GLOBAL JOURNAL OF BIO-SCIENCE AND BIOTECHNOLOGY

www.scienceandnature.org

FOURIER TRANSFORM INFRARED SPECTROSCOPY (FTIR) ANALYSIS OF SOME ISOLATED ENDOPHYTIC FUNGI

M. Kanjana*, G. Kanimozhi and A. Panneerselvam
Post Graduate and Research Department of Botany and Microbiology, A.V.V.M. Sri Pushpam College (Autonomous), Poondi - 613 503, Thanjavur, Tamilnadu, India.
*Corresponding author e-mail: kanjimsm@yahoo.com

ABSTRACT
The present study was aimed to identify the functional groups present in the ethyl acetate extracts of isolated endophytic fungi *Chaetomium globosum*, *Cladosporium tenuissimum* and *Penicillium janthinellum by* FTIR spectroscopic analysis**.** The leaves of medicinal plants *Passiflora foetida*, *Memecylon edule* and *Justicia adhatoda* were used in this study for the isolation of endophytic fungi *Chaetomium globosum*, *Cladosporium tenuissimum* and *Penicillium janthinellum*, respectively. The isolated fungal endophytes were mass cultured and prepared the extracts using the solvent ethyl acetate. The ethyl acetate extracts of endophytic fungi were subjected to FTIR spectroscopic analysis to detect the characteristic peak values with their corresponding functional groups. The FTIR spectroscopic analyses exhibited the different characteristic peaks with various functional groups of compounds**.** The results showed the presence of functional groups of alcohol, alkanes, alkenes, aromatics, amines, amides, carboxylic acids, phenol, nitromethane, nitrotoluene and alkyl halides in the ethyl acetate extracts of endophytic fungi *Chaetomium globosum*, *Cladosporium tenuissimum* and *Penicillium janthinellum* by the existence of different peaks with corresponding wave numbers in the spectral region of 4000-400 cm^{-1}. The FTIR spectrum profile of this study concluded that the presence of various phytocompounds in the endophytic fungi *Chaetomium globosum*, *Cladosporium tenuissimum* and *Penicillium janthinellum.* Further study is needed to identify the name of compounds of endophytic fungi with their biological activity.

KEYWORDS: FTIR spectroscopy; Endophytic fungi; Phytocompounds; *Chaetomium globosum*; *Cladosporium tenuissimum*; *Penicillium janthinellum.*

INTRODUCTION

Endophytic fungi are microorganisms which live within the plant tissues without causing any noticeable symptoms of disease (Chanway, 1996; Wilson, 2000). Endophytic fungi have a mutualistic relationship with the host, protecting the host against pathogen and in some cases may be an opportunistic pathogen (Stierle *et al*., 1993). Fungi are the second largest group after insects and key component of tropical ecosystems throughout the world. They are present in most plant parts, especially the leaves, where the tissue is apparently healthy. An endophyte is a bacterial or fungal microorganism, which spends the whole or part of its life cycle colonizing inter and/or inside the healthy tissues (intracellular) of the host plant, typically causing no apparent symptoms of disease (Tan and Zou, 2001).

Endophytic fungi colonise plant tissue and are remain within the tissue, except that fruiting structures may emerge through the surface of the plant tissue. Indeed, leaves may be fully colonised by a variety of fungi within a few weeks of leaf emergence. So, the endophytic fungi that are residing asymptomatically in internal tissues of all higher plants are of growing interest as promising sources of biologically active agents. Endophytic fungi are one of the most creative groups of secondary metabolite producers that play important biological roles for human life. It also considers and their medicinal applications especially in the production of anticancer, antimicrobial, antioxidant, and antiviral compounds. The endophytic fungi from medicinal plants have received much attention in recent years as they are excellent source of biologically active compounds. The presence of novel secondary metabolites in endophytic fungi and some of which possess biological activities were reported (Strobel *et al*., 2004). Endophytic fungi have been found in nearly all plant families examined to date (Arnold, 2000). A large number of secondary metabolites have been extracted, isolated and characterized from endophytic microbes (Jianglin Zhao *et al*., 2000). So, the present study was aimed to analyse the functional groups of phytocompounds in the extracts of isolated endophytic fungi *Chaetomium globosum*, *Cladosporium tenuissimum* and *Penicillium janthinellum* from the selected medicinal plants *Passiflora foetida*, *Memecylon edule* and *Justicia adhatoda*, respectively.

