

**Research Article**

## **POLLEN IDENTIFICATION OF *HIBISCUS ROSA-SINENSIS* AND *SIDA ACUTA* THROUGH FTIR SPECTROSCOPY**

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### **ABSTRACT**

Present study includes the FTIR characterization of pollen grains of two taxa of family Malvaceae. Pollen grains have received more attention in taxonomical and forensic studies. Their structural characterization through scanning electron microscopy allows their differentiation on cellular level; however, their molecular profile can prove more reliable method for discriminating different pollen species as required for medical, forensic or taxonomical studies. Pollen samples of *Hibiscus rosa-sinensis* and *Sida acuta* were found to have variable molecular IR peaks which can be used as their spectral signatures.

**Keywords:** FTIR, Malvaceae, *Hibiscus Rosa-sinensis*, *Sida Acuta*, Pollen Grains

### **INTRODUCTION**

Infrared signals of different biochemicals are highly specific molecular fingerprints which can be used for probing the identity of a plant species (Naumann *et al.*, 1991; Parodi *et al.*, 2013). Use of pollen grains in forensic studies has gained momentum since past two decades yet their correlation with parent plant and native region would become more rapid and thorough using techniques like FTIR (Fourier Transform Infrared) spectroscopy. This technique is rapid and reproducible to differentiate and identify plants at subspecies level for forensic studies, community ecology and taxonomical studies. Accurate measurement of pollen biochemical composition by IR spectroscopy should offer unique new perspective on reproductive success and total plant fitness.

Another interesting result of the IR study is the correlation between pollination strategy and the protein-to-carbohydrate ratio (Zimmermann and Kohler, 2014). Conventional methods of pollen analysis include Hirst Spore Trap which is time consuming; therefore, FTIR spectroscopy can serve rapid identification of pollen grains (Dell'Anna *et al.*, 2009).

Malvaceae is a family of wide distribution but with no regular pattern or habitat leap. Its members are found in all continents except Antarctica. Members of Family Malvaceae range from ephemerals to tall rain-forest trees. In the present study, pollen grains of two members of this family *Hibiscus rosa-sinensis* and *Sida acuta* have been analyzed using FTIR.

*Hibiscus rosa-sinensis* and *Sida acuta*, both species are native to Central America. *Hibiscus rosa-sinensis* is an evergreen shrub with brilliant red flowers, numerous monadelphous stamens and red velvety stigmas.

*Sida acuta* is a weedy species adopting pantropical distribution, small herbaceous plant with yellow flowers having five sepals, five petals, numerous stamens and branched stigma.

Pollen grains of both species have not been analyzed by FTIR spectroscopy earlier, therefore this study was planned to develop their IR Spectral signatures for future database.

### **MATERIALS AND METHODS**

#### **Samples**

Pollen samples of test species were obtained through fieldwork in areas adjoining DAV College, Jalandhar, Punjab, India. Flowers were collected and separated anthers. Pollen grain were collected from mature anthers and kept in dry vials at 4°C till used for microscopic and FTIR analysis.

#### **Light Microscopy**

Pollen grains of both the species were mounted in 10% Glycerin and observed under Olympus microscope equipped with MIPs imaging system.

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### FTIR Spectroscopy

Pellets of FTIR spectroscopy were prepared by grinding 1 mg of sample with 100 mg of KBr approximately using Pestle and mortar. Prepared matrix was pressed into a disc for each pollen sample and scanned at room temperature  $25\pm 2^\circ\text{C}$  at  $4000\text{-}400\text{cm}^{-1}$  spectral range. FTIR analysis was performed using Perkin Elmer spectrophotometer system.

### RESULTS AND DISCUSSION

Light Microscopy of the pollen grains of both species revealed different structural characteristics (Figure 1). The surface of *Hibiscus* pollen was colporate having numerous round tip spines and opercula whereas pollen grains of *Sida* were also colporate almost half the size of *Hibiscus* pollen grains.

