IDENTIFICATION AND COMPARISON OF BIOMOLECULES IN MEDICINAL PLANTS OF *TEPHROSIA TINCTORIA* AND *ATYLOSIA ALBICANS* BY USING FTIR

J. KOMAL KUMAR, A.G. DEVI PRASAD

Department of Environmental Science, University of Mysore, Mysore – 570006, Karnataka, India,
e mail: kompaddy@yahoo.com

Abstract. The aim of this study is to adopt the approach of metabolic fingerprinting through the use of Fourier Transform Infrared technique to understand the composition, chemical structure and discrimination of biomolecules in medicinal plants of *Tephrosia tinctoria* and *Atylosia albicans*. IR spectrum in mid infrared region (4000–400cm^{-1}) was used for discriminating and indentifying various functional groups present in two different species of medicinal plants belonging to the family Leguminosae. Presence of C=O, C–H, C=C and C–O, C–C, 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. In the present study FTIR Spectroscopy was used as a sensitive and effective assay for the detection and comparison of biomolecules between the two species of medicinal plants. The results showed that *Tephrosia tinctoria* and *Atylosia albicans* are rich in phenolic compounds.

Key words: FTIR, biomolecules, *Atylosia albicans, Tephrosia tinctoria*, leguminosae.

INTRODUCTION

Medicinal plant research includes much more than the discovery of new drugs. This field has been expanding to also include diverse subjects as negotiation of power based on medicinal plant knowledge [11]. A wide range of our recently used medicines had their roots directly or indirectly from plants. Some of these medicines are no longer synthesized in large quantities by competitors because they have shown toxicity to humans and other animals. This has made possible for more investigations to be carried out on plants so as to enable us to know the therapeutic status of newly discovered drugs of plant origin. In this respect, plant based research has made promising results in the fields of anticancer and anti-malarial therapies [5].

Received: November 2010;
in final form January 2011.

ROMANIAN J. BIOPHYS., Vol. 21, No. 1, P. 63–71, BUCHAREST, 2011

Attempts to apply IR technology to biology began as early as the 1910s, when the use of IR spectroscopy for the analysis of biological samples was first suggested. By the late 1940s, the technique was being successfully explored for the study of biological materials and, in fact, IR spectroscopy has become an accepted tool for the characterization of biomolecules [16]. FTIR has been proven to be useful in studying compositional changes in plant cell walls during development. Therefore, it can possibly be used to determine changes in cell wall architecture upon exposure to organic contaminants [18]. Initially, the use of infrared spectroscopical method is restricted only for structural elucidation of isolated compounds from the herbal matrices. It was also found to be useful in phytochemical studies as a fingerprinting device, for comparing natural with synthetic sample [14]. Drug discovery from the medicinal plants continues to provide new and important leads against various pharmacological targets including cancer, HIV/AIDS, Alzheimer's, malaria, infections and pain [1].

A number of events preceding the interaction of a drug with biological target and/or pharmacological effects have to be considered, and these involve absorption, distribution, metabolism and elimination. In order to assess the importance of each of these factors on drug action, both structural and physico-chemical properties of the drug should be taken into account [12]. In biological systems, properties such as electrostatic bonds, hydrogen bonds, van der Waals bonds, as well as effects related to electron-transfer and hydrophobic effects are of major importance. Although the hydrogen bond is fairly weak compared to other interactions, it is of paramount importance in biological systems. Investigations of drug metabolism, its biotransformation pathways and structure of formed metabolites are of toxicological, pharmacological and biomedical interest [25].

In the present study FTIR spectroscopy was used for identification and comparison of biomolecules in two different species of medicinal plants.

MATERIALS AND METHODS

COLLECTION AND IDENTIFICATION OF PLANT MATERIAL

Two medicinal plants *Atylosia albicans* and *Tephrosia tinctoria* were collected from the Western Ghats region of Hassan District, Karnataka, India. The leaf, flower, fruit and stem were carefully excised from the plant. These cleaned plant parts were shade dried and placed in polythene bags. The Herbaria of these plants are kept in the Department of Environmental Science, University of Mysore.