MATERIALS AND METHODS

Study Area

The medicinal plants *Justicia adhatoda*, *Memecylon edule* and *Passiflora foetida* were selected based on the medicinal importance for this study and collected from the natural habitats of Narthamalai hills at Pudukkottai District, Tamilnadu, India. Pudukkottai is one of the small districts of Tamil Nadu with an area of 4661 square kilometres. The district lies between 78 degrees 25' to 79 degrees 15' of the eastern longitude and 9 degrees 50' to 10 degrees 40' of the northern latitude. It is bounded by Tiruchirappalli in the north, Thanjavur in the north-east,

Bay of Bengal in the east and Ramanthapuram in the south. In this district, Narthamalai is located 11 miles Northeast West of Pudukottai in the state of Tamil Nadu, India. The temple complex is located on a gently sloping rock, the path winding through shrub jungle. *Narthamalai* region consists of nine small hillocks, *Mela malai*, *Kottai malai*, *Alurutti malai*, *Kadambar malai*, *Paraiyan malai*, *Uvachchan malai*, *Man malai*, *Bommadi malai* and *Pon malai*. There is a thin forest surrounding south east part of the region and hence it comes under the forest reserve area. As per a mythological legend when *Hanuman* was carrying *Sanjeevani* hill, few shrubs fell over this part of region making it famous for a variety of magic shrubs. An another legend stated in *Perungalur Sthala puranam* states that the name *Narthamalai* is derived from the sage *Narada*, and calls it *Naradar malai*. *Narthamalai*, which at present is a very small village, was a sprawling trading center in olden days. The name *Narthamalai* is probably derived from *Nagarattar malai* where *Nagarattar* refer to a mercantile community, also known as *Nattukkottai Chettiyars*. Narthamalai is lies on 10^0-30' E latitude, and 78^0-30' longitude. The temperaturee of Narthamalai is moderate, in winter season 24^0C and 38^0C in summer season. Heavy rainfall received only in October and November months.

Collection of Plant Material

Mature healthy and disease free leaves of medicinal plants *Justicia adhatoda*, *Memecylon edule* and *Passiflora foetida* were collected from the natural habitats of Narthamalai hills at Pudukkottai District, Tamilnadu, India during the months of January and February 2014. The collected plant was identified by Rev. Dr. S. John Britto, Director, Rapinat Herbarium and Centre for Molecular Systematics, St. Joseph's College, Tiruchira-ppalli, Tamilnadu, India. The leaf samples from each plant were placed separately in sterile polythene bags and stored in an icebox. The stored samples in chilled condition were used for the isolation of endophytic fungi within 48 h of collection.

Isolation of Endophytic Fungi

The medicinal plants *Passiflora foetida*, *Memecylon edule* and *Justicia adhatoda* were selected for this study and found colonized with endophytic fungi *Chaetomium globosum*, *Cladosporium tenuissimum* and *Penicillium janthinellum*, respectively. Isolation of endophytic fungi was carried out by the modified method of Hallman *et al*. (2007). The collected leaf samples were washed with mild detergent and thoroughly with running tap water to remove the soil particles and adhered debris and then finally washed with sterile distilled water. The leaf samples were subjected to surface sterilization with 70% ethanol for one minute. For further surface sterilization and to remove adhering microorganisms, the leaf samples were immersed in 4% sodium hypochlorite for 3 minutes. The leaf samples were then rinsed with 70% ethanol for one minute. Finally the leaf samples were rinsed with deionised water and blot dried on sterile tissue paper. The leaf samples were cut into 5-10 x 5-10 mm in size using a sterile scalpel. The leaf explants were cultured in petridishes containing Potato Dextrose Agar (PDA) medium supplemented with 100µg/mL of streptomycin to suppress bacterial growth. Petridishes were sealed with parafilm and incubated at 27 ±2°C for 15 days under dark condition and monitored every day. Fungi growing out of the plant explants were subcultured on separate PDA plates and maintained at 4°C. Fungi were identified in their sporulation state by staining with Lactophenol blue. The fungal isolates were identified based on the colony colour, morphology, hyphal structure, spore size and spore bearing structures and compared with standard manuals of endophytic fungi (Kenneth and Charles, 1949; Gilman, 1957; Kenneth and Dorothy, 1965; Petrini, 1986).

Mass Culture of Endophytic Fungi

The fungal endophytes *Chaetomium globosum*, *Cladosporium tenuissimum* and *Penicillium janthinellum* were mass cultured by placing agar blocks of actively growing pure culture (5mm in diameter) in 250ml Erlenmeyer flasks containing 100ml of potato dextrose broth. The flasks were incubated at 27 ±2°C for 14 days with periodical shaking at 150 rpm. After incubation period, the cultures were taken out and used for further study.

