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Research Article

Rapid Structural Characterization of Plant Extracts – a FT-IR study

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Abstract

Plant natural product chemistry have ensued numerous pharmaceutical intermediates or drug precursors utilized for synthetic drugs. However lack of convenient qualitative tool for incessantly monitoring the potency of plant drugs, hinders application of herbal products. Molecular structure is the major theme in chemistry. A molecule absorbs a unique set of Infra Red light frequencies and as such beholds its own characteristic spectrum indicating characteristic functional group bands that assert a character to any phyto-compound. The application of FTIR spectroscopy in crude medicine authentication and quality evaluation is the most convenient tool to sort medicinal plants holistically at molecular level. I have tried to exemplify the fact with wetland macrophytes from my work viz; *Vallisneria spiralis* and *Ipomoea aquatic* as anti-dermatitis and anti-bacterial respectively. The two prime regions are to studied *Functional group region* (4000 cm^{-1}-1450 cm^{-1}) and *Finger print region* (1450 cm^{-1} – 500 cm^{-1}). The bands of spectra could be interpretative by scanning the sample table.

Keywords: FT-IR, Functional group, Finger print region, macrophytes, medicinal property.

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Introduction

The invention of Fourier Transform Infra-Red Spectroscopy (FT-IR) has revolutionized the natural product chemistry research. It not only detects very minute quantity of sample but also allows recovery of the sample. The fraction upon extraction followed by chromatographic purification or in a semi purified state can be subjected to IR spectrum analysis for construal of function groups or quality assurance fingerprints. Most of these instrumental analysis are high cost oriented and beyond the extend of most Indian students for multiple shots of a single samples in case of research need demands, despite that FT-IR could facilitate out compounds asserting the biotic activity of the fraction.

In this review paper an attempt has been made to illustrate the assisting IR spectra of purified compounds and their predictive chemical constituents of two aquatic macrophytes viz; *Vallisneria spiralis* (Hydrocharitaceae) and *Ipomoea aquatic* (convolvulaceae). Briefing about the plants - *Vallisneria spiralis* L. is a common submerged rooted macrophytes found in many wetlands, shallow ponds, lakes, marshes and streams of West Bengal. It is an invasive colonizer and of immense significance to maintaining an aquarium. *Ipomoea aquatica* Forsk (Water Spinach) is a commonly consumed leafy vegetable. It usually grows in wild areas in wetlands and confined water bodies. Well known for dermal medical relevance and a source of antioxidants.

Experimental

Seclusion of target compounds

Vallisneria leaves were endured to influx extraction process plunged for chemical leaching in glass jars with perforated lids in increasing ratios from 0%, 20%, 40% and 80% ethanol using milipore water. The amount of solvent added was in the ratio of 10:1 with respect to the fresh weight of the plants. The jars were kept in room temperature with sufficient sunlight initially for a span of not more than three days to prevent auto toxicity resulting into decolouration and foul odour. The crude extracts of the four fractions were collected and concentrated to dryness by rota vap and subjected to the following biochemical analysis. The fourth fraction was found to contain highest antioxidants with a resonance with phenol curves. Henceforth the compound was ideally isolated using solvent system of 1% methanol in ethyl acetate by column chromatography using silica gel (100-230).The samples upon room

temperature retention for a week shaped as crystals which could be isolated with chloroform: methanol washes and finally flushed with toluene. Mass spectrum depicts molecular weight of the isolated compound to be m/z (%) = 359 $[M]^+$ [1, 2, 3].

For *Ipomoea aquatica*, following the preparative steps of sterilized wash, around 5kg leaves were processed by shade dry and powered to obtain a dry weight of approximately 1.7kg. It was soaked in methanol which was recovered after 24hours, concentrated in rota vap at 40^0C. The fraction was mixed with equal proportion of methanol: water for liquid extraction and mixed with 250 ml hexane to obtain the hexane fraction. The presence of both methanol and water in the same chemical ambience is necessary for extraction of hexane or other solvents with low polarity index. The chloroform fraction was obtained from the aqueous fraction after methanol was evaporated. It was dried using anhydrous sodium sulphate. The ethyl acetate fraction was obtained in the similar manner. The methanol and the aqueous fraction were made free from non polar compounds and the aqueous fraction upon repetitive acetone precipitation [4] was almost pure and retained in freezer which was later purified by Pet. Ether: chloroform wash [5, 6].The chloroform fraction was column purified with increasing percentage of methanol and along with the aqueous fraction subjected to IR spectrometric reading prepared in KBr plates under high pressure (solids) without absorptions from mulling agent. The FT-IR analysis was done partially from IICB, Kolkata and Kalyani University, Nadia.

