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Microalgae *Chlorella Vulgaris*isolation and GCMS Analysis of Phytocomponent Methanolic Extracts

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***Abstract*:** This study focuses on the isolation of *Chlorella vulgaris*, a unicellular microalga with therapeutic and nutritional properties, from a freshwater pond in West Bengal, India. The methanolic extract of *C. vulgaris* was analyzed using Gas Chromatography-Mass Spectrometry (GC-MS) to identify its phytochemical components. Eight fatty acid methyl esters were identified, with compounds 4, 5, 6, and 7 being the major constituents. Compound 4 exhibited properties such as preventing hair loss and acting as an antioxidant, while compound 7 showed hepatoprotective, anti-inflammatory, and anti-cancer properties. Compound 5 displayed antifungal and antioxidant activities, while compound 6 showed anti-inflammatory and cholesterol-lowering effects. Compound 2 was also identified, known for its presence in various oils. This study sheds light on the diverse bioactive compounds presents in *C. vulgaris*, highlighting its potential applications in various fields including medicine and nutrition.

Keywords:*Microalgae, Chlorella vulgaris, isolation, GC-MS analysis, phytocomponents, nutritional properties*

1. Introduction:

Microalgae are the lower eukaryotic microorganisms containing only one single cell produces photosynthesis with a wide range of field. Generally, they are grown in aquatic environments. Microalgae has various advantages for large-scale sustainable production, such as high biomass yields per unit area and the capacity to grow on non-arable land with non-potable water or even seawater[1]. In this present situation global warming and carbon dioxideissue is the biggest problem of environment. In current decennial,based on microalgae,carbon dioxideadoption has arisen because of its long-term viability and the conversion of carbon dioxide into oxygen. Also, the scientific community concentrated on improving microalgae's ability to repair carbon dioxide [2,3,4]. Microalgae, like any other phytoplankton, are medicinally valuable.

Chlorella vulgaris, a green, unicellular microalgae that is recognized for its therapeutic properties and biological properties. Because of its unique combination of functional macro- and micronutrients, inclusive as Proteins, minerals, vitamins, polysaccharides, and omega-3 polyunsaturated fatty acids. It is also a useful source of nourishment for humans. Its characteristics include those against cancer, imbalance of free radicals and antioxidants in the body, and COPD. It widely used as food supplement.[5,6,7] Additionally, it fights against hyperglycemia and hyperlipidemia and reduce cardiovascular risk factors [8]. *C. vulgaris* shows anti-inflammatory, anti-oxidant and anti-tumor activity [9,10]. Many businesses make use of microalgae effectively. The biofuel business is presently one of the most investigated fields. For the purpose of producing biodiesel, extracted lipids are primarily transformed to fatty acid methyl esters [11]. According to GC-MS data, *C. vulgaris* has a large production capacity for fatty acid methyl ester.

2. Material and Methods:

2.1. Sample collection:

Microalgal sample is collected from freshwater pond of Uluberia locality near Bharat Technology, Howrah district, West Bengal. Sample is collected in the time of summer when the pond is in blooming condition. Local wastage is drained out inside the pond.

2.2. Microalgal Algal sp. Isolation:

The water sample of microalgae containing different sp. of microalgae. From them one single species is isolated by washing out and subculturing the algae in Algae culture media in aseptic condition. By this purification of strain is done. Help them to grow in suitable atmospheric condition in laboratory method. Under temperature and light control chamber.

2.3. Sp. identification:

Microalgal sample*Chlorella vulgaris* is identified by Dr. R. K. Gupta, Scientist- 'E', in the voucher no CNH/Tech.II/2023/195 atCentral National Herbarium, Botanical survey of India, Howrah, 711103. ThePharmacognosy Department at Bharat Technology, Uluberia, Howrah, 711316, has preserved of the algal species.

2.4. Extract preparation:

*Chlorella vulgaris*growing algal culture media is centrifuged in cold centrifuge.Total mass is collected and supernatant fluid is thrown out. Perform maceration by methanol and filtered out the methanolic extract.

