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Fourier transform infrared (Ft-Ir) spectroscopic analysis of *Nicotiana plumbaginifolia* (Solanaceae)

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Abstract

The present study deals with the Fourier Transform Infrared (Ft-Ir) Spectroscopic analysis of *Nicotiana plumbaginifolia*, an ethnomedicinally important plant. The phytochemical group analysis of the plant has been performed by Ft-Ir method. Methanolic extraction of dried leaf along with dry KBr. salt has been employed in this method. FTIR spectral data indicates specific fingerprint region for the species. The results shows different frequencies, ranges from 896.90cm^{-1} to 3352.28cm^{-1} , confirmed the presence of phenolics, protein, acid, alcohol, ether etc in this plant. It is also observed that the plant is rich in amino acids content. Different phytochemical groups present in the plant sample give an indication of its medicinal potency. Findings of this paper will be useful for identification, quality control of crude drugs obtained from this plant and pharmacological studies in future.

Keywords: Ft-Ir, *Nicotiana plumbaginifolia*, identification, ethnomedicine, phytochemistry, pharmacognosy

Introduction

Plants are known to contain large spectrum of biochemical substances synthesized by both primary and secondary metabolic processes. Such metabolites often play an important role in plant's defense, signaling, interfere with enzymatic and hormonal activities, and cure diseases etc. Now a days it is essential to validate such medicinal plants for their phytochemical profile using different sophisticated techniques and scientific methods. FT-IR spectroscopic analysis is one of such powerful techniques used as an effective tool in phytochemical group investigation by identifying and characterizing chemical bonds present in biological samples including plant parts. Earlier studies on FTIR of some Indian medicinal plants have also proved the importance of this technique [1, 2, 3, 4].

The primary reason to use this technique is that several biomolecules, such as nucleic acids, proteins, lipids and carbohydrates are known to have vibrational fingerprints of molecular bonds that could possible to analyze by IR spectroscopy. Chemical bonds are very crucial in the structure of any chemical which determine the fate of a metabolic reaction in biological system *in vivo* [5] since functional group in a biomolecule, like hydroxyl, methyl, carbonyl, sulfhydryl groups can participate in specific biochemical reaction. The presence of functional group affects the chemical and physical properties of molecules such as melting point, boiling point, polarity, dipole moment, solubility etc. For example, a molecule with a strongly polar functional group can be predicted to have a higher melting point and a higher boiling point than a molecule with a nonpolar functional group. In addition a polar molecule would preferentially dissolve in a polar solvent rather in a nonpolar solvent [6].

Plants belonging to the family Solanaceae are reported to have several therapeutic, food and agricultural importances [7, 8]. FTIR studies in some members of the family Solanaceae has also been reported earlier [1, 3, 4, 7, 9]. However, literature survey reveals that there is no study done on FTIR of the taxon *Nicotiana plumbaginifolia*. With this aim, an attempt has been made to investigate the main functional components of different phytochemical groups present in the leaves of this important ethnomedicinal plant by FTIR method.

Material and Methods

Collection of the plant

The plant material is collected during the field surveys in Joypur forest of Bankura District, West Bengal (23.053061° N, 87.443512° E) the Burdwan University Campus, of Golapbag

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area of Burdwan District, West Bengal (23.2392° N, 87.8513° E).

Extract preparation

Dried *Nicotiana plumbaginifolia* leaves were weighted (8g.) after crushed in a mortar with pestle and sieved to obtain coarse powder. 80 ml methanol is then added to this powder. Extraction was done by magnetic stirrer. These solutions were filtered using Whatman filter paper (Number 40).

FT-IR analysis

The FT-IR analysis is done by following a standard method¹⁰. The methanol extract (30ml) of the plant (2g.) was mixed with dry potassium bromide (KBr) (300 mg) of spectroscopic grade purity using a mortar pestle and compressed into thin tablets. subjected to a pressure of about 5x10⁶ Pa in an evacuated die to produce a clear transparent disc of diameter 13 mm and thickness 1mm. IR spectra region and peaks were recorded at room temperature on a Perkin-Elmer Fourier Transform spectrometer (model RX1, Norwalk, CT, USA) between 4000- 400 cm⁻¹. equipped an air cooled DTGs were recorded (deuterated triglycine sulfate) detector. Each analysis was twice done for confirmation.

Results

Taxonomic description of the plant- *Nicotiana plumbaginifolia* Viv., Elench. Pl. Hort. Bot. 26, t. 5, 1802; P.K. Bhattacharyya & Sarkar, Fl. West Champaran Dist. Bihar 3:277.1998. Cl. In Hook. f., Fl. Brit. India 4: 246.1883; Prain, Bengal Pl. 2: 559.1903; Haines, Bot. Bihar & Orissa 2: 647.1922(1961).

Herb, stem: solid, cylindrical, hairy, erect, branched. Leaves simple, alternate, entire, repand, acute, hairy, unicostate reticulate, lanceolate, cuniate; shortly petiolate, hairy, swollen, upto 1mm, exstipulate. Flowers cymose, complete, bisexual, actinomorphic, diplochlamydous, violetish, hypogynaous, infundibuliform, soft, pedicillate, hairy, ebracteate; sepals-5, gamosepalous, hairy, valvate; petals-5, gamopetalous, soft, infundibuliform, valvetish, imbricate; stamens 5(2+2+1), epipetalous, alternate to corolla lobe, filament slender, anthers bithecal, globose or slightly elongated, dorsifixed; carpels-2, syncarpous; stigma-1, bilobed, style-1, slender; ovary superior, 2-chambered with many ovule, placentation axile. Fruit capsule with persistent calyx.

Herbarium documentation- Burdwan University Campus, 02.03.2017, Sayani Chandra, 08 (BURD); Joypur forest, 26.03.2017, Sayani Chandra, 10 (BURD).

Ethnomedicinal use

The tribal people of Joypur Forest of Bankura is used the leaf

paste of *Nicotiana plumbaginifolia* to cure sores in mouth and foot of cow and buffalo.

FT-IR analysis

The FTIR spectrum was used to identify the functional groups of organic and inorganic active components in plant samples based on the peak value in the region of infrared radiation. The methanolic plant extracts was passed into the FTIR and the functional groups of the components were separated based on its peak ratio. The results of FTIR peak values and functional groups are represented in Figs.1 and Tables 1. Various functional groups of different compounds present in the plant sample were investigated.

The FTIR analysis of leaf powder of the plant revealed the following functional groups in which the frequency ranges are 3600-3200 cm⁻¹, 3000-2850 cm⁻¹, 1740-1720 cm⁻¹, 1680-1620 cm⁻¹, 1400-1000 cm⁻¹, 1320-1000 cm⁻¹, 1250-1080 cm⁻¹, 910-665 cm⁻¹. The analysis revealed 12 different types of functional groups viz., phenolic group, alkanes, alkenes, aldehyde, alkyl halide, alcohols, carboxylic acids, esters, ethers, aliphatic amines, primary amines, secondary amines which belongs to 7 different types of compounds viz., aromatic compound, aliphatic compound, aldehyde compound, alkyl halide, acid, alcohol and protein. The results of FTIR peak values and functional groups along with the biological roles are tabulated in Table 1.

The infra-red spectrum shows a frequency range 3600–3200 cm⁻¹, is represented the O-H stretching vibration, that confirms the presence of phenolics. The peak at 3352.28 cm⁻¹ in this range represented presence of phenolics. The bands at 2962.66 cm⁻¹, 2920.23 cm⁻¹ and 1622.13 cm⁻¹ under frequency ranges 3000–2850 cm⁻¹ and 1680 cm⁻¹- 1620 cm⁻¹ represented C-H stretching vibration of alkanes containing aliphatic compounds. The peak range 1680 cm⁻¹–1620 cm⁻¹ is indicated the –C=C– stretching vibration, confirmed the presence of alkenes. The frequency range 1740 cm⁻¹-1720 cm⁻¹ is represented the C=O stretching vibration, confirmed the presence of aldehyde group at 1728.22 cm⁻¹ peak value. The peaks from 1400 cm⁻¹-1000 cm⁻¹ are denoted C-F stretching vibration for alkyl halide group. This group is detected at two different peak values – at 1396.46 cm⁻¹ and at 1334.74 cm⁻¹. Presence of alcohol, carboxylic acids, esters and ethers are confirmed in frequency range 1320 cm⁻¹–1000 cm⁻¹. This range showed C-O stretching vibration at 1049.28 cm⁻¹ peak. Peaks observed at 1240.23 cm⁻¹ and 896.90 cm⁻¹ indicated amine groups. Specifically, the C–N stretching at 1240.23 cm⁻¹ confirmed the presence of aliphatic amines and the N–H symmetric stretching vibration at 896.90 cm⁻¹, confirmed the presence of primary and secondary amines. The absorption spectra of *N. plumbaginifolia* dry leaf sample extract are in presented Fig.1.

Table 1: FTIR peak values and functional groups in leaves of *N. plumbaginifolia*

S. No.	Frequency ranges(cm ⁻¹)	Frequency peak value (cm ⁻¹)	Stretching vibration and specific functional groups	Relevant chemical compounds	Uses / Importance (References in superscripts)
1.	3600-3200	3352.28	O-H stretching vibration, presence of phenolic group	Aromatic compound	Helps in defense and used as disinfectant ^[11]
2.	3000-2850	2962.66	C-H stretching vibration, presence of alkanes	Aliphatic compound	Major component of fuel, used as antiseptic ^[12]
3.	3000-2850	2920.23	C-H stretching vibration, presence of alkanes	Aliphatic compound	Major component of fuel, used as antiseptic ^[12]
4.	1740-1720	1728.22	C=O stretching vibration, presence of aldehyde group	Aldehyde compound	Used as insecticide and fungicide ^[13]
5.	1680-1620	1622.13	-C=C stretching vibration, presence of alkenes	Aliphatic compound	Used in fruit ripening ^[14]

