramachandran C. Anticancer effects of Annona glabra plant extracts in human leukemia cell lines. Anticancer Research. 2008;28(2):965–971.
27. Bechelli J, Coppage M, Rosell K, Liesveld J. Cytotoxicity of algae extracts on normal and malignant cells. Leukemia Research and Treatment. 2011;2(1):p.
28. Talib WH, Mahasneh AM. Antiproliferative activity of plant extracts used against cancer in traditional medicine. Scientia Pharmaceutica. 2010;78(1):33–45.
29. Reed JC. Apoptosis-based therapies. Nature Reviews Drug Discovery. 2002;1(2):111–121.
30. Fulda S, Debatin KM. Extrinsic versus intrinsic apoptosis pathways in anticancer chemotherapy. Oncogene. 2006;25(34):4798–4811.
31. Ooi KL, Muhammad TST, Sulaiman SF. Growth arrest and induction of apoptotic and non-apoptotic programmed cell death by, Physalis minima L. chloroform extract in human ovarian carcinoma Caov-3 cells. Journal of Ethnopharmacology. 2010;128(1):92–99.
32. Kim JY, Yoon MY, Cha MR, et al. Methanolic extracts of plocamiumtelfairiae induce cytotoxicity and caspase-dependent apoptosis in HT-29 human colon carcinoma cells. Journal of Medicinal Food. 2007;10(4):587–593.
33. Tait L, Soule HD, Russo J. Ultrastructural and immunocytochemical characterization of an immortalized human breast epithelial cell line, MCF-10. Cancer Research. 1990;50(18):6087–6094.
34. Ramachandran C, Rodriguez S, Ramachandran R, et al. Expression profiles of apoptotic genes induced by curcumin in human breast cancer and mammary epithelial cell lines. Anticancer Research. 2005;25(5):3293–3302.
35. Nagaraja GM, Othman M, Fox BP, et al. Gene expression signatures and biomarkers of noninvasive and invasive breast cancer cells: comprehensive profiles by representational difference analysis, microarrays and proteomics. Oncogene. 2006;25(16):p. 2328.
36. Ebrahimi Nigjeh S, Yusoff FM, Mohamed Alitheen NB, Rasoli M, Keong YS, Omar AR. Cytotoxic effect of ethanol extract of microalga, Chaetoceroscalcitrans, and its mechanisms in inducing apoptosis in human breast cancer cell line [published correction appears in Biomed Res Int. 2016;2016:8693826]. Biomed Res Int. 2013;2013:783690. doi:10.1155/2013/783690
37. Jemal A, Bray F, Center MM, Ferlay J, Ward E, Forman D. Global cancer statistics. CA Cancer J Clin. 2011;61:69–90.

38. Mayer AM, Gustafson KR. Marine pharmacology in 2003-2004: Anti-tumour and cytotoxic compounds. Eur J Cancer. 2006;42:2241–70.
39. Mayer AM, Gustafson KR. Marine pharmacology in 2005-2006: Antitumour and cytotoxic compounds. Eur J Cancer. 2008;44:2357–87.
40. Mishima T, Murata J, Toyoshima M, Fujii H, Nakajima M, Hayashi T, et al. Inhibition of tumor invasion and metastasis by calcium spirulan (Ca-SP), a novel sulfated polysaccharide derived from a blue-green alga, Spirulina platensis. Clin Exp Metastasis. 1998;16:541–50.
41. Goh SH, Loh SP, Yusoff FM. A comparison of the antioxidant properties and total phenolic content in a diatom, Chaetoceros sp. and a green microalga, Nannochloropsis sp. J Agric Sci. 2010;2:123–30.
42. Athukorala Y, Kim KN, Jeon YJ. Antiproliferative and antioxidant properties of an enzymatic hydrolysate from brown alga, Ecklonia cava. Food Chem Toxicol. 2006;44:1065–74.
43. Bank HL. Rapid assessment of islet viability with acridine orange and propidium iodide. in vitro Cell Dev Biol. 1988;24(Suppl 4):266–73.
44. Hoppe HA, Levring T, Tanaka Y. Marine Algae in Pharmaceutical Science. New York: Walter de Gruyter; 1979. Marine algae and their products and constituents in pharmacy; pp. 25–119.
45. Fautin DG. Biomedical Importance of Marine Organisms. San Francisco: California Academy of Sciences; 1988. New pharmaceuticals from cultured blue-green algae; pp. 143–50.
46. Simmons TL, Andrianasolo E, McPhail K, Flatt P, Gerwick WH. Marine natural products as anticancer drugs. Mol Cancer Ther. 2005;4(Suppl 2):333–42.
47. Mayer AM, Hamann MT. Marine pharmacology in 2001 - 2002: Marine compounds with anthelmintic, antibacterial, anticoagulant, antidiabetic, antifungal, anti-inflammatory, antimalarial, antiplatelet, antiprotozoal, antituberculosis, and antiviral activities; affecting the cardiovascular, immune and nervous systems and other miscellaneous mechanisms of action. Comp BiochemPhysiol C ToxicolPharmacol. 2005;140:265–86.
48. Mosmann T. Rapid colorimetric assay for cellular growth and survival: Application to proliferation and cytotoxicity assays. J Immunol Methods. 1983;65:55–63.
49. Huang HL, Wu SL, Liao HF, Jiang CM, Huang RL, Chen YY, et al. Induction of apoptosis by three marine algae through generation of reactive oxygen species in human leukemic cell lines. J Agric Food Chem. 2005;53:1776–81.
50. Khanavi M, Nabavi M, Sadati N, Shams Ardekani M, Sohrabipour J, Nabavi SM, et al. Cytotoxic activity of some marine brown algae against cancer cell lines. Biol Res. 2010;43:31–7
51. NIH Publication No. 01-4499. U.S. Public Health Service:NIEHS, Research Triangle Park, N; 2001. National Institute of Environmental Health Sciences (NIEHS). Report of the International Workshop on In Vitro Methods for Assessing Acute Systemic Toxicity.
52. Dimas K, Papadaki M, Tsimplouli C, Hatziantoniou S, Alevizopoulos K, Pantazis P, et al. Labd-14-ene-8,13-diol (sclareol) induces cell cycle arrest and apoptosis in human breast cancer cells and enhances the activity of anticancer drugs. Biomed Pharmacother. 2006;60:127–33.
53. Chen WY, Wu CC, Lan YH, Chang FR, Teng CM, Wu YC. Goniothalamin induces cell cycle-specific apoptosis by modulating the redox status in MDA-MB-231 cells. Eur J Pharmacol. 2005;522:20–9.
54. Ding L, Liu B, Qi LL, Zhou QY, Hou Q, Li J, et al. Anti-proliferation, cell cycle arrest and apoptosis induced by a natural xanthone from Gentianopsispaludosa Ma, in human promyelocytic leukemia cell line HL-60 cells. Toxicol in vitro. 2009;23:408–17.
55. Hanahan D, Weinberg RA. The hallmarks of cancer. Cell. 2000;100:57–70]
56. Chan KM, Rajab NF, Ishak MH, Ali AM, Yusoff K, Din LB, et al. Goniothalamin induces apoptosis in vascular smooth muscle cells. Chem Biol Interact. 2006;159:129–40.