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Endophytic Fungi from Four Indonesian Medicinal Plants and Their Inhibitory Effect on Plant Pathogenic *Fusarium oxysporum*

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ABSTRACT

The medicinal plants *Centella asiatica*, *Curcuma xanthorrhiza*, *Guazuma ulmifolia*, and *Hydrocotyle verticillata* are widely used in Indonesian traditional medicine, but little is known about their associated endophytic fungi. This research aimed to study the diversity of endophytic fungi derived from functional parts of these plants and to evaluate their potential as antifungal agents against the plant pathogenic fungus *Fusarium oxysporum*. A total of 17 isolates of endophytic fungi were obtained: nine from leaves of *G. ulmifolia*, three each from leaves of *C. asiatica* and *H. verticillata*, and two from rhizomes of *C. xanthorrhiza*. The genus *Colletotrichum* was found in all plants studied, but each plant was associated with different species. *Colletotrichum aeschynomenes* was associated with *C. xanthorrhiza*, *C. siamense* was associated with *C. asiatica*, and *C. tropicale* was associated with *G. ulmifolia* and *H. verticillata*. The species *Curvularia affinis*, *Diaporthe tectonae*, *Lasiodiplodia mahajangana*, *Parengyodontium album*, *Talaromyces trachyspermus*, and *Speiropsis pedatospora* were found only in *G. ulmifolia*; while *Didymella coffeae-arabicae* and *Muyocopron laterale* were found only in *H. verticillata*. The endophytic fungi showed inhibition activity against *F. oxysporum* with inhibition values of 6.0-78.9%, *T. trachyspermus* JBd10 and *C. affinis* JBd14 gave the highest inhibition activity.

1. Introduction

The medicinal plants *Centella asiatica* L. (asiatic pennywort), *Curcuma xanthorrhiza* Roxb. (java turmeric), *Guazuma ulmifolia* Lamk. (bay cedar), and *Hydrocotyle verticillata* Thunb. (whorled pennywort) are well known for their usage and medicinal properties. The raw material for medicines can be obtained from different parts of a medicinal plant based on their various active ingredients. The dried sample can be obtained from a particular part of the medicinal plant such as the leaf, stem, rhizome, or root that contains a high concentration of the active compound, or from the whole plant. In traditional medicines, *Curcuma* dried sample is derived from rhizomes, while *Centella*, *Guazuma*, and *Hydrocotyle* dried samples are derived from leaves.

Endophytes are microorganisms that live within plant tissues for at least part of their life cycle without causing apparent disease. Fungi and bacteria are the most common microbes living as endophytes, but the most commonly isolated are fungi (Hardoim *et al.* 2015). There are relatively few reports on the endophytic fungi associated with medicinal plants. Among them, Hammerschmidt *et al.* (2015) isolated *Xylaria* sp. from healthy leaves of plants collected on the island of Timor, Indonesia, and the fungus produced a new compound (resacetophenone). Septiana *et al.* (2017) successfully isolated eleven

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endophytic fungi from the turmeric plant, some of which showed antibacterial and anti-histamine activities. Endophytic fungi reported for *C. asiatica* included *Colletotrichum higginsianum, Guignardia mangiferae*, and *Glomerella cingulata* (Rakotoniriana *et al.* 2008). *Penicillium* sp. derived from turmeric leaves produced alkaloids, phenols, flavonoids, tannin, glycosides, and cellulase enzyme (Devi *et al.* 2012). Endophytic fungi associated with *G. ulmifolia* were *Muscodor albus* (Strobel *et al.* 2007), *Pestalotiopsis* sp. (Russell *et al.* 2011), and *Nigrograna mackinnonii*, which produced limonene compound (Shaw *et al.* 2015). Strobel *et al.* (2007) reported that *Muscodor albus* E-6 obtained from *G. ulmifolia* branches could produce many secondary metabolites. So far, there are no reports of endophytic fungi associated with the *H. verticillata* plant.

Endophytic fungi are widely accepted to be able to synthesize bioactive natural compounds (Strobel and Daisy 2003), including compounds with antimicrobial activity for defense against pathogens (Kusari *et al.* 2013), which may be used as biocontrol agents in agriculture. Endophytic fungi inhibit the growth or stop the reproduction of pathogens by many mechanisms such as antagonism, mycoparasitism, antibiosis, and competition (Cook 1993). They produce bioactive compounds in two ways, either from precursors initiated by their metabolism or from precursors produced by the plant's metabolism. This research aimed to obtain endophytic fungi from the medicinally used organs of *C. asiatica, C. xanthorrhiza, G. ulmifolia*, and *H. verticillata*; to study their diversity in these organs; and to evaluate their antifungal activity against the plant pathogenic fungus *Fusarium oxysporum*.

