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Bio chemical analysis of selected red algal seaweeds

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Abstract
Seaweeds are potential renewable resources in the marine environment. About 6000 species of seaweeds have been identified and are grouped into different classes like green (Chlorophytes), Brown (Phaeophytes) and Red (Rhodophytes) algae. They rich in biologically active compounds against pest management, three major seaweeds of *Gracilaria corticata*¸ *Acanthaphora specifera* and *Liagora Ceranoides* showed insecticidal activity against insects and it was explored through the thin layer chromatography, FT-IR spectrometry and ^{1}H Nuclear magnetic resonance (NMR). Thin layer chromatographic movement confirmed that the selected seaweeds of *G. corticata*, *L. ceranoides* and *A. specifera* had definite organic chemical moiety. FT-IR spectral analysis showed that *A. specifera* had polysaccharides or flavanoids. In *G. corticata* extensively hydrated carbonyl compound or polysaccharides and presence of arylated aliphatic ether or anα, β saturated carbonyl compound or its derivatives in *L. ceranoides* were identified. The ^{1}H NMR spectral analysis of *A. specifera* had that major functional component of aromatic protons and alcoholic proton. Whereas, it confirmed the presence of alcoholic and methine proton in *G. corticata* and *L. ceranoides* constituted the presence of methine proton and methyl protons.

Keywords: Bio active compound, pest management, *Gracilaria corticata*¸ *Acanthaphora specifera*, *Liagora ceranoides*, TLC, FT-IR and 1H NMR spectral analysis

1. Introduction
Bio-stimulant properties of seaweeds are explored for use in agriculture and the insecticidal activities for the development of novel insecticides. Seaweeds have some valuable medicinal components such as antibiotics, laxatives, anticoagulants, anti-ulcer products and suspending agents in radiological preparations. Seaweeds have recently received significant attention for their potential as natural antioxidants. Most of the compounds of marine algae show anti-bacterial activities. (Vlahos *et al*., 2010 and Vairappan *et al*., 2001) [7, 6].
To date, there are quite a lot of reports on antibacterial activity of solvent extracts from marine algae. However, there are very few reports pertaining to insecticidal activity of crude solvent extracts from the seaweeds. In this study shows seaweeds had an insecticidal properties which is highly alternative to the chemical insecticides with harmful effects.

2. Materials and Methods
2.1 Collection of samples
Red algal seaweeds were found near the Mandapam coastal Rameshwaram, India. The seaweeds collected by hand picking method were immediately washed in fresh sea water. Collected seaweeds were carefully washed thoroughly three times with the tap water to remove the excess salt, sand and epiphytes (Sahayaraj and Mary Jeeva, 2012) [1]. To drain off the water, the algae were wiped with a blotting sheet and air – dried under shade (Kombiah and Sahayaraj, 2012) [2].

2.2 Extract preparation
Thirty gram partially powdered seaweeds of *Acanthaphora specifera, Gracilaria corticata* and *Liagoras ceranoides*, were packed in Soxhlet apparatus (in 10grams of three packets) and refluxed with acetone individually for 36 hours continuously. Extracted solvent was evaporated and dried in desiccator under vacuum.
The final extract was elucidated with corresponding solvent and used for the experiments. The crude extracts were stored at -20 ^{0}C (Kombiah and Sahayaraj, 2012) [2].

2.3 Thin layer chromatographic analysis

Thin layer chromatography or TLC, is a method for analyzing mixtures by separating the compounds in the mixture. TLC can be used to help determine the number of components in a mixture, the identity of compounds, and the purity of a compound. Diluted solution of AS, GC and LC were dropped in one end of TLC plate and it was evaporated. Samples were placed in screw capped jar with small amount of ethyl acetate. It helps for development of sample spots, spot movements were clearly visualized under the UV lamp (Fall, 2009) [3].

