International Journal of Science and Research (IJSR)
ISSN (Online): 2319-7064
Index Copernicus Value (2013): 6.14 | Impact Factor (2013): 4.438

Analysis of *Cladophora Glomerata* in High Performance Liquid Chromatography-Mass Spectrometry (HPLC –MS)

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Abstract: ***In this study is mainly focused on the analysis of Cladophora Glomerata with the help of High performance liquid chromatography-mass spectrometry. Algae exhibit a wide range of reproductive strategies, from simple, asexual cell division to complex forms of sexual reproduction. Algae lack the various structures that characterize land plants, such as the phyllids (leaf-like structures) of bryophytes, rhizoids in nonvascular plants, and the roots, leaves, and other organs that are found in tracheophytes. The study is specifically carried in the medicinal availability of algae. Thus its usage can be encouraged in human nutrition and in disease treatment.***

Keywords: *Cladophora Glomerata;* HPLC; MS; Algae; chromatography.

1. Introduction

Algae are a very large and diverse group of simple, typically autotrophic organisms, ranging from unicellular to multicellular forms, such as the giant kelp (large brown algae), that may grow up to 50 meters in length. Most are photosynthetic and "simple" because they lack many of the distinct cell organelles and cell types found in land plants. (P J Keeling et al, 2004) Cladophora is a genus of reticulated filamentous green algae. Cladophora glomerata is a fresh water algae and it is used as edible type used in many dishes as a dry sheets in Japan. The genus Cladophora contains many species that are very hard to tell apart and classify, mainly because of the great variation in their appearances, which is affected by habitat, age and environmental conditions. (Gestinari et al, 2010.)

In this work help to analysis, availability and identification antioxidant and phytochemical component in *Cladophora Glomerata* ethanol extract. The ethanol extract algae was analysis with the in high performance liquid chromatography-mass spectrometry (HPLC – MS).

2. Materials and Methods

2.1. Identification of the Algae Sample

The algae which is taken from the pond of a village near Vellore district, Tamil Nadu, India, is been identified by the Centre for Advance Studies (CAS) in Botany Department, University of Madras. The algae are identified as Cladophora glomerata.

2.2. Preparation Extract

The algae is washed in the fresh water and the impurities was been removed. Algae sample is dried in the hot air oven. The dried sample is weighed (10 grams) and soaked in the 100 ml of ethanol for about two weeks in room temperature (RT). After two weeks the extract is been filtered using Whatman No. 1 filter paper. The extract samples has been stored at 2 ^{0}C until use.

2.3. Purification of Algae extract

The crude algae extract has been purified for the further analysis. The crude extract is been deproteinated by using TCA (Trichloroacetic acid). Further the deproteinated extract is been purified using column chromatography. The column was made of ion exchange buffered resin.

2.4. High Performance Liquid Chromatography

2.4.1. Principle

The purified extract is been analyzed using High Performance Liquid Chromatography- Mass Spectrometry (HPLC). This is been done in the IIT of madras. The basis of all chromatography is the partition or distribution coefficient (K_d), which describes the way in which a compound distribution itself between two immiscible phase. For two such immiscible phase A and B the values for this coefficient is a constant at a given temperature and is given by the expression:

$$K_d = \frac{concentration \quad in\ phase\ A}{concentration \quad in\ phase\ B}$$

The term effective coefficient is defined as the total amount, as distinct from the concentration, of substance present in one phase divided by the total amount present in the other phase. It is in fact the distribution coefficient of a compound between two phases A and B in one, and if this compound is distributed between 10cm^3 of B, the concentration in the two phases will be the same, but the total amount of the compound in phase B.

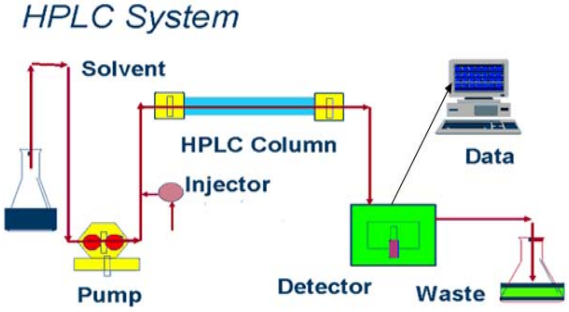
2.4.2. Procedure

The sample was added to the HPLC chamber, the HPLC chamber is made of the column which is made up of silica gel. The sample runs in the column along with the mobile phase. The mobile phases consist of two different buffers with one of high pH and other of low pH. When the high pH buffer is added first and the then the buffer with low pH is added slowly to decrease the pH of the mobile phase. As the

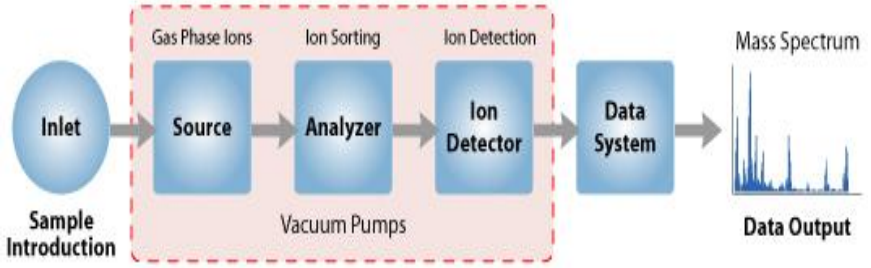
pH changes the samples will be separated accordingly and the elutant is observed using a UV spectroscopy and the graph is plotted based on the concentration of the elutant. The elutant graph is shown in the figure 6. The elutant is identified along with the standard graph and the elutant is further analyzed in the mass spectroscopy to identify the structure of the compound. The compound with the maximum area is identified and further analyzed for the antibacterial and anticancer activity assay.

2.5 Mass Spectroscopic

A mass spectrometer generates multiple ions from the sample under investigation; it then separates them according to their specific mass-to-charge ratio (m/z), and then records the relative abundance of each ion type. The first step in the mass spectrometric analysis of compounds is the production of gas phase ions of the compound, basically by electron ionization. This molecular ion undergoes fragmentation. Each primary product ion derived from the molecular ion, in turn, undergoes fragmentation, and so on. The ions are separated in the mass spectrometer according to their mass-to-charge ratio, and are detected in proportion to their abundance. A mass spectrum of the molecule is thus produced. It displays the result in the form of a plot of ion abundance versus mass-to-charge ratio. Ions provide information concerning the nature and the structure of their precursor molecule. In the spectrum of a pure compound, the molecular ion, if present, appears at the highest value of m/z (followed by ions containing heavier isotopes) and gives the molecular mass of the compound.



(a)



(b)

Figure 1: (a) The schematic diagram–HPLC system (b) Mass Spectroscopic block diagram