2. Materials and Methods

2.1. Plant Materials

Endophytic fungi were isolated from the medicinally used organs of *C. asiatica, C. xanthorrhiza, G. ulmifolia*, and *H. verticillata*. All the medicinal plant samples were obtained from the living collections of the Indonesian Medicinal and Aromatic Crops Research Institute, Bogor, Indonesia. In traditional Indonesian remedies, the medicinally used organs of *C. asiatica, G. ulmifolia*, and *H. verticillata* are the leaves, while for *C. xanthorrhiza* are the rhizomes.

Five clumps of fresh and healthy plants of *C. asiatica, G. ulmifolia*, and *H. verticillata* were randomly selected and harvested by cutting the three leaves from the third to fifth leaf from the top of the plants. For *C. xanthorrhiza*, three clumps of fresh and healthy plants were carefully harvested by digging up the plant and cutting the rhizomes. Samples were then put in clean plastic bags, transported to the laboratory, and processed within 24 hours of collection. Each sample was washed thoroughly with running tap water and followed up by rinsing with sterilized reverse osmosis water three times and pooling them to make a composite sample.

2.2. Isolation of Endophytic Fungi

All preparations and isolation processes were carried out in a biosafety cabinet. Leaf samples were cut into small pieces of 2 x 2 cm^2 size. The rhizomes of *C. xanthorrhiza* were peeled and then cut into 2 x 2 x 2 cm^3 pieces. Surface sterilization was conducted by immersing the sample in 70% ethanol for 1 min, soaking in 0.5% hypochlorite solution for 5 min, and 70% ethanol for 1 min, and finally washing with sterilized distilled water six times. Then the samples were blotted on sterile Whatman filter paper for 12 hours. Four pieces from each cutting sample were randomly chosen and cultured on potato dextrose agar (PDA, difco) plates containing rose bengal (30 mg L^{-1}) and chloramphenicol (0.5 g L^{-1}). Media plates were sealed and incubated at 28°C over 21 days, during which time they were checked daily for hyphal growth (Hallmann *et al.* 2007). The hyphal tips arising from the colonies having different characteristics were picked and transferred onto new PDA plates without being supplemented with either rose bengal or chloramphenicol. Each fungal isolate was purified to obtain a single colony.

2.3. Identification of Endophytic Fungal Isolates

The pure isolates having different characteristics were identified by a combination of morphological characteristics (Barnett and Hunter 1998) and molecular analyses. DNA extraction was prepared according to the CTAB-based extraction method (Sambrook and Russel 2000). The fungi were cultured in potato dextrose broth (PDB, difco) and incubated in a shaker at 120 rpm 28°C for seven days. Mycelia were harvested through sterilized filter paper by vacuum filtration. The mycelia were frozen in liquid nitrogen and ground in a sterile mortar. About 0.5 g of mycelia powder was mixed with warm extraction buffer (600 µl PVP and 1.2 µl

CTAB) in a 1.5-ml Eppendorf tube. It was inverted and incubated at 65°C for 30 min. The tube was then incubated on ice for 5 min and then 600 µl of a mixed solution of chloroform: alcohol (24:1) was added. It was then inverted and centrifuged for 10 min at 10°C, 25,000×g. The aqueous phase was removed carefully to a new tube, then added with an equal volume of phenol: chloroform: isoamyl alcohol (25:24:1). The tube was inverted and centrifuged again for 5 min at 4°C, 25,000×g. The supernatant was mixed with an equal volume of 2M NaOAc pH 5.2 and 2x volume of cold EtOH in a new tube, and incubated for 30 min at 20°C, and then centrifuged at 25,000×g, at 4°C for 30 min. DNA pellets were collected and washed with 500 µl 70% cold ethanol and then centrifuged for 5 min at 4°C, 25,000×g. DNA pellets were dried briefly using a vacuum, resuspended in 20 µl of sterilized double-distilled water, added with 0.2x volume of RNAse, and incubated for 10 min at 37°C. The DNA was then incubated for 10 min at 70°C to inactivate the RNAse. Fungal DNA was then stored in a freezer until used.