2.4 FT-IR spectral analysis

Infrared spectroscopy used to identifying various types of bonds within in a molecule which determine the functional groups present or absent. In liquid spectrum take a small amount of spectrum of a liquid without adding any added solvent. Add 2 ml of our liquid sample (AS, GC and LC) to the centre of one face to a clean dry salt plate, and it makes sandwich for band formation. This same procedure was carried out for each one of our sample. Then it was slowly and evenly spread through between the plates.

Then they were placed in the sample beam in the spectrophotometer. The infrared rays slowly passed through the sample and we get significant peaks at particular wavelength. For a strong peak our samples were made into pellets (2µm) with the help of KBR and it was placed under the salt plate and remaining procedure was same as liquid sample processing. And finally, the graph was recorded and it was shows the peaks which we get in particular range for determining the group of compounds (Pouchert, 1985) [4].

2.5 ^{1}H NMR spectral analysis

Spectroscopy determines the physical and chemical properties of atoms or the molecules in which they are contained in respective samples. Dissolve the starting material (AS, GC and LC) in 0.7ml deuterated solvent ($CDCl_3$) with 4.5-5 cm in a tube and it was capped and it was placed it into a spinner. After completion of the NMR measurement, process the spectrum and assign the peaks in the spectrum. Process the spectrum with a suitable program. Correlate the different peaks to the NMR shifts in. The chemical shifts gives a hint of what type of environment the protons exists in our samples (Ounch *et al.*, 2013) [5].

3. Results and Discussion

3.1 Thin layer Chromatographic analysis

As there is movement from the solvent extract samples it was concluded that all the three seaweed extracts were containing chemical moiety which would be further confirmed through IR and ^{1}H NMR spectroscopic and they were done as per the protocols and presented.

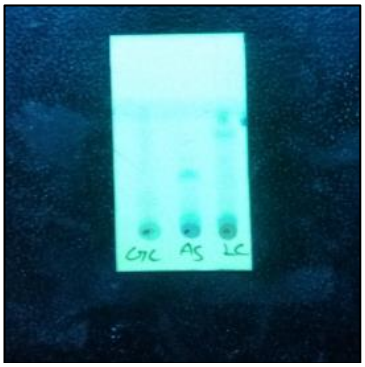

Fig 1: Chromatographic movement of selected red algal seaweeds

3.2 FT- IR Spectral analysis of selected red algal seaweeds

3.2.1 *Acanthophora spicifera*

The representative FT-IR spectrum of an analyte sample extracted from *Acanthaphora specifera* using acetone as a solvent is shown in the Fig.2

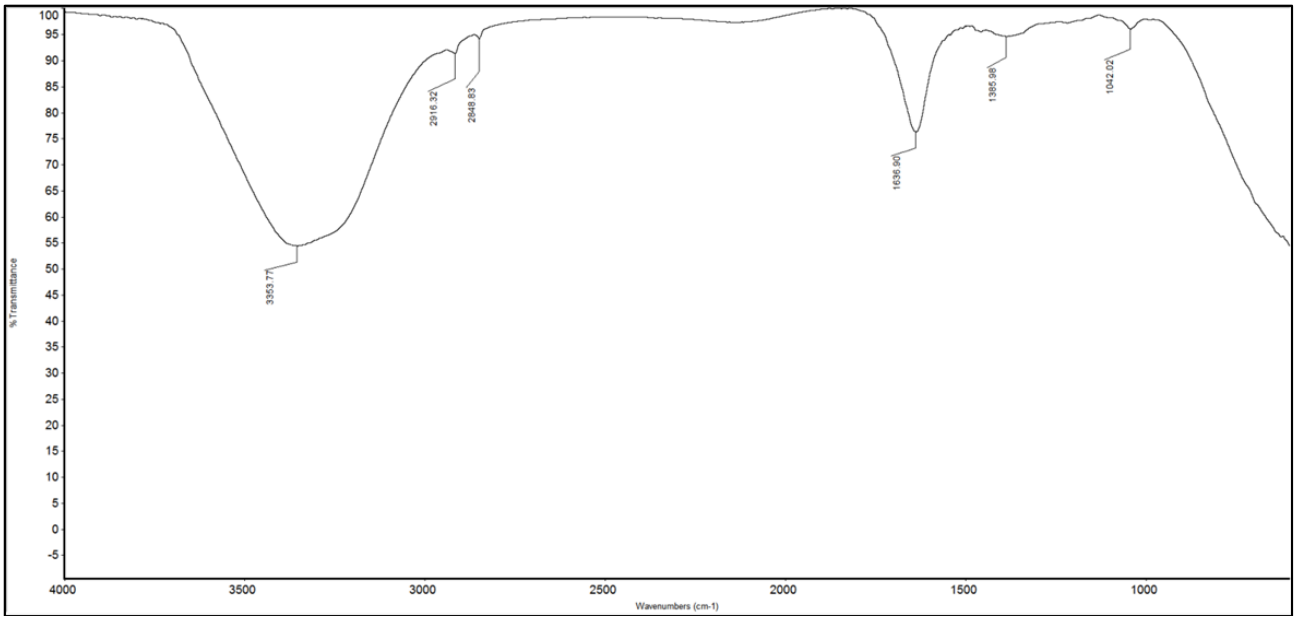

Fig 2: FT-IR Spectral analysis of *Acanthaphora specifera*

As it is shown, the spectrum contains a broad peak spreads over a range of 3200-3400 cm^{-1} could most probably be the vibrational stretching frequency of O-H bond. The less intense peak observed at 1385 cm^{-1} and 2916 cm^{-1} was attributed more likely due to the bending and stretching modes of C-H bonds. The peak appearing at 1636 cm^{-1} could be a stretching frequency of carbonyl group (C=O) involved in conjugation or bond resonance. A less intense peak at 1042 cm^{-1} would be most probably arises due to the breathing mode of aromatic ring. And its respective aromatic C-H stretching mode seems to be obscured due to the broad peak that stretches over an entire region between 3000 -3500cm^{-1}.

Furthermore, the peak appearing at 2848 cm^{-1} could be a stretching mode of C-H vibrations involved in aldehydic functional moiety. With all these vibrational assignments and from combined literature sources, it has been concluded that the major constituent is more likely present in the non-polar solvent extract of *A. specifera* could be an extensively hydrated carbonyl compound/polysaccharides or flavanoids like poly phenolic compounds.