Preparation of Fungal Extracts

The fungal extracts were prepared according to the modified method of Raviraja *et al*. (2006). After mass culture of endophytic fungi, the cultures were filtered through four layers of sterile cheesecloth to separate the mycelial mats. Then the culture filtrate was extracted with equal volume of the filtrate and solvent ethyl acetate were taken individually in separating funnels and shaken vigorously for 15 min. The solutions were then allowed to stand, the cell mass got separated and the organic phase of solvents so obtained, were collected. The solvent ethyl acetate was evaporated and the resultant residue was dried in vacuum evaporator to yield the crude extract (Culture filtrate extract). Mycelial mats were also used for the preparation of ethyl acetate extracts followed the same procedure by taking mats instead of filtrate (Mycelial mat extract). After evaporation, the dried extracts of culture filtrate and mycelial mat were equally mixed. The ethyl acetate extracts of *Chaetomium globosum*, *Cladosporium tenuissimum* and *Penicillium janthinellum* were subjected to FTIR analysis.

FTIR-Spectroscopic Analysis

Fourier Transform Infrared Spectrophotometer (FTIR) is the most powerful tool for identifying the types of chemical bonds/functional groups present in the phytochemicals. The wave length of light absorbed is salient feature of the chemical bond. By interpreting the infrared absorption spectrum, the chemical bonds in a compound can be determined. Dried powder of ethyl acetate extracts of endophytic fungi *Chaetomium globosum*, *Cladosporium tenuissimum* and *Penicillium janthinellum* was used for FTIR analysis. 10 mg of the dried ethyl acetate extract of endophytic fungi *Chaetomium globosum*, *Cladosporium tenuissimum* and *Penicillium janthinellum* was encapsulated in 100 mg of potassium bromide (KBr) individually and pellet was prepared. The pellet was used to detect the characteristic peaks and their functional groups. The pellet of each endophytic fungal extract was loaded in FTIR spectroscope (Shimadzu, Japan), with a scan range from 400 to 4000 cm^{-1} with a resolution of 4 cm^{-1} and the

obtained spectra were recorded and analyzed using the standard methods (Lin *et al*., 1994; Yin *et al*., 2008).

RESULTS

FTIR spectroscopy analysis carried out to identify the functional groups present in the ethyl acetate extracts of endophytic fungi *Chaetomium globosum*, *Cladosporium tenuissimum* and *Penicillium janthinellum*. Figures 1, 2 and 3 refer FTIR spectra of ethyl acetate extracts of endophytic fungi *Chaetomium globosum*, *Cladosporium tenuissimum* and *Penicillium janthinellum*, respectively. The FTIR spectroscopic analyses of ethyl acetate extracts of endophytic fungi showed different form of peaks revealed the presence of different functional groups of the bioactive compounds (Tables 1, 2 and 3).

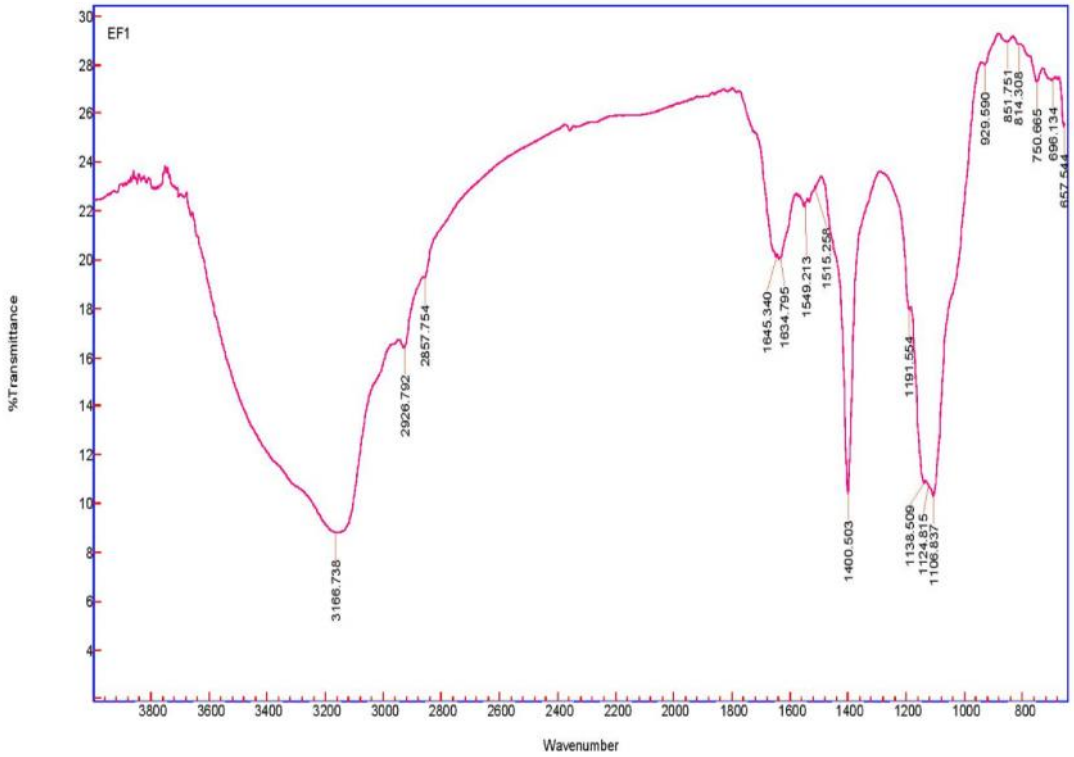

FIGURE 1. FTIR spectra of the ethyl acetate extract of endophytic fungus *Chaetomium globosum*

The functional groups were separated based on its peak ratio. The bands between 650-1000 cm^{-1} assigned to =C-H bend indicates the presence of aromatics, alkyl halides, carboxylic acids, amines and amides. In this study, the bands at 657.544, 696.134, 750.665, 814.308, 851.751 and 929.590cm^{-1} assigned to =C-H bend which means aromatics, alkyl halides, carboxylic acids, amines and amides are present in ethyl acetate extracts of *Chaetomium globosum*. The C-N stretches from 1020-1250cm^{-1} indicate the aliphatic amines. So, the bands at 1106.837, 1124.815, 1138.509 and 1191.554 cm^{-1} assigned to the C-N stretch which means aliphatic amines are present in *Chaetomium globosum*. The peak value at 1400.503 cm^{-1} assigned to the O-H bending vibration confirms carboxylic acid. The peak at 1515.258 cm^{-1} assigned to the N-O asymmetric stretching confirms Nitromethane and m-Nitrotoluene compounds. The peak at 1549.213 cm^{-1} was observed in the extract of endophytic fungus *Chaetomium globosum*, but the functional group is unknown. The peak at 1634.795 cm^{-1} assigned to N-H bend confirms primary amines. The peak at 1645.340 cm^{-1} assigned to the C=C stretching confirms alkenes. The peak at 2857.754 and 2926.792 cm^{-1} assigned to the C-H stretching indicates the alkane compounds. The results of FTIR spectra analyses showed the presence of peak at wave number of 3166.738 cm^{-1} in *Chaetomium globosum* is corresponded to O-H stretch frequency indicates the presence of an alcohol or phenol (Figure 1 and Table 1).

The Fourier Transform Infrared (FTIR) spectra of ethyl acetate extract of endophytic fungus *Cladosporium tenuissimum* revealed that the most important adsorption bands located at various wave numbers. In this study, the bands at 657.265, 695.450, 778.695 and 797.350 cm^{-1} assigned to the =C-H bend indicates the presence of aromatics, alkyl halides, carboxylic acids, amines and amides in the extracts of *Cladosporium tenuissimum*. The bands at 11088.538 cm^{-1} assigned to the C-N stretch which means aliphatic amines are present in *Cladosporium tenuissimum*. The peak value at 1401.071 assigned to the O-H bending vibration confirms carboxylic acid. The peak at 1552.300 cm^{-1} was observed in the extract of endophytic fungus *Cladosporium tenuissimum*, but the functional group is unknown. The peak at 1625.953 cm^{-1} assigned to N-H bend confirms primary amine. The peak at 1745.086 cm^{-1} assigned to C=O stretching confirms ketones, aldehydes and esters. The peak at 1874.898 cm^{-1} showed unknown functional group. The peak at 2855.080 and 2926.528 cm^{-1} assigned to the C-H stretching indicates the alkane compounds. The bands between 3200-3500 cm^{-1} indicates the presence of an

alcohol or phenol. The FTIR spectra of *Cladosporium tenuissimum* showed peaks at various wave numbers 3230.752, 3291.245 and 3320.352 cm^{-1} assigned to O-H stretch frequency indicates the presence of an alcohol or phenol (Figure 2 and Table 2).

The FTIR spectra of the ethyl acetate extracts of endophytic fungus *Penicillium janthinellum* were recorded in the spectral region of 4000-400 cm^{-1}. In this study, the bands at 702.208, 817.221 and 850.517 cm^{-1} are assigned to=C-H bend confirms aromatics, alkyl halides, carboxylic acids, amines and amides in ethyl acetate extracts of *Penicillium janthinellum*. The C-O stretches from 1000-1300 cm^{-1} indicates ether or ester. The bands at 1044.370, 1081.112,1143.904, 1196.133 and 1228.383 cm^{-1} assigned to C-O stretch indicates ether or ester in *Penicillium janthinellum*. The peak value at 1401.112 cm^{-1} assigned to the O-H bending vibration confirms carboxylic acid. The peak at 1550.852 cm^{-1} assigned to -NO2 confirms nitrocompounds. The peak at 1637.429 cm^{-1} assigned to N-H bend confirms primary amines or amides. The bands at 2315.492 and 2353.767 cm^{-1} showed unknown functional groups. The peaks at 2857.799 and 2925.868 cm^{-1} assigned to C-H stretching indicates alkanes. The FTIR spectra of *Penicillium janthinellum* showed peak at 3299.954 cm^{-1} correspond to O-H stretch indicates an alcohol or phenol (Figure 3 and Table 3).

TABLE 1. FTIR spectral peak values and corresponding functional groups of the ethyl acetate extract of endophytic fungus *Chaetomium globosum*

S.No.	Wave number (cm^{-1})	Functional Group	Compounds
1.	657.544	=C-H bend	Aromatics, Alkyl halides, Carboxylic acids, Amines and Amides
2.	696.134		
3.	750.665		
4.	814.308		
5.	851.751		
6.	929.590		
7.	1106.837	C-N stretch	Aliphatic amines
8.	1124.815		
9.	1138.509		
10.	1191.554		
11.	1400.503	O-H bend	Carboxylic acid
12.	1515.258	N-O asymmetric stretch	Nitromethane and m- Nitrotoluene
13.	1549.213	Unknown	Unknown
14.	1634.795	N-H bend	Primary amines
15.	1645.340	C=C stretch	Alkenes
16.	2857.754	C-H stretch	Alkanes
17.	2926.792		
18.	3166.738	O-H stretch	Alcohol or Phenol

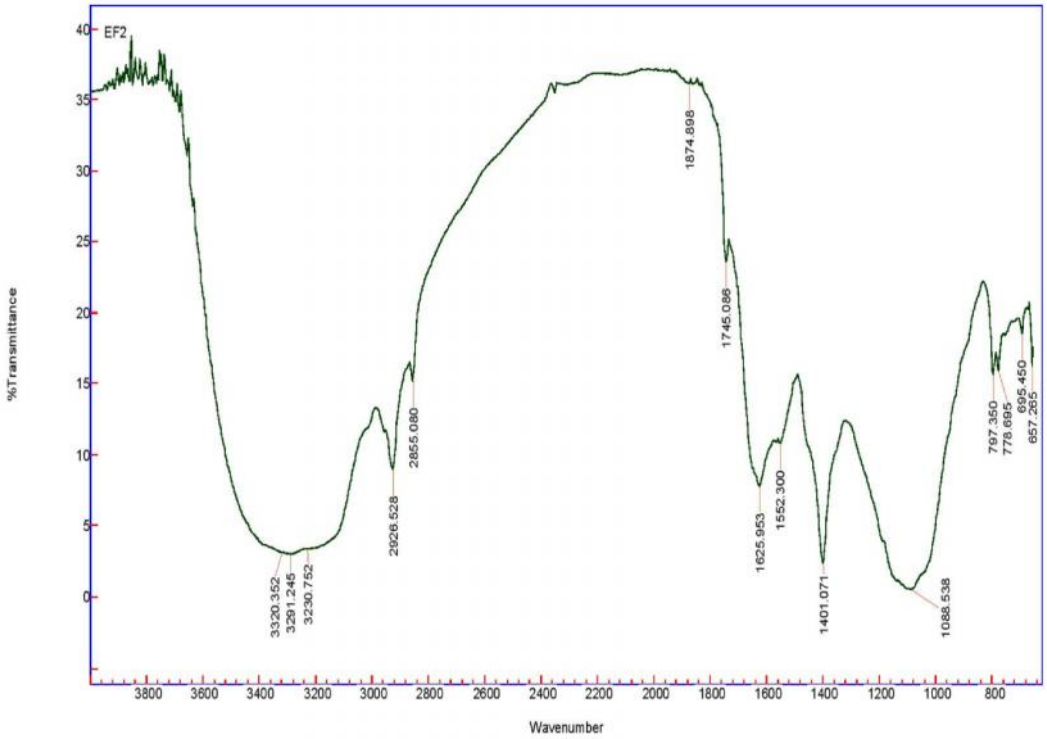

FIGURE 2. FTIR spectra of the ethyl acetate extract of endophytic fungus *Cladosporium tenuissimum*

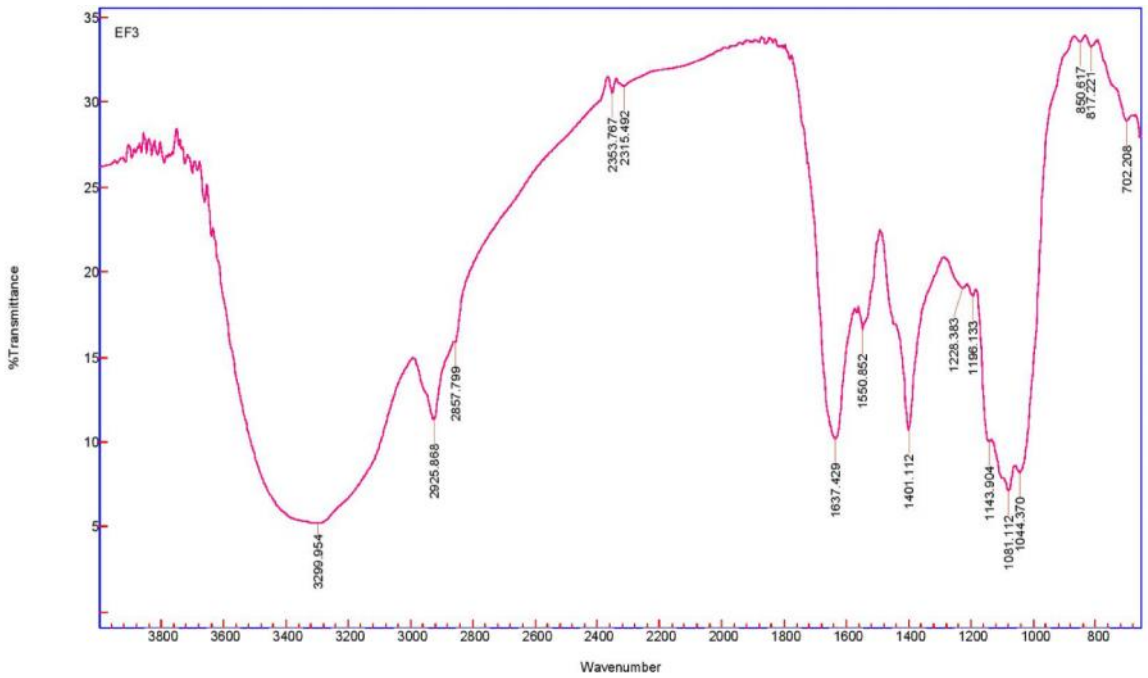

FIGURE 3. FTIR spectra of the ethyl acetate extract of endophytic fungus *Penicillium janthinellum*

TABLE 2. FTIR spectral peak values and corresponding functional groups of the ethyl acetate extract of endophytic fungus *Cladosporium tenuissimum*

S.No.	Wave number (cm^{-1})	Functional Group	Compounds
1.	657.265	=C-H bend	Aromatics, Alkyl halides,
2.	695.450		Carboxylic acids, Amines and
3.	778.695		Amides
4.	797.350		
5.	1088.538	C-N stretch	Aliphatic amines
6.	1401.071	O-H bend	Carboxylic acid
7.	1552.300	Unknown	Unknown
8.	1625.953	N-H bend	Primary amines
9.	1745.086	C=O stretch	Ketones, Aldehydes and Esters
10.	1874.898	Unknown	Unknown
11.	2855.080	C-H stretch	Alkanes
12.	2926.528		
13.	3230.752	O-H stretch	Alcohol or Phenol
14.	3291.245		
15.	3320.352		

TABLE 3. FTIR spectral peak values and corresponding functional groups of the ethyl acetate extract of endophytic fungus *Penicillium janthinellum*

S.No.	Wave number (cm^{-1})	Functional Group	Compounds
1.	702.208	=C-H bend	Aromatics, Alkyl halides,
2.	817.221		Carboxylic acids, Amines and
3.	850.517		Amides
4.	1044.370	C-O stretch	Ether or Ester
5.	1081.112		
6.	1143.904		
7.	1196.133		
8.	1228.383		
9.	1401.112	O-H bend	Carboxylic acid
10.	1550.852	-NO2	Nitro compound
11.	1637.429	N-H bend	Primary amines or Amide
12.	2315.492	Unknown	Unknown
13.	2353.767	Unknown	Unknown
14.	2857.799	C-H stretch	Alkanes
15.	2925.868		
16.	3299.954	O-H stretch	Alcohol or Phenol

DISCUSSION

FTIR is an important method to identify the functional groups of chemical constituents and elucidate the compounds (Gopalakrishnan *et al*., 2012). FTIR allows infrared spectrum simultaneously providing speed and accuracy in measurements of whole range of biological specimens (Griffiths *et al*., 1986), and has been used as a requisite method to identify medicines in pharmacopoeia of many countries (Liu *et al*., 2006). The ethyl acetate extracts of endophytic fungi *Chaetomium globosum*, *Cladosporium tenuissimum* and *Penicillium janthinellum* were subjected to FTIR spectroscopic analysis under IR region in the range of 400-4000 cm^{-1}.