The DNA was then subjected to PCR amplification using the universal primers pair of ITS1 (forward) (5'-TCC GTA GGT GAA CCT GCG G-3') and ITS4 (reverse) (5′-TCC TCC GCT TAT TGA TAT GC-3′) (White *et al.* 1990). The amplified fragments consisted of the internal transcribed spacer (ITS) regions of the extracted DNA, including the 5.8S rDNA. The PCR reaction was performed in a 60 µl reaction mixture which consisted of 42.6 µl sterilized ddH2O, 6 µl buffer (10x), 1.2 µl 2 mM dNTP, 1.5 µl 10 pmol of each forward and reverse primer, 1.2 µl 5 U *Taq* DNA polymerase, and 6 µl DNA template. The PCR amplification reaction was carried out under the following conditions: initial denaturation for 5 min at 95°C, followed by 35 cycles of denaturation, annealing, and extension at 72°C for 1 min, 94°C for 30 seconds, and 52°C for 30 seconds, respectively. This process was followed by a final re-extension step of 72°C for 5 min and finally stored at 25°C for 10 min using a Gene Amp 9700 thermal cycler (Applied Biosystems, USA).

The PCR products were purified and sequenced by First Base (Malaysia) using the same primers. The sequence was analyzed using the BioEdit Ver.7 (Hall 1999) and aligned using Clustal W (Thompson *et al.* 1994). The sequence similarity was determined by using available DNA fungal sequences at MycoBank (https://www.mycobank.org) and GenBank (BLAST) (https://blast.ncbi.nlm.nih.gov/Blast.cgi). Phylogenetic analyses were conducted using Maximum Likelihood methods in MEGA6 (Tamura *et al.* 2013). The reference GeneBank accession was used to construct the phylogenetic tree of *Aspergillus* based on Samson *et al.* (2014), *Colletotrichum* based on Cannon *et al.* (2012), *Curvularia* based on Marin-Felix *et al.* (2020), *Diaporthe* based on Dissanayake *et al.* (2017), *Didymella* based on Scarpari *et al.* (2020) and Chen *et al.* (2017), *Neocosmospora* based on Sandoval-Denis and Crous (2018), *Lasiodiplodia* based on Abdollahzadeh *et al.* (2010), *Parengyodontium* based on Tsang *et al.* (2016), and *Talaromyces* based on Adhikari *et al.* (2015). Sequences used for phylogenetic analysis are shown in Table 1. The maximum likelihood tree was constructed using the best DNA model (Nei and Kumar 2000). The genera *Aspergillus, Colletotrichum, Curvularia, Neocosmospora*, and *Lasiodiplodia* used the K2+G model; *Diaporthe* used the K2+G+I model; *Parengyodontium* used the T92+G model; and the genera *Didymella* and *Talaromyces* used the T92+G+I (G Gamma distributed, I evolutionarily invariable, K2 Kimura 2-parameter, T92 Tamura 3-parameter). Phylogenetic analyses of the genera *Muyocopron* and *Speiropsis* were not done due to the limited availability of DNA sequences in the database. Gaps and missing data were treated as complete deletions. Initial trees for ML were made by the NJ/BioNJ algorithm and the branch swap filter was set very strong. Support for specific nodes on the ML tree was estimated by bootstrapping 1,000 replications. The nucleotide sequences generated in this study were deposited in GenBank (https://www.ncbi.nlm.nih.gov/).

2.4. *In Vitro* Antagonistic Bioassay

The antagonistic activities of the endophytic fungi isolates were evaluated against the plant pathogenic fungus *F. oxysporum* (IPBCC.88.0.12 or CBS 254.52) using the antagonist assays method of Morton and Stroube (1955) by using a dual culture technique *in vitro* assay on PDA. First, the endophytic fungi isolates and pathogenic fungus *F. oxysporum* were grown on separate PDA plates for seven days at 28°C. A 5 mm diameter mycelial plug of endophytic fungus was placed 1 cm away from the periphery at one end of a Petri dish containing PDA and incubated at room temperature for 4 days. A 5 mm in diameter culture plug of *F. oxysporum* was placed in the same Petri dish at a distance of 1 cm from the edge, at the opposite side to the endophytic fungus. In the control treatment, an agar disc (5 mm in diameter) was placed 1 cm away from the periphery

Table 1. DNA sequence accession numbers of the isolates included in this study

Species	Isolates	ITS GenBank accession numbers	Sources
DNA sequence accession numbers for phylogenetic analysis of *Aspergillus*			
Aspergillus aculeatus	NRRL 5094^{T}	EF661221	Soil
A. avenaceus	CBS 109.46^{NT}	AF104446	*Pisum sativum* seed
A. calidoustus	CBS 121601^{T}	HE616558	Bronchoalveolar lavage specimen
A. clavatus	NRRL 1^{T}	EF669942	Soil
A. fischeri	NRRL 181^{NT}	EF669936	Canned apples
A. flavipes	NRRL 302^{LT}	EF669591	Soil
A. flavus	NRRL 1957^{NT}	AF027863	Moldy cellophane
A. fumigatus	NRRL 163^{T}	EF669931	Chicken lung
A. funiculosus	NRRL 4744^{T}	EF661223	Soil
A. glaucus	NRRL 116^{NT}	EF652052	House lumber
A. montevidensis	NRRL 108^{NT}	EF652077	Tympanic membrane
A. nidulans	NRRL 187^{NT}	EF652427	Soil
A. ochraceus	NRRL 398^{NT}	EF661419	Unknown
A. penicillioides	NRRL 4548^{NT}	EF652036	Human skin
A. pseudoterreus	NRRL 4017^{HT}	NR_137472	Soil
	IPBCC 11.758	*SUB9428972*	Rhizomes of *Curcuma xanthorrhiza*
A. sparsus	NRRL 1933^{LT}	EF661181	Soil
A. terreus	NRRL 255^{T}	EF669586	Soil
A. togoensis	CBS 272.89^{T}	AJ874113	Seed
A. versicolor	NRRL 238^{NT}	EF652442	Unknown
	IPBCC 11.760	*SUB9403371*	Leaves of *Centella asiatica*
	IPBCC 11.749	*SUB9427202*	Leaves of *Guazuma ulmifolia*
Talaromyces flavus	CBS 310.38^{T}	JN899360	Unknown
DNA sequence accession numbers for phylogenetic analysis of *Colletotrichum*			
Colletotrichum acerbum	CBS 128530^{HT}	JQ948459	Bitter rot of *Malus domestica* fruit
C. aeschynomenes	ICMP 17673^{HT}	JX010176	*Aeschynomene virginica*
	IPBCC 11.757	*SUB9432747*	Rhizomes of *Curcuma xanthorrhiza*
C. anthrisci	CBS 125334^{T}	GU227845	*Anthriscus sylvestris*
C. boninense	CBS 123755^{HT}	AB051400	*Crinum asiaticum* var. *sinicum*
C. cliviae	CBS 125375^{HT}	JX519223	*Clivia miniata*
C. curcumae	IMI 288937^{ET}	GU227893	*Curcuma longa*
C. dracaenophilum	CBS 118199^{HT}	JX519222	*Dracaena sanderana*
C. fructi	CBS 346.37^{ET}	GU227844	*Malus sylvestris*
C. jasminigenum	MFLUCC 10-0273^{HT}	HM131513	*Jasminum sambac*
C. kahawae	ICMP 17816^{HT}	JX010231	*Coffea arabica*
C. lindemuthianum	CBS 144.31^{ET}	JQ005779	*Phaseolus vulgaris*
C. lineola	CBS 125337^{ET}	GU227829	*Apiaceae, dead stem*
C. orbiculare	CBS 514.97^{HT}	JQ005778	*Cucumis sativus*
C. pseudoacutatum	CBS 436.77^{HT}	JQ948480	*Pinus radiata*
C. pyricola	CBS 128531^{HT}	JQ948445	*Pyrus communis*, fruit rot
C. rhombiforme	CBS 129953^{HT}	JQ948457	*Olea europaea*
C. siamense	ICMP 18578^{T}	FJ972613	*Coffea arabica*
	IPBCC 13.1092	*SUB9446440*	*Leaves of Centella asiatica*
C. torulosum	CBS 128544^{HT}	JQ005164	*Solanum melongena*
C. tropicale	ICMP 18653^{HT}	GU994331	*Theobroma cacao*
	IPBCC 11.752	*SUB9446618*	Leaves of *Hydrocotyle verticillata*
	IPBCC 11.747	*SUB9433263*	Leaves of *Guazuma ulmifolia*
C. truncatum	CBS 151.35^{ET}	GU227862	*Phaseolus lunatus*
C. yunnanense	CBS 132135^{HT}	EF369490	*Buxus sp.*
Monilochaetes infuscans	CBS 869.96	GU180626	*Ipomoea batatas*

Table 1. Continued

Species	Isolates	ITS GenBank accession numbers	Sources
DNA sequence accession numbers for phylogenetic analysis of *Curvularia*			
Curvularia affinis	CBS 154.34^{SynT}	KJ909780	Unknown
	IPBCC 13.1088	*SUB9445050*	Leaves of *Guazuma ulmifolia*
C. asiatica	MFLUCC 10-0711^{T}	JX256424	*Panicum* sp.
C. beerburrumensis	BRIP 12942^{T}	MH414894	*Eragrostis bahiensis*
C. cactivora	CBS 580.74	MN688803	Member of *Cactaceae*
C. chiangmaiensis	CPC 28829^{T}	MF490814	*Zea mays*
C. coicis	CBS 192.29^{SynT}	JN192373	*Coix lacryma*
C. crassiseptata	CBS 503.90^{T}	LT631310	Plant material
C. cymbopogonis	CBS 419.78^{T}	HG778985	*Yucca* sp.
C. dactyloctenii	BRIP 12846^{T}	KJ415545	*Dactyloctenium radulans*
C. ellisii	CBS 193.62^{T}	JN192375	Air
C. eragrosticola	BRIP 12538^{HT}	MH414899	*Eragrostis pilosa*
C. gladioli	CBS 210.79	HG778987	*Gladiolus* sp.
C. heteropogonis	CBS 284.91^{T}	JN192379	*Heteropogon contortus*
C. intermedia	CBS 334.64	HG778991	*Avena versicolor*
C. ischaemi	CBS 630.82^{T}	JX256428	*Ischaemum indicum*
C. microspore	GUCC 6272^{T}	MF139088	*Hippeastrum striatum* leaf spot
C. mosaddeghii	IRAN 3131^{CT}	MG846737	*Syzygium cumini* leaf spot
C. neergaardii	BRIP 12919^{IsoT}	KJ415550	*Oryza sativa*
C. nodosa	CPC 28800^{T}	MF490816	*Digitaria ciliaris*
C. nodulosa	CBS 160.58	JN192383	*Eleusine indica*
C. oryzae	CBS 169.53^{IsoT}	KP400650	*Oryza sativa*
C. ovariicola	CBS 470.90^{T}	MN688809	*Eragrostis interrupta*
C. pallescens	CBS 156.35^{T}	KJ922380	Air
C. palmicola	MFLUCC 14-0404^{T}	MF621582	*Acoelorrhaphe wrightii*
C. papendorfii	CBS 308.67^{T}	KJ909774	*Acacia karroo*
C. paterae	CBS 198.87^{T}	MN688810	*Triticum durum* seed
C. perotidis	CBS 350.90^{T}	JN192385	*Perotis rara*
C. petersonii	BRIP 14642^{T}	MH414905	*Dactyloctenium aegyptium*
C. portulacae	BRIP 14541^{IsoT}	KJ415553	*Portulaca oleracea*
C. pseudointermedia	CBS 553.89^{T}	MN688819	Cultivated pasture soil
C. soli	CBS 222.96^{HT}	KY905679	Soil
C. sorghina	BRIP 15900^{IsoT}	KJ415558	*Sorghum bicolor*
C. sporobolicola	BRIP 23040^{HT}	MH414908	*Sporobolus australasicus*
C. thailandica	MFLUCC 15-0747^{HT}	MH275057	Dead leaf of *Pandanus* sp.
C. trifolii	CBS 173.55	HG779023	*Trifolium repens*
C. tuberculate	CBS 146.63^{IsoT}	JX256433	*Zea mays*
C. xishuangbannaensis	MFLUCC 17-2271^{T}	MH275058	*Dead leaf of Pandanus* sp.
Bipolaris maydis	CBS 136.29^{T}	KJ909769	*Zea mays*
B. sorokiniana	CBS 110.14	KJ922381	*Hordeum* sp.
DNA sequence accession numbers for phylogenetic analysis of *Diaporthe*			
Diaporthe ambigua	CBS 114015^{T}	KC343010	*Pyrus communis*
D. aquatica	IFRDCC 3051^{T}	JQ797437	Aquatic habitat
D. brasiliensis	CBS 133183^{T}	KC343042	*Aspidosperma tomentosum*
D. caatingaensis	CBS 141542^{HT}	KY085927	*Tacinga inamoena*
D. citriasiana	ZJUD 30^{HT}	JQ954645	*Citrus unshiu*
D. compacta	CGