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COMPREHENSIVE METABOLITE PROFILING OF *HAEMATOCARPUS VALIDUS* (MIERS) BAKH.f. EX FORMAN LEAF AND FRUIT SAMPLES USING FTIR SPECTROSCOPIC ANALYSIS

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Abstract

Haematocarpus validus (Khoon Phal) is a member of the family Menispermaceae with folklore medicinal and nutraceutical value. As there are no metabolome studies on this underutilized edible fruit crop, the present study was undertaken to generate a snapshot of plant specific metabolic fingerprint employing Fourier Transform Infrared spectroscopy. FTIR is an ideal tool for assessing the various chemical bonds in a molecule utilizing infrared absorption spectrum. Dried crude powder of khoon phal leaf and fruit samples were made into thin pellets using KBr and IR spectra was obtained for wavenumber ranged from 4000 cm^{-1} to 450 cm^{-1}. From the results obtained it was inferred that the leaf and fruit samples had more or less similar spectra of functional groups and fingerprint regions. The spectra included the functional groups region containing alcohols/phenols (OH- stretch), alkanes (aliphatic C-H stretching band), alkenes (-C=C- stretch) and 1^0 amines (N-H bend) and the fingerprint regions that represent aromatics (C-C stretch - in ring, C-H loop), aromatic amines (C-N stretch), alcohols, carboxylic acids, esters, ethers (C-O stretch) and alkyl halides (C-Cl stretch, C-Br stretch). The FTIR analysis of leaf and fruit samples of *H. validus* gives an insight into the plant and plant part specific chemical profile and the unique spectra obtained can be used as a marker system for chemotaxonomic investigations.

Key words : *Haematocarpus validus,* Khoon phal, FTIR, phytoconstituents, metabolite finger printing.

Introduction

Khoon phal [*Haematocarpus validus* (Miers) Bakh. f. ex Forman] is an underutilized edible fruit crop with known ethnomedicinal and nutraceutical value. It is a perennial woody climber belonging to the moonseed family Menispermaceae and found in limited geographical distribution in the hot and humid tropics of Asian continent. Various parts of this plant is traditionally used by different ethnic groups in India (North Andaman, Assam, Meghalaya, Southern Mizoram, Tripura), Bangladesh and West Java (Hooker, 1872; Balakrishnan, 1968; Forman, 1972; Flora of Andaman-Nicobar Islands, 1999; Lalmuansangi and Lalramnghinglova, 2014) for treating hepatic disorders (tender shoot extract), anemic conditions (fruit and seeds) and skin diseases (root extract). The fruits are rich in iron content and beta carotene. From the phytochemical point of view, there is only limited information available on this plant (Singh *et al*., 2014; Rahim *et al*., 2015; Bohra *et al*., 2016).

Fourier Transform Infrared Spectroscopy (FTIR) is a valuable analytical tool to provide a comprehensive metabolite profile of the biological system under investigation. The FTIR method measures predominantly the vibrations of bonds within chemical functional groups and generates a spectrum that can be regarded as a biochemical or metabolic fingerprint of the sample (Mariswamy *et al*., 2012). This analytical tool is inevitable for bioprospecting studies of potential medicinal herbs. The present study is the first attempt to elucidate the spectrum of functional groups present in both leaf and fruit samples of *H. validus* through FTIR analysis.

Materials and Methods

Infra red spectra were obtained by using FTIR spectrophotometer (Model: Perkin Elmer Spectrum 2). Approximately 10 mg of powdered leaf and fruit material

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were made separately into pellet form (Mode M-15) with KBr (50Kg/cm^2 and 40Kg/cm^2 pressure applied respectively for leaf and fruit). Infra red spectra were generated for the wavelengths ranging from 4000 cm^{-1} to 450 cm^{-1} with the two regions *viz.,* functional group region (4000-1450 cm^{-1}) and fingerprint region (1450 – 450 cm^{-1}). Cleaned blank crystal was placed over the sample chamber each time before sample analysis for obtaining reference spectra.

Results and Discussion

The FTIR spectrum of leaf sample showed the presence of alcohols, phenols, aromatic amines, alkanes, alkenes, carboxylic acids, esters, ethers and alkyl halides with major peaks at 3411.83, 2923.95, 1656.14, 1440.99, 1259.85, 1048.41 and 604 cm^{-1} (table 1, fig. 1). Similar spectrum was obtained for fruit samples with major peaks at 3358.9, 2920.64, 1615.29, 1419.17, 1280.34, 1047.77, 1014.08, 899.93, 775.06 and 594.93 cm^{-1} (table 2, fig. 2) confirming the presence of same class of compounds in both leaf and fruit extracts of *H. validus*.

Functional group region : The FTIR spectra exhibited three peaks each in the functional group region for leaf and fruit samples. A strong and broad band with a peak at 3411.83 cm^{-1} in leaves and 3358.9 cm^{-1} in fruit samples corresponding to alcohols and phenols (O-H stretch, H-bonded) was observed. Sharp intensity bands at 2923.95 cm^{-1} and 2920.64 cm^{-1} respectively was observed in leaf and fruit samples, which is a characteristic of aliphatic C-H stretch present in alkanes. A unique, medium intensity band at 1656.14 cm^{-1} was observed in the leaf sample denoting –C=C- stretch in alkenes. A corresponding unique band was observed in fruit sample at 1615.29 cm^{-1} denoting the presence of 1^0 amines.

Fingerprint region : The peaks appearing between 1500-1400 cm^{-1} (1440.99 cm^{-1} in leaf and 1419.17 cm^{-1} in fruit samples) indicates the presence of aromatic cycles in the samples analyzed. The presence of aromatic amines was revealed due to the presence of peaks at 1259.85 cm^{-1} and 1280.34 cm^{-1} in leaf and fruit samples

Table 1 : FTIR data of leaf sample.

Peak name	Peak area/height		Molecules
	X (cm^{-1})	Y (%T)	
Functional group region of the spectra			
1	3411.83	31.21	O-H stretch, H-bonded; alcohols, phenols
2	2923.95	36.35	Aliphatic C-H stretching band; Alkanes
3	1656.14	40.13	-C=C- stretch; alkenes
Fingerprint region of the spectra			
4	1440.99	44.11	C-C stretch (in ring); Aromatics
5	1259.85	46.55	C-N stretch; Aromatic amines
6	1048.41	43.15	C-O stretch; Alcohols, carboxylic acids, esters, ethers
7	604	54.43	C-Br stretch; Alkyl halides

Table 2 : FTIR data of fruit sample.

Peak name	Peak area/height		Molecules
	X (cm^{-1})	Y (%T)	
Functional group region of the spectra			
1	3358.9	16.8	O-H stretch, H-bonded; alcohols, phenols
	2	2920.64	24.85 Aliphatic C-H stretch; Alkanes
3	1615.29	25.77	N-H bend; 1^0 amines
Fingerprint region of the spectra			
4	1419.17	26.97	C-C stretch (in ring); Aromatics
5	1280.34	29.01	C-N stretch; Aromatic amines
6	1047.77	20.98	C-O stretch; Alcohols, carboxylic acids, esters, ethers
7	1014.08	22.25	C-O stretch; Alcohols, carboxylic acids, esters, ethers
8	899.93	34.44	C-H loop; Aromatics
9	775.06	34.02	C-Cl stretch; Alkyl halides
10	594.93	30.06	C-Br stretch; Alkyl halides

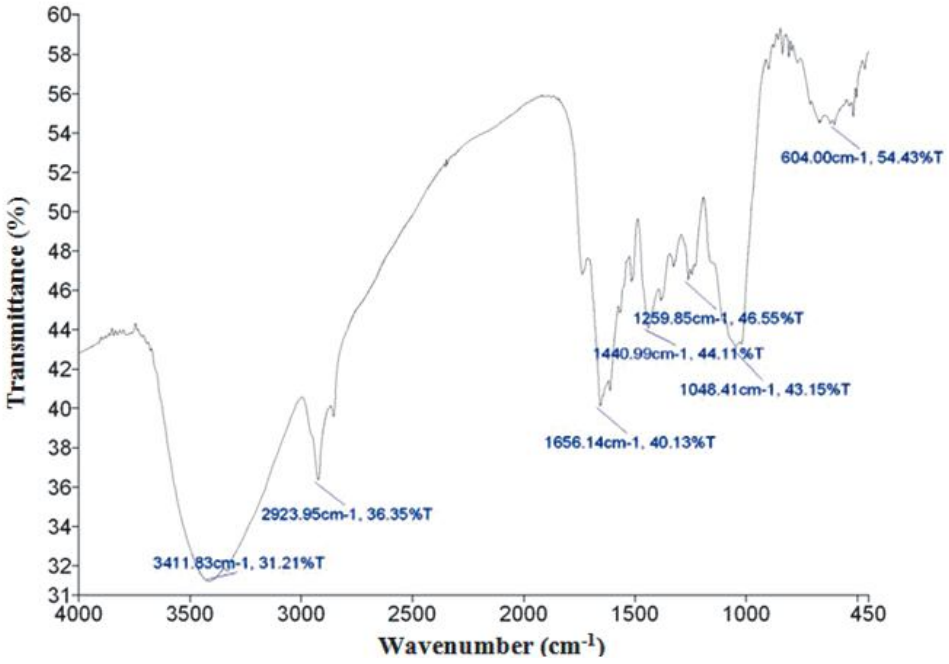

Fig. 1 : FTIR spectra of leaf sample.

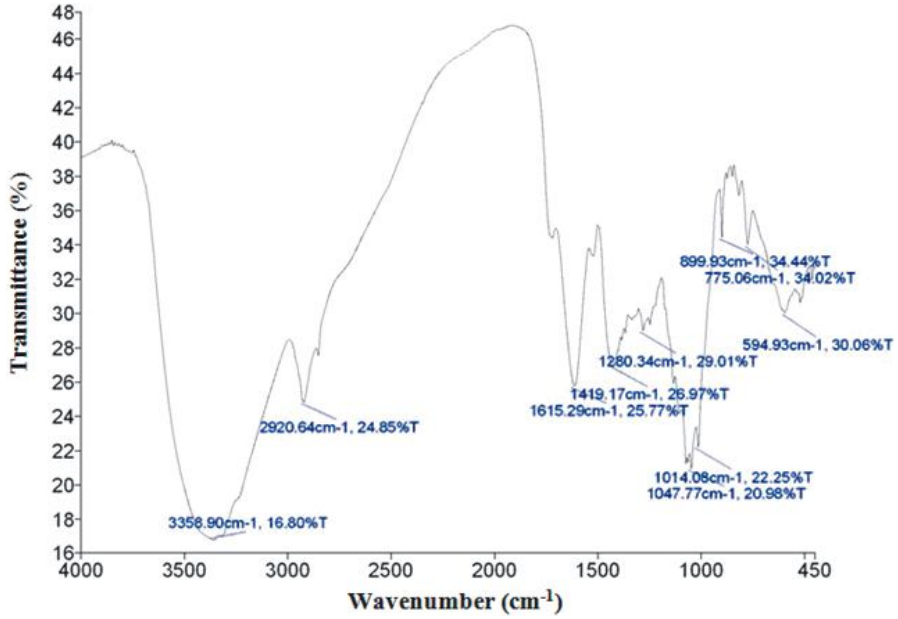

Fig. 2 : FTIR spectra of fruit sample.

respectively. The peaks observed at 1048.41 (in leaf), 1047.77 and 1014.08 cm^{-1} (in fruit) represents C-O stretch vibrations in alcohols, carboxylic acids, ethers and esters. The bands observed at 604 cm^{-1} and 594.93 cm^{-1} in leaf and fruit samples denotes to C-Br stretch of alkyl halides. In addition to the four common peaks present in leaf and fruit samples, the fruit sample had two peaks which are unique. At 899.93 cm^{-1}, a sharp band corresponding to C-H loop of aromatics was detected assuming the presence of an aromatic cycle. The band at 775.06 cm^{-1} correlating to the presence of C-Cl stretch of alkyl halides was also detected.

The corresponding peak heights (%T) of the various functional groups of the leaf samples ranged from 31.21 to 40.13, whereas the peak heights of the various functional groups of the fruit samples ranged from 16.8 to 25.77. The peak heights of the fingerprint region of the leaf samples varied from 43.15 to 54.43 and the fruit samples varied from 20.98 to 34.44.

As an effective and inexpensive analysis method to entirely monitor the whole constituents of the medicinal

materials and their corresponding extract products, FTIR has emerged as a valuable tool in pharmaceutical analysis during last two decades (Ellis *et al*., 2002; Liu *et al*., 2006; Thenmozhi *et al*., 2011). There are many reports available on FTIR analysis which supports its usefulness in comparing, classifying and differentiating various samples on the basis of molecular fingerprint obtained (Mariswamy *et al*., 2012; Ashokkumar and Ramaswamy, 2014; George and Shanmugam, 2014; Joseph and George, 2014).

FTIR analysis of leaf and fruit samples of *H. validus* revealed the spectrum of chemical constituents present in these materials. The functional group similarity of both the leaf and fruit samples corroborates the fact that the molecular fingerprint generated through FTIR spectroscopy can differentiate samples or identify materials precisely and efficiently on the basis of their origin. The functional group region contained relatively few peaks as expected which are generally associated with the stretching vibrations of the functional groups. While the fingerprint region of bending vibrations provided a unique and complicated pattern of peaks facilitating identification to each plant part (*i.e.*, leaf and fruit) and checking the authenticity of plant materials. The fingerprint region has its significance in having unique peak patterns, which can be further utilized for chemotaxonomy, surveillance of herbal drug adulteration, pharmacognosy, etc. Thus FTIR can be used as an effective tool to rapidly obtain a comprehensive profile of metabolome with relatively low analytical cost and technical simplicity.

In conclusion, the FTIR spectral analysis of the leaf and fruit samples of *H. validus* revealed the presence of characteristic functional groups ascribed to alcohols, phenols, aromatic amines, alkanes, alkenes, carboxylic acids, esters, ethers and alkyl halides. Further studies are required for the identification and characterization of medicinally active principles of this valuable ethnomedicinal plant by employing high throughput chromatographic separation methods hyphenated to mass spectroscopic techniques and detailed structural analysis.

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