3. GC-MS analysis:

The Shimadzu QP 2010 Ultra (Shimadzu, Tokyo, Japan) was applied for the Gas Chromatography Mass Spectroscopy study of the microalgae *Chlorella vulgaris* extracted by methanol. The GC-MS consists with an MS, ECD, and FID detector. GC-MS detection is carried out utilizing an equipment which has electron ionization power with ionization energy of 70 eV that operates in an electron impact mode. A split ratio of 1:10 was chosen to regulate the flow rate of helium (99.999%, AGA Lithuania) carrier gas to 14.1 mL/min with a 1.00µl injection volume. The injector and ion source temperatures remained at 250°C and 200°C, respectively.The oven temperature was set at 70°C (hold for 5 minutes), then increased by 10°C every minute until 310°C. Mass spectra of particles ranging in size from 45 to 450 Da were acquired at 70 eV throughout a 0.5-second scan period. The GC/MS run lasted sixty-three minutes, and the solvent delay ranged from zero to 4.5 minutes.

4. Phytoconstituents Identification:

Interpretation using mass spectrum GC-MS was conducted. The database of the Central Instrumentation Laboratory (CUPB, Ghudda, Bathinda) has patterns of 62000. The unknown components emission spectrum was compared with known components comprised in the NIST library. The test materials' components were recognized based on their chemical names, molecular mass, and structure.

5. Result:

The GC-MS chromatogram of methanolic extract of *Chlorella vulgaris*figured out 8 peaks, gives the evidence of phytochemical component. Table 1 shows the 8 phytocompounds identified by cross-referencing with the CUPB library's mass spectra. Table 2 exhibits the mass spectrum of all phytocompounds found in the methanolic extract of *Chlorella vulgaris*.

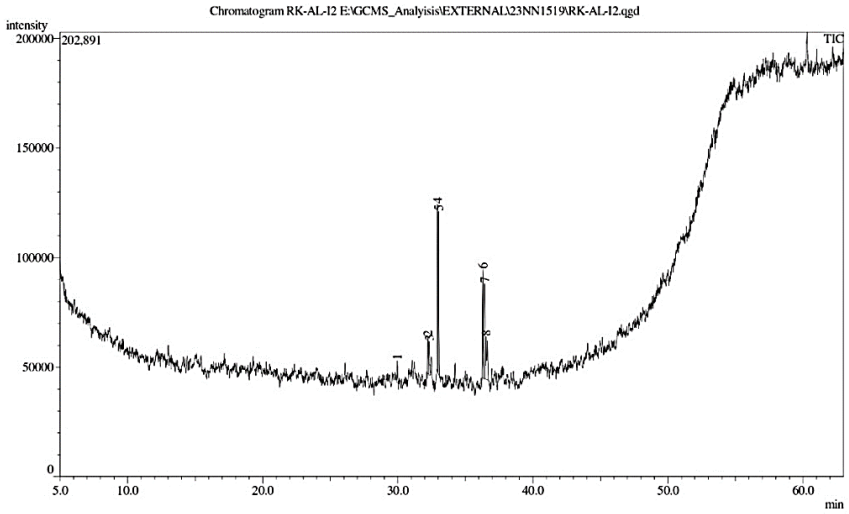


Figure 1: GC-MS chromatogram of*Chlorella vulgaris* methanolic extracts.

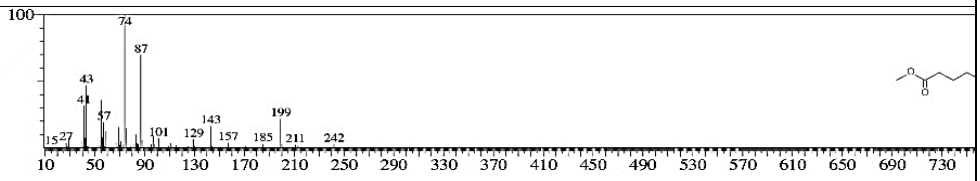
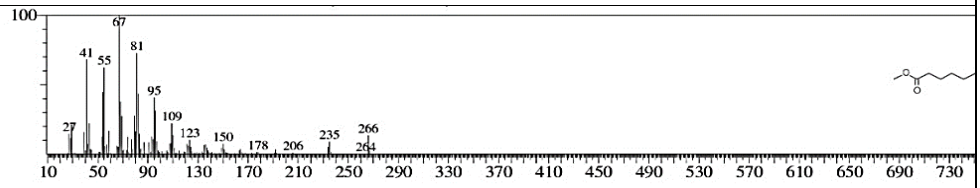
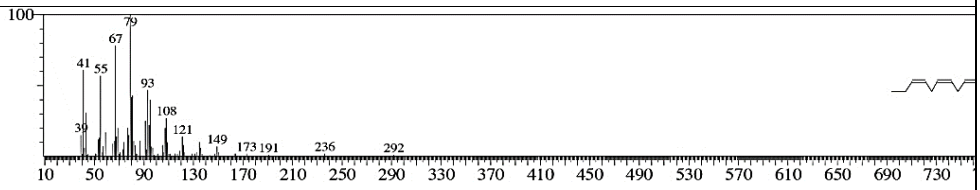
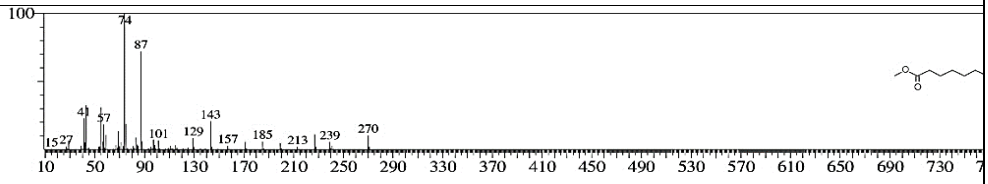
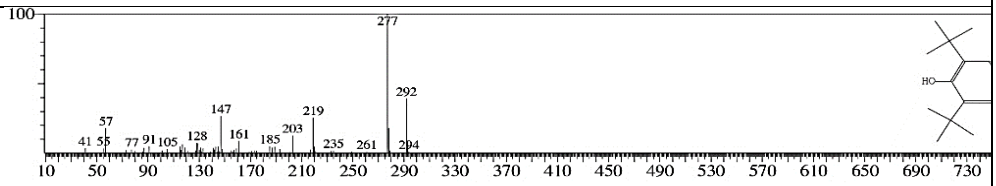
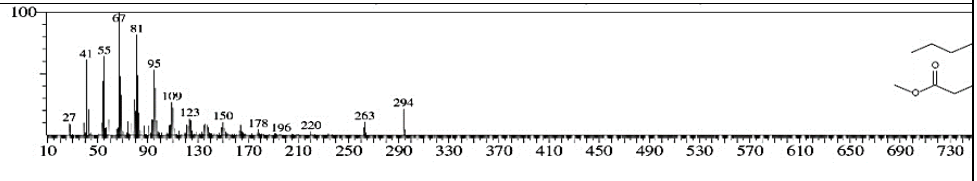
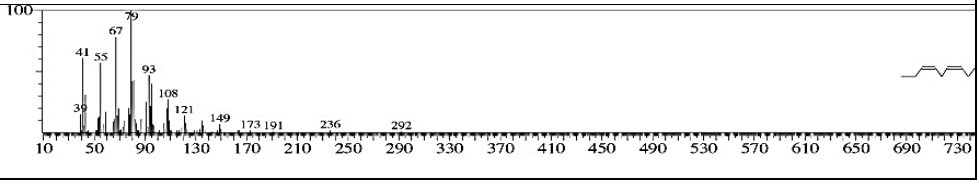
Table 1: Analysis of *Chlorella vulgaris* phytocomponents by GC-MS

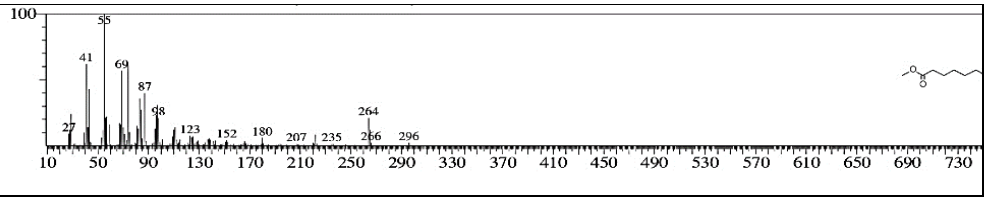
Peak number	R. time	Height %	Peak area %	A/H	Mark name	Chemical formula	Molecular weight	Compound number	Biological activities
1	29.968	3.24	2.29	3.21	Tridecanoic acid, 12-methyl-, methyl ester	C15H30O2	242	1	-
2	32.219	5.49	4.99	3.24	9,12-Hexadecadienoic acid, methyl ester	C17H30O2	266	2	-
3	32.326	5.10	5.92	4.15	9,12,15-Octadecatrienoic acid, methyl ester	C19H32O2	292	3	Hepatoprotective, antihistaminic, antieczemic, antiacne, antiarthritic, antiandrogenic, anti-inflammatory, anti-cancer, and cholesterol lowering [13]
4	32.936	24.94	21.45	3.07	Hexadecanoic acid, methyl ester	C17H34O2	270	4	Prevent hair loss, 5-Alpha reductase inhibitor antioxidant, anti-

