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GCMS Determination of Anticancer, Anti inflammatory and Anti bacterial compounds from salt tolerance Microalgae (Lyngbya sp. Nostoc sp. and Phormidium sp.) Isolated from Marakkanam Salt Pan, Tamil Nadu, India.

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Abstract:

Microalgae are prokaryotic or eukaryotic autotrophic microorganisms that can grow slowly and live in harsh condition due to their unicellular or simple multi cellular structure. Example of prokaryotic microorganisms is cyanobacteria and eukaryotic microalgae are green algae and diatoms. The present investigation was carried out to determine the novel bioactive anticancer compound from salt tolerance microalgae (*Lyngbya sp., Nostoc sp., and Phormidium sp.*) by using GCMS analysis. Microalgae are playing an important role in production of various secondary metabolites such as alkaloid (Quinolin, 5-nitro-, 1-oxide),flavones were identified from *Nostoc sp*. flavones were identified from *Phormidium sp.* alkaloid (caprilic acid acid or Octanoic acid, 4-nitrophenyl ester)and Steroid (ethyl iso allocholate) compounds were identified from *Lyngbya sp. Nostoc sp.*, and *Lyngbya sp.* contains more anticancer activity than the phormidium sp. because presence of the number of secondary metabolites. The improving of high quality anticancer compound can be achieved via genetic and metabolic engineering.

Key words: Microalgae; GCMS analysis; alkaloid; flavones; Steroid and anticancer activity.

Introduction - Anticancer compound:

Cyanobacteria are considered as a rich source of novel compounds. A huge number of antitumor

Compounds are natural products and their metabolic derivatives commonly produced by blue-green algae. Microalgae are able to produce the various numbers of secondary metabolites and its biological mechanisms characterized such as anti tumor, anti inflammatory, anti viral and anti bacterial activities. Further investigation is essential to reveal the molecular mechanisms and anticancer activities of the blue-green algae.

Quinazolin-4(3H)-one:

Quinazoline is a heterocyclic compound, soluble in water. It is chemically called as Quinazolin-4(3H)-one. Quinazoline derivatives are more important in the field of organic chemistry and medicinal chemistry because of their therapeutic applications such as anticancer, antibacterial, anti diabetic, hypnotic, sedative, analgesic, anticonvulsant, antitussive, anti inflammatory and several others. Approximately 150 new natural quinazoline have been isolated from various natural species.

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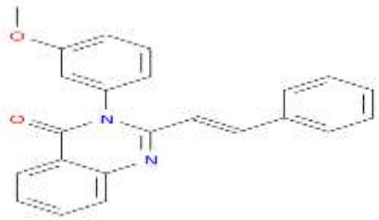
Quinazolin-4(3H)-one

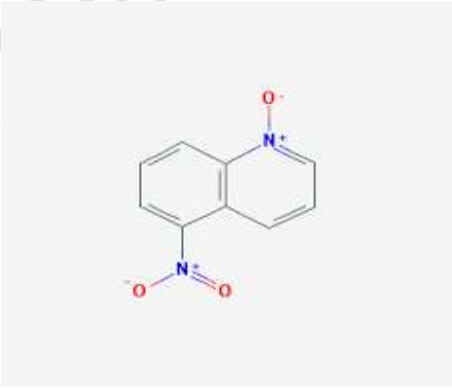


Quinazoline is a planar molecule with isomeric compounds are 1, 3-diazanaphthalene, phenmiazine, benzo-1, 3-diazine, 5, 6-Benzopyrimidine. Quinazolinones are commonly partition in to three major classes 2-substitutes, 3-substituted and 2, 3-disubstituted-quinazolinone. Screened quinazolinone alkaloids are one of the highly engaging natural products leading to drug developments. The identifiable characteristic feature of quinazoline mechanisms would make a good template for library preparation. Some artificial quinazoline are available in the maret such as Raltitrexed, Ispinesib and Tempostatin.

Quinoline:

Quinoline is a heterocyclic base and was first isolated in 1834 by Runge. Large number of quinoline derivatives have also isolated from petroleum products. Naturally and pharmacologically important alkaloids are Echinopsine, Cusparine, Galipine, uspareine, Quinine etc. Quinoline is a hygroscopic liquid with a strong odour, exposed to light, become yellow and later brown. Quinoline is slightly weaker base than pyridine. It forms N-oxide and quaternary salt like methyl iodide, methyl sulphate, benzoyl chloride etc. Quinoline compounds showed antimicrobial activity, potential analgesic, antipyretic, anti-inflammatory, anti-rheumatic properties, antidepressant drug, tumor inhibiting agents, bactericidal and fungicidal activities.

Quinoline



Quinoline derivatives were found to inhibit the growth of Ehlich ascites carcinoma and ascites heptoma AH13 in animals. The effect of these compounds on cancer cell was reported to be due to the cell injury and inhibition of nucleic acid synthesis. Styryl quinolines and its quaternary iodides act as tumor suppressant agent, anti bacterial and antifungal. Liniquincin is used as a hypotensive drug and it was prepared by condensing veratraldehyde with 4-amino-6, 7- dimethoxyquinoline. Quinolines containing thiourea derivatives showed a significant antibacterial activity against S. aureus and E. coli. Lavendamycin a pentacyclic quinoline was isolated from Streptomyces lavendalaeim act as an antitumour antibiotic and another quinoline was streptonigrin

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Table 1: Microalgae producing Quinoline derivatives.