Infra-red spectrum Interpretations

Infrared spectroscopy deals with the interaction of infrared light with theme. Matter of fact, the energy of infrared photon can be calculated using the Planck energy relation and Wave equation which states energy is directly proportional to frequency and wave number. A molecule absorbs infrared radiation when the vibration of atoms in the moleculeproduces waver electric field with the same frequency as the frequency of incident IR radiations. All of these motions can be described in terms of two basic types of molecular vibrations - stretching and bending. Stretch has higher energy requirement than bending and hence corresponds to higher wave number in a spectrum. However, the energy of the stretching mode is inversely proportional to the mass of the atoms which shifts the bands towards the lower wavelengths and as well dependent on its hybridization in the order of sp > sp2 > sp3 . The Hooke's Law model for bond stretching frequencies helps us to understand various modes; it is useful to compare a vibrating bond to the physical model of a vibrating spring system. "*The spring system as described by Hooke's Law is a good working approximation".* The stronger the bonds the higher are the corresponding frequency. A stretch mode can be symmetric (ν_s) or asymmetric (ν_{as}) of which usually asymmetric stretch corresponds to higher energy than symmetric. Bending can occur in the plane of the molecule (scissoring, rocking) or out of plane (wig-wag and twisting). The number of basic stretching and bending modes also increases with the number of atoms in the molecule. For non-linear molecules 3N-6 (2N-5 bending, N-1 stretching) vibrations are observed; hence for linear its 3N-5 number of modes [7]. In Fourier transform of the Infra red Spectroscopy (FT-IR) coverts the raw data in readable sample spectrum [8]. A primary requisite for this analysis must contain vivid sequence of the peaks corresponding to exact wavenumber from the spectrum (integer), the intensities (w/m/s/br) and scanning two prime regions: Functional group region, Fingerprint regions and Bohlmann bands for amines. The functional group region runs from 4000 cm^{-1} to 1450 cm^{-1}, and the fingerprint region from 1450 cm^{-1} to 500 cm^{-1}[9]. The functional group contains fewer peaks and is coupled with stretching mode of the atoms and the finger print region is much complicated and outlines the bending vibrations which are unique to every compound. Elucidation of the complete chemical structure is a long winded method and requires sophisticated instrumentations or financial aspects. In contrary, the aim of detailed IR spectral study is for forecasting of natural compounds which could further be compared with standard spectral database or even serve as attribute factor for unknown compounds [10, 11].

Results and Interpretations

Ipomoea aquatica Methanol fraction

Description: Compound status: Aggregate of white prismatic crystals.

The Infra Red spectra seem to be of a much pure compound (purity < 90%) (Figure1). There is a sp3 C-H stretching at 2929 cm^{-1}. This is an alkane (νas CH2) stretching mode with medium strength. 3000 cm^{-1} can be assigned as a bar liner. Similar stretching mode would be absorbed left to 3000 cm^{-1} at around 3200 cm^{-1} – 3000 cm^{-1} if it would be of alkenes/aromatics. The narrow sharp peak at 3395 cm^{-1} corresponds to hydrogen bonded hydroxyl moiety. In this case

there is not much trough in the hydroxyl band. The prominent and sharp peak of non-conjugated C=O band appears at 1750 cm^{-1}. The corresponding C-O-H peak appears 1450 cm^{-1}. The compound is an ester group derivative (Table 1) and probable to be in monomeric state [12, 13].

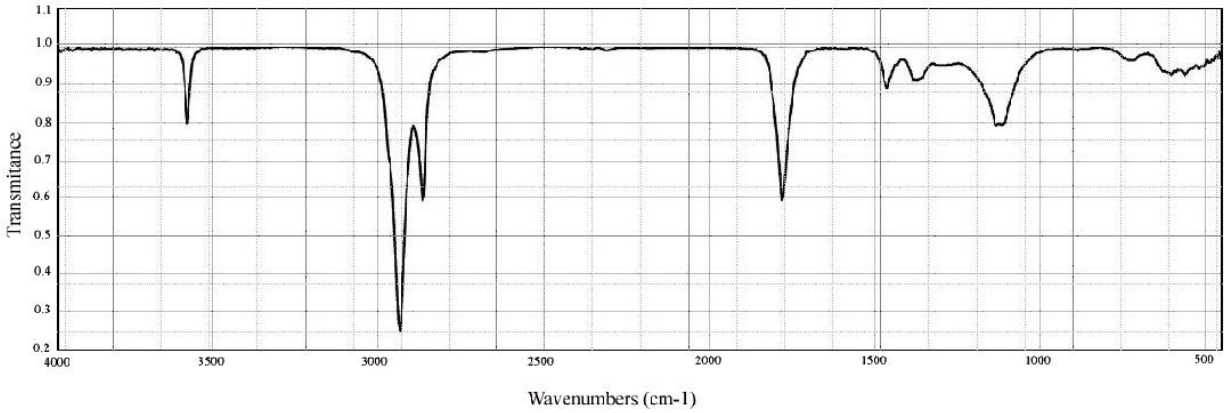

Figure 1 IR spectra of Methanol Fraction of *Ipomoea* leaves

Table 1 Peak Value with Corresponding Functional groups of Methanol Fraction of *Ipomoea* leaves

SL. No.	Peak value (cm^{-1})	Chemical groups
1	3385	OH
2	2929	C-H
3	2343	C-H
4	1750	C=O
5	1450	C-O-H
6	1382	C-O

Ipomoea aquatica Water fraction

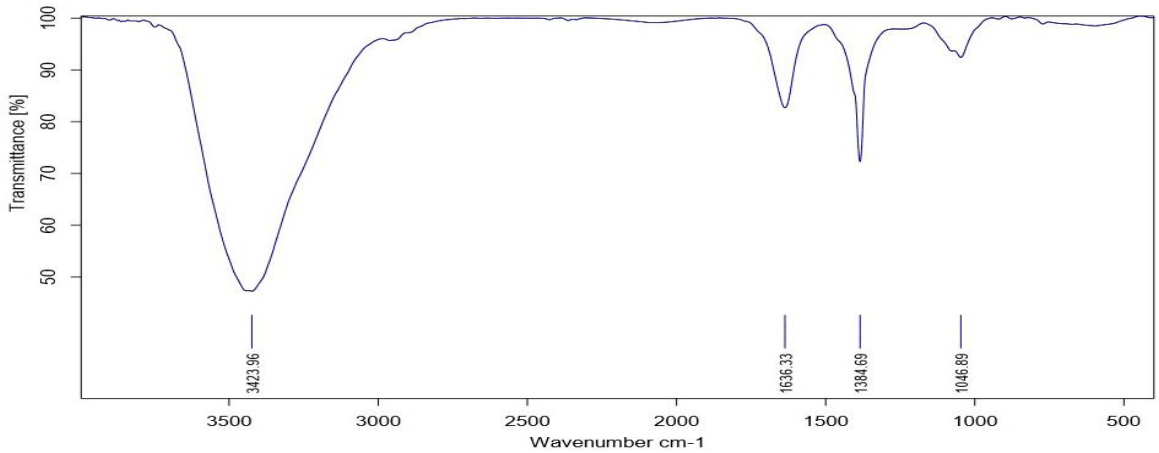

Figure 2 IR spectra of Water Fraction of *Ipomoea* leaves

Table 2 Peak Value with Corresponding Functional groups of Water fraction of *Ipomoea* leaves

SL. No.	Peak value (cm^{-1})	Chemical groups
1	3423.96	OH
2	1636.33	Presence of water molecules.
3	1384.69	C-H (bending/scissoring)
4	1046.89	C-O stretch

Description: Compound status: Aggregate of white cubic crystals.

