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ORIGINAL RESEARCH ARTICLE

Fourier Transform Infrared (FT-IR) Spectoroscopic Analysis of *Spirulina*

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ABSTRACT

FT-IR spectra of *Spirulina* have been recorded in the region of 3428-3320 cm^{-1} to 620-490 cm^{-1} in the different frequency ranges. In the present study, observation of the total protein, lipid, glycogen and amino acid content was identifying of the *Spirulina*. The different frequency ranges and their different functional groups are analyzed during the study period. *Spirulina* having essential vitamins like Vitamin A (in the form of β -carotene), Vitamin B_{12}. It is also a very rare source of GLA (Gamma Linolenic Acid) an essential fatty acid. Moreover *Spirulina* is a good source of phytochemical. *Spirulina* has potential benefits in the areas of immunomodulation, biochemical, antioxidant and anti-inflammatory protection, cardiovascular health, cellular protection, detoxification from toxicants and drugs probiotic effects.

Key words: FT-IR, *Spirulina*, GLA, Vitamin B_{12}, protein, lipid and amino acid.

1. INTRODUCTION

FT-IR is one of the most widely used methods to identify the chemical constituents and elucidate the compounds structures and has been used as a requisite method to identify medicines in Pharmacopoeia of many countries [1]. The use of IR spectroscopy for the analysis of biological samples was first suggested on 1940s the technique was being successfully explored for the study of biological materials and infect. IR spectroscopy has become an accepted tool for the characterization of biomolecules [2]. The revival of IR-spectroscopy as a means for characterizing microbial samples were initiated after the development of modern interferometric IR spectroscopy, the availability of low-cost minicomputers and powerful new algorithms for multivariate statistical analysis and pattern recognition methodologies. FT-IR spectroscopy has been shown to be a powerful technique for the study of biological macromolecules and of complex biological systems such as tissues and cells [3].

Fourier Transform Infrared (FT-IR) spectrometer is a routine analytical technique [4]. The spectrometers are sophisticated and which use a blackbody radiator as an infrared (IR) photon source, infrared studies are multidisciplinary but more and more attention is paid to biological as well as biochemical investigation. The primary reason is that many common bimolecular, such as nucleic acids, proteins, lipids and carbohydrates have characterized and a known vibrational fingerprints, which has led to several important and extensive investigations of biological samples were analyzed by IR spectroscopy. The field of chemical diversity has become fashionable in drug discovery research on which the development of high-throughput screening and combinatorial chemistry. A major step in the lead generation phase is the ability to quantify the chemical similarity between compounds [5]. Based on the review of the literature there is no study recorded in the *Spirulina*. Hence, an attempt has been made to investigate the phytochemical constituents' of *Spirulina* by FTIR methods.

2. MATERIALS AND METHODS

Collection and preparation of *Spirulina*

The dried *Spirulina* was collected from aurospirul commercial form, Aurovill Village (away from 15 km) near to Pondicherry. The *Spirulina* was kept carefully.

Taxonomy

Empire	Prokaryota
Kingdom	Bacteria
Subkingdom	Negibacteria
Phylum	Cyanobacteria
Class	Cyanophyceae
Subclass	Synechococcophycideae

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Order Pseudanabaenales
Family Pseudanabaenaceae
Subfamily Spirulinoideae
Genus *Spirulina*

FT-IR analysis

The FT-IR studies have been followed by the method described by [6]. The lyophilized resin or powered samples were mixed with dry potassium bromide pellet (KBr) and subjected to a pressure of about $5x10^6$ Pa in an evacuated die to produce a clear transparent disc of diameter 13 mm and thickness 1mm. IR spectra region 4000-400 cm^{-1} were recorded at room temperature on a perkin-Elmer fourier transform spectrometer equipped an air cooled DTGs (deuterated triglycine sulfate) detector. For each spectrum, 100 scans were CO-added at a spectral resolution of 4cm^{-1}. The frequencies for all sharp bands were accurate to 0.01 cm^{-1}.

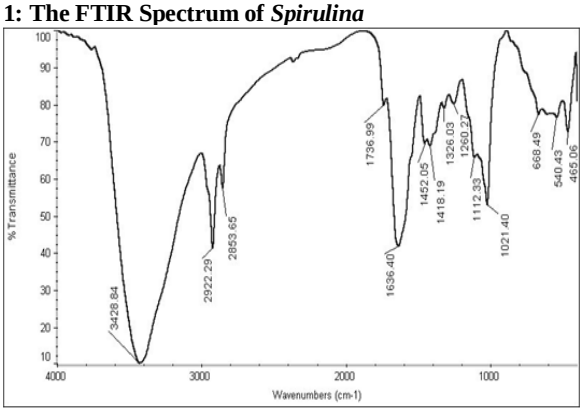
3. RESULTS

The FT-IR analyze of freshwater algae *Spirulina* powder, represent the following functional groups in which the frequency ranges 3560-3500 cm^{-1}, 3500-3300 cm^{-1}, 2925-2875 $^{-1}$, 1750-1735 cm^{-1}, 1650-1580 cm^{-1}, 1435-1405 cm^{-1}, 1350-1260 cm^{-1}, 1300-1250 cm^{-1}, 1120-1030 cm^{-1}, 1080-1010 cm^{-1}, 1030-990 cm^{-1}, 700-600 cm^{-1} and 620-490cm^{-1} The functional groups responsible for improving the health status and normalized in atrazine toxicity on *Spirulina* species were confirmed by FTIR spectra of *Spirulina* species shown (**Fig 1 & Table 1**).