									fibrinolytic, cholesterol-lowering, androgen antagonist, destroy red blood cell, and nematicide action [14]
5	33.025	24.33	20.99	3.08	Benzenepropanoic acid, 3,5-bis(1,1-dimethylethyl)-4-hydroxy-, methyl ester	C18H28O3	292	5	Antifungal, antioxidant [15]
6	36.300	16.27	14.26	3.13	9,12-Octadecadienoic acid (Z,Z)-, methyl ester	C19H34O2	294	6	Anti-inflammatory, anti-histaminic, antiarthritic, anti-coronary, and androgen antagonist activity, lowers cholesterol level, prevent cancer, protect liver from damage, nematicide activity [16]
7	36.419	14.21	16.51	4.14	9,12,15-Octadecatrienoic acid, methyl ester, (Z,Z,Z)	C19H32O2	292	7	lowers cholesterol and possesses hepatoprotective, antihistaminic, antieczemic, antiacne, antiarthritic, antiandrogenic, anti-inflammatory, and anti-cancer properties. [13]

8	36.544	6.42	12.96	7.20	16-Octadecenoic acid, methyl ester	C19H36O2	296	8	-

Table 2: Phytocomponents mass spectrum and structure of identified by Gas chromatography mass spectroscopy in the methanolic extracts of *Chlorella vulgaris*

	Compound name	Mass spectrum and structure
1	Tridecanoic acid, 12-methyl-, methyl ester	
2	9,12-Hexadecadienoic acid, methyl ester	
3	9,12,15-Octadecatrienoic acid, methyl ester	
4	Hexadecanoic acid, methyl ester	
5	Benzenepropanoic acid, 3,5-bis(1,1-dimethylethyl)-4-hydroxy-, methyl ester	
6	9,12-Octadecadienoic acid (Z,Z)-, methyl ester	
7	9,12,15-Octadecatrienoic acid, methyl ester	

8	16-Octadecenoic acid, methyl ester	

6. Conclusion:

Study of Gas chromatography and mass spectroscopy methanolic extract of *Chlorella vulgaris* gives 8 compounds, which are all fatty acid methyl esters. The highest peak of area calculated Compound **4**, (21.50%).Compound**5,** gives peak area of 20.99%; Compound**7,**gives 16.51%; Compound **6**present gives 14.26%; Compound **8**gives 12.96%; Compound **3**gives 5.92%; Compound **2**(4.99%); Compound **1**gives 2.29% of peak area. Compound **4**helps to prevent hair loss, antioxidant, anti-fibrinolytic, cholesterol-lowering, antiandrogenic, hemolytic, 5-Alpha reductase inhibitor, and nematicide action. [14]. Compound **7**, (16.51%) is essential component of a diet that lowers cholesterol and possesses hepatoprotective, antihistaminic, antieczemic, antiacne, antiarthritic, antiandrogenic, anti-inflammatory, and anti-cancer properties. [13]. Compound **5**gives antifungal, antioxidant activity[15]. Compound **6**, present (14.26%) shows anti-inflammatory, anti-histaminic, antiarthritic, anti-coronary, and androgen antagonist activity, lowers cholesterol level,prevent cancer, protect liver from damage, nematicide activity[16]. Compound **2**(4.99%) is a unique fatty acid, found in various fish oil and others oils [12].

7. Acknowledgement:

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8. References:

[1] Torres-Tiji Y, Fields FJ, Mayfield SP. Microalgae as a future food source. Biotechnology advances. 2020 Jul 1; 41:107536.

[2] Goswami RK, Mehariya S, Verma P. Advances in microalgae-based carbon sequestration: Current status and future perspectives. Environmental Research. 2024 Feb 2:118397.

[3] Goswami, R. K., Mehariya, S., Karthikeyan, O. P., Gupta, V. K., & Verma, P. (2022). Multifaceted application of microalgal biomass integrated with carbon dioxide reduction and wastewater remediation: A flexible concept for sustainable environment. Journal of Cleaner Production, 339, 130654.

[4] Hu X, Zhou J, Liu G, Gui B. Selection of microalgae for high CO2 fixation efficiency and lipid accumulation from ten Chlorella strains using municipal wastewater. Journal of Environmental Sciences. 2016 Aug 1; 46:83-91.

[5] Safi, C., Zebib, B., Merah, O., Pontalier, P. Y., & Vaca-Garcia, C. (2014). Morphology, composition, production, processing and applications of Chlorella vulgaris: A review. Renewable and Sustainable Energy Reviews, 35, 265-278.

[6] Panahi, Y., Darvishi, B., Jowzi, N., Beiraghdar, F., & Sahebkar, A. (2016). Chlorella vulgaris: a multifunctional dietary supplement with diverse medicinal properties. Current pharmaceutical design, 22(2), 164-173.

[7] Sherafati, N., Bideshki, M. V., Behzadi, M., Mobarak, S., Asadi, M., & Sadeghi, O. (2022). Effect of supplementation with Chlorella vulgaris on lipid profile in adults: A systematic review and dose-response meta-analysis of randomized controlled trials. Complementary Therapies in Medicine, 66, 102822.

[8] Barghchi, H., Dehnavi, Z., Nattagh-Eshtivani, E., Alwaily, E. R., Almulla, A. F., Kareem, A. K., ... & Pahlavani, N. (2023). The effects of Chlorella vulgaris on cardiovascular risk factors: A comprehensive review on putative molecular mechanisms. Biomedicine & Pharmacotherapy, 162, 114624.

[9] Drossinakis, C., Baiti, S., Karadzhov, S. D., Valcheva, N., Angushev, I., Angelcheva, M., ... & Popova, T. P. (2024). Research on the structuring of water clusters in Chlorella vulgaris water suspension. Plant Science Today, 11(1), 258-265.

[10] Prabakaran, G., Moovendhan, M., Arumugam, A., Matharasi, A., Dineshkumar, R., & Sampathkumar, P. (2019). Evaluation of chemical composition and in vitro antiinflammatory effect of marine microalgae Chlorella vulgaris. Waste and Biomass Valorization, 10, 3263-3270.

[11] Biller, P., & Ross, A. B. (2014). Pyrolysis GC–MS as a novel analysis technique to determine the biochemical composition of microalgae. Algal Research, 6, 91-97.

[12] Nakano, N., Shirasaka, N., Murakami, T., SHIMIZU, S., & YOSHIZUMI, H. (1999). Formation of 8, 11, 14-octadecatrienoic acid (18: 3 n-4) from naturally occurring unique fatty acid, 9, 12-hexadecadienoic acid (16: 2 n-4), in animal cell cultures. Journal of nutritional science and vitaminology, 45(2), 203-212.

[13] Devi, J., & Muthu, A. K. (2014). Gas chromatography-mass spectrometry analysis of bioactive constituents in the ethanolic extract of Saccharum spontaneum Linn. International Journal of Pharmacy and Pharmaceutical Sciences, 6(2), 755-759.

[14] Selvan, P. S., & Velavan, S. (2015). Analysis of bioactive compounds in methanol extract of cissus vitiginea leaf using GC-MS. Rasayan J Chem, 8(4), 443.

[15] Ahmad, B., Khan, I., Bashir, S., & Azam, S. (2012). Chemical composition and antifungal, phytotoxic, brine shrimp cytotoxicity, insecticidal and antibacterial activities of the essential oils of Acacia modesta. J. Med. Plants Res, 6(31), 4653-4659.

[16] Khanday, S., & Sharma, G. D. (2021). GC-MS analysis and antifeedant activity of Azaridiachta indica-leaf extract. Stechnolock Plant Biol Res, 1, 1-15.