JEB
Website : www.jeb.co.in
E-mail : editor@jeb.co.in

Journal of Environmental Biology

ISSN: 0254-8704 (Print)
ISSN: 2394-0379 (Online)
CODEN: JEBIDP

Distribution and antimicrobial potential of endophytic fungi associated with ethnomedicinal plant *Melastoma malabathricum* L.

Vineet Kumar Mishra[1], Garima Singh[1], Ajit Kumar Passari[1], Mukesh Kumar Yadav[2], Vijai Kumar Gupta[3] and Bhim Pratap Singh[1]*

[1]Department of Biotechnology, Mizoram University, Aizawl-796 004, India
[2]Korea University College of Medicine, Korea University, Seoul, 02841, South Korea
[3]Molecular Glyco-biotechnology Group, Department of Biochemistry, National University of Ireland Galway, Galway, H91CF50, Ireland
*Corresponding Author E-mail: bhimpratap@gmail.com

Publication Info

Paper received:
31 December 2014

Revised received:
09 September 2015

Accepted:
06 October 2015

Abstract

Distributions of endophytic fungi associated with ethnomedicinal plant *Melastoma malabathricum* L. was studied and 91 isolates belonging to 18 genera were recovered. The isolates were distributed to sordariomycetes (62.63%), dothideomycetes (19.78%), eurotiomycetes (7.69%), zygomycetes (4.19%), agaricomycetes (1.09%), and mycelia sterilia (4.39%). Based on colony morphology and examination of spores, the isolates were classified into 18 taxa, of which *Colletotrichum*, *Phomopsis* and *Phoma* were dominant, their relative frequencies were 23.07%, 17.58% and 12.08% respectively. The colonization rate of endophytic fungi was determined and found to be significantly higher in leaf segments (50.76%), followed by root (41.53%) and stem tissues (27.69%). All the isolates were screened for antimicrobial activity and revealed that 26.37% endophytic fungi were active against one or more pathogens. Twenty four isolates showing significant antimicrobial activity were identified by sequencing the ITS1-5.8S-ITS2 region of rRNA gene. Results indicated that endophytic fungi associated with leaf were functionally versatile as they showed antimicrobial activity against most of the tested pathogens. The endophytic fungi *Diaporthe phaseolorum* var. *meridionalis* (KF193982) inhibited all the tested bacterial pathogens, whereas, *Penicillium chermesinum* (KM405640) displayed most significant antifungal activity. This seems to be the first hand report to understand the distribution and antimicrobial ability of endophytic fungi from ethno-medicinal plant *M. malabathricum*.

Key words

Antimicrobial activity, Colonization frequency, Endophytic fungi, *Melastoma malabathricum*

Introduction

Bioprospection of functional microbial diversity from unusual and pristine habitat for the search of new secondary metabolites is an emerging area of research. Endophytic fungi which reside and colonize diverse atypical niche of plant tissues are one of the important source for searching of new novel bioactive agents (Strobel and Daisy, 2003; Qadri *et al.*, 2014). Endophytes are ubiquitously present in broad range of host plant species and have been reported from various climatic zones (Ghimire *et al.,* 2011; Li *et al.*, 2012; Verma *et al.,* 2014). It has been shown that the endophytic population among different host plants varies (Naik *et al.*, 2008). However, researchers till date are able to explore only limited plants for the isolation and characterization of endophytic fungal population (Hyde and Soytong, 2008; Tejesvi *et al.*, 2011). Interestingly, some of the endophytic fungi isolated from a particular host was shown to produce same chemical compound as produced by their respective host (Aly *et al.,* 2010), which undoubtedly showed their importance. These observations led to isolation of Paclitaxel (Taxol™) from an endophytic fungi *Penicillium aurantiogriseum* NRRL 62431 isolated from the bark of yew *Taxus brevifolia* (Yang *et al.*, 2014). Furthermore, several

studies have demonstrated the potential of endophytic fungi associated with medicinal plants which synthesizes plethora of secondary metabolites with diverse biological activities like antimicrobial, anti-cancerous, immunosuppressive, insecticidal and anti-parasitic (Daisy *et al.,* 2002; Vieira *et al.,*2012; Martinez-Luis *et al.*, 2012).

Melastoma malabathricum L. belongs to family Melastomaceae, and it is widely distributed in south-East Asian region. This plant is a small shrub, which has an important place in Chinese, Indian, Malay and Indonesian ethno-medicine. Traditionally, leaves are used as a preventive scarring against smallpox, piles, gastric ulcer, dysentery, skin scars and as a tonic; shoots are taken to treat puerperal infection, high blood pressure and diabetes; while roots are used to diminish rheumatism, arthritis and toothache, whereas flowers are used to treat cancer (Sharma *et al.,* 2001; Rai and Lalramnghinglova, 2010; Joffry *et al.*, 2012). Emerging scientific findings also supports an array of medicinal and pharmacological activities of various parts of *M. malabathricum* such as antibacterial, anti-inflammatory, wound healing, anti diarrheal, cytotoxic, antiviral, antiparasitic, antioxidant and antipyretic (Wiart *et al.,* 2004; Zakaria *et al.,* 2006; Susanti *et al.,* 2007; Sunilson *et al.,* 2009). Endophytic fungi associated with *Melastoma malabathricum* have also been reported to have antifungal activities against fungal plant pathogens and their ability to decolorize synthetic dyes (Kokaew *et al.,* 2007; Ngieng, 2013).

Keeping in view the medicinal properties of the host and its unexplored nature, the present study was focused to investigate the diversity and antimicrobial potential of endophytic fungi associated with traditional ethno-medicinal plant *M. malabathricum*.

Materials and Methods

Sample collection and isolation of endophytic fungi : The plant samples were collected from Phawngpui National Park, Mizoram (22°40'N; 93°03'E), the highest mountain peak in Mizoram, northeast, India, rising about 2,157 meters high near the Myanmar border and famous for orchids and Rhododendrons. This plant is abundantly found in North Eastern region of India. To understand the diversity of endophytic fungi associated with *M. malabthricum* (n=10), plants were randomly selected. To avoid identical population of endophytes, distance of at least 100 m was taken into consideration between two plants. Herbarium specimen was prepared and submitted to the Department of Forestry, Mizoram University, India. The plant samples were placed in labeled polyethylene bags, transported to laboratory and placed in refrigerator at 4 ^{0}C. The samples were processed within 24 hr of collection.