PREPARATION OF THE PLANT MATERIAL

The plant parts 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 plant powders were kept in a Lyophilizer to remove water. The samples were again ground in an agate mortar and pestle in order to obtain fine powder. Each powdered plant material was mixed with completely dried potassium bromide (at a ration of 1/100), and the mixture was subjected to a pressure of 5×10^6 pa in an evacuated die to produce a Kbr pellet for use in a FTIR spectrometer.

TEST CHEMICALS

The AnalaR grade Alcohol and Kbr 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 samples of both *Atylosia albicans* and *Tephrosia tinctoria* were mixed with dried potassium bromide and prepared as pellets, scanned at room temperature (25±2 ºC) at 4000–400cm^{-1} spectral range. To improve the signal to noise ratio for each spectrum, 100 interferograms with a spectral resolution of ±4cm^{-1} were averaged. Background spectra, which were collected under identical conditions, were subtracted from the sample spectra. Each sample was scanned under the same conditions with six different pellets. Special care was taken to prepare the pellets at the same thickness by taking the same amount of sample and applying the same pressure. Therefore, in the present study it was possible to directly relate the intensities of the absorption bands to the concentration of the corresponding functional groups [3].

RESULTS AND DISCUSSION

The results of functional group analysis using FTIR revealed the existence of various characteristic functional groups in leaves, stem, flower and fruit of *A. albicans* and *T. tinctoria* (Fig. 1 to Fig. 5). The absorption bands, the wave number (cm^{-1}) of dominant peak obtained from absorption spectra were defined in Table 1.

The dominant bands at 1655cm^{-1} and 1546 cm^{-1} were attributed to protein amide I and II bands [8]. The shoulder at about 1750 cm^{-1} was attributed to lipid C=O stretching vibration [8]. The band at 1465 cm^{-1} was assigned to the CH_2 bending mode of the cell lipids. The band at 1460 cm^{-1} represents asymmetric CH_3 bending modes of end ethyl group proteins [26]. The band at 1402 cm^{-1} represents C=O symmetric stretching of COO^- and assigned to lipids [26 and 17]. And band at 1377 cm^{-1} represents C–H bending mode of CH_2 [2]. 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 [8]. The peak at 1064 cm^{-1} resulted from the overlap of several bands, including absorption due to the vibration modes of CH_2OH and the C–O stretching vibration coupled to the C–O bending mode of cell carbohydrates [27]. The very strong absorption band observed around 3373–3422 cm^{-1} may be due to the presence of bonded N–H/C–H/O–H stretching of amines and amides [19]. The very strong absorption at 3400 cm^{-1} shows the presence of amino acids and the very strong absorption band appearing in the region 2933–2922 cm^{-1} is due to N–H stretching. The lone C=O stretching vibration band corresponding to saturated aliphatic ester 1743 cm^{-1} is present in all parts of the plants. The bands at 900–1350cm^{-1}, 1020 cm^{-1}, 1024 cm^{-1} and 1050–100 cm^{-1} are attributed to phosphodiester stretching bands region (for absorbances due to collagen and glycogen), DNA, glycogen (C–O stretch associated with glycogen, phosphate and oligosaccharides PO^{-2} stretching modes), P–O–C antisymmetric stretching mode of phosphate ester, and C–OH stretching of oligosaccharides respectively. A band at 1051 cm^{-1} is attributed to C–O–C stretching of DNA and RNA [10]. The more intense bands occurring at 3419 cm^{-1}, 2927 cm^{-1}, 2853 cm^{-1}, 1633 cm^{-1},1421 cm^{-1}, 1260 cm^{-1}, 1073 cm^{-1}, 816 cm^{-1}, and 635 cm^{-1} corresponding to O–H/N–H, C–H, C–O and C–CI/C–S stretching/bending vibrations respectively indicate the presence of amino acids, alkenes, nitrates, ethers, organic halogen compounds and carbohydrates in *A. albicans* and *T. tinctoria* [21].