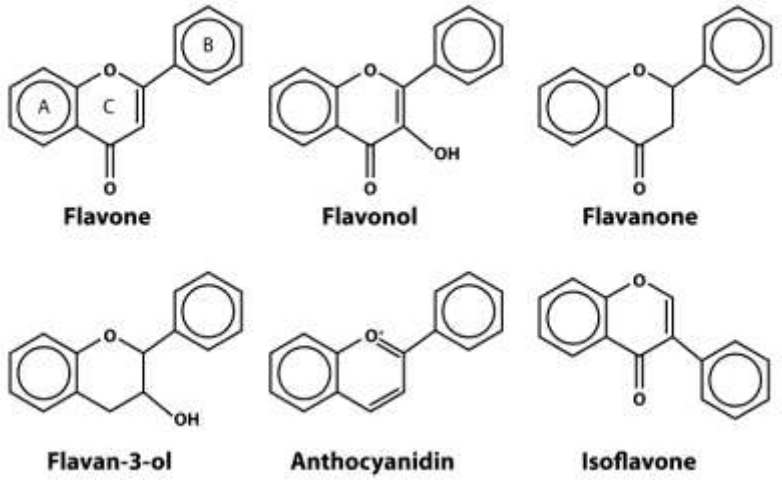
Algae	Quinoline derivatives	Function	References
Anabaena .sp	Echinopsine	Antidepressant	Mascavage *et al*., 2010
Nostoc.sp	Cusparine	Antileishmanial	Amritpal Singh Saroya, 2011
Chlorella vulgaris	Galipine	acute antimigraine agent	Silva *et al*., 2013
Scenedesmus dimorphus	Chloroquinoline	antidepressant drug	Bhuvaneshwari *et al*., 2015
Lyngbya majuscule	Styryl quinolines	tumour inhibiting property	Kumar *et al*., 2011
Scytonema sp *Nostoc punctiforme*	Quinolines containing thiourea derivatives	antibacterial activity	Garcia-Pichel and Castenholz, 1993
Chaetoceros abnormis	pentacyclic quinoline	Anti tumour	Zanella *et al*., 2018
Micromonospora sp *Lyngbya sp* *Microsystis sp*	Tri cyclic antitumour quinoline	Anti tumour	Demay *et al*., 2019
chaetoceros calcitrans	synthesized 7-substituted methyl 8-hydroxy quinolines	Anti cancer	Zanella *et al*., 2018
Chlorella vulgaris	Quinine	Anti arthritis and anti malaria	Suvidha *et al*., 2019

Flavone:

The flavones are phenol molecules which are obtained from secondary metabolites of plants. It is present in the flowers, leaves, vegetables and fruit tissues of living plants as flavonoid. Flavones are important members of the flavonoid family. Algae are one of the oldest living organisms of planet earth. Microalgae can grow in preferably different environments, like sea, and desert. In recent years algae is a rich source of bioactive compounds, like phenolic compounds, fatty acids, amino acids, and carotenoids. Our data clearly showed that microalgae contain a wide range of flavonoids. Flavonoids molecules are act as a plant growth regulator. Flavonoids are either bitter or sweet in taste and recent chemical tests have proved that minor altercations in their cell structure can change their taste from bitter to sweet. Flavonoids may be found in almost all plants, but their concentration varies depending on the plant family.

For example: citrus fruits, all white and yellow flowers have very high concentration of flavonoids.

Structure of flavone



Flavonoid aglycones can be classified into five groups - flavones, isoflavone, flavonols, flavononesand xanthones. The pharmacological properties of different flavonoids include - diuretic, anti-inflammatory, antiseptic, antispasmodic and also anti-tumor. Flavonoids, however, are most useful in treating the vascular system and this has become evident from the research done on bio-flavonoids or 'vitamin P', especially hesperidin and rutin. Medically, rutin has various and effective roles in treating hypertension, diabetes, arsenic poisoning and allergies. In the modern day of allopathic treatment, rutin is also used to treat hemorrhages due to radiation; while in herbal medicine buckwheat is used for the same purpose. Multiple numbers of natural, semi-synthetic and synthetic derivatives of flavones have been synthesized and estimated for pharmacological effects like antitumor and cytotoxic, anti-allergic, antioxidant, and anti-inflammatory, antiestrogenic and antimicrobial. Oxidative stress is known to be associated with a number of metabolic diseases. Current studies are available in the article showing the application and various mechanisms of flavones in diseased conditions like cancer, diabetes, Alzheimer's disease and several others.

Flavone ($C_{15}H_{10}O_2$) is a kind of flavonoids, chemically called as 2-phenylchromen-4-one (2-phenyl-1-benzopyran-4-one). Its functional group like hydroxy, carbonyl group and associated double bond which are responsible for giving exact reactions, these colourless-to-yellow crystalline substances are soluble in water and ethanol. Yellow color solution is obtained up on their dissolution in alkali and mild to-strong oxygen bases when solubilised in to acid are result in the formation of oxonium salts with pKa values in the range of 0.8 to 2.45. Cancer is one of the major causes for mortality and morbidity in the world. It is characterized by cell proliferation, differentiation, angiogenesis, and loss of apoptosis. Currently available therapy for the treatment of this deadly disease suffers from the major drawback of associated side effects along with emergence of resistance to these drugs. Lack of selectivity towards cancerous cells is also one of the major issues. This results in the continuous engagement in the development of novel anticancer agents. Several studies have demonstrated that algae are capable of forming p-coumaric acid, the precursor of the flavonoid synthesis as the enzyme PAL has been detected in the microalgae *Chlorella pyrenoidosa* (Chen et al. 2003) as well as in the *Anabaena variabilis* and *Nostoc punctiforme*, suggesting that this enzyme was already present in the ancestors of the chloroplasts (Moffitt et al. 2007). Scientific evidence suggests that antioxidants reduce the risk for chronic diseases including cancer and heart disease. Flavones (apigenin) are found in celery seed and parsley, luteolin in Equisetum spp. (the