FTIR analysis of the extracts of endophytic fungi *Chaetomium globosum*, *Cladosporium tenuissimum* and *Penicillium janthinellum* revealed that the presence of various chemical constituents. The Infra-Red of extracts of *Chaetomium globosum*, *Cladosporium tenuissimum* and *Penicillium janthinellum* showed absorption bands with the wave number (cm^{-1}) of prominent peaks. Several bands are pertained to functional groups represent chemical components or metabolic products in the endophytic fungi. Similarly, Ragupathi Raja Kannan (2011) reported that the FTIR spectrum as an effective tool for differentiating, classifying and discriminating closely related plants and other organisms. Zavoi *et al*. (2011) used FTIR technique and reported the polyphenolic composition of medicinal herbs *Cynara scolimus*, *Taraxacum officinalis*, *Chelidonium majus*, *Hypericum perforatum*, *Silybum marianu* and *Lycopodium clavatu*. Csernatoni *et al*. (2013) applied FTIR screening to characterize and identify the main biomarkers of food supplement by the analysis of plant ingredients comparatively with the final product. Ramamurthy and Kannan (2007) used FTIR spectroscopy and reported the qualitative characters regarding the organic molecules in *Calotropis gigantea*.

The spectra wavelength observed in the ethyl acetate extracts of endophytic fungi *Chaetomium globosum*, *Cladosporium tenuissimum* and *Penicillium janthinellum* served as a characteristic medium to elucidate the inherent functional group and organic compounds. The Infra-Red of ethyl acetate extracts of endophytic fungi showed different peaks, indicating transitions between vibration levels of different molecules. Similarly, the presence of different functional groups of compounds was identified with a variation in the peaks ratio (Kalaiselvi *et al*., 2012).

In this study, the FTIR spectral analyses showed important absorption bands at various wave number (cm^{-1}) indicates the presence of chemical structures. So, the FTIR spectroscopy revealed the absorption peaks of different functional groups of the chemical compounds present in the endophytic fungi. Up to date of our knowledge, it may be the first FTIR report on endophytic fungi *Chaetomium globosum*, *Cladosporium tenuissimum* and *Penicillium janthinellum.* The FTIR analysis showed the presence of phenolic compounds in the extracts of endophytic fungi *Chaetomium globosum*, *Cladosporium tenuissimum* and *Penicillium janthinellum*. which can be isolated and screened for biological active compounds. Medicinal plants have served as a constant source of medicaments, which have a great efficacy and demand for the treatment of various diseases.

Within a decade, there were a number of dramatic advances in analytical techniques, including FTIR and GC-MS for identification and determination of phytochemicals (Roberts and Xia, 1995). The FTIR spectrum was used to identify the functional groups of the active components present in the endophytic fungi based on the peak values in the region of IR radiation. When the extract was passed into the FTIR, the functional groups of the components were separated based on its peaks ratio. The results of FTIR analyses confirmed the presence of alcohol, alkanes, alkenes, aromatics, carboxylic acids, phenol, amines, amides, nitromethane, nitrotoluene and alkyl halides in the ethyl acetate extracts of endophytic fungi *Chaetomium globosum*, *Cladosporium tenuissimum* and *Penicillium janthinellum*, which may provide an insight in its use of traditional medicine.

CONCLUSION

The present study concluded that the presence of bioactive compounds in the extracts of endophytic fungi *Chaetomium globosum*, *Cladosporium tenuissimum* and *Penicillium janthinellum*. Further study is needed on isolation and identification of active compounds in *Chaetomium globosum*, *Cladosporium tenuissimum* and *Penicillium janthinellum.* This study may also provide a better source for developing new therapeutic agents from the extracts of endophytic fungi.

CONFLICT OF INTERESTS

The authors have declared that there is no conflicts of interests exist.

ACKNOWLEDGEMENT

The authors are thankful to the Management of A.V.V.M. Sri Pushpam College (Autonomous), Poondi -613 503, Thanjavur, Tamilnadu, India for provided the facility to complete this research work.

REFERENCES

Arnold, A.E., Maynard, Z., Gilbert, G.S., Coley, P.D. and Kursar, T.A. (2000) Are tropical fungal endophytes hyperdiverse?. Ecology Letters, 3, 267–274.

Chanway, C.P. (1996) Endophytes: they are not just fungi. Canadian Journal of Botany, 74, 321- 322.

Csernatoni, F., Socaciu, C., Pop, R.M., Fetea, F. and Bunghez, F. (2013) Application of FT-IR spectroscopy for fingerprinting bioactive molecules in a nutraceutical PROMEN, comparatively with plant ingredients. Bulletin UASVM Food Science and Technology, 70(1), 68-69.

Gilman, J.C. (1957) A manual of soil fungi. 2nd Ed., The Iowa State College Press, Ames, Iowa.

Gopalakrishnan, V.K., Starlin, T., Arul Raj, C. and Ragavendhran, P. (2012) Phytochemical screening, functional groups and elemental analysis of *Tylophora Pauciflora* wight and arn. International Research Journal of Pharmacy, 3, 6.

Griffiths, P.R. and De Haseth, J.A. (1986) Fourier transform infrared spectroscopy. John Wiley and Sons, New York, 656.

Hallmann, J., Berg, G. and Schulz, B. (2007) Isolation procedures for endophytic microorganisms, In: Schulz, B., Boyle, C. and Sieber, T., Microbial root endophytes. Springer Berlin Heidelberg, New York, pp. 299-319.

Jianglin Zhao, Yan Mou, Tijiang Shan , Yan Li , Ligang Zhou, Mingan Wang and Jingguo Wang. (2000) Antimicrobial metabolites from the endophytic fungus *Pichia guilliermondii* isolated from *Paris polyphylla* var. Yunnanensis. Molecules, 15, 7961-7970.

Kalaiselvi, M., Gomathi, D., Vidya, B. and Uma, C. (2012) Evaluation of antioxidant potential and fourier transform infrared spectroscopy analysis of *Ananus comosus* . merr peel. International Research Journal of Pharmacy, 3, 237-242.

Kenneth B. Raper and Charles Thom. (1949) A manual of the penicillia. Williams and Wilkins Co., Baltimore.

Kenneth B. Raper and Dorothy I. Fennell. (1965) The Genus *Aspergillus*. Williams and Wilkins Co., Baltimore.

Lin, S.C., Carswell, K.S., Sharma, M.M. and Georgiou, G. (1994) Continuous production of the lipopeptide biosurfactant of *Bacillus licheniformis* JF-2. Appl Microbiol Biotechnol., 41, 281–285.

Liu, H.X., Sun, S.Q., Lv, G.H. and Chan, K.K. (2006) Study on Angelica and its different extracts by fourier transform infrared spectroscopy and two-dimentional correlation IR spectroscopy. Mol Biomol Spec., 64, 321-326.

Petrini, O. (1986) Taxonomy of endophytic fungi of aerial plant tissues. In: Microbiology of Phyllosphere; Fokkema, N.J., Van Den Heuvel, J., Eds., Cambridge University Press, Cambridge, UK, pp. 175–187.

Ragupathi Raja Kannan, Rengasamy Arumugam, Rajasekaran, Anantharaman, Perumal. (2011) Fourier Transform Infrared Spectroscopy analysis of sea grass polyphenols. Current Bioactive Compounds, 7(2), 118-125.

Ramamurthy, N. and Kennan, S. (2007) Fourier transform infrared spectroscopic analysis of a plant *(Calotropis gigantea* Linn.) from an industrial village, Cuddalore District, Tamilnadu, India. Romanian Journal of Biophysics, 17 (4), 269-276.

Raviraja, N.S., Maria, G.L. and Sridhar, K.R. (2006) Antimicrobial evaluation of endophytic fungi inhabiting medicinal plants of the Western Ghats of India. Engineering of Life Sciences, 6(5), 515–520.

Roberts, J.K.M. and Xia, J.H. (1995) High-resolution NMR methods for study of higher plants. Methods Cell Biol., 49, 245–258.

Stierle, A., Strobel, G. and Stierle, D. (1993) Taxol and taxane production by *Taxomyces andreanae*, an endophytic fungus of Pacific yew. Science, 260, 214-216.

Strobel, G.A., Daisy, B., Castillo, U. and Harper, J. (2004) Natural product from endophytic microorganisms. J Nat Prod., 67, 257-268.

Tan, R.X. and Zou, W.X. (2001) Endophytes: a rich source of functional metabolites. Prod. Rep., 18, 448–459.

Wilson, D. (2000) Ecology of woody plant endophytes, In: Microbial endophytes, Marcel Dekker, New York, NY, USA.

Yin, H., Qiang, J., Jia, Y., Ye, J., Peng, H., Qin, H., Zhang, B. and He. (2008) Characteristics of biosurfactants produced by *Pseudomonas aeruginosa* S6 isolated from oil-containing wastewater. Process, 7, 262-266.

Zavoi, S., Fetea, F., Ranga, F., Pop, R.M., Baciu, A. and Socaciu, C. (2011) Comparative fingerprint and extraction yield of medicinal herb phenolics with hepatoprotective potential, as determined by UV-Vis and FT-MIR spectroscopy. Notulae Botanicae Horti Agrobotanici Cluj–Napoca, 39(2), 82-89.