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Original Article

FTIR Based Metabolomics Profiling and Fingerprinting of Some Medicinal Plants: An Attempt to Develop an Approach for Quality Control and Standardization of Herbal Materials

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ABSTRACT

Background: The medicinal plants are used for their various therapeutic effects in treatment and prevention of various diseases. Recently herbal medicines are playing an important role in health care across the world. With increased global acceptance of the herbal drugs proper quality control of the herbal medicines have become important to ensure the safety and efficacy of the herbal products. Herbal-based FT-IR metabolomics is a suitable method for quick and reliable quality control and metabolite profiling to ensure quality and reproducibility of herbal medicine. The FT-IR analysis is relatively easy to use, reproducible, non-destructive, and can be used for quick analysis and verification of the herbal medicines. **Materials and Methods:** In the present work FTIR fingerprint was obtained for extracts and powders of some of the selected medicinal plants. The samples were characterized by using FTIR metabolomics profiling on basis of the diagonostic peaks. **Results:** Various functional groups, such as phenolics (-OH), carbonyl (C=O), aldehyde (CH=O), ether (C-O-C), aromatic (C=C), and alkyl groups -CH, were identified. Various metabolites e.g. liquiritin, glycyrrhizic acid, glabridin, shogoal, piperine were successfully identified on basis of the diagonostic FTIR peaks. **Conclusion:** FTIR was found to be a simple, rapid and convenient analytical method and fingerprinting technique for quality control of herbal materials.

Keywords: Medicinal Plants, Fingerprinting, Metabolomics, Secondary metabolites, FTIR, Quality control.

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INTRODUCTION

The Herbal medicines have long been used for the treatment and prevention of various diseases. The natural based medicines have evolved across different parts of world through traditional knowledge and experience. Nowadays the trust on herbal and traditional medicines has increased in the global health care. The Ayurveda an Indian traditional medicine system has become very popular in world due to its long history of uses in treatment and prevention of various diseases. Due to increase in awareness about the herbal ingredients in healthcare and wellness the quality control and standardization of the herbal medicines has become an essential part to ensure the safety and efficacy of the products.[1] Quality control of the herbal raw materials is carried out by various organoleptic tests of color, odour, test and various physicochemical evaluations mentioned in pharmacopoeial monographs. But these approaches are having some of the disadvantages like lack of specificity.[2] Nowadays a metabolomics is emerging as a versatile and holistic approach for quality control of herbal drugs.[3] Metabolomics is defined as the simultaneous study of group of plant metabolites or group of phytochemicals present in an herbal sample.[4] Metabolomics study help in comprehensive analysis, characterization, identification and quantification of metabolites present in biological samples like animal or plant origin.[5] It helps in overall analysis of chemical nature of the metabolites present in the sample.[6,7] Most frequently used techniques used in herbal metabolomics are proton nuclear magnetic resonance spectroscopy (^{1}H-NMR), Fourier transform infrared (FTIR) spectroscopy and hyphenated techniques like e.g. gas chromatography-mass spectrometry (GC-MS), Liquid chromatography-mass spectrometry (LC-MS), ultra-performance liquid chromatography-mass spectrometry (UPLC-MS), and high performance thin layer chromatography (HPTLC).[8,9]

The FT-IR spectrometry can be used as a valuable tool for the metabolic fingerprinting that simultaneously analyzes a wide range of primary metabolites like carbohydrates, amino acids, proteins, polysaccharides and secondary metabolites like phenolics, alkaloids and steroids, flavonoids etc in the plants. The FTIR fingerprint provides information about the functional

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Table 1: Interpretation of FTIR fingerprint of *Terminalia chebula* aqueous extract.

FTIR Frequencies (IR_{max} in cm^{-1})	Related functional groups
3737.79, 3614.35, 3150.50 cm^{-1}	Hydroxyl and phenolic OH groups
2878.56 cm^{-1}	CH, CH_2, CH_3
1718.46 cm^{-1}	Carbonyl group from carboxylic acid
1608.52, 1511.12, 1452.30 cm^{-1}	Aromatic C=C stretching, amide C-H C-N and N-O stretching groups
1339.47, 1208.81, 963.38, 871.76, 760.87, 640.32, 517.85 cm^{-1}	C-O stretching and OH bending