horsetails), while isoflavones (genistein) are common in clover, gorse and other legumes, which is a pro-oestrogen with oestrogenic properties. Examples of flavonols (guercitol or glycoside rutin) are buckwheat, rue and over half of all plants tested kaempferol and in around half all plants tested myricetin in woody plants only. Similarly, flavonones (eridictyol / methyleriodictyol) jointly form the 'citrin' of citrus fruit glycoside and hesperidin liquiritigetol in licorice. Xanthones (gentisin) are normally found in gentian.

Table 2: Microalgae producing flavone derivatives.

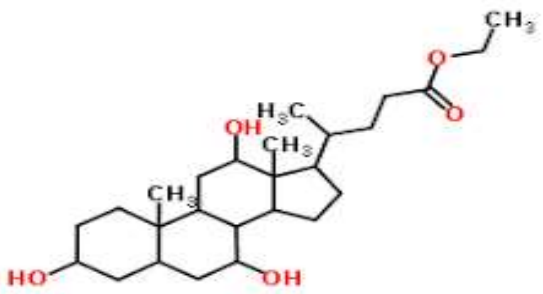
Algae	**Flavones**	**Activity**	**References**
Chlorella zofingiensis	Wogonin	cytotoxic efficacy against HepG2, BCG-823 and A549 cancer cell lines	Sun *et al.*, 2011
Spirulina maxima	flavone derivatives	inhibit the production of nitric oxide	Hanaa *et al.*, 2009
Botryococcus braunii	ferrocene embedded into chalcone, aurone and flavone skeletons	anticancer activity against resistant tumor cells.	Turu *et al.*, 2016
Chaetomium cupreum	alpha-naptho flavone derivatives	inhibition of human CYP1B1 enzyme	Nazir and Sharmila, 2018
Stigonema sp	1,2,3-triazole linked chalcone and flavone hybrid compounds	cytotoxic activity	Kant *et al.*, 2016
Spirulina subsalsa	offlavone- 7-phosphoramidate derivatives	antiproliferative activity.	Atta-ur- Rahman, 2005
Plectonema boryanum	polyamine conjugates of flavonoids with a naphthalene derivatives	anti-hepatocellular carcinoma	Yongfeng Guo, 2018
Anabaena cylindrical	3,5-dihydroxy- 7,8-dimethoxy-2-(4-methoxyphenyl)benzopyran-4-one derivatives	anti-cancer potential	Das *et al.*, 2011
Acanthophora spicifera	3-arylflavone-8-acetic acid derivatives	anticancer activity against A549 cell lines	Gihan *et al.*, 2016
Synechocystis sp.	flavone-triazole-	against human cancer cell	Vimal *et al.*, 2019

	tetrahydropyran conjugates	line activity	
Moorea bouillonii	7-methoxy-3-arylflavone-8-acetic acids derivatives.	anti-cancer activity.	Shaheen *et al.,* 2016
Nannochloropsis.sp	flavone and isoflavone derivatives	anti-cancer activity	Nur *et al.,* 2018

Ethyl iso- allocholate:

Ethyl iso- allocholate (C26H44O5) molecular weight is 436. Our recent studies are demonstrates that ethyl iso-allocholate compound isolated from medicinal rice "Karungkavuni" can serve as a potent inhibitor for dihydropteroate synthase. The barks, leaf and roots of Bersama engleriana plants containing ethyl iso-allocholate compound are widely used in the medicinal treatment of stomach disorders, such as abdominal pain, colic, diarrhea, cholera, intestinal worms, amoebiasis and dysentery. Feronia elephantum is one of the most important medical plant belongs to Rutaceae family, it's commonly known as wood apple. Various parts of wood–apple have been used against various diseases in ethnomedicine studies. Juice of young leaves is mixed with milk and sugar candy given as remedy for biliousness and intensive troubles of children. A powdered gum mixed with honey, is given to overcome dysentery and diarrhea in children. leaf of F. elephantum showed more potent anti- inflammatory acitivity at the dose of 400 mg/Kg when compared with standard drug indomethacin.Phenol, 4[2(dimethylamino)ethyl], 2, 3Dimethylquinolin4(1H)one(Alkaloid), Ethylisoallocholate (Steroid) and Phenol, 4-(3-hydroxy-1-propenyl)-2-methoxy-,3-2-N-Acety l-N-methylaminoethyl) indol (Steroid), Cholesta-8,24-dien-3-ol,4-methyl-, (3a,4a')-(Steroid) were reported in the ethanol extract of F. elephantum leaf and bark by GC-MS analysis17. These compounds may have the role in inanti-inflammatory.