Tissues (leaves, stems and roots) were separated from plants for surface sterilization and isolation of endophytic fungi with minor modifications (Cannon and Simmons, 2002). The plant samples were washed thoroughly in running tap water for 10 min. The samples were surfaced sterilized with 70% ethanol for 1 min, 3% sodium hypochlorite for 1 min and subsequently rinsed thrice with sterile distilled water. The outer surface was removed with a sterile scalpel and samples were excised into 1.0 cm^{2} pieces. Tissues were thoroughly rinsed using sterile distilled water followed by drying on sterile blotters to ensure complete drying. Prior to plating, the tissues were washed again with distilled water. The water obtained from the last wash was spreaded on PDA medium to ensure the effectiveness of surface sterilization and fingerprints of the tissues were also taken on media plates as control to cross check the epiphytic fungal growth. One hundred and ninety five tissues were selected from each leaf, stem and root region of the plant. Tissues were placed on petri dishes containing Potato Dextrose Agar (PDA), Malt Yeast Extract Agar (MYA) and Czapex Dox Agar (CDA) medium supplemented with streptomycin (100 mg l^{-1}) to suppress bacterial growth. Inoculated Petri plates were incubated at 28 ^{0}C under 12 hr white light: 12 hr dark cycles (Bills and Polishook, 1991). Petri plates were observed daily till 2-3 weeks. Mycelium coming out from sterile tissues was transferred to fresh plates free from antibiotics for further purification. Pure cultures were left to sporulate for one month on PDA media and then microscopic identification was carried out for vegetative and reproductive structures (Riddel, 1950). Morphology based taxonomy was used for identification of sporulating fungi (Barnett and Hunter, 1972).

Preparation of fungal extracts : The endophytic fungi were cultured in Potato Dextrose Broth (PDB) medium for three weeks at 28 ^{o}C. The cultures were extracted by using methanol for mycelial mat and ethyl acetate for aqueous extract. Both the extracts were air dried by using rotary evaporator system (BUCHI, Switzerland) and mixed together to get a crude solid extract. The crude extract was dissolved in methanol and filtered through Whatman filter paper to remove residues which were not dissolved in methanol; then the filtrate was diluted to 10 mg ml^{-1} with methanol.

Evaluation of antimicrobial activity of fungal extracts : Antimicrobial activity of the extract from endophytic fungi was tested against fungal and bacterial pathogens by agar cup diffusion method (Tayung and Jha, 2010). The test organisms include two Gram negative bacteria: *Pseudomonas aeruginosa* (MTCC 2453) and *Escherichia coli* (MTCC 739), one Gram positive bacteria: *Staphylococcus aureus* (MTCC 96) and three fungal pathogens: *Fusarium*

oxysporum (CABI 293942), *Fusarium graminearum* (MTCC 1893) and *Fusarium culmorum* (MTCC 2090). The bacterial cultures were grown on nutrient agar (NA) medium, and fungi were cultured on potato dextrose agar. NA and PDA plates were spread inoculated with 0.2 ml of overnight grown bacterial culture containing 1.0×10^9 cfu (colony forming unit), and 1.0×10^9 spores of fungi respectively. A cork borer (7mm in diameter) was used to prepare agar cups in the plates. Each cup was loaded with 100 µl of crude fungal extracts dissolved in methanol. The control cup was filled with methanol only. The plates were incubated at 37 °C for 24 hr for antibacterial and 28 °C for seven days for antifungal activity. Three replicates were maintained in groups. The magnitude of antimicrobial action was assessed by the diameter (mm) of inhibition zones and compared with co-assayed antibiotics tetracycline as antibacterial and fluconazole as antifungal agent.

DNA extraction amplification and sequencing : The fungal isolates that showed significant antimicrobial activity were identified and subjected to DNA isolation, amplification and sequencing of ITS region of rRNA gene. The genomic DNA was extracted by procedure previously reported (Cenis, 1992). The universal primers ITS1 (5'-TCCGTAGGTGAACCTGCGG-3') and ITS4 (5'-TCCTCCGCTTATTGATATGC-3') (White *et al.,* 1990) were used for amplification of ITS region. Reaction was performed in a total volume of 25 µl containing 1X PCR assay buffer, 2.5mm dNTPs, 10 pmols of each primer, 50 ng of template DNA and 1 unit of Taq DNA polymerase. Amplification was performed in Veriti thermal cycler (Applied Biosystems, Singapore) with initial denaturation at 95 °C for 5 min, followed by 35 cycles of 1 min denaturation at 94 °C, annealing at 50 °C for 1 min, extension at 72 °C for 1 min 20 sec, followed by final extension at 72 °C for 10 min. The PCR products were visualized by running on 1.2 % (w/v) agarose gel in 1X TAE buffer stained with ethidium bromide (0.5 µg ml^{-1}) and visualized under gel documentation system (Bio-Rad Gel Doc XR+ gel documentation system, California, USA). A negative control reaction mixture without DNA template of fungi was included with each set of PCR reactions.

The ITS1-5.8S-ITS2 region was sequenced, and the sequences were analyzed by visual inspection of chromatograms using Finch TV v1.40v (http://www.geospiza.com/finchtv). Sequences were compared with available sequences in NCBI database by BLASTn search. The sequences were aligned using CLUSTAL W software packaged with MEGA 5.05 (Tamura *et al.*, 2011) with default settings. Appropriate model was selected using BIC scores (Bayesian Information Criterion) and AICc value (Akaike Information Criterion, corrected) . The phylogenetic tree was constructed by maximum likelihood method using Molecular Evolutionary Genetics Analysis (MEGA 5.05 with Kimura 2-parameter model (Kimura, 1980). The robustness of phylogenetic tree was tested by bootstrap analysis using 1,000 replicates. Taxonomic assignment was based on similarity with reference sequences retrieved from GenBank. The sequences used in this study were deposited in

Table 1 : Number, taxa, colonization frequency and % dominance of endophytic fungi

Endophytic Fungi	No. of endophytes	Colonization Frequency (CF)%	Dominance (%)	Class
Acremonium sp.	5	2.56	5.49	Sordariomycetes
Diaporthe sp.	3	1.53	3.29	
Pilidiella sp.	2	1.02	2.19	
Chaetomium sp.	2	1.02	2.19	
Colletotrichum sp.	21	10.76	23.07	
Phomopsis sp.	16	8.20	17.58	
Fusarium sp.	4	2.05	4.39	
Gibberella sp.	2	1.02	2.19	
Nodulisporium sp.	2	1.02	2.19	
Penicillium sp.	7	3.58	7.69	Eurotiomycetes
Leptosphaeria sp.	3	1.53	3.29	Dothidiomycetes
Corynespora sp.	2	1.02	2.19	
Phoma sp.	11	5.64	12.08	
Alternaria sp.	2	1.02	2.19	
Phanerochaete sp.	1	0.51	1.09	Agaricomycetes
Mortierella sp.	2	1.02	2.19	Zygomycetes
Gongronella sp.	2	1.02	2.19	
Mycelia sterilia	4	2.05	4.39	Mycelia sterilia
Total no. of isolates	**91**	**46.66**		

Note: Based on 195 tissue segments for analysis

the NCBI GenBank and accession numbers were obtained.

