# IDENTIFICATION OF CELLULAR COMPONENTS OF MEDICINAL PLANTS USING FTIR

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*Abstract*. FTIR spectroscopy was used to identify the cellular components of the medicinal plants of *Mimosa pudica* and *Caesalpinia pulcherrima.* FTIR spectroscopy is proved to be a sophisticated instrument to analyse the components of the plant cells. The cellular constituents in the leaves and stem of these plants were monitored for the qualities of medicinal applications. Various functional groups present in the medicinal plants were identified. The results indicate that plants contain carotenoids, polysaccharides, carbohydrates, and glycogen.

*Key words*: FTIR, functional groups, cellular components, medicinal plant.

## INTRODUCTION

Infrared (IR) spectroscopy has the potential to provide biochemical information without disturbing the biological sample. Consequently, the spectroscopic study of biological cells and tissue is an active area of research, its primary goal being to elucidate how accurately infrared spectroscopy can determine whether cells or tissue are damaged. Fourier transform infrared spectrometers, with their high signal-to-noise ratio and high precision in absorbance and wave number measurements, have caused a resurgence of interest in the use of infrared spectra for identification of biomolecules. FTIR 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. Owing to the fingerprint characters and extensive applicability to the samples, FTIR has played an important role in pharmaceutical analysis in recent years [3, 8, 10, 12]. Recently, spectroscopy has emerged as one of the major tools for biomedical applications and has made

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significant progress in the field of clinical evaluation. Research has been carried out on a number of natural tissues using spectroscopic techniques, including FTIR spectroscopy. These vibrational spectroscopic techniques are relatively simple, reproducible, nondestructive to the tissue, and only small amounts of material (micrograms to nanograms) with a minimum sample preparation are required. In addition, these techniques also provide molecular-level information allowing investigation of functional groups, bonding types, and molecular conformations. Spectral bands in vibrational spectra are molecule specific and provide direct information about the biochemical composition. These bands are relatively narrow, easy to resolve, and sensitive to molecular structure, conformation and environment. It is strongly believed that in studies related to spectroscopic techniques, both the reliable experimental procedure and characterization of spectral peak position and their assignment along with accurate peak detection and definition are of crucial importance [6]. Infrared spectroscopy is a powerful method for the study of molecular structure and intermolecular interaction in biological tissues and cells [9, 11].

Therefore we made an attempt to study the bimolecular constituents of the medicinal plants.

## MATERIALS AND METHODS

### COLLECTION AND IDENTIFICATION OF PLANT MATERIAL

Two medicinal plants *Mimosa pudica* and *Caesalpinia pulcherrima* were collected from the Mysore University campus, Karnataka, India. The leaves and stem were carefully excised from the plants and kept in polythene bags in shade for further analysis.

### PREPARATION OF THE PLANT MATERIAL

The leaves and stem were shade dried at room temperature in a clean environment to avoid contamination for 14 days and powdered in a domestic grinder. The powdered samples were stored in air tight glass bottles at room temperature for further analysis.

### SAMPLE PREPARATION

The powdered samples were kept in a lyophilizer to remove moisture. The samples were ground in an agate mortar and pestle in order to obtain fine powder. Powdered leaves and stem samples were mixed with paraffin liquid (at a ratio of

1/100) completely, and subsequently the mixture of each plant was subjected to FTIR spectroscopic analysis.

### TEST CHEMICALS

The AnalaR grade Alcohol and paraffin was obtained from Sigma Aldrich Company, Bangalore, India, and were used without further purification for the experiment.

### SPECTROSCOPIC ANALYSIS

FTIR spectra were recorded with a FTIR 460 plus Jasco. The powdered plant samples of *Mimosa pudica* and *Caesalpinia pulcherrima*, were scanned at room temperature (25±2 ºC) and a spectral range of 4000–400cm$^{-1}$. To improve the signal to noise ratio for each spectrum, 100 interferograms with a spectral resolution of ±4cm$^{-1}$ were averaged. Background spectra collected under identical conditions were subtracted from the sample spectra. Therefore, in the present study it is possible to directly relate the intensities of the absorption bands to the concentration of the corresponding functional groups [2].

## RESULTS AND DISCUSSION

Results of FTIR spectroscopic studies have revealed the existence of various chemical constituents in leaves and stem of *Mimosa pudica* and *Caesalpinia pulcherrima* (Fig. 1 to Fig. 4). The absorption bands, the wave number (cm$^{-1}$) of dominant peaks obtained from absorption spectra were defined in Table 1. Although the interferences of plant fibres inherent in medicinal materials, we can still see the IR spectrum of medicinal plants shows lots of structural information of major and minor constituents. It should be pointed that we did not find the peak at 1635 cm$^{-1}$ due to the absence of moisture content in the samples investigated, as shown in Figs. 1–4. The weak peak at 3400 cm$^{-1}$ assigned to the O–H stretching vibration, the medium stronger peak appear in the range of 1130–997 cm$^{-1}$ mainly attributed to the stretching vibration of C–O. Those characters of peaks intensities and position at 2930, 1130, 1104, 997, 925 and 832 cm$^{-1}$ in the IR spectrum display the characteristic absorption of polysaccharides especially the peak at 832 cm$^{-1}$ belongs to the characteristic peak of α-glucose [3]. The infrared spectra of protein are characterized by a set of absorption regions known as the amide region and the C–H region. The most widely used modes in protein structure studies in the amide region are amide I, amide II and amide III. The amide I band arises principally

from the C=O stretching vibration of the peptide group. The amide II band is primarily N–H bending with a contribution from C–N stretching vibrations. The amide III absorption is normally weak and arises primarily from N–H bending and C–N stretching vibrations. The amide absorptions are considered sensitive to protein conformation; hence an increase or a decrease in the ratio of the intensities of the bands at ~1541 $cm^{-1}$ (amide II) and ~1653 $cm^{-1}$ (amide I) could be attributed to a change in the composition of the whole protein pattern. Polysaccharides are one of the obvious functions on enhancing body immunity, antitumor and resisting radiation damage. In addition, the peak at 1744 $cm^{-1}$ assigned to the C=O stretching vibration means that some carbonyl compounds existed in these rare medicinal plants. So, depending on the fingerprint characters of the peaks positions, shapes and intensities, the fundamental components indwell in the materials can be seen clearly [3]. The hydroxyl peak at 3400 $cm^{-1}$ and the peak situated at 1769 $cm^{-1}$ assigned to the absorption of carbonyl becomes the medium strong, as well as the peaks at 2958, 2929 and 2856 $cm^{-1}$ medium stronger, which belong to the C–H stretching vibrations of methyl and methylene; meanwhile, the peak intensity at 1052 $cm^{-1}$ is very weak. Those characters are the typical absorption of lipophilic components [14]. The medium strong peak of νO–H at 3413 $cm^{-1}$ and stronger peak of νC–O at 1054 $cm^{-1}$ are the typical absorption peaks of cellulose or hemicellulose, which are obviously different from the peaks of polysaccharides in the medicinal plants [6]. From information obtained from previous studies we assigned the remaining IR bands as follows: the peaks at 1237 $cm^{-1}$ and 1082 $cm^{-1}$ were attributed to $PO_{-2}$ asymmetric and symmetric stretching vibrations and phospholipids [1]. Presence of C=O, C–H, C=C and C–O, C–C and C–O were identified. These bonding structures are responsible for the presence of alkyl groups, methyl groups, alcohols, ethers, esters, carboxylic acid, anhydrides and deoxyribose [4, 13]. The more intense bands occurring at 3419 $cm^{-1}$ 2927, 2853, 1633, 1421, 1260, 1073, 816, and 635 $cm^{-1}$ corresponding to O–H/N–H, C–H, C–O and C–CI/C–CS stretching/bending vibrations respectively indicate the presence of amino acids, alkenes, nitrates, ethers, organic halogen compounds and carbohydrates in plants [7]. Protein plays a vital role in the physiology of living organisms. All the functions of an organism are regulated by enzymes and hormones, which are proteins. If any alteration takes place in the protein turnover, it may have an adverse effect on the important and complex groups of biological materials, comprising the nitrogenous constituents of the body and food intake and thus performing different biological events to maintain homeostasis of the cell. Therefore, the protein content of a cell can be considered a diagnostic tool to determine the physiological phases of a cell [8].

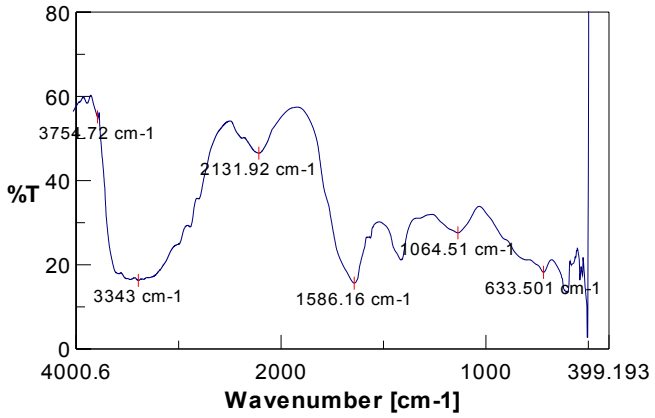


Fig. 1. FTIR spectra of *Mimosa pudica* leaves.

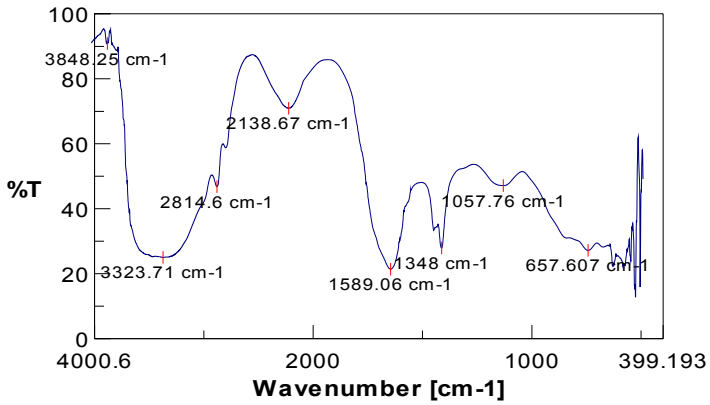


Fig. 2. FTIR spectra of *Mimosa pudica* stem.

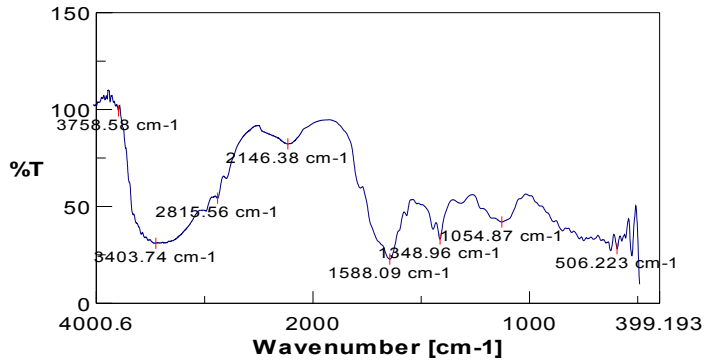


Fig. 3. FTIR spectra of *Cesalpina pulcherrima* leaves.

*Table 1*

Assignment of IR absorption bands in the spectra of the medicinal plants [5]

| S. No. | Peak ($cm^{-1}$) | Assignment |
|---|---|---|
| 1 | 472/5 | Cα=Cα torsion and C–$OH_3$ torsion of methoxy group |
| 2 | 521 | Torsion and ring torsion of phenyl |
| 3 | 600–900 | CH out-of-plane bending vibrations |
| 4 | 892 | C–C, C–O deoxyribose |
| 5 | 900–1300 | Phospodiester region |
| 6 | 940 | Carotenoid |
| 7 | 1000–140 | Protein amide I absorption |
| 8 | 1150–200 | Phosphodiester stretching bands |
| 9 | 1000–200 | C–OH bonds in oligosaccharides such as mannose & galactose |
| 10 | 1000–350 | Region of the phosphate vibration carbohydrate residues attached to collagen and amide III vibration (in collagen) |
| 11 | 1020–50 | Glycogen |
| 12 | 1030 | Collagen |
| 13 | 1105 | Carbohydrates |
| 14 | 1145 | Phosphate & oligosaccharides |
| 15 | 1180–300 | Amide III band region |
| 16 | 1206 | Amide III Collagen |
| 17 | 1244/5 | $PO^{-2}$ asymmetric (phosphate I) |
| 18 | 1255 | Amide III |
| 19 | 1312–1317 | Amide III band components of proteins Collagen |
| 20 | 1369/70 | Stretching C–N cytosine, guanine |
| 21 | 1375 | Lignins |
| 22 | 1456 | $CH_3$ bending vibration (lipids and proteins) |
| 23 | 1482 | Benzene |
| 24 | 1504 | In-plane CH bending vibration from the phenyl rings |
| 25 | 1600–800 | C=O stretching (lipids) |
| 26 | 2800–3000 | C–H Lipid region |
| 27 | 2930, 113, 1104, 997, 925, 832 | Characteristic absorption of polysaccharides |
| 28 | 3000–3600 | N–H stretching |
| 29 | 3500–600 | OH bonds |
| 30 | 3000–700 | O–H stretching (water) |

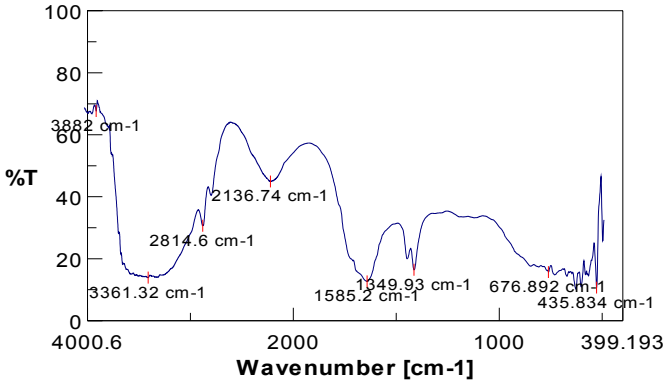


Fig. 4. FTIR spectra of *Ceasalpinia pulcherrima* stem.

## CONCLUSIONS

The present investigation shows the presence of carbohydrates, carotenoid, glycogen, amino acids, amides, starch, calotropin, calotropogenin, phosphates, lipids, glycogen and cellulose. So, FTIR spectrum reflecting objectively the panorama of chemical constituents in a complex system is the most credible method to validate and identify the mix-substance systems such as traditional medicine and herbal medicine. Further research may help for the identification of new bioactive compounds in these medicinal plants.

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