Ethyl iso- allocholate



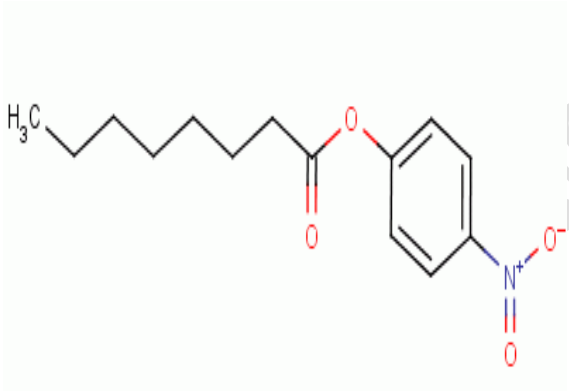
Trigonella foenum graecum L. is also known as fenugreek belongs to family Fabaceae. It is an annual herb indigenous to Mediterranean countries and is cultivated throughout Asia.the steroidal compound, ethyl iso-allocholate isolated from T. foenum graecum L. seeds are opposed to A549 lung cancer cells in vitro and in vivo. the isolated compound ethyl iso-allocholate showed the cytotoxicity against HepG2, hepatocellular cancer cells which was mediated by upregulation of PCNA, Bax, and caspase 3activation . The seed extract also

induced apoptosis in breast and colon cancer cells via intrinsic and extrinsic pathway.

Octanoic acid:

Octanoic acid is linear saturated fatty acids, ubiquitous in nature. Structure of Octanoic acid and decanoic acids are similar but differ in 2 C-atoms. It is biodegradable. Fatty acids are nutrients for microorganisms and are mineralized to CO_2 and water through β-oxidation by microbial activity. Caprylic acid is chemically known as octanoic acid. Caprylic acid acts as antibacterial, antiviral, antifungal and anticancer agent. Octanoic acid is found naturally in the milk of various mammals like Goat buffalo, female yak, zebu, sheep and camel, small amount in coconut oil and palm kernel oil. The medicinal value of goat milk contains three fatty acids, namely capric, caprylic and caproic acids.

Octanoic acid



The fatty acid compounds are against to the human colorectal, skin and mammary gland cancer cells. It is used for the treatment of vaginal yeast infections, candida, ringworm, thrush, simply managing the high blood pressure, treatment of Crohn's disease and reduce the cholesterol levels, it is sold in many foods store, medical store and dietary supplements health care center. Octanoic acid producing microalgae are *Scenedesmus* sp., *Desmodesmus communis*., *Chlorella emersonii*., *Neodesmus* sp., *Nannochloris* sp., *Chlorococcum* sp., *Picochlorum oculatum*., *Phormidium* sp., *Spirogyra* sp. and plant *Lawsonia inermis Linn*. Caprylic acids have a various important role in anti-cancer, anti-aging, anti-Alzheimer's, anti-Autism, anti-infection and general circulatory improvement. Caprylic Acid with Omega-3 Fish Oil is used as antiviral agent; caprylic acid increases the normal cell telomere (NCT). Caprylic acid act as a blood-brain barrier, because of their application in anticonvulsant, slowed progression of weakness and protected spinal cord motor neuron loss. Caprylic acid can create an acidic environment to inactivate bacteria. Caprylic acid suppresses the IL-8 secretion.

Materials and Methods:

Nile Red Staining

A stock solution of Nile red (0.1 to 10 µg/ml) in acetone was prepared and stored in dark condition to protect from light. Staining could be carried out on either fixed (1.5% glutaraldehyde for 5 min) or unfixed cells. Isolated cells (1-2 * 10^{6}/ml) were suspended in phosphate buffered saline; attached cultured cells were covered with PBS. The dye was then added directly to the preparation of effect a 1:50/ 1: 100 dilution and the preparation were incubated for a minimum of 5-10 minutes in the darkness at room temperature.

Extraction of essential compounds from Microalgae

The cells were obtained from 40ml medium containing culture flask, centrifuged at 8000X g for 5 min, at 15c and washed once with distilled water. The fresh culture cells were well homogenized using a mortar and pestle for 20 min at room temperature. Extraction of lipids from wet biomass was performed according to the procedure of Sharif Hossain & Salleh (2008).The algal cells were spread over a clean glass plate for air drying. The dried biomass was mixed with citric acid for making

algal beads. Allow it for oven drying at 120c for 1 min. Fatty acid presenting in the algal cell contents were extract using petroleum ether, catalyst such as NaOH and methanol. The dried biomass was soaked with petroleum ether (1:1 by vol) solvent in a beaker overnight. The yellow colored oil extracts were collected on the top of the solution and mixed with catalyst (0.30g NaOH and ^{2ml} of methanol). Then allow that the solution for 16h to settle their sediments clearly.

GC-MS studies

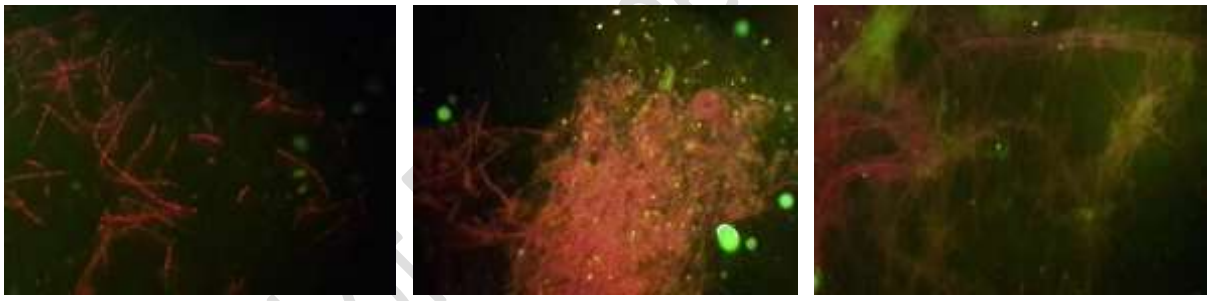
The collected samples were processed with JEOL GCMATE II (GC-MS with Data system is a high resolution, double focusing instrument. Maximum resolution: 6000 Maximum calibrated mass: 1500 Daltons. Source options: Electron impact (EI); Chemical ionization (CI)). The sample was evaporated in a split less injector at 300°c. The fatty acids were quantified by a gas chromatography. The column (HP5) were fused silica 50m x 0.25 mm I.D. Analysis in 20 minutes at 100°C the 3°/ min to 235°C for column temperature, 240°C for injector temperature, helium was the carrier gas. The weight percentages of extracted sample containing compound were approximated by the area of the detector response. The compound was identified by GCMS.

Results and Discussion

Nile Red Staining

Nile red fluorescence can be viewed at 515-560nm. The fluorescence intensity of the stained micro algal cells was measured by using fluorescence microscope with imaging software. The chlorophylls present in algal cells have the ability to auto fluorescence as red color; lipids present in algal cells have the ability to auto fluorescence as yellow color (Fig: 1).

Fig. 1: Morphologically identified microalgae strains from salt pan - Nile Red Staining



1.) ***Nostoc sp.*** **2.)** ***Lyngbya sp.*** **3.)** ***Phormidium sp.***

GC MS:

Gas chromatography is used to identify the anticancer compound. The chromatograms show several compounds at various retention periods. The spectrum data base software installed in GC-MS. The anticancer comounds such as Quinolin, 5-nitro-, 1-oxide - (15.53 Retention time), Quinazolin-4(3H)-one,3(3-methoxyphenyl)-2-(2-phenylethenyl)- - (24.25 Retention time) and Flavone - (16.87 Retention time) were observed in *Nostoc sp* (Fig 2 and Table 3). Ethyl iso- allocholate- (45.88 Retention time) and Octanoic acid, 4-nitrophenyl ester (45.352 Retention time) present in *Lyngbya sp* ((Fig 3 and Table 4) and Flavone - (15.07 Retention time) present in *Phormidium sp* (Fig 4 and Table 5).

Fig 2: The GC spectrum of *Nostoc* sp.

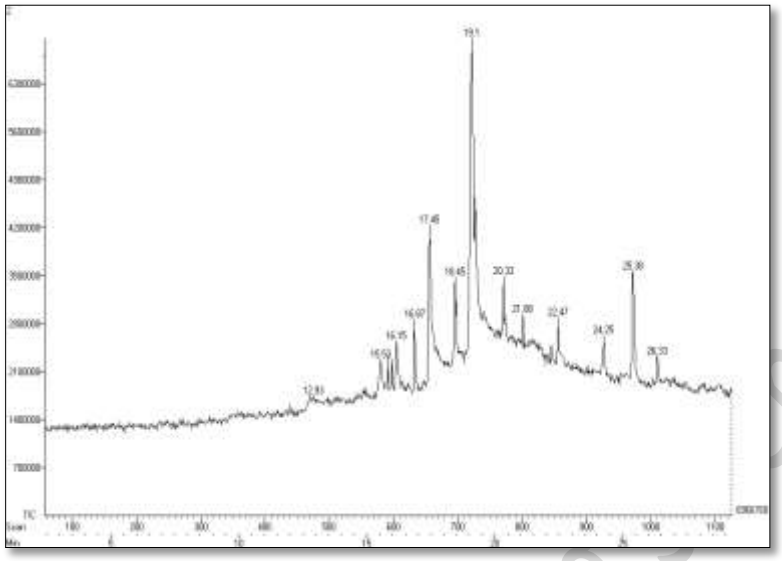


Table 3: Molecular weight and retention time of anticancer compound obtain from GC-MS of *Nostoc* sp.

Retention time	Compound	Chemical formula	Molecular weight
12.93	Allyl (2-methylphenyl) sulfide	$C_{10}H_{12}S$	164.266
15.53	Quinolin , 5-nitro-, 1-oxide	$C_9H_6N_2O_3$	190.158
16.15	2H-Indeno[1,2-b]furan-2-one, 3,3a,4,5,6,7,8,8b-octahydro-8,8-dimethyl	$C_{13}H_{18}$	206.281
16.87	Flavone	$C_{15}H_{10}O_2$	222.243
17.45	Cetylic acid	$C_{16}H_{32}O_2$	256.43
18.45	16-Octadecenoic acid, methyl ester	$C_{19}H_{36}O_2$	296.495
19.1	Oleic acid	$C_{18}H_{34}O_2$	282.468
22.47	Tricosan-12-ol	$C_{23}H_{48}O$	340.636
24.25	Quinazolin-4(3H)-one,3(3-methoxyphenyl)-2-(2-phenylethenyl)-	$C_{20}H_{14}N_2O$	298.345