3.2.2 *Gracilaria Corticata*

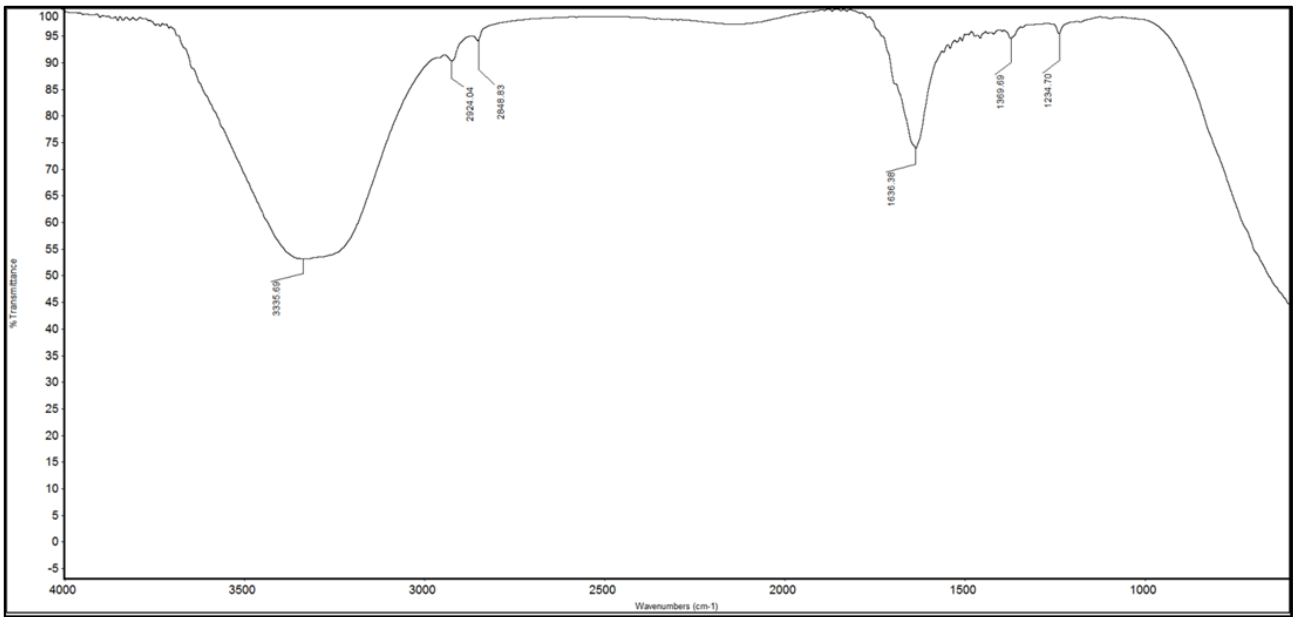

Fig 3: FT-IR spectral analysis of *Gracilaria corticata*

The spectrum showed much simpler vibrational frequency absorption for an organic leaf extract which is generally more unlikely for a multi component system. Though, it is presumed that the spectral peaks were arises due to the vibrational stretching/bending modes of the major chemical ingredient constituting the vast majority of the leaf extracts. Evidently, the spectrum contains a broad peak which spreads over a range of 3200-3400 cm^{-1} could most probably be the vibrational stretching frequencies of O-H bond. The less intense peak observed at 1369 and 2964 cm^{-1} was attributed more likely due to the bending and stretching modes of C-H bonds.

Similarly, an intense peak at 1636 cm-1 would be a stretching frequency of carbonyl group (C=O) involved in conjugation or some kind of bond resonance leading to a considerable decrease in the force constant of this stretching mode. A less intense peak at 1234 cm^{-1} was probably due to vibrational mode of alkoxy C-O bond. Furthermore, the peak appearing at 2848 cm^{-1} could be a stretching mode of C-H vibrations present in an aldehydic functional moiety. With all these vibrational assignments and from combined literature sources, it has been concluded that the major constituent is more likely present in the non-polar solvent extract of *G. corticata* could be an extensively hydrated carbonyl compound or polysaccharides.

3.2.3 *Liagora Ceranoides*

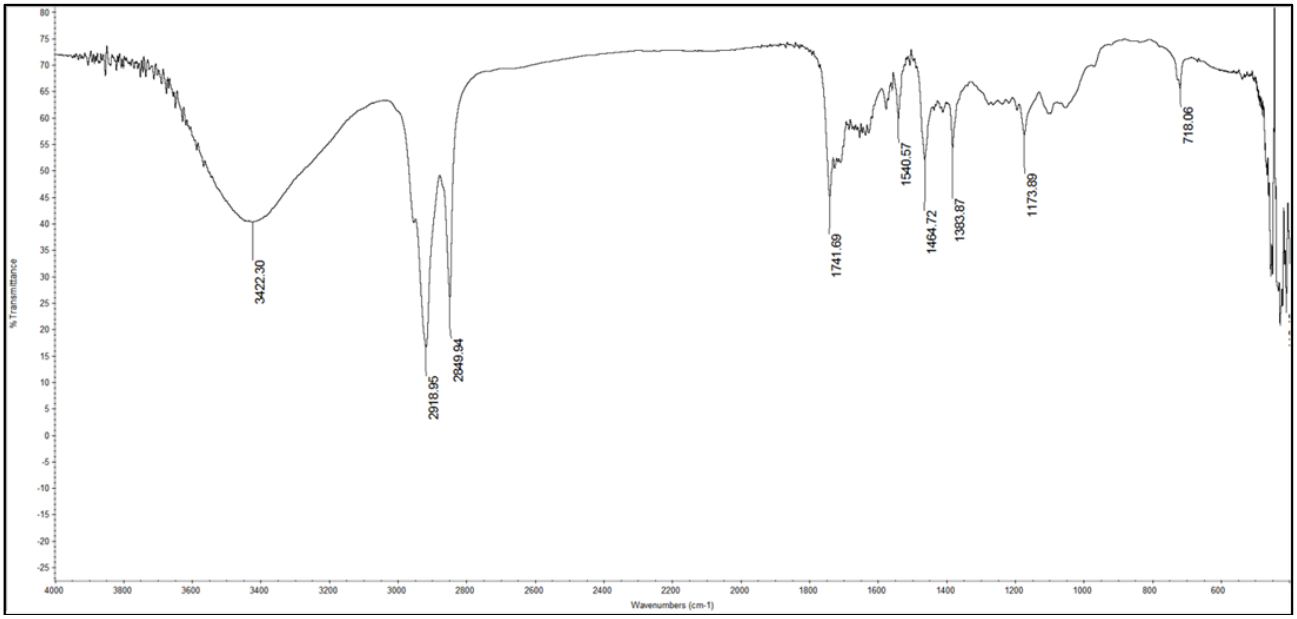

Fig 4: FT-IR spectral analysis of Liagora Ceranoides

It is evident that the vibrational spectrum of *L. ceranoides* leaf extract reveals a series of narrow peaks and no peaks were found to appear in the region between 1800 -2800 cm^{-1} which ruled out the presence of sp hybridized carbon atom in the substrate molecule. A slightly intense peak appearing at 1741 cm^{-1} could be attributed to the stretching vibrational mode of carbonyl group and the frequency is slightly shifted towards the higher wave number which generally belongs to an ester functional group.

Similarly, the two peaks observed at 2918 cm^{-1} and 1464 cm^{-1} could probably be corresponding to the stretching and bending modes of C-H bonds respectively. An intense peak arising at 2849 cm^{-1} could be due to the stretching mode of methine C-H bond which could possibly be attached to an allylic or carbonyl carbon atom. Furthermore, a broad peak that stretches over the region of 3400 cm^{-1} could belong to the O-H stretching vibration. Finally the peak appearing at 1173 cm^{-1} and 1540 cm^{-1} attributed more likely to the stretching and breathing vibrational modes of an aromatic ring and C=C bond respectively.

With all these vibrational assignments and from the known literature sources, it has been concluded that the major chemical component assumed to posses unsaturated, arylated aliphatic ester or a α, β- unsaturated carbonyl compounds or its derivatives.

3.3 ^{1}H NMR Spectral analysis of selected red algal seaweeds

3.3.1 *Acanthaphora specifera*

The experimental ^{1}H NMR spectrum of *Acanthaphora specifera* leaf extract dissolved in CDCl3 is shown in the Fig 5**.**