6.	1400-1000	1396.46	C-F stretching vibration, presence of alkyl halide	Alkyl halide	Enhance bioactivity through both steric and electronic effects; used as refrigerants and propellants [15]
7.	1400-1000	1334.74	C-F stretching vibration, presence of alkyl halide	Alkyl halide	Enhance bioactivity through both steric and electronic effects; used as refrigerants and propellants [15]
8.	1320-1000	1049.28	C-O stretching vibration, presence of alcohols, carboxylic acids, esters, ethers	Acid, Alcohol	Important component of some commonly used food [16]
9.	1250-1080	1240.23	C-N stretching vibration, presence of aliphatic amines	Protein	Important part of amino acids [17]
10.	910-665	896.90	N-H stretching vibration, presence of primary, secondary amines	Protein	Important part of amino acids [17]

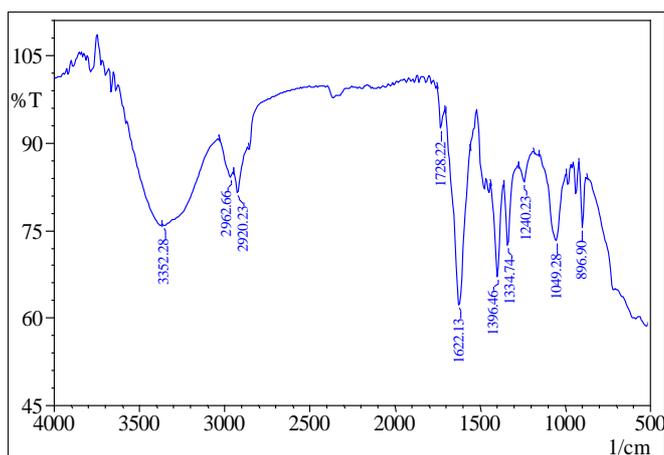


Fig 1: FT-IR spectrum analysis of methanolic leaf extract of *Nicotiana plumbaginifolia*

Discussion

FTIR technique is very useful to reveal different types of organic and inorganic compounds present in plants. In this present study, the analysis was carried out with dried leaf sample of *Nicotiana plumbaginifolia*. From the FT-IR spectra we can see clearly that each band represent characteristic absorption peaks of functional groups present in the sample. Screening of functional groups represents the presence of phenolic group, alkanes, aliphatic amines, alcohols, carboxylic acids, esters, ethers, primary amines, secondary amines. The presence of these functional groups are indicating various medicinal properties of *N. plumbaginifolia*. Presence of bioactive compounds in Solanaceae family has already been proved in earlier studies [18, 19]. The ethnomedicinal claim of this plant by the tribal people is therefore seems to be justified.

Phenolic compounds play important role in plant lignin biosynthesis and development. Phenolics are aromatic benzene ring compounds with one or more hydroxyl groups produced by plants mainly for protection against stress and pathogen attack. However, phenolics rich plants are good source of antimicrobial agents. Not only that, they also plays important role in part in other processes like incorporating phytochemical substances to accelerate pollination, coloring for camouflage and defense against herbivores [20]. Phenolics are also important agent to control oxidative damage by ROS. Thus presence of phenolics in any plant sample indicates it as a potent source of therapeutic agents for different human disorders. The antioxidant properties of phenolics in Solanaceous plants has already been proved in earlier studies [21, 22, 19]. Plant tissues containing phenols act as stimulant agents of leucocyte. Leucocyte is a type of WBC known to have immune regulatory functions against different diseases.

Hydroquinone, the simple phenol, is usually used in drugs as urinary antiseptic. It is also used in different skin related problems like hyperpigmentation in the skin [23].

Presence of large proportion of alkenes in volatile oil of *Solanum aculeastrum* has been investigated [24]. It is well-known that plant sample having biochemicals of alkene group is used as an antiseptic for external use. Presence of alkene group in the leaf of *N. plumbaginifolia* indicates it might have those good properties. Alkanes, on the other hand are present in plenty in more or less all biological organism. It confers ecological and metabolic functions by proving source of carbon and energy.

Amines are the important part of amino acids, building blocks of the protein of living beings. So, amino group play important role in both plants and animals. Ether is very essential component for human body. Heart, kidney, skin, pancreas etc contain ether of high percentage [25]. The present investigation proves the presence of amines, ether etc.

Plant sample in crude form contains different minerals. The human body requires a number of minerals in order to maintain good health. A number of minerals essential to human nutrition are accumulated in different parts of plants [26, 27]. So the leaf drug of the plant in crude form here also, surely contain several minerals, although it is not investigated in the present study.

Conclusion

The biochemical or metabolic fingerprint of the leaf extract of *N. plumbaginifolia* is generated by FT-IR technique which is very unique and therefore useful as a standard in quality control of the plant drug in its crude form. By attaining IR spectra from plant samples, it might be possible to detect the minor changes of primary and secondary metabolites. Further advanced phytochemical studies with this plant will explore its phytochemical potential and pharmaceutical implications.

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