Fig 3: The GC spectrum of *Lyngbya sp.*

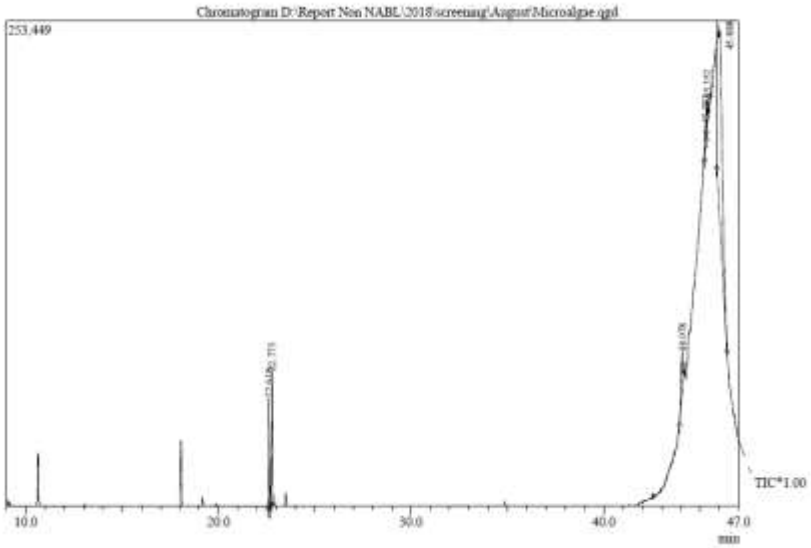


Table 4: Molecular weight and retention time of anticancer compound obtain from GC-MS of *Lyngbya sp.*

Retention time	Compound	Chemical formula	Molecular weight
22.618	6-Tridecane	6-$C_{13}H_{24}$	180
22.775	10-Nonadecenoic acid, methyl ester	$C_{20}H_{38}O_2$	310
44.078	4-Nitrophenyl laurate	$C_{18}H_{27}NO_4$	321
45.283	2-Dodecylcyclohexanone	$C_{18}H_{34}O$	266
45.352	Octanoic acid, 4-nitrophenyl ester	$C_{14}H_{19}NO_4$	265
45.888	Ethyl iso- allocholate	$C_{26}H_{44}O_5$	436

Fig 4: The GC spectrum of *Phormidium sp.*

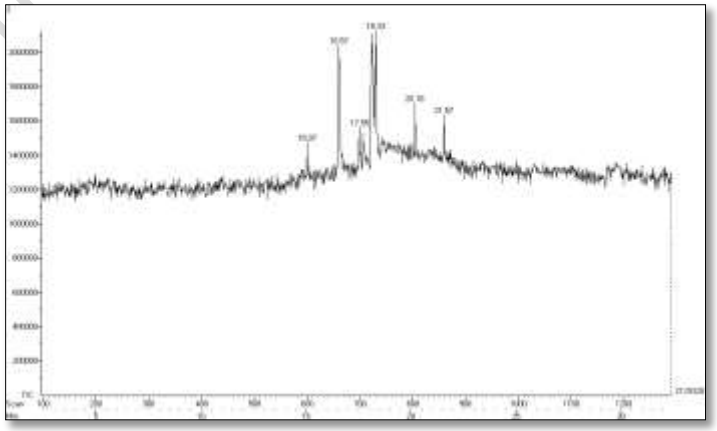


Table 5: Molecular weight and retention time of anticancer compound obtain from GC-MS of *Phormidium sp.*

Retention time	Compound	Chemical formula	Molecular weight
15.07	Flavones	$C_{15}H_{10}O_2$	222.243
16.57	Palmitic acid	$C_{16}H_{32}O_2$	256.42
18.33	Octadecanoic acid	$CH_3(CH_2)_{16}COOH$	284.5
20.18	Nonadecane-2,4-dione	$C_{19}H_{36}O_2$	296.5
21.57	Methoxyacetic acid, octadecyl ester	$C_{21}H_{42}O_3$	342.6

Conclusion:

Microalgae are playing an important role in production of various secondary metabolites such as alkaloid (Quinolin, 5-nitro-, 1-oxide),flavones were identified from *Nostoc sp*. flavones were identified from *Phormidium sp.* alkaloid (caprilic acid acid or Octanoic acid, 4-nitrophenyl ester)and Steroid (ethyl iso allocholate) compounds were identified from *Lyngbya sp. Nostoc sp.*, and *Lyngbya sp.* contains more anticancer activity than the phormidium sp. because presence of the number of secondary metabolites. The improving of high quality anticancer compound can be achieved via genetic and metabolic engineering.

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References

1. Amritpal Singh Saroya, 2011. "Herbalism, Phytochemistry and Ethnopharmacology". *Science Publishers*, Enfield, New Hampshire.
2. Atta-ur- Rahman, 2005. "Cumulative general subject index volumes 1-30". *Studies in Natural Products Chemistry*, *Elsevier*, **31**:3-869.
3. Bhuvaneshwari M., Iswarya V., Archanaa S., Madhu G.M., Kumar G.K.S., Nagarajan R., Chandrasekaran N and Mukherjee A, 2015. "Cytotoxicity of ZnO NPs towards fresh water algae Scenedesmus obliquus at low exposure concentrations in UV-C, visible and dark conditions". *Aquatic Toxicology*, **162**, p 29-38.
4. Chen K, Feng H, Zhang M & Wang X, 2003. "Nitric oxide alleviates oxidative damage in the green alga Chlorella pyrenoidosa caused by UV-B radiation". *Folia microbiol*. **48**: 389–93.
5. da Silva M.F..G.F., Fernandes J.B., Forim M.R., Vieira P.C., de Sá I.C.G, 2013. "Alkaloids Derived from Anthranilic Acid: Quinoline, Acridone, and Quinazoline". In: Ramawat K., Mérillon JM. (eds) *Natural Products*. *Springer*, Berlin, Heidelberg.
6. Demay, J., Bernard, C., Reinhardt, A and Marie B, 2019. "Natural Products from Cyanobacteria: Focus on Beneficial Activities". *Marine drugs*, **17**(6), 320.
7. Garcia-Pichel, F and Castenholz, R.W, 1993. "Occurrence of UV-absorbing, mycosporine-like compounds among cyanobacterial isolates and an estimate of their screening capacity".