The broad and strong peak at 3423.96 corresponds to free hydroxyl group (Figure 2). The compound is of low molecular weight and probable to be inorganic salt lacking functional groups. Due its hydrophilic nature it absorbed water in room temperature which gave a peak at 1636 cm^{-1}. The spectrum is see-through in the 1480-1850 cm^{-1} region and 3500-3000 cm^{-1} region indicating absence of C=C and C-heteroatom (Table 2). The IR spectrum therefore indicates the compound to be of aliphatic [14, 15]

***Vallisneria spiralis* Ethanol - Water fraction**

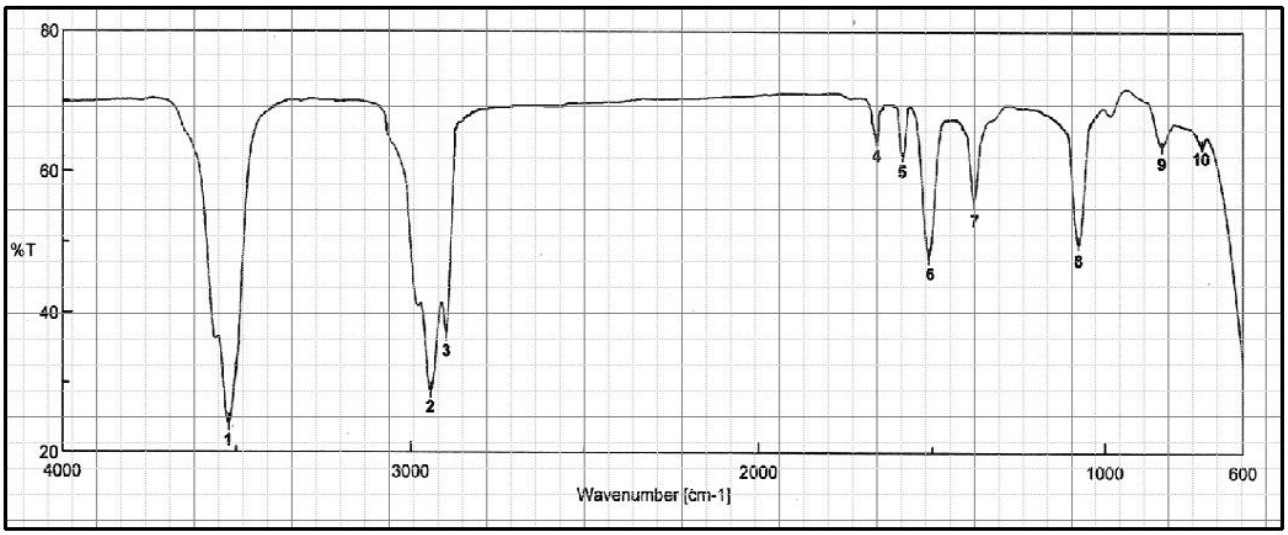

Figure 3 IR spectra of Ethanol Fraction of *Vallisneria* leaves

Table 3 Peak Value with Corresponding Functional groups of Ethanol fraction of *Vallisneria* leaves

SL No.	Peak value (cm^{-1})	Chemical group
1	**3642.10**	O-H stretching (free hydroxyl)
2	**2965.43**	C-H stretching
3	**2928.54**	C-H stretching
4	**1665.22**	C=O bending
5	**1600.78**	C=C stretching
6	**1554.80**	C=C bending of aromatic ring
7	**1360.00**	S=O, sym
8	**1080.04**	C-O
9	**1076.89**	C-O
10	**779.64**	C-H bending
11	**675.16**	C-Cl stretching

Description: Compound status: Aggregate of white cubic crystals.

The absorption spectrum of the 80% ethanol fraction shows ten major bands; the band at 3642.10cm^{-1} corresponds to broad hydroxyl group band usually free hydroxyls. 2965.43 cm^{-1} and 2928.54 cm^{-1} represents the sp3 C-H stretching with corresponding bending at 1432cm^{-1}. The other dominating bands at 1665.22 cm^{-1} and 1554.80 cm^{-1} are those of carbonyls and aromatic compounds. The presence of aromatic compound is further confirmed at 779.64 cm^{-1} with C-H stretching. The S=O bond signifies the presence of the sulphoxides (Figure 3 & Table 3)[16, 17].