Data analysis : The relative colonization frequency (% CF) was calculated as the number of tissues colonized by a specific fungus divided by the total number of tissues x 100. Dominance of endophytes was calculated as percent of colony frequency divided by sum of percentage of colony frequency of all endophytes x 100.

Colonization rates of fungal diversity were also calculated as percentage of segments colonized by one or more fungal isolates from the total number of segments of each tissue plated (Fisher and Petrini, 1987). Isolation rate (IR) was evaluated as number of isolates obtained from plant segments divided by total number of segments observed x 100 (Poole, 1974; Groth and Roelfs, 1987)

The percentage data is arcsine transformed before being analyzed for significance using ANOVA (analysis of variance, $P < 0.05$). Furthermore, the differences in means were contrasted using Duncan's new multiple range test following ANOVA. All other statistical analyses were carried out using SPSS statistical software package version 16.0. Similarity index calculations were carried out by using computer program PAST version 1.86 (Hammer *et al.,* 2001). All the experiments were repeated at least twice and three replicates were taken into consideration. Relative abundance of endophytic fungal isolates was compared among different tissues (leaves, stems and roots) of plants by using Sigma Plot 12.0.

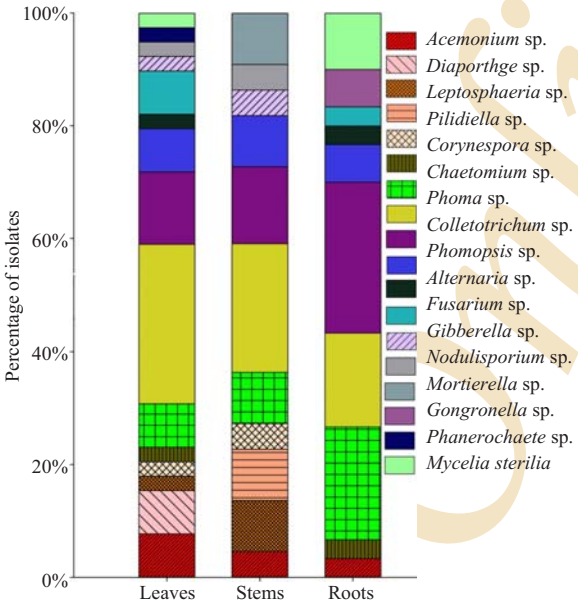

Fig. 1: Relative abundance of endophytic fungi associated with different tissues of *M. malabathricum* at genus level

Results and Discussion

A total of 91 endophytic fungi were recovered from 195 tissue segments belonging to 18 genera and 7 classes. Maximum number of endophytes were isolated by using PDA (n=49, 53.84%) followed by MYA (n=27, 29.67 %) and CDA (n=15, 16.48 %). The colonization rate of endophytic fungi in leaf (50.76 %) was higher than in root (41.53 %) and stem (27.69%) tissues, which is consistent with the findings of many researchers. Like Gond *et al.* (2012) reported highest CR in leaf tissues of *Nyctanthes arbor-tristis*, an important medicinal plant of India. Similarly, Kumar and Hyde (2004) stated that the overall colonization rate of endophytic fungi was found to be significantly higher in leaves than those in root bark, root xylem, flowers and twig bark of *Tripterygium wilfordii*, which might be due to large surface area exposed to the environment and the stomata provides passage for entry of fungal mycelium. This might also be one of the reasons why colonization frequency and colonization rate in leaf tissues used to be higher than stem and root. Similarity, isolation rate was also found to be highest in leaf tissues (0.60) followed by root (0.46) and stem (0.33) tissues. The dominance of *Phomopsis* in root tissues is in accordance with the results of Indian neem tree *Azadirachta indica* (Verma *et al.*, 2007), while dominance of *Colletotrichum gloeo sporioides* is as per findings of Huang *et al.* (2008).

The overall colonization frequency (% CF) of endophytic fungi associated with tissues of *M. malabathricum* was 46.66%. The composition of fungal endophytes included of sordariomycetes (62.63%), dothideomycetes (19.78%), eurotiomycetes (7.69%), zygomycetes (4.39%), agaricomycetes (1.09%), and mycelia sterilia (4.39%) (Table 1). Among all the selected plant segments, *Colletotrichum* (23.07%), *Phomopsis* (17.58%), *Phoma* (12.08%) and *Penicillium* (7.69%) were significantly distributed. *Colletotrichum* was most dominant in leaf and stem tissues, whereas, *Phomopsis* and *Phoma* showed dominance in root tissues (Fig. 1). *Phanerochaete* represented scarce phylotype within endophytic fungal community, being the only isolated agaricomycete (Table 1). The diversity of endophytic fungal community recovered from all three types of selected tissue was compared by using indices of α-diversity (Shannon-Wiener and Simpson's indexes and their components). Simpsons diversity index, Shannon-Wiener index and species richness were higher in leaf tissues followed by root and stem tissues. But when evenness component of Shannon-Wiener Index were compared it was more in stem tissue followed by root and leave tissues. Diversity indices can provide significant information about the rarity and commonness of a specie in a community. Hence, it is an important tool for biologists trying to predict community structure. Simpson's dominance or concentration of taxa was higher in stem tissues; similar