International Nuclear Information System (INIS).

8. Gihan A. El Shoubaky, Mohamed M. Abdel-Daim, Mohamed H. Mansour and Essam A. Salem, 2016. "Isolation and Identification of a Flavone Apigenin from Marine Red Alga Acanthophora spicifera with Antinociceptive and Anti-Inflammatory Activities". *J. Exp. Neurosci.*, **10**: 21-29.
9. Hanaa H. Abd El-Baky, Farouk K. El Baz and Gamal S. El-Baroty, 2009. "Production of phenolic compounds from Spirulina maxima microalgae and its protective effects in vitro toward hepatotoxicity model". *African Journal of Pharmacy and Pharmacology*, **3**(4). pp. 133-139.
10. K.C. Das, Thomas T. Adams, Mark A. Eiteman, John Stickney, Joy Doran Peterson, James R. Kastner, Sudhagar Mani and Ryan Adolophson, 2011. "Biorefinery and Hydrogen Fuel Cell Research". *Georgia Environmental Facilities Authority, University of Georgia*, Athens GA.
11. Kant R, Kumar D, Agarwal D, Gupta RD, Tilak R, Awasthi SK and Agarwal A, 2016. "Synthesis of newer 1,2,3-triazole linked chalcone and flavone hybrid compounds and evaluation of their antimicrobial and cytotoxic activities". *Eur. J. Med. Chem.*, **113**: 34-49.
12. Linda M. Mascavage, Serge Jasmin, Philip E. Sonnet, Michael Wilson and David R. Dalton, 2010. *Ullmann's Encyclopedia of Industrial Chemistry*.
13. Lorenzo Zanella, Venice, Paolo Pertile, San Pietro Viminario, Michele Massironi, Padua, Marco Massironi, Padua, Elisa Caviola and Pieve d ' Alpago, 2018. "Extracts of Microalgae and Their Application". *United States Patent*, US009974819B2.
14. K. m, , Anand Anbarasu and Sudha Ramaiah, 2016. "Ethyl Iso-allocholate from a Medicinal Rice Karungkavuni Inhibits Dihydropteroate Synthase in Escherichia coli: A Molecular Docking and Dynamics Study". *Indian J Pharm Sci*;**78**(6):780-788.
15. Moffitt, M. C., Louie, G. V, Bowman, M. E., Pence, J., Joseph P & Moore BS, 2007. "Discovery of two cyanobacterial phenylalanine ammonia lyases: kinetic and structural characterization". *Biochemistry*, **46**: 1004–12.
16. Nazir Ahmad Wani and Sharmila Tirumale, 2018. "Phytochemical analysis and biomass estimation of Chaetomium cupreum extracts through submerged fermentation (SmF)". *Research Journal of Pharmacognosy and Phytochemistry*, **10** (1).
17. Nur Husna Azim, Atiqah Subki and Zetty Norhana Balia Yusof, 2018. "Abiotic stresses induce total phenolic, total flavonoid and antioxidant properties in Malaysian indigenous microalgae and cyanobacterium". *Malaysian Journal of Microbiology*, **14**(1): 25-33.
18. Rajnish Kumar, Ashwani Kumar, Sandip Jain and Darpan Kaushik, 2011. "Synthesis, antibacterial evaluation and QSAR studies of 7-[4-(5-aryl-1,3,4-oxadiazole-2-yl)piperazinyl] quinolone derivatives". *European Journal of Medicinal Chemistry*, **46** (9).
19. Runge F, 1834. "Pogg. Ann.", *J. Prakt. Chem.*, **31**, p. 68.
20. Shaheen Begum, Bharathi K and Prasad KV, 2016. "Mini Review on Therapeutic Profile of Phenoxy Acids and Thier Derivatives". *Int. J. Pharm. Pharm. Sci*., **8**(10): 66-71.
21. Sun, Zheng & Liu, Jin & Zeng, Xiaohui & Huangfu, Jieqiong & Jiang, Yue & Wang, mingfu & Chen and Feng, 2011. "Astaxanthin is responsible for antiglycoxidative properties of microalga Chlorella zofingiensis". *Food Chemistry*, **126**, 1629-1635.
22. Suvidha Gupta, Sanjay B. Pawar and R.A. Pandey, 2019. "Current practices and challenges in using microalgae for treatment of nutrient rich wastewater from agro-based industries". *Science of The Total Environment*, **687**, p 1107-1126.
23. Turu, I.Cemre, Turkcan-Kayhan C, Kazan A, Yildiz-Ozturk E, Akgol S, Yesil-Celiktas O, 2016. "Synthesis and characterization of cryogel structures for isolation of EPSs from Botryococcus braunii". *Carbohydrate Polymers*, **150**: 378-384.
24. Vimal Katiyar, Raghvendra Gupta and Tabli Ghosh, 2019. "Advances in Sustainable Polymers Processing and Applications". *Springer*.
25. Yongfeng Guo, 2018. "Plant Senescence Methods and Protocols". *Methods in Molecular Biology, Humana Press, Springer Science+Business Media.*

26. Hariram .V and Mohan Kumar .G, 2012. Identification and characterization of FAME's algal oil methyl ester using FT IT, NMR and GC/MS. International Journal of Emerging trends in Engineering and Development, 2: 5.