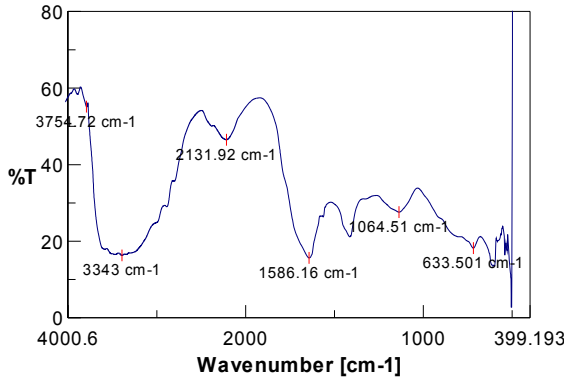

Fig. 1. FTIR spectra of *Tephrosia tinctoria* leaves.

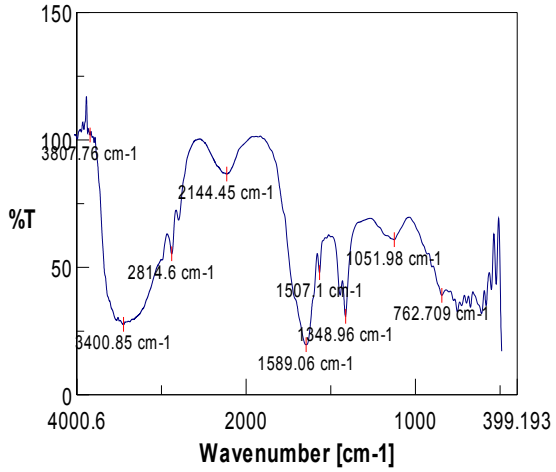

Fig. 2. FTIR spectra of *Atylosia albicans* leaves.

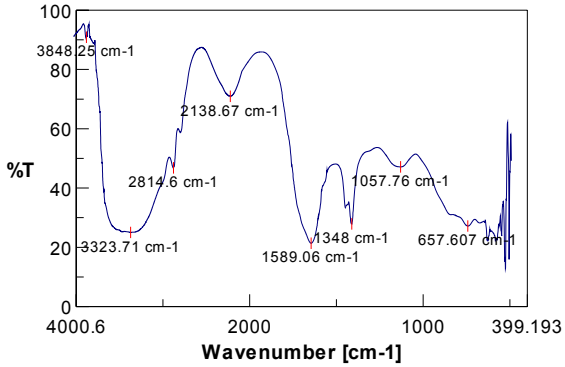

Fig. 3. FTIR spectra of *Tephrosia tinctoria* flower.

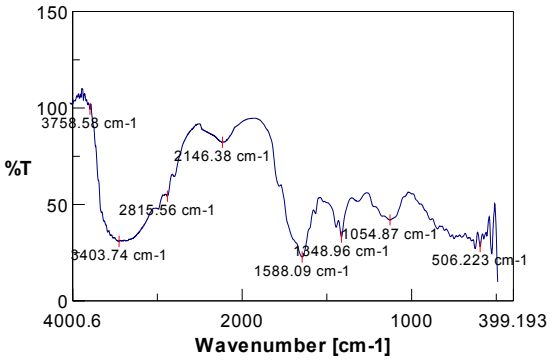

Fig. 4. FTIR spectra of *Atylosia albicans* fruit.

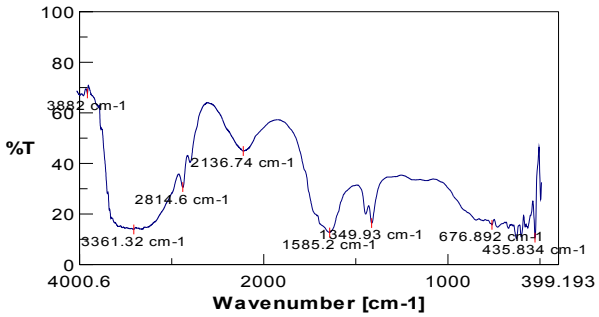

Fig. 5. FTIR spectra of *Atylosia albicans* stem.