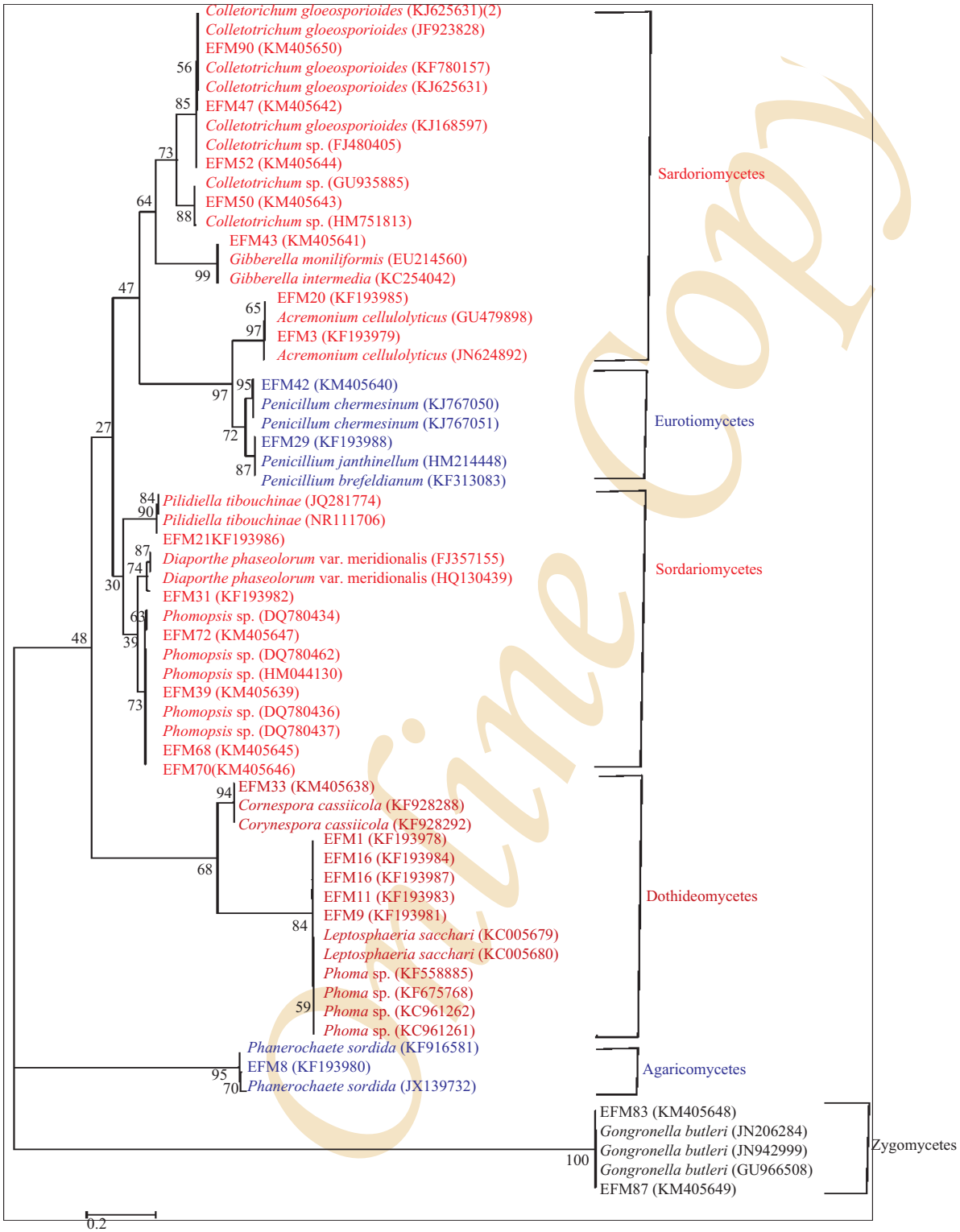

Fig. 2 : Maximum likelihood tree (-lnL=2973.3508) of fungal community having antimicrobial activity associated with *M. malabathricum* based on ITS rDNA sequences. Numbers above branches indicate bootstrap support

trend was found in *Nycanthes-arbortristis* (Gond *et al.,* 2012). Sordariomycetes were dominant in all the tissues of plant and other classes of fungi were randomly spreaded in different tissues. *Colletotrichum* sp. was the most dominant fungi in both leaves and stem tissues, whereas *Phoma* sp. was found to be the most dominant fungi in root tissues. Similarly, Vieira *et al.* (2011) concluded that the existence of endophytic fungal population in medicinal plants vary from tissue to tissue. Most endophytic fungi were isolated from leaf tissues which were also reported by Kharwar *et al.* (2011). Common occurrence of genera *Penicillium* is also reported from healthy tissues of *Melia azedarach* (Santos *et al.* 2003).Henceforth, for finer resolution of diversity both species richness and evenness component's should taken into consideration. The leaf tissues harboured higher number of taxa but root and stem regions were more diverse with respect to evenness distribution of endophytic fungal taxa.

The bioactive compounds produced by endophytic fungi, recovered from medicinal plants are potential candidates for curing many diseases (Tejesvi *et al*. 2007).The results of antimicrobial activity indicated that out of 91 endophytic isolates, 24 strains (26.37%) showed antimicrobial activity by inhibiting at least one of the test pathogen (Table 2). Among all the tested fungal isolates, 20 isolates (21.97%) showed antibacterial activity against *E. coli*, 18 isolates (19.78%) against *P. aeruginosa* and 19 isolates (20.87%) against *S. aureus*. Similar findings of antimicrobial activity of endophytic *Neurospora crassa* examined in crude extract was reported by Joel and Bhimba (2013). 23 isolates (25.27%) displayed antifungal activity against *F. culmorum* and 22 isolates (24.17%) against both *F. oxysporum* and *F. graminearum*. Among all the tested endophytic isolates, metabolites produced by *Diaporthe phaseolorum* var. *meriodionalis* (KF193982) displayed significant antibacterial activity against the entire range of test pathogens. Two new benzopyranones diaportheone A and B were also isolated from endophytic *Diaporthe* sp. associated with *Pandanus amaryllifolius* leaves which have shown inhibitory activity against virulent strains of *Mycobacterium tuberculosis* (Bungihan *et al*., 2011). Specian *et al.* (2012) isolated bioactive compounds from endophytic *Diaporthe* sp. associated with *Luehea divaricata* which showed antibacterial activity against human pathogenic bacteria including *Enterococcus hirae*, *Escherichia coli*, *Staphylococcus aureus* and *Salmonella typhi*.