A review of Bioassay

These are the bioassay [18-20] done against most troubleshoot dermatitis fungi *Malassezia globosa* (Figure 4)

- The left figure is the inhibition zone assay by 80% ethanol extract of *Vallisneria* leaves. It displays an early zone of inhibition at 100µg/ml with a noteworthy zone at 1000 µg/ml. The MIC was found to be at 156.25µg/ml and MFC at 5000µg/ml.
- The right figure is a non-monotonic dose response curve plotted for concentration of the water fraction of the Ipomoea leaves against inhibition zone diameter with MIC at 625 µg/ml and MFC at 5000 µg/ml.

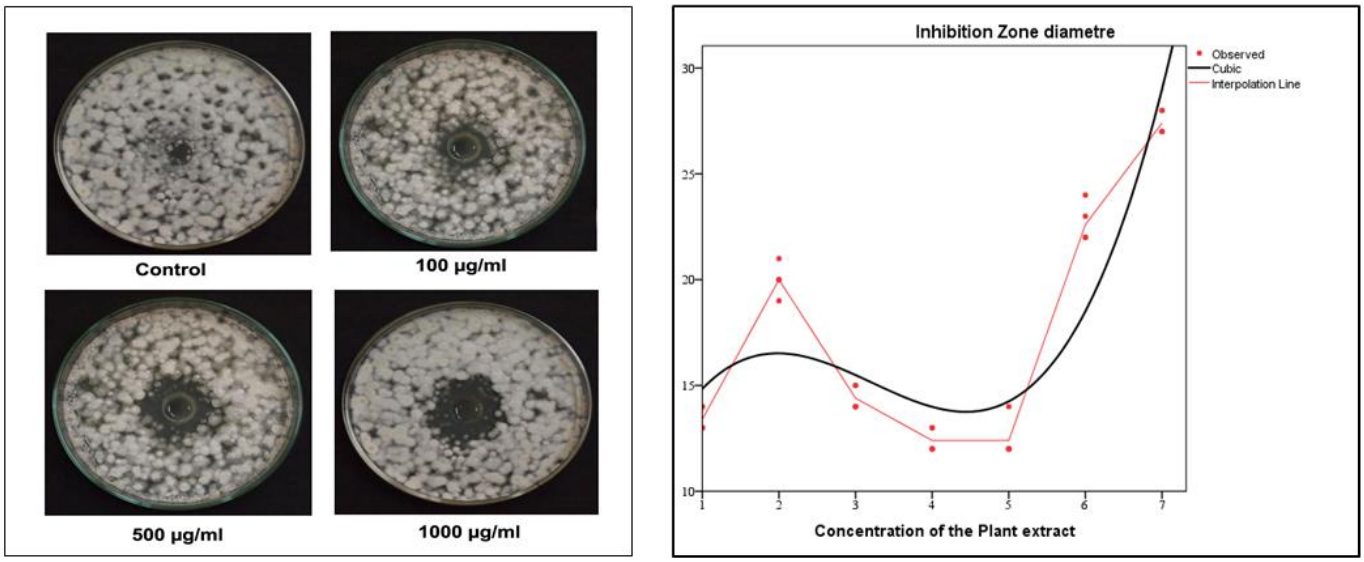

Figure 4: (A): Inhibition Zone activity of *Vallisneria* leaf extract at four different concentrations on *Malassezia globosa*.

(B): Non-Monotonic Dose Response Curve.

Conclusion

A mere diverse and versatile approach for interpretation of structure peak relationship in Infra-red spectroscopy could be accomplished by recognizing characteristic shapes and patterns or different modes of vibration within the spectrum, and by applying the information obtained from authentic database along with other attributes of the sample. Identification of the chemical groups of phytochemical compounds present in plants provides some information on the different functional groups accountable for their pharmacological traits. The presence of aromatic compound at 779.64 cm^{-1} in *Vallisneria* leaves with C-H stretching which if in case of flavonoids, corresponds to the first aromatic ring. The S=O bond includes compounds which shows radical trapping antioxidant property and act as antimicrobial, antiparasitic and antitumor agents. The FTIR analysis of methanolic and aqueous leaf extracts of *Ipomoea* revealed the presence of phenolic compounds, flavonoids and esters as major functional groups which are indeed present in broad spectrum antimicrobial compounds [21].

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