27. Benemann, J. R, Goebel. R. P, Weissman. J. C and Augenstein. D. C , 1982. Microalgae as a source of liquid fuels, final technical report to US dept. of energy. Washington DC, US dept. of energy; SAN-**003**-4-2.

28. Tadashi Matsunaga., Mitsufumi Matsumoto., Yoshiaki Maeda., Hiroshi Sugiyama., Reiko Sato and Tsuyoshi Tanaka., Characterization of marine micro alga, *Scenedesmus* sp. Strain JPCC GA0024 toward biofuel production 2009. Biotech Let DOI 10.1007/s10529-009-0029-y.

29. Sharif Hossain ABM and Salleh A (2008) Biodiesel fuel production from algae as renewable energy. Amer. J. Biochem. **4(3)**, 250-254

30. Indhumathi Ponnuswamy, Soundararajan Madhavan and Syed Shabudeen, 2013. Isolation and Characterization of Green Microalgae for Carbon Sequestration,Waste Water Treatment and Bio-fuel Production. International Journal of Bio-Science and Bio-Technology. **Vol 5**.

31. C. Hoek and D. Mann, 1997.Algae an introduction to physiology, Cambridge University press, 32: 203±205.

32. Sonntag, N. O. V. 1982. Glycerolysis of Fats and Methyl esters. Journal of American Oil Chemists Society 59, n° 10 p. 759.

33. Tadashi Matsunaga., Mitsufumi Matsumoto., Yoshiaki Maeda., Hiroshi Sugiyama., Reiko Sato and Tsuyoshi Tanaka., 2009, Characterization of marine micro alga, *Scenedesmus* sp. Strain JPCC GA0024 toward biofuel production . Biotech lett DOI 10.1007/s10529-009-0029-y.

34. Zhu M, Zhou P. P, Yu L. J. 2002. Extraction of lipids from Mortierella alpina and enrichment of arachidonic acid from the fungal lipids. Bioresource Technology. **84**: 93-95.

35. J. De la Noue and N. De Pauw, 1988. The potential of micro algal biotechnology: a review of production and uses of micro algae, **vol 6**, pp. 725–70.

36. R. A. Andersen, 2005. Algal cultural techniques. Elsevier academic press, London.

37. W. Becker, 2004.Microalgae in human and animal nutrition. A. Richmond, Ed., Handbook of micro algal culture: biotechnology and applied phycology, Blackwell Publishing, pp. 312-346**.**

38. L. G. Benning, V. R. Phoenix, N. Yee and M. J. Tobin, 2004. Molecular characterization of cynaobacterial signification using synchrotron infrared micro-spectroscopy, Geocimica et Cosmochimica acta. **vol. 68**, pp. 729-741**.**

39. J. De la Noue and N. De Pauw, 1988. The potential of micro algal biotechnology: a review of production and uses of micro algae, **vol 6**, pp. 725–70.

40.Andersen, O. . M. and Markham, K. R. 2006. *Flavonoids: Chemistry, Biochemistry and applications.* CRC Press, Boca Raton, USA.

41.Harborne, J. B. & Williams, C.A. 2000. Advances in flavonoid research since 1992. *Phytochemistry* **55**: 481–504.

42.López, A., Rico, M., Rivero, A. & Suárez de Tangil, M. 2011. The effects of solvents on the phenolic contents and antioxidant activity of Stypocaulon scoparium algae extracts. *Food Chem.* **125**: 1104–9.

43.Markham, K.R. 2006. Preface In Andersen, O. M. & Markham, K. R. Flavonoids: Chemistry, Biochemistry and applications. CRC Press, Boca Raton, USA.

44.Markham, K. R. and Porter, L. J. 1969. Flavonoids in the green algae (chlorophyta). *Phytochemistry* **8**: 1777–81.

45.Moore, B. S., Hertweck, C., Hopke, J. N., Izumikawa, M., Kalaitzis, J. A., Nilsen, G., O'Hare, T., Piel, J., Shipley, P. R., Xiang, X. L., Austin, M. B. & Noel, J. P. 2002. Plant-like Biosynthetic Pathways in Bacteria : From Benzoic Acid to Chalcone. *J. Nat. Prod.* **65**: 1956–62.

46.K. Malathi, Anand Anbarasu and Sudha Ramaiah (2016). Ethyl Iso-allocholate from a Medicinal Rice Karungkavuni Inhibits Dihydropteroate Synthase in

Escherichia coli: A Molecular Docking and Dynamics Study. Indian J Pharm Sci;**78(6)**:780-788.

47.Never Zekeya, Musa Chacha, Francis Shahada and Abdul Kidukuli (2014). Analysis of phytochemical composition of Bersama abyssinica by gas chromatography – mass spectrometry. Journal of Pharmacognosy and Phytochemistry ; **3(4)**: 246-252