Among the tested fungal pathogens, the most susceptible pathogen was *Fusarium culmorum*, inhibited by 23 isolates. An endophytic fungus isolated from leaf, identified as *Penicillium chermesinum* (KM405640) showed most prominent activity against plant pathogens. Shen *et al.* (2014) showed that *Penicillium* sp. displayed broad range of antifungal activity against clinical yeasts (*R. rubra*, *S. cerevisiae* and *C. albicans*); whereas Wang *et al.* (2008) isolated pure compounds from *Penicillium* sp. which displayed antifungal activity against *C. albicans*, *T.rubrum* and *A.niger.*

Twenty four isolates, which showed antimicrobial ability, were identified by amplification of Internal Transcribed Spacers (ITS) region of rRNA gene. Sequence analysis based on ITS region has been widely used for taxonomical studies and phylogenetic analysis (Gehlot *et al.,* 2012). Amplicon generated was sequenced commercially and the closest match (> 98% homology) was searched by using NCBI BLASTn program and considered for phylogenetic analysis. For the construction of phylogenetic tree, ITS sequences were aligned by using multiple sequence alignment software (CLUSTAL W) by using MEGA 5.05 software. A discrete Gamma distribution was used to model evolutionary rate differences among sites 5 categories (+G, parameter = 0.3575) and the model was selected based on the lowest BIC scores (Bayesian Information Criterion) which considered to describe the substitution pattern. Phylogenetic tree of 24 endophytic isolates along with 38 ITS sequences retrieved from GeneBank was constructed by using maximum likelihood matrix (Fig. 2).

The phylogenetic tree constructed by using ITS sequences classified endophytic fungal population into 3 groups: Ascomycota, Basidiomycota and Zygomycota comprising of five classes and twelve genera. There were 3 classes of ascomycota namely Sordariomycetes, Dothideomycetes and Eurotiomycetes among identified isolates. Sordariomycetes occupied 50% of total identified isolates containing six genera *Colletotrichum*, *Fusarium*, *Acremonium*, *Pilidiella*, *Diaporthe* and *Phomopsis*. Whereas, only *Penicillium* came under Eurotiomycetes (Fig. 2). Sequences were submitted to NCBI GenBank and accession numbers were obtained (KF193978-KF193988 and (KM405638-KM405650). Their close association is also evident in the tree despite *Penicillium* was now placed under Eurotiomycetes. Class dothideomycetes contained three genera namely, *Corynespora*, *Leptosphaeria* and *Phoma* sp. Agaricomycetes (Basidiomycota) and Mucorales (Zygomycota) contained only one genera each, *Phanerochaete* and *Gongronella* respectively. *Fusarium verticillioides* (EFM 43) was found to be clustered with *Gibberella moniliformis* which is an anamorph of *F. verticillioides* (Jurgenson *et al.,* 2002). According to previous reports most endophytic fungi were identified as ascomycetes and their anamorphs whereas endophytes belonged to basidiomycota have only been reported in limited number of studies (Wang *et al.,* 2005; Rivera-orduna *et al.,* 2011). In the present study only one endophyte identified as *Phanerochaete sordida* (KF193980) belonged to agaricomycetes.

Table 2 : A.ntimicrobial activities of endophytic fungi isolated from *Melastoma malabathricum*

Endophytic Fungi	Zone of inhibition (mm ± SD)					
	Gram-negative bacteria		Gram-positive bacteria		Fungal pathogens	
	P. aeruginosa	*E. coli*	*S. aureus*	*F. oxysporum*	*F. graminearum*	*F. culmorum*
Negative control	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}
Phoma sp.	6.60 ± 0.33^{bc}	3.00 ± 0.00^{bc}	0.00 ± 0.00^{a}	11.33±0.57^{bc}	11.00±1.00^{bc}	10.66±0.57^{bc}
Acremonium cellulolyticus	2.60 ± 0.33^{bde}	4.30 ± 0.33^{bde}	7.60 ± 0.55^{bc}	14.00±0.5^{bde}	14.33±0.57^{bde}	13.83±0.28^{bde}
Leptosphaeria sacchari	4.60 ± 0.33^{bdfg}	0.00 ± 0.00^{a}	3.10 ± 0.44^{bde}	12.33±0.57^{bdfg}	12.5±0.5^{bdfg}	13.33±0.57^{bde}
Phoma sp.	0.00 ± 0.00^{a}	4.30 ± 0.33^{bde}	9.30 ± 0.33^{bdfg}	13.16±0.76^{bdfhi}	13.5±0.5^{bdfhi}	14.83±0.76^{bdfg}
Pilidiella tibouchinae	10.30 ± 0.33^{bdfhi}	2.30 ± 0.33^{bdfg}	2.60 ± 0.33^{bdfhi}	6.66±0.76^{bdfhjk}	0.00±0.00^{a}	6.83±0.76^{bdfhi}
Phanerochaete sordida	10.00 ± 0.00^{bdfhi}	0.00 ± 0.00^{a}	2.00 ± 0.00^{bdfhi}	13.83±0.28^{bde}	11.16±0.5^{bc}	11.33±0.57^{bdfhjk}
Phoma sp.	9.30 ± 0.33^{bdfhjk}	5.30 ± 0.33^{bdfhi}	5.30 ± 0.33^{bdfhjk}	11.00±1.00^{bc}	11.16±0.5^{bc}	11.33±0.57^{bdfhjk}
Leptosphaeria sacchari	10.60 ± 0.33^{bdfhi}	6.30 ± 0.33^{bdfhjk}	0.00 ± 0.00^{a}	12.50±0.5^{bdfg}	12.33±0.28^{bdfg}	11.16±0.5^{bdfhjk}
Diaporthe phaseolorum	11.00 ± 0.57^{bdfhjlm}	9.00 ± 0.57^{bdfhjlm}	8.10 ± 0.44^{bc}	14.33±0.57^{bde}	13.66±0.57^{bdfhi}	14.16±0.28^{bde}
Acremonium cellulolyticus	0.00 ± 0.00^{a}	9.00 ± 0.57^{bdfhjlm}	4.60 ± 0.33^{bdfhjlm}	15.5±0.5^{bdfhjlm}	14.16±0.28^{bde}	16.00±0.5^{bc}
Penicillium brefeldianum	6.30 ± 0.33^{bc}	4.60 ± 0.33^{bde}	4.60 ± 0.33^{bdfhjlm}	15.16±0.28^{bdfhjlm}	15.5±0.5^{bdfhjk}	14.00±0.76^{bde}
Corynespora cassicola	8.00± 0.5^{bdfhjlno}	0.00 ± 0.00^{a}	6.00 ± 0.5^{bdfhjln}	6.83±0.76^{bdfhjk}	5.00±0.5^{bdfhjlm}	8.16±0.28^{bdfhjlm}
Phomopsis sp.	0.00 ± 0.00^{a}	3.60 ± 0.33^{bc}	3.80 ± 0.16^{bdfhi}	13.66±0.57^{bde}	13.33±0.57^{bdfhi}	13.83±0.76^{bde}
Penicillium chermesinum	3.80 ± 0.16^{bdfhjlnpq}	2.60 ± 0.33^{bdfg}	8.00 ± 0.00^{bc}	16.83±0.28^{bdfhjlno}	16.00±0.5^{bdfhjk}	16.83±0.28^{bc}
Fusarium verticillioides	5.30 ± 0.33^{bdfhjlnprs}	4.30 ± 0.33^{bde}	6.30 ± 0.33^{bdfhjlno}	11.16±0.5^{bc}	12.33±0.57^{bdfg}	12.56±0.5^{bdfhjlno}
Colletotrichum gloeosporioides	3.50 ± 0.28^{bdfhjlnpq}	4.50 ± 0.28^{bde}	4.50 ± 0.28^{bdfhjlm}	14.83±0.76^{bdfhjlnpq}	14.16±0.76^{bde}	14. ±0.50^{bde}
Colletotrichum sp.	4.00 ± 0.00^{bdfg}	5.00 ± 0.57^{bdfhi}	2.60 ± 0.33^{bde}	14.66±0.57^{bde}	14.16±0.28^{bde}	14.00±0.5^{bde}
Colletotrichum gloeosporioides	0.00 ± 0.00^{a}	3.30 ± 0.33^{bc}	0.00 ± 0.00^{a}	15.5±0.5^{bdfhjlm}	14.66±0.76^{bde}	14.00±0.5^{bde}
Phomopsis sp.	1.80 ± 0.16b^{bde}	5.00 ± 0.00^{bdfhi}	0.00 ± 0.00^{a}	13.83±0.76^{bde}	12.50±0.5^{bdfg}	13.16±0.76^{bdfhjlnp}
Phomopsis sp.	0.00 ± 0.00^{a}	6.50± 0.5^{bdfhjk}	4.83± 0.28^{bdfhjlm}	14.16±0.57^{bde}	14.33±0.28^{bde}	14.00±0.5^{bde}
Phomopsis sp.	4.16 ± 0.28^{bdfg}	0.00 ± 0.0^{a}	3.83±0.28^{bdfhi}	13.33±0.57^{bdfhi}	12.33±0.76^{bdfg}	12.5±0.5^{bdfhjlno}
Gongronella buleri	5.3 ± 0.28^{bdfhjlnprs}	7.16± 0.5^{bdfhjlno}	0.00 ± 0.0^{a}	0.00±0.00^{a}	3.50±0.5^{bdfhjlno}	0.00±0.00^{a}
Gongronella butleri	0.00 ± 0.0^{a}	5.83 ± 0.76^{bdfhi}	6.33± 0.57^{bdfhjlno}	0.00±0.00^{a}	0.00±0.00^{a}	6.83±0.76^{bdfhi}
Colletotrichum gloeosporioides	4.66 ± 0.28^{bdfg}	4.83± 0.28^{bde}	2.83± 0.28^{bde}	14.5±0.5^{bde}	15.5±0.5^{bdfhjk}	15±0.5^{bdfg}
Total N (%)	**18 (19.78)**	**20 (21.97)**	**19 (20.87)**	**22 (24.17)**	**22 (24.17)**	**23 (25.27)**
Tetracycline	12.66 ± 0.57^{bdfhjlnprt}	11.00 ± 0.00^{bdfhjlnp}	10.66 ± 0.57^{bdfhjlnp}	0.00±0.00^{a}	0.00±0.00^{a}	0.00±0.00^{a}
Fluconazole	0.00±0.00^{a}	0.00±0.00^{a}	0.00±0.00^{a}	0.00±0.00^{a}	0.00±0.00^{a}	0.00±0.00^{a}

Note: Tetracycline (30mg/disc), Fuconazole (10mg/disc), Negative control; methanol (solvent used to dissolve the crude metabolite) 100µl/cup. Mean (±) followed by the same letter(s) in each column are not significantly different at P<0.05 using Duncan's new multiple range test. Total N (%): Total number of isolates showing antimicrobial activity against each pathogen

The findings suggest that endophytic fungi isolated from *M. malabathricum* possess antimicrobial activity. The fungal extracts showed significant inhibitory effect against both bacterial and fungal pathogens. Most of the isolated endophytic fungi showed antimicrobial activity was ascomycetes, suggesting the potential of this group to fight against bacterial and fungal pathogens which hold promise for future development of antimicrobial natural products.

Acknowledgments

The senior author is thankful to University Grant Commission (UGC) for financial support as major research project (69/2014 F.No. 10-11/12 dated 26/03/2014) to BPS. VKM is thankful to Department of Science and Technology (DST), New Delhi for providing fellowship under DST INSPIRE fellowship (IF 130374). Authors are also thankful to the Department of Biotechnology, New Delhi for establishment of DBT-BIF centre and DBT-State Biotech hub in the Department of Biotechnology, Mizoram University which has been used for the present study.

References

Aly, A.H., A. Debbab, J. Kjer and P. Proksch: Fungal endophytes from higher plants: A prolific source of phytochemicals and other bioactive natural products. *Fungal. Divers.*, **41**, 1-16 (2010).

Barnett, H.L. and B.B. Hunter: Illustrated genera of imperfect fungi. APS press, Minnesota, USA (1972).

Bills, G.F. and I.D. Polishook: Microfungi from *Carpinus caroliniana*. *Can. J. Bot.*, **69**, 1477-1482 (1991).

Bungihan, M.E., M.A. Tan, M. Kitajima, N. Kogure, S.G. Franzblau, T.E. Dela Cruz, H. Takayama and M.G. Nonato: Bioactive metabolites of *Diaporthe* sp. P133, an endophytic fungus isolated from *Pandanus amaryllifolius*. *J. Nat. Med.,* **65**, 606-609 (2011).

Cannon, P.F. and C.M. Simmons: Diversity and host preference of leaf endophytic fungi in the Iwokrama Forest Reserve, Guyana. *Mycologia*, **94**, 210-220 (2002).

Cenis, J.L.: Rapid extraction of fungal DNA for PCR amplification.

Nucleic Acids Res., **20**, 2380 (1992).

Daisy, B.H., G.A. Strobel, U. Castillo, D. Ezra, J. Sears, D.K. Weaver and J.B. Runyon: Naphthalene, an insect repellent is produced by *Muscodor vitigenus*, a novel endophytic fungus. *Microbiology*, **148**, 3737–3741(2002).

Fisher, P.J. and O. Petrini: Location of fungal endophytes in tissue of *Suaeda fruticosa*: A preliminary study. *T. Brit. Mycol.Soc.*, **89**, 246–249 (1987).

Gehlot, P., S.K. Singh and R. Pathak: Morphometric and molecular characterization of fungus *Pestalotiopsis* using nuclear ribosomal DNA analysis. *J. Environ. Biol*., **33**, 897-901 (2012).

Ghimire, S.R., N.D. Charlton, J.D. Bell, Y.L. Krishnamurthy and K.D. Craven: Biodiversity of fungal endophyte communities inhabiting witchgrass (*Panicum virgatum* L.) growing in the native tallgrass prairie of northern Oklahoma. *Fung. Divers*., **47**, 19–27 (2011).

Gond, S.K., A. Mishra, V.K. Sharma, S.K. Verma, J. Kumar, R.N. Kharwar and A. Kumar: Diversity and antimicrobial activity of endophytic fungi isolated from *Nyctanthes arbor-tristis*, a well-known medicinal plant of India. *Mycoscience*, **53**, 113-121 (2012).

Groth, J.V. and A.P. Roelfs : The concept of measurement of phenotypic diversity in *Puccinia graminis* on wheat. *Phythopathology,* **77**, 1394-1399 (1987).

Hammer, O., D.A.T. Harper and P.D. Ryan: PAST: Palaeontological statistics software packages for education and data analysis. *Palaeontol. Electron.,* **4**, 9 (2001).

Huang, Z., X. Cai, C. Shao, C.Z. She, X. Xia, Y. Chen, J. Yang, S. Zhou and Y. Lin: Chemistry and weak antimicrobial activities of phomopsins produced by mangrove endophytic fungus *Phomopsis sp*. ZSU-H76. *Phytochemistry*, **69**, 1604-1608 (2008).

Hyde, K.D. and K. Soytong. The fungal endophyte dilemma. *Fungal divers.*, **33**, 163-173 (2008).

Joel, E.L. and B.V. Bhimba: Biological activity of secondary metabolites isolated from mangrove fungi *Neurospora crassa*. *J. Environ. Biol*., **34**, 729-732 (2013).

Joffry, S.M., N.J. Yob, M.S. Rofiee, M.M.R. MeorMohd., M.M. Affandi, Z. Suhaili, F. Othman, A. Md. Akim, M.N.M. Desa and Z.A Zakaria.: *Melastoma malabathricum* (L.) smith ethnomedicinal uses, chemical constituents, and pharmacological properties: A Review. *eCAM*, **2012**, 1-48 (2012).

Jurgenson, J.E., K. A. Zeller and J.F. Leslie: Expanded genetic map of *Gibberella moniliformis* (*Fusarium verticillioides*). *Appl. Environ. Microbiol.,* **68**, 1972–1979 (2002).

Kharwar, R.N., S.K.Verma, A. Mishra, S.K. Gond, V.K. Sharma, T. Afreen and A. Kumar: Assessment of diversity, distribution and antibacterial activity of endophytic fungi isolated from a medicinal plant *Adenocalymma alliaceum* Miers. *Symbiosis*, **55**, 39–46 (2011).

Kimura, M.: A simple method for estimating evolutionary rate of base substitutions through comparative studies of nucleotide sequences. *J. Mol. Evol*., **16**, 111-120 (1980).

Kokaew, J., L. Manoch, J. Worapong, N. Visarathanonth, N. Singbooraudom, A. Eamvijarn and V. Punnatrakul: Endophytic fungi in medicinal plant and studies on antagonistic effect against plant pathogenic fungi *in vitro*. Proceedings of the 45th Kasetsart University Annual Conference, Bangkok, Thailand, Subject: Plants, pp. 571-578 (2007).

Kumar, D.S.S. and K.D. Hyde: Biodiversity and tissue-recurrence of endophytic fungi in *Tripterygium wilfordii*. *Fungal Divers.,* **17**, 69–90 (2004).

Li, H.Y., M. Shen, Z.P. Zhou, T. Li, Y. Wei and L.B. Lin: Diversity and cold adaptation of endophytic fungi from five dominant plant species collected from the Baima Snow Mountain , south west China. *Fung. Divers*., **54**, 79–86 (2012).

Martínez-Luis, S., L. Cherigo, E. Arnold, C. Spadafora , W.H. Gerwick and L. Cubilla-Rios: Antiparasitic and anticancer constituents of the endophytic fungus *Aspergillus* sp. strain F1544. *Nat. Prod. Commun*., **7**, 165-8 (2012).

Naik, B.S., J. Shashikala and Y.L. Krishnamurthy: Diversity of fungal endophytes in shrubby medicinal plants of Malnad region, Western Ghats, Southern India. *Fungal. Ecol.*, **1**, 89-93 (2008).

Ngieng, N.S., A. Zulkharnain, H.A. Roslan and A. Husaini: Decolourisation of synthetic dyes by endophytic fungal flora isolated from senduduk plant (*Melastoma malabathricum*). *ISRN Biotechnology,* **2013**, 1-12 (2013).

Poole, R.W.: An introduction to quantitative ecology. McGraw-Hill, New York, USA (1974).

Qadri, M., R. Rajput, M.Z. Abdin, R.A. Vishwakarma and S. Riyaz-Ul-Hassan: Diversity, molecular phylogeny, and bioactive potential of fungal endophytes associated with the Himalayan blue pine (*Pinus wallichiana*). *Microb. Ecol.*, **67**, 877−887 (2014).

Rai, P.K. and H. Lalramnghinglova: Ethnomedicinal plant resources of Mizoram, India: implication of traditional knowledge in health care system. *Ethnobot. Leaflets*, **14**, 274 (2010).