Table 1: the FT-IR frequency range and the following functional groups are present in the *Spirulina*

S. No	Frequency ranges (cm^{-1})	Functional groups
1	3560-3500	O-H Stretching vibration presence of carbohydrate amino acid
2	3500-3300	N-H Stretching vibration presence of secondary amines (protein, lipid)
3	2925 – 2875	Aliphatic C-H Stretching vibration
4	1750-1735	C=O Stretching vibration (esters and amino acids)
5	1650-1580	N-H bending vibration Carbonyl β-Unsaturated Ketone amide
6	1435-1405	CH_2 bending vibration CH_2-CO- presence of carbonyl compounds
7	1350-1260	C-O Stretching, O-H bending vibration presence of alcohol
8	1300-1250	C-O asymmetric C-O-C Stretching presence of esters.
9	1120-1030	Symmetric C-H Stretching vibration, presence of Antioxidant enzyme
10	1080-1010	SO_3 symmetric stretching vibration presence of acid and RSO_3, ionic sulphonates
11	700-600	S-O Stretching vibration presence of sulphonic acid
12	620-490	C-I Stretching vibration presence of Iodo compounds

Fig 1: The FTIR Spectrum of *Spirulina*



4. DISCUSSION

FT-IR technique was used for evaluation the type of organic and inorganic complexes in plants. The analyze were carried out on drying and low aching temperature material of different parts of plants. The FT-IR analyzes of *Spirulina* represent the following functional groups. The infra red spectrum shows a frequency ranges from 3560-3500 cm^{-1} representing the O-H stretching vibration, presence of carbohydrate and amino acid. The frequency ranges from, 3500-3300 cm^{-1} peaks are representing in the N-H stretching vibration presence of secondary amines (protein, lipid) and frequency ranges from 2925-2875 cm^{-1} peak are representing aliphatic C-H stretching vibration. The frequency ranges from 1750-1735 cm^{-1} peak are representing C=O stretching vibration (ester and amino acid). The following peaks 1650-1580 cm^{-1} are present in the N-H bending vibration present in the carbonyl β unsaturated ketone amide. The frequency ranges from 1435-1405 cm^{-1} peak are present in the CH_2 bending vibration. The particular frequency ranges from 1350-1260 cm^{-1} C-O stretching, O-H bending vibration presence of alcohol. The following frequency ranges from 1300-1250 cm^{-1}, presence of C-O asymmetric C-O-C stretching presence of esters, the peak range 1120-1030 cm^{-1} present in symmetric C-H stretching, presence of antioxidant enzymes, the peak value representing 1050-1010 cm^{-1} present in of SO_3 symmetric stretching vibration, presence of acids, RSO_3 and ionic sulphonates. The frequency ranges from 700-620 cm^{-1} peaks are representing the S-O stretching vibration of sulphonic components. The frequency ranges from 620-490 cm^{-1} peaks are representing in C-I stretching vibration presence of Iodo compounds.

Most FT-IR studies on algae and seaweeds and algal extracts revealed the toxic interaction sites of carboxyl, amino acid and hydroxyl groups on the algal surface [7]. Biological molecules such as algae show complex vibrational spectra that

include overtones and combination all bands. But metal-legend stretching frequencies and properties of functional groups coordinated to toxic centers offer useful information. C–O stretching, NH_2 rocking C-O and CH_2 stretching bands are metal sensitive and are shifted as the metal is changed, but NH_2 vibrations are very sensitive to the effect of intermolecular interactions (e.g., hydrogen bonding) which makes it difficult to discuss the strength of the metal-nitrogen bond from the frequency shift. [8] Reported a blue shift of about 600 cm^{-1} of the band at 3450 cm^{-1} assigned to NH_2 coupled with hydrogen bonded hydroxyl stretching in *Spirulina* sp. upon treatment with metal ions. Alcoholic groups in the glucose ring may play a role in metal binding, although [9] considered it constant and used it as an internal standard for calculating band intensities.

Spirulina has high protein content (60%-70%). This is useful in human nutrient due to the high quality and quantity of its protein. The nutritive value of protein is related to the quality of amino acid digestibility coefficient as well as by its biological value [10, 11]. *Spirulina* contains essential amino acids the highest values of leucine (10.9% of total amino acids), Valaine (7.5%) and isoleucine (6.8%), [12]. Denaturation of *Spirulina* protein is observed when algae are heated above 67^{o}C. Hydrophobic regions interaction during heating and hydrogen bonds formation during cooling are aggregation and gelation factors of *Spirulina* protein [13].

Spirulina contains numerous characteristic peripheral inclusions associated to thylakoids, cyanophycin, polyhedral bodies, polyglucon, lipid and polyphosphate [14]. The cyanophycin granules or reserve granules are important due to their chemical nature and a series of chemical compound.

The FT-IR analyzed of *Spirulina* having high quantity of proteins, vitamins, phycocyanin and antioxidants substances. The *Spirulina* acting as protective role of atrazine toxicity and gradually recovered in treated fish at the time of supplementation period.

CONCLUSION

Based on the systematically analysis of *Spirulina* contains in protein, lipid, carbohydrate, aliphatic (C-H), Carbonyl (esters and acid), Carbonyl Beta-Unsaturated Ketone amide (C=N), ester, symmetric C-H stretching vibration, halogen compounds (C-Cl) and Iodo compound (C-I). So, the FT-IR spectrum shows more characteristic features. These are phytochemicals may responsible for the medicinal property of the micro algae *Spirulina* further toxicological study.

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