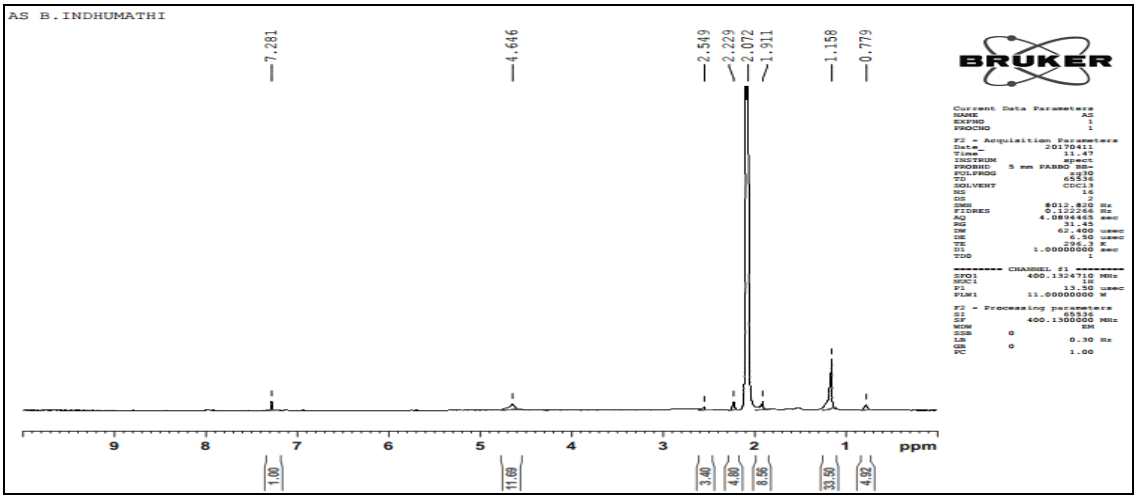

Fig 5: ^{1}H NMR Spectral analysis of *Acanthaphora specifera*

The spectrum revealed the radio frequency absorption of red algal powder extracted with acetone primarily over the much shielded region except a less intense peak appearing at 7.28 ppm which could be arises due to the presence of an aromatic protons. The singlet appearing at 4.64 ppm could be attributed to the presence of an alcoholic proton which is more likely to be present in the red algal powder as a major functional component. Similarly, the peak emerged at 2.54 ppm would be due to the presence of methine proton which can be located in an α- position to a carbonyl group or it could be an allylic proton in conjugation with an aromatic ring or with a double bond. Moreover the peak appearing at 1.04ppm reveals the presence of proton which could be attached to a primarily or secondary carbon atoms and an intense peak appearing at 2.07ppm attributed to the protonated solvent molecule during a proton- Deuterium exchange reaction involving an acidic or labile protons of the substrate molecule.

3.3.2 *Gracilaria corticata*

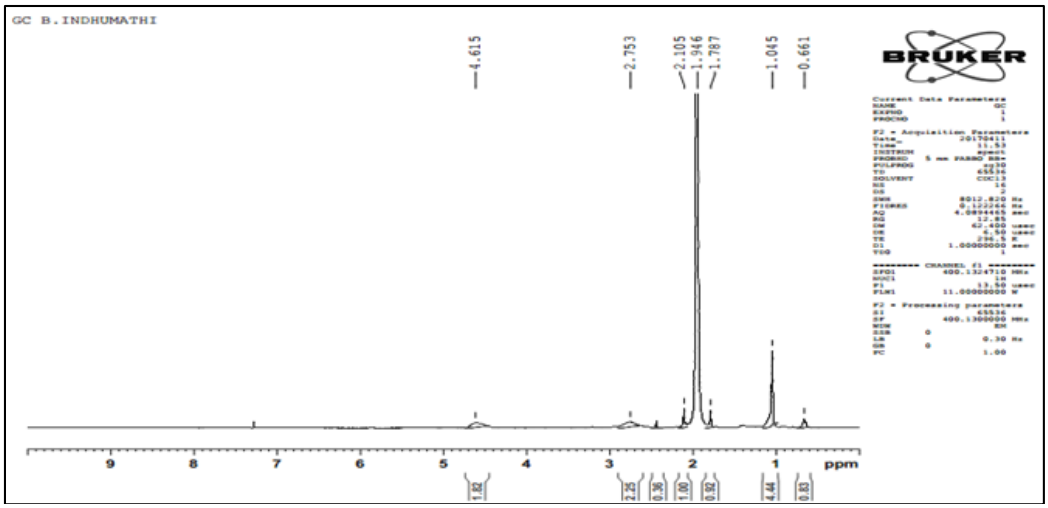

Fig 6: ^{1}H NMR Spectral analysis of *Gracilaria corticata*

The spectrum of radio frequency absorption of red algal powder extracted with acetone only in the up field region as major peaks. No peaks seem to be appeared in the down field or the shielded region which ruled out the possibility of the presence of aromatic carboxyl or aldehydic protons in the multi-component leaf extract. The singlet appearing at 4.61ppm could be attributed to the presence of alcoholic proton, which is more likely to be present in the red algal powder as a major functional component.

Similarly, the peak observed at 2.75ppm could be due to the presence of methine proton which can be located in an α-position to a carbonyl moiety or it could be due to the presence of epoxide C-H proton, which can also be more likely to be present in the extract as a form of carbohydrates present in a major proportion. Moreover the peak at 1.07ppm clearly showed the presence of proton localized on sp^3 hybridized carbon centre and an intense peak appearing at 1.9ppm was attributed to the solvent molecule which could be easily protonated in a Proton- Deuterium exchange reaction involving labile protons on the substrate analyte molecule.

3.3.3 *Liagora Ceranoides*

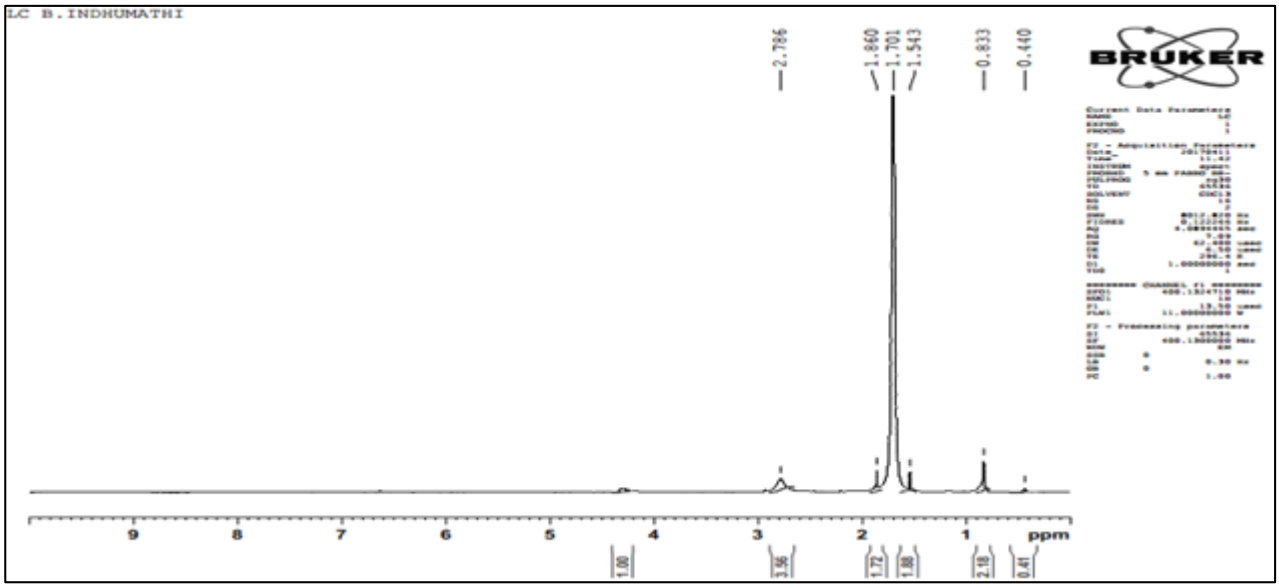

Fig 7: [1]H NMR Spectral analysis of *Liagora Ceranoides*

The radio frequency absorption of red algal powder extracted with acetone only in the up field region as major peaks. No peaks seem to be appeared in the down field or the shielded region which ruled out the possibility of the presence of aromatic, carbonyl or aldehydic protons in the multi-component leaf extract. The singlet appearing at 2.78ppm could be due to the presence of methine proton which can be located in an α- position to a carbonyl moiety or it could simply be a part of an unsaturated aliphatic hydrocarbon. Similarly, the peak appearing at 0.83ppm revealed the presence of methyl protons and an intense peak appearing at 1.7ppm was attributed to the protonated solvent molecule which could produced during the proton-Deuterium exchange reaction between the acidic protons on the analyte substrate molecules and the deuterated solvent molecules.

4. Acknowledgment

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