Riddell, R.W.: Permanent stained mycological preparations obtained by slide culture. *Mycologia*, **42**, 265-270 (1950)

Rivera-Orduna, F., R. Suarez-Sanchez, Z. Flores-Bustamante, J. Gracida-Rodriguez and L. Flores-Cotera: Diversity of endophytic fungi of *Taxus globosa* (Mexican yew). *Fung. Divers.,* **47**, 65–74 (2011).

Santos, R.M.G., E. Rodrigues-Fo, W.C. Rocha and M.F.M. Teixeira: Endophytic fungi from *Melia azedarach*. *World. J .Microb. Biot.*, **19**, 767–770 (2003).

Sharma, H.K., L. Chhangte and A.K. Dolui: Traditional medicinal plants in Mizoram, India. *Fitoterapia.* **72**, 146–161 (2001).

Shen, X.Y., Y.L. Cheng, C.J. Cai, L. Fan, J. Gao and C.L. Hou: Diversity and antimicrobial activity of culturable endophytic fungi isolated from Moso Bamboo seeds. *Plos One,* **9**, 1-7 (2014).

Specian, V., M.H. Sarragiotto, J.A. Pamphile and E. Clemente: Chemical characterization of bioactive compounds from the endophytic fungus *Diaporthe helianthi* isolated from *Luehea divaricata*. *Braz. J. Microbiol.,* **43**, 1174-1182 (2012).

Strobel, G. and B. Daisy: Bioprospecting for microbial endophytes and their natural products. *Microbiol. Mol. Biol. R.,* **67**, 491-502 (2003).

Sunilson, J.A.J., K. Anandarajagopal, A.V.A.G. Kumari and S. Mohan: Antidiarrhoeal activity of leaves of *Melastoma malabathricum* linn. *Indian. J. Pharmaceut. Sci.,* **71**, 691–695 (2009).

Susanti, D., H.M. Sirat, F. Ahmad, R.M. Ali, N. Aimi and M. Kitajima : Antioxidant and cytotoxic flavonoids from the flowers of *Melastoma malabathricum* L. *Food. Chem*., **103,** 710–716 (2007).

Tamura, K., D. Peterson, N. Peterson, G. Stecher, M. Nei and S. Kumar: MEGA5: Molecular evolutionary genetics analysis using maximum likelihood, evolutionary distance, and maximum parsimony methods. *Mol. Biol. Evol.*, **28**, 2731-9 (2011).

Tayung, K. and D.K. Jha: Antimicrobial endophytic fungal assemblages inhabiting bark of *Taxus baccata* L. of Indo-Burma megabiodiversity hotspot. *Indian. J. Microbiol.,* **50**, S74–S81 (2010).

Tejesvi, M.V., Nalini, M.S., Mahesh, B., Parkash ,S.H., Kinni, R.K.,

Shetty, H.S. and V. Subbiah :New hopes from endophytic fungal secondary metabolite. *Bol. Soc. Quim .Mex .,* **1**,19–26 (2007).

Tejesvi, M.V., M. Kajula, S. Mattila and M.A. Pirttila: Bioactivity and genetic diversity of endophytic fungi in *Rhododendron tomentosum* harmaja. *Fung. Divers.*, **47**, 97-107 (2011).

Verma, S.K., S.K. Gond, A. Mishra, V.K. Sharma, J. Kumar, D.K. Singh, A. Kumar, J. Goutam and R. N. Kharwar: Impact of environmental variables on the isolation, diversity and antibacterial activity of endophytic fungal communities from *Madhuca indica* Gmel. at different locations in India. *Ann. Microbiol*., **64**, 721-734 (2014).

Verma, V.C., S.K. Gond, A. Kumar, R.N. Kharwar and G. Strobel: The endophytic mycoflora of bark, leaf and stems tissues of *Azadirachta indica* A. Juss (Neem) from Varanasi (India). *Microbial Ecol.,* **54**,119–125 (2007).

Vieira, M.L., A.F. Hughes, V.B. Gil, A.B. Vaz, T.M. Alves, C.L. Zani, C.A. Rosa and L.H. Rosa: Diversity and antimicrobial activities of the fungal endophyte community associated with the traditional Brazilian medicinal plant *Solanum cemum* Vell. (Solanaceae). *Can. J. Microbiol*., **58**, 54-66 (2012).

Wang, F.W., Z.M. Hou, C.R. Wang, P. Li and H. Shi: Bioactive metabolites from *Penicillium* sp., an endophytic fungus residing in *Hopea hainanensis. World. J .Microb. Biot.,* **24**, 2143-2147 (2008).

Wang, Y., L.D. Guo and K.D. Hyde: Taxonomic placement of sterile morphotypes of endophytic fungi from *Pinus tabulaeformis* (Pinaceae) in northeast China based on rDNA sequences. *Fung. Divers.,* **20**, 4876-4882 (2005).

White, T., T. Burns, S. Lee and J. Taylor: Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. In: PCR protocols: a guide to methods and applications. (Eds.: M.A. Innis, D.H. Gelfand, J.J. Sninsky, T.J. White) Academic Press, San Diego, New work, pp. 315–322 (1990).

Wiart, C., S. Mogana, S. Khalifah, M. Mahan, S. Ismail, M. Buckle, A.K. Narayana and M. Sulaiman: Antimicrobial screening of plants used for traditional medicine in the state of Perak, Peninsular Malaysia. *Fitoterapia,* **75**, 68–73 (2004).

Yang, Y., H. Zhao, R.A. Barrero, B. Zhang, G. Sun, I.W. Wilson, F. Xie, K.D. Walker, J.W.Parks, R. Bruce, G. Guo, L. Chen, Y. Zhang, X. Huang, Q. Tang, H. Liu, M.I. Bellgard, D. Qiu, J. lai and A. Hoffman: Genome sequencing and analysis of the paclitaxel-producing endophytic fungus *Penicillium aurantiogriseum* NRRL 62431.*BMC Genomics*, **15**, 1-14 (2014).

Zakaria, Z.A., R.N.S. Raden Mohd. Nor, G.H. Kumar, Z.D.F. Abdul Ghani, M.R. Sulaiman, G. Rathna Devi, A.M. Mat Jais, M.N. Somchit and C.A. Fatimah: Antinociceptive, anti-inflammatory and antipyretic properties of *Melastoma malabathricum* leaves aqueous extract in experimental animals. *Can. J. Physiol. Pharmacol.,* **84**, 1291–1299 (2006).