3. Result & Discussions

3.1. Purification of Algae Extracts

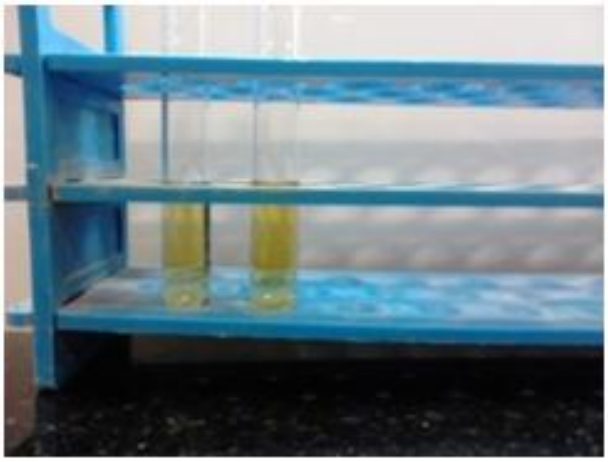


Figure 2: Deproteinated algae extract

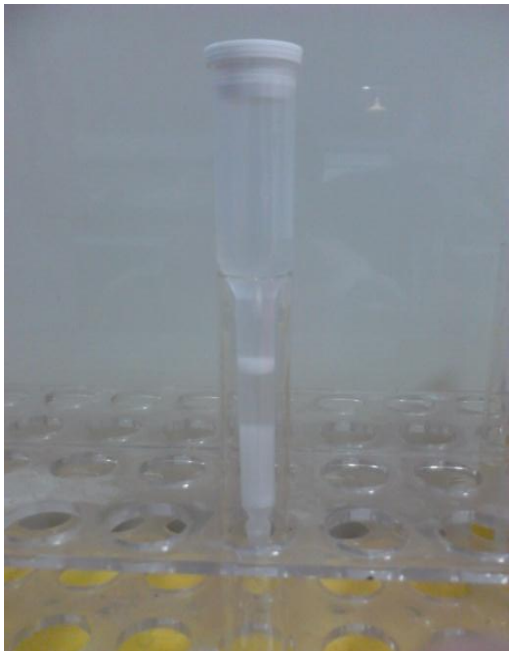


Figure 3: The resin column used for the purification Deproteinated column

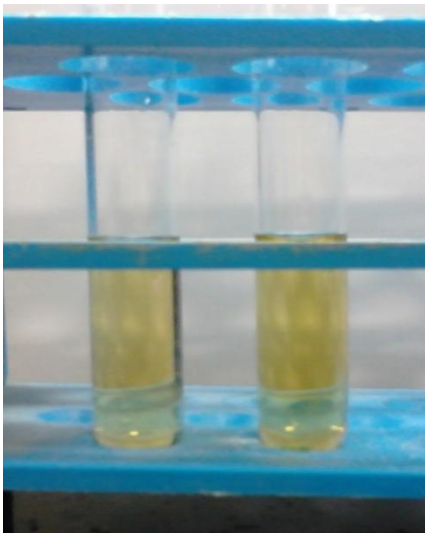


Figure 4: Purified Extract

The purified extract is used of the further HPLC analysis.

3.2. HPLC-MS Analysis

The purified fraction from column chromatography is subjected to HPLC-MS to analyze the chemical constituents. In the second active fraction, Octacosane was found to be a major compound (11.66%), followed by pentadecane, hexly- (10.01%), tridecane, 8-hexyl- (8.76%), Heptadecane hexyl (3.93%), Heptadecane, 3-methy- (3.45%), tridecane, 7-hexyl (3.43%) and Heptadecane, 9-hexyl shown in the table 3. The hydrocarbons distribution pattern mainly in Cladophora glomerata is closely similar to prokaryotic *Anacystis montane and Botryococcus braunii* belonging to Cyanophycophyta and chrysophycopgyta respectively done by Tellez et al, 2001.