groups of molecules on the basis of specific wavelengths. So it supports in identification of the multiple phytochemicals present in the herbal extracts or powders on the basis of diagonostic functional group patterns. Further the FTIR spectrometry is having several advantages like it is a non-destructive, relatively simple, cost effective, easier method and needs minimum samples preparation. The FTIR fingerprint is also possessed high reproducibility and specificity.[10,11] In the present study the extracts and powders of various common and useful medicinal plants like *Ocimum sanctum, Curcuma longa, Terminalia chebula, Glycyrrhiza glabra, Zingiber officinale*, and *Piper longum* were analyzed using FTIR-based metabolomics for characterizing secondary metabolites. The metabolites were identified by comparing FTIR spectra data with the data from the published literatures. The objective of this study we have attempted to develop FTIR technique based metabolic fingerprinting of the various herbal extracts and herbal powder samples.

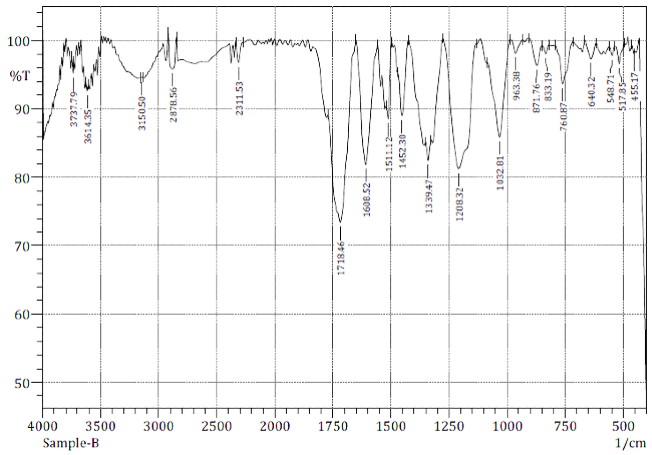
MATERIALS AND METHODS

The FTIR spectra of the samples were recorded FTIR instrument (Shimadzu, 8400S). A small amount of sample was made into pellets using KBr for FTIR analysis. The data of infrared transmittance was collected over a wave number ranged from 4000 cm^{-1} to 500 cm^{-1}. The spectra were compared with reference to identify the characteristic functional groups present. FTIR spectra used for metabolite profiling of the herbal samples.

RESULTS

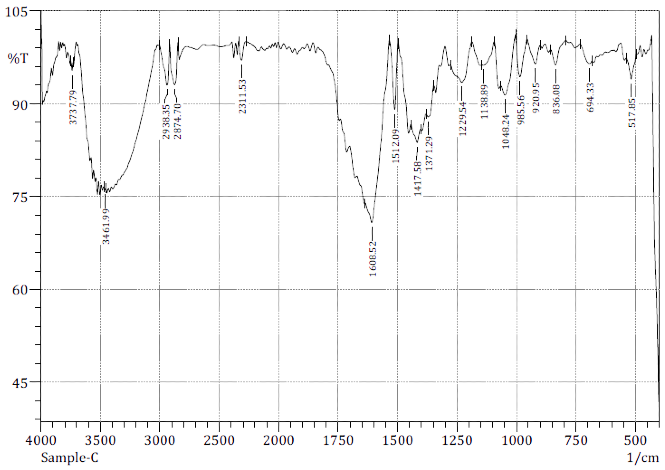
FTIR analysis of *Terminalia chebula* aqueous extract

The FTIR frequencies of the aqueous extract of *Terminalia chebula* is presented in the Table 1. The pattern of the IR fingerprint was in accordance with that of reported for compounds like gallic acid and arabinogalactan.[12-14]



FTIR analysis of *Glycyrrhiza glabra* root aqueous extract

IR_{max} in cm^{-1} around 3500, 3350, 2600, 1608.52, 1512.09, 1138.89, 836.08 matches with the peak for glabridin in accordance with previous IR published information. Peaks at 1612 and 1512 cm^{-1} were the characteristic peaks of liquiritin, peak around 1100 to 1000 cm^{-1} represent for polysacchraides, peaks at 745 and 1386 corresponds to glycyrrhizic acid.[15-16]

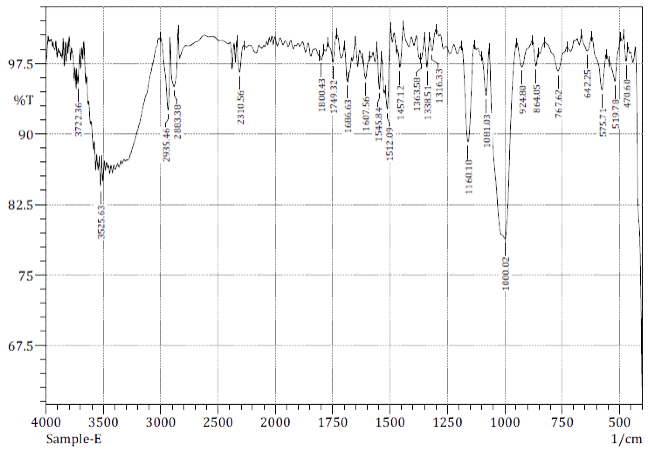


FTIR analysis of *Zingiber officinale* powder

IR_{max} (KBr) in cm^{-1}: 2935.46, 2883.38 (CH-stretch), 3525.63 (OH-stretch), 3722.36 (OH-stretch), 1686.63 (C=O-stretch), 1607.56 (C=O stretch), 1545.84, 1512.09, 1457.12 (C=C stretch), 1000.2 (C-O stretch), 1160.10 (C-O stretch), 1081.03 (C-O stretch). These characteristic absorption bands in the infrared absorption spectrum was in accordance with that of reported for gingerol. The pattern of the peaks represent presence of flavonoids, phenolic and ketone type of compounds in ginger such as gingerol, paradol, shogoal, gingerone A, zingerone, quercetin.[17]

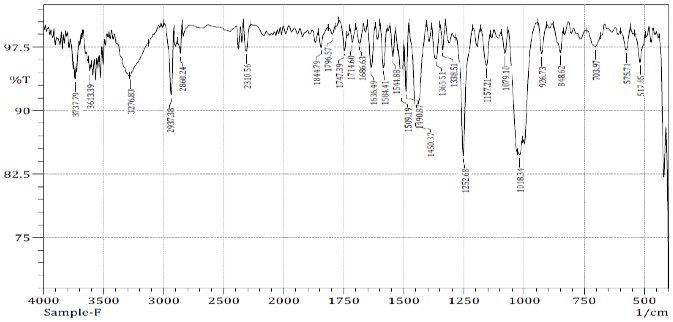
Table 2: Characteristic FTIR peaks of identified metabolites in selected herbs.

FTIR Frequencies (IR_{max} in cm^{-1})	Metabolites identified	Name of the Herb	Reference
3614.35, 3150.50, 1718.46, 1608.52, 1452.30, 1339.47, 1208.81, 760.87 cm^{-1}	Gallic acid	*Terminalia chebula*	[14,26]
2878.56, 1511.12, 1608.52, 963.38, 1032.81 cm^{-1}	Arabinogalactan protein		
2935.46, 1607.56, 1607.56, 1081.03, 1160.10 cm^{-1}	Gingerols	*Zingiber officinale*	[27-28]
2935.46, 3525.63, 1545.84, 1512.09, 1686.63, 1316.33, 1081.03, 1000.20 cm^{-1}	Shogaols		
3526.63, 2935.46, 1749.32, 1545.84, 1316.33,1081.03 cm^{-1}	Paradol		
3525.63 (br-OH), 1749.32 (C=O)	Phenylalkanoids		
2937.38 (C-H),1636.49 (C=O), 1490.87 (C-O)	Piperine	*Piper longum*	[29]
1604.66, 1511.12, 1362.61, 1315.36, 1029.92 cm^{-1}	Curcumin	*Curcuma longa*	[23]



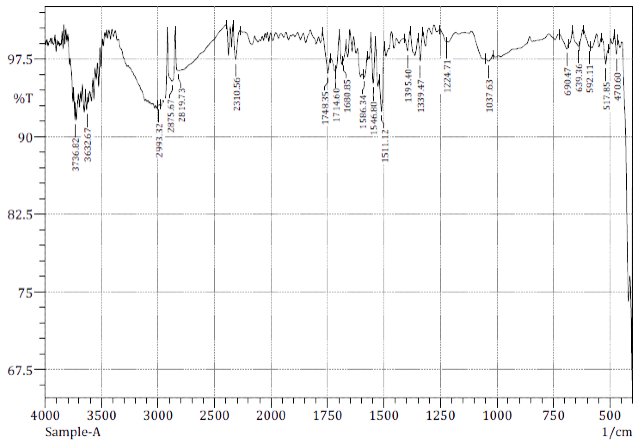
FTIR analysis of *Piper longum* powder

IR_{max} (KBr) in cm^{-1}: 3737.79 (OH), 3613.39 (OH), 3276.83 (OH), 2937.38 (CH), 2860.24 (CH), 2310.56(CH), 1844.79 (C=O), 1796.57 (C=O), 1747.39(C=O), 1714.60(C=O), 1686, 63 (C=O), 1636.49 (C=O), 1584.41 (N-H bending), 1509.19,1490.87 (C=C), 1450.37, 1365.51, 1338.51, 1252.68, 1157.21, 1079.10 (C-O), 1018.34 (C-O), 926.73 (C-O), 848.62 (C-O), 703.97 (C-O). IR spectrum showed shows peaks corresponding to the functional groups present in piperine.[18]



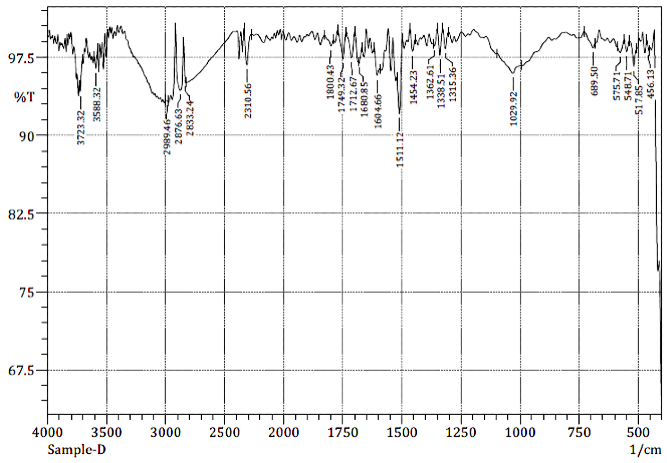
FTIR analysis of *Ocimum sanctum* leaf aqueous extract

IR_{max}(KBr) in cm^{-1} : 3736.82 (OH), 3632.67(OH), 2993.32 (CH), 2875.67 (CH), 2819.73 (CH), 1748.35 (ester C=O), 1714.60(ester C=O), 1680.85 (aldehyde CH=O), 1636, 1586.34, 1546.80 and 1511.12 (aromatic C=C), 1224.71 (C-O-C) 1395.40, 1339.47 (OH bending), 690.47, 639.36 (CH bending). The pattern of the spectra infers presence of flavonoid, phenolic and glycoside class of phytochemicals in the extract.[19,20]



FTIR analysis of *Curcuma longa* powder

IR_{max} in cm^{-1}**:** 3723.32, 3588.32, and 3580 (phenolic OH stretching), 2989.46, 2876.63, 2833.24, 2310.56 (C-H), 1800.56 (C=O), 1749.32 (C=O), 1712.67(C=O), 1680.85 (C=O), 1604.66 (aromatic C=C stretching), 1597 (benzene ring stretching), 1511.12(C=O and C=C vibration), 1029.92 (C-O-C stretching), 1454.23 (olefinic C-H stretching).[22,23]



DISCUSSION

Medicinal plants continued to contribute enormously to drug discovery and development in modern medicine. The plants are rich source of versatile types of complex bioactive metabolites of different chemical scaffolds. Spectroscopic characterization of metabolites present in medicinal plants is a useful technique for understanding their phytochemical constituents.[23] Metabolomics study helps in simultaneous identification several metabolites present in the plants without prior complex and time consuming chromatographic purifications. The metabolomics can be used as a promising technique for dereplication of metabolites present in the medicinal plant extracts.[24-25] In the above study various phytochemical marker constituents like liquirtin, piperine, gingerols and curcumin were identified on basis of the diagonostic FTIR peaks in the spectra. The characteristic peaks of some of the identified metabolites are given in the Table 2.

CONCLUSION

In this study we have investigated fingerprinting properties and metabolite profiling of the complex mixtures of the herbal samples like extracts and powders using FTIR based metabolomics approach. The characteristic FTIR spectra of some of the popular Indian medicinal plants were obtained. Various functional groups, such as -CHO, -COOH, -NO_2, -NH, and –OH were identified on basis of FTIR frequencies. The application of FTIR analyses was successful in analysis and identification of various secondary metabolites present in the selected medicinal plants. FTIR was found to be a simple, easy to use, rapid and inexpensive method for identification and detection of adulteration and for checking in any variation in herbal raw material. So it can be a very useful and supportive tool in the quality control and standardization of the herbal raw materials that are useful for phytopharmaceuticals and nutraceuticals industries.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

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