Fourier Transform Infrared (FTIR) spectroscopy is a rapid, non-invasive, high resolution analytical tool for identifying different types of chemical bonds in a molecule by producing an infrared absorption spectrum that is like a molecular fingerprint. In the present study, FTIR spectral data revealed specific absorption peaks for test species (Figure 2 & 3). Peaks at  $3400.3$ ,  $2955.1$ ,  $2921.4$ ,  $2850.9$ ,  $1629.5$ ,  $1442.9$ ,  $1324.5$ ,  $1245.4$ ,  $1107.1$ ,  $1047.6$ ,  $928.8$ ,  $897.1$ ,  $783.1$ ,  $716.6$ ,  $634.3$  and  $586.2\text{ cm}^{-1}$  were characteristic of *Hibiscus* pollen and  $3418.3$ ,  $2955.1$ ,  $2920.9$ ,  $2850.7$ ,  $1732$ ,  $1651.1$ ,  $1538.5$ ,  $1455.3$ ,  $1273.9$ ,  $1159.9$ ,  $1049.5$ ,  $1023.7$ ,  $723$ , and  $465.5\text{ cm}^{-1}$  were characteristic of *Sida acuta*. Peaks at  $2920\text{-}21$  and  $2850\text{ cm}^{-1}$  correspond to C – H and CH (Methoxy compounds) bond vibrations (Murugan *et al.*, 2013). Broad band in the range of  $1270\text{-}1150\text{ cm}^{-1}$  is characteristic of Ester Carbonyls (Murugan *et al.*, 2013). By visual recognition, there is no significant difference in characteristic absorption bands but the intensity of certain wavelengths do differ from each other viz. sharp peak at  $1107.1$ ,  $928.8$ ,  $897.1$  and  $783.1\text{ cm}^{-1}$  in *Hibiscus* pollens and  $1732.0\text{ cm}^{-1}$  in *Sida* pollen.

Recently and Kohler (2014) have employed IR spectra of pollen grains to identify plant species but no previous study has been conducted for *Hibiscus rosa-sinensis* and *Sida acuta*. Therefore, present study provides IR fingerprints of pollen grains of both species which may prove significant in their characterization.

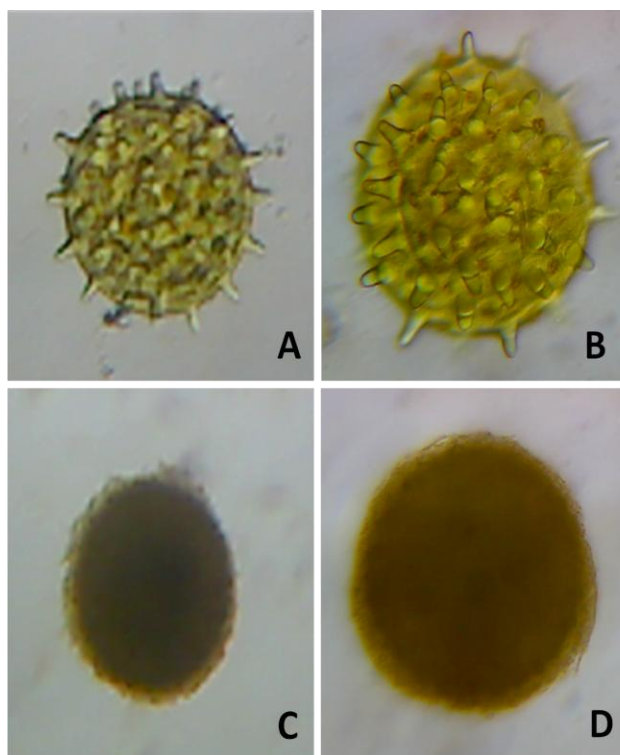
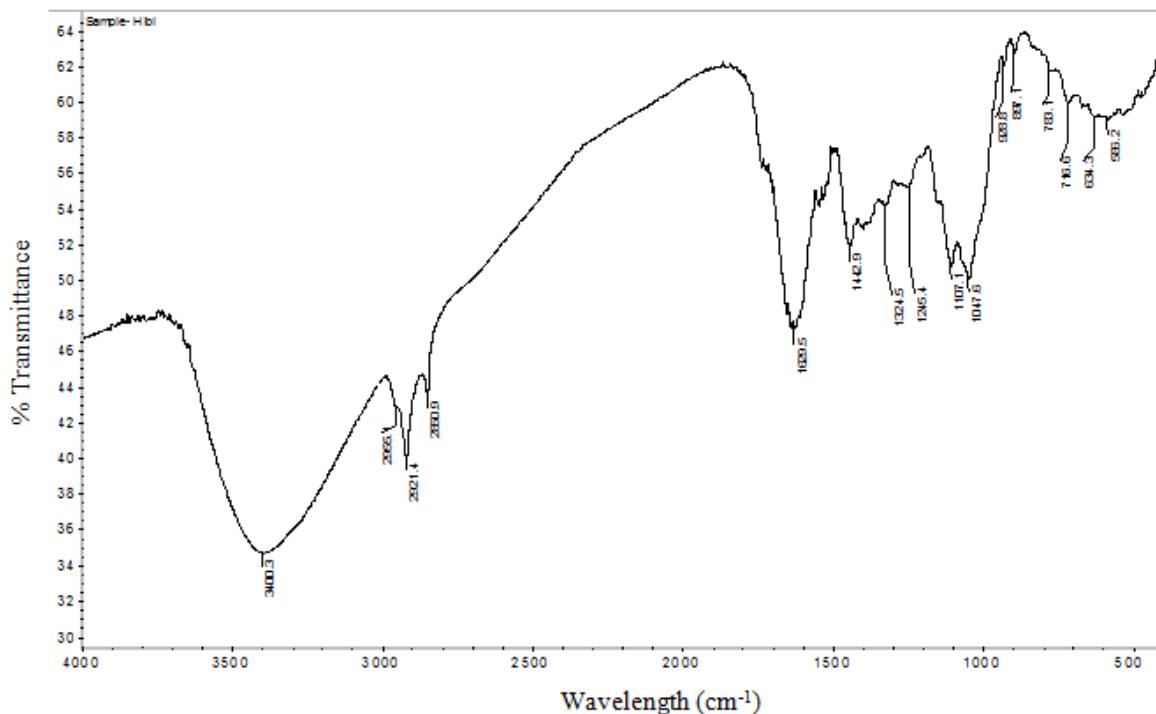
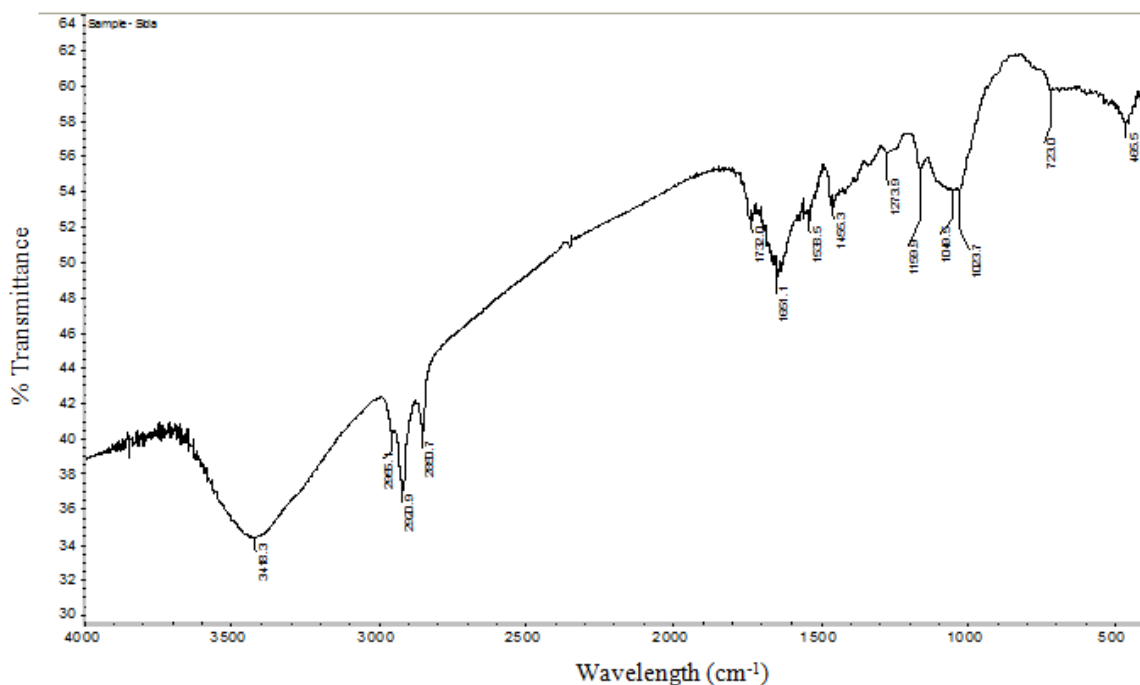


Figure 1: Surface view of Pollen grains of *Hibiscus rosa-sinensis* (A, B) and *Sida acuta* (C, D) at 10 and 40X

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**Figure 2: FTIR spectra of Pollen Grains of *Hibiscus rosa-sinensis***



**Figure 3: FTIR spectra of pollen grains of *Sida acuta***

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