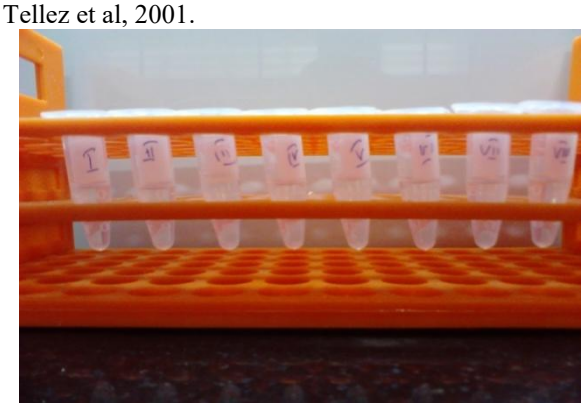


Figure 5: HPLC elutant

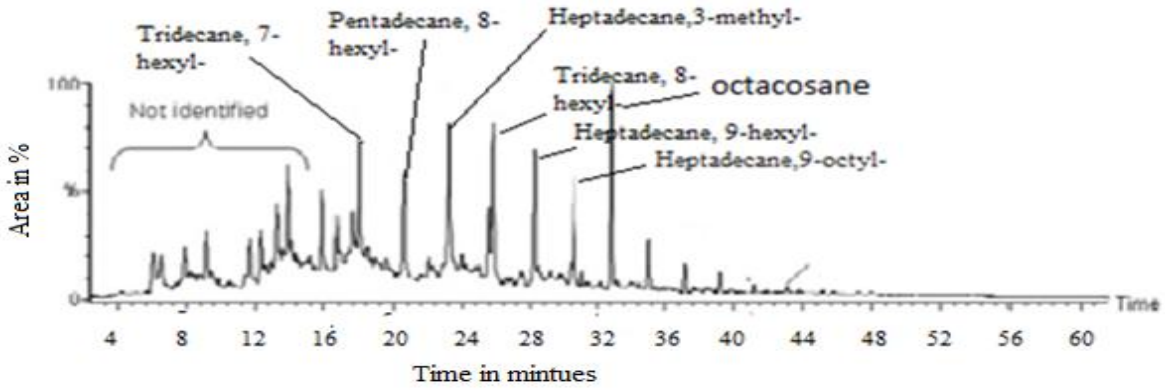


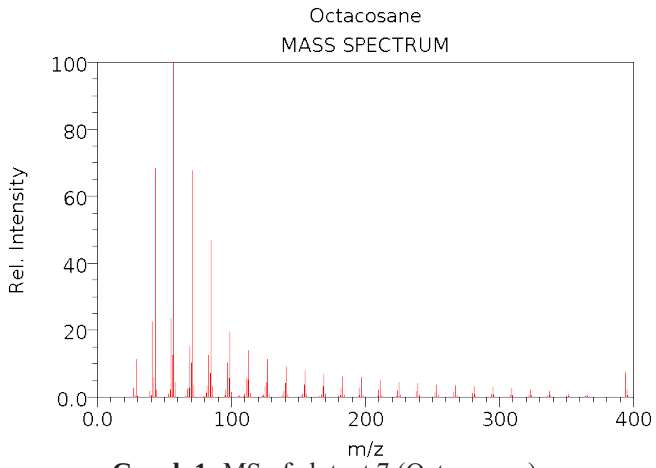
Figure 6: Peaks showned in HPLC

The HPLC elutant no. 7 (Octacosane) shows higher area content, it is further used for antibacterial and anticancer activity.

Table 3: HPLC details for the purified extract of Cladophora glomerata

E.No.	Rt time (min)	Compound name	Area (%)
1	18.12	Tridecane, 7-hexyl-	3.43
2	21.32	Pentadecane, 8-hexyl-	10.01
3	23.45	Heptadecane,3-methyl-	3.45
4	26.54	Tridecane, 8-hexyl-	8.76
5	28.25	Heptadecane, 9-hexyl-	3.93
6	30.35	Heptadecane,9-octyl-	3.21
7	33.53	Octacosane	11.66

The MS of the compound Octacosane, its breakdown is given in the Graph 1. Based on this the compound is identified. It is a linear structure of molecular formula $C_{28}H_{58}$.



Graph 1: MS of elutant 7 (Octacosane)

4. Conclusion

In the current study, the algae from a pond of village are identified as Cladophora glomerata. Then the crude ethanol extract of *Cladophora glomerata* is prepared. The crude extract is been purified using HPLC method and the compound Octacosane is purified. The purified Octacosane is been analyzed for Mass Spectrometry to find the structure.In the *Cladophora glomerata* analyzed with the help of HPLC found the seven components has been isolated. Its help to carry out further studies like antioxidant and phytochemical analysis of *Cladophora glomerata.*

Acknowledgement

The one of the author, L. Dhanasekar are grateful to Mr. P. Senthamil Selvan, Research scholar PG & Research Department of Biochemistry, Maruthupandiyar College, Thanjavur, Tamil Nadu for preparation of manuscript. Grateful thanks are extended to the secretary and correspondent, Principal, Dean of Science, University of Madras, Chennai, My sincere thanks to Head of the Department of Biochemistry for constant encouragement of my work.

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