Table 1

General band assignments of the FTIR spectra of biological tissue based on literature [20]

S. No.	PEAK	ASSIGNMENT
1	521 cm^{-1}	torsion and ring torsion of phenyl
2	600–900 cm^{-1}	CH out-of-plane bending vibrations
3	892 cm^{-1}	C–C, C–O deoxyribose
4	940 cm^{-1}	Carotenoid
5	1000–140 cm^{-1}	Protein amide I absorption
6	1000–200 cm^{-1}	C–OH bonds in oligosaccharides such as mannose & galactose
7	1000–350 cm^{-1}	Region of the phosphate vibration carbohydrate residues attached to collagen and amide III vibration (in collagen)
8	1020–50 cm^{-1}	Glycogen
9	1030 cm^{-1}	Collagen
10	1105 cm^{-1}	Carbohydrates
	1145 cm^{-1}	Phosphate & oligosaccharides
11	1180–300 cm^{-1}	Amide III band region
12	1206 cm^{-1}	Amide III Collagen
13	1244/5 cm^{-1}	PO^{-2} asymmetric (phosphate I)
14	1255 cm^{-1}	Amide III
15	1312–1317 cm^{-1}	Amide III band components of proteins collagen
16	1456 cm^{-1}	CH_3 bending vibration (lipids and proteins)
17	1482 cm^{-1}	Benzene
19	1504 cm^{-1}	In-plane CH bending vibration from the phenyl rings
20	2800–3000 cm^{-1}	C–H Lipid region
21	3500–600 cm^{-1}	OH bonds
22	3000–700 cm^{-1}	O–H stretching (water)

A symmetrical stretching of NO_2 group results in strong absorption in the region 1660–1625 cm^{-1}. The observed absorption band at 1630 cm^{-1} indicates the presence of amines (protein) [15]. This gives the evidence that the plants *A. albicans* and *T. tinctoria* are rich in proteins. The weak absorption band

observed between 1421 and 1415 cm^{-1} in the plant parts of *A. albicans* and *T. tinctoria* may be due to the presence of bonded C–O/O–H bending. The medium absorption band of 620 cm^{-1} indicates the presence of sulphate. A strong absorption band occurs at 597 and 580 cm^{-1} in the stem, leaves, fruits and flower of two species which is possibly due to aliphatic C–CI absorption and brominate compounds. The brominate compounds show an infrared band region at 600–500 cm^{-1} [24]. The weak absorption band at 539 cm^{-1} indicates the presence of phosphates in the leaves, fruits, flowers and stem in the examined plants. The very weak band occurring at 780 cm^{-1} in the flowers, leaves, stem and fruits of these plants can be attributed to out-of-plane N–H wagging, primary and secondary amide and nitrite group.

Five major peaks 1590 cm^{-1}, 1348 cm^{-1}, 1051, 3385 cm^{-1}, 1063 cm^{-1} and 456 cm^{-1} were observed in the FTIR spectra. A weak absorption peak at 453 cm^{-1} has appeared which is attributed to the absorption of Y–O bond [28]. This is a significant observation made in *A. albicans* as it is not reported in any legumes so far. A weak absorption band at 940 cm^{-1} is attributed to carotenoid being present only in the fruit of *A. albicans* [13]. A weak absorption band at 965 cm^{-1} is attributed to C–O stretching of the phosphodiester and the ribose and is present only in *A. albicans* [4]. A very weak peak at 892 cm^{-1} is attributed to C–C, C–O deoxyribose and seen only in *A. albicans* [7]. A medium peak at 1340 cm^{-1} is due to CH_2 wagging collagen present in *A. albicans* [9, 27]. A very strong peak at 1581 cm^{-1}, 1358 cm^{-1}, 520 cm^{-1} is attributed to ring C–C stretch of phenyl, stretching C–O, deformation C–H, deformation N–H and phenyl group respectively, are present in fruits, leaves and the stem of *A. albicans* and leaves and flowers of *T. tinctoria* [6, 23]. A medium peak at 3300 cm^{-1} and 1020–1050 cm^{-1} is attributed to amide I bands stemming from N–H stretching modes in proteins, nucleic acids and glycogen [12].

CONCLUSIONS

By the above investigations, it can be concluded that *A. albicans* and *T. tinctoria* are rich in phenolic compounds and also show the presence of oligosaccharides, phosphates, proteins, carbohydrates and carotenoid. The present work also indicates the presence of biomolecule concentration is different in different parts of the plants. This work offers scope for further research in phytochemical analysis and biological activity of medicinal plants. Fourier transform infrared spectroscopy is proved to be a reliable and sensitive method for detection of biomolecular composition of cells. In the present study we examined the potential of FTIR spectroscopy for easy and rapid discrimination and identification of various functional groups responsible for medicinal properties. Spectral area ranged between 4000–400 cm^{-1} could be considered as an important area for an easy and reliable discrimination between different plant species based on biomolecules, as it provides a unique fingerprint for the biomolecules.

REFERENCES

1. BALUNAS, M.J., A.D. KINFHORN, Drug discovery from medicinal plants, *Life Scien.*, 2005, **78,** 431–441.
2. BRANDENBURG, K., U. SEYDEL, Vibrational spectroscopy of carbohydrates and glycoconjugates, in *Handbook of Vibrational Spectroscopy*, John Willey and Sons, Chichester, UK, 2002, pp. 3481–3507.
3. CAKMAK, G., I. TOGAN, F. SEVERCAN, 17β-estradiol induced compositional, structural and functional changes in rainbow trout liver, revealed by FT-IR spectroscopy: A comparative study with nonylphenol, *Aquatic Toxicology*, 2006, **77**, 53–63.
4. CHIRIBOGA, L., P. XIE, H. YEE, V. VIGORITA, D. ZAROU, D. ZAKIM, M. DIEM, Infrared spectroscopy of human tissue. I. Differentiation and maturation of epithelial cells in the human cervix, *Biospectroscopy*, 1998, **4**, 47–53.
5. DE SMET, P.A., The role of plant derived drugs and herbal medicines in healthcare. *Drugs*, 1997, **54**, 801–804.
6. DOVBESHKO, G.I., N.Y. GRIDINA, E.B. KRUGLOVA, O.P. PASHCHUK, FTIR spectroscopy studies of nucleic acid damage, *Talanta*, 1997, **53**, 233–246.
7. DOVBESSHKO, G.I., V.I. CHEGEL, N.Y .GRIDINA, O.P. REPNYTSKA, Y.M. SHIRSHOV, V.P. TRYANDIAK, I.M. TODOR, G.I. SOLYANIK, Surface enhanced IR absorption of nucleic acids from tumor cells: FTIR reflectance study, *Biopolymer Biospectroscopy*, 2002, **67**, 470–486.
8. DUKOR, R.K., Vibrational spectroscopy in the detection of cancer, in *Handbook of Vibrational Spectroscopy*, John Wiley and Sons, Chichester, 2002, pp. 3335–3360.
9. ECKEL, R., H. HUO, H.W. GUAN, X. HU, X. CHE, W.D. HUANG. Characteristic infrared spectroscopic patterns in the protein bands of human breast cancer tissue, *Vibrational Spectroscopy*, 2001, **27**, 165–173.
10. FABIAN, H., M. JACKSON, L. MURPHY, P.H. WATSON, I. FICHTNER., H.H. MANTSCH, A comparative infrared spectroscopic study of human breast tumors and breast tumor cell xenografts, *Biospectroscopy*, 1995, **1(**1**)**, 37–45.
11. GARRO, L.C., Intercultural variation folk medicinal knowledge: A comparison between curers and noncurers, *American anthropologist*, 1986, **88**, 351–370.
12. HACKHELL, U., Structural and physicochemical factors in drug action, in *A Textbook of Drug Design and Development,* 2nd ed., Harwood Academic Publishers, Amsterdam, 1996, pp. 35–59.
13. HANLON, E.B., R. MANOHARAN, T.W. KOO, K.E. SHAFER, J.T. MOTZ, M. FITZMAURICE , J.R KRAMER, I. ITZKAN, R.R. DASARI, M.S. FELD, Prospects for *in vivo* Raman spectroscopy, *Physics in Medicine and Biology*, 2000, **45**, 1–59.
14. HARBORNE, J.B., *Phytochemical Methods. A Guide to Modern Techniques of Plant Analysis*, 3rd Edition, Chapman and Hall, UK, 1998.
15. JONES, R.N., B. CLEVERLEY, *Chemical Application of Spectroscopy*, Wiley Inter Science, New York, 1956.
16. MARGARITA P. R. Q., Fourier transform infrared (FTIR) technology for the identification of organisms, *Clinical Microbiology Newsletter*, 2000, **22**(8), 57–61.
17. MAQUEELIN, K., L.P. CHOO-SMITH, C. KIRSCHNER, N.A. NGO-THI. D. NAUMANN, G.J. PUPPLES, Vibrational spectroscopic studies of micro organisms, in *Handbook of Vibrational Spectroscopy*, John Wiley and Sons, Chichester, UK, 2002, pp. 3309–3334.
18. MC CANN, M.C., L. CHEN, K. ROBERTS, E.K. KEMSLEY, C. SENE, N.C. CARPITA, N.J. STACEY, R.H. WILOSON, Infrared micro spectroscopy: Sampling heterogeneity in plant cell wall composition and architecture, *Physiol. Plantar.*, 1997, **100**, 729–738.
19. MOHAN. J, *Organic Spectroscopy. Principle and Applications*, Nasrosa Publishers, New Delhi, 2001.
20. MOVASAGHI, M., S. REHMAN, I.U. REHMAN. Fourier transform infrared (FTIR) spectroscopy of biological tissues, *Applied Spectroscopy Reviews*, 2008, **43**, 134–179.

21. MURUGANANTHAM, S., G, ANBALAGAN, N. RAMAMURTHY, FTIR and SEM-EDS comparative analysis of medicinal plants, *Eclipta alba* Hassk and *Eclipta prostate* Linn, *Romanian J. Biophs.*, 2009, **19(**4**),** 285–294.
22. SANDHYA, B., S. THOMAS, W. ISABEL, R. SHENBUGARATHAI, Ethnomedicinal plants used by the valaiyan community at Piranmalai Hills (reserved forest) Tamil Nadu India. A pilot study, *Afr. J. Trad. Comp. Alt. Med*., 2006, **3(**1), 101–114.
23. SCHULZ, H., M. BARANSKA. Identification and qualification of valuable plant substances by IR and Raman spectroscopy, *Vibrational Spectroscopy*, 2007, **43**, 13–25.
24. SILVERSTEIN R.M., F.X. WEBSTER, *Spectroscopic Identification of Organic Compounds*, John Wiley and Sons, New York, 1996.
25. VERMUELEN, N.P.E., Role of metabolism in chemical toxicity, in: C. Ioanides, ed., *Cytochrome p450: Metabolic and Toxicological Aspects*, CRC Press, Boca Raton, FL, 1995, pp. 29–53.
26. WONG, P., S. GOLDSTEIN, R. GREKIN, A .GODWIN, C. PIVIK, B. RIGA , Distinct infrared spectroscopic pattern of human basel cell carcinoma of the skin, *Cancer Research*, 1993, **53**, 762–765.
27. YANG, D., D. CASTRO, I. EI-SAYED, M. EL-SAYED, R. SAXON, Y. NANCY, A Fourier transforms infrared spectroscopic comparison of cultured human fibroblast and fibrosarcoma cells, *SPIE*, 1995, **2389**, 543–550.
28. YU, M., J. LIN, J. FANG, Silica spheres coated with YVO4:Eu3+ layers via sol-gel process: A simple method to obtain spherical core-shell phosphors, *Chem. Mater.*, 2005, **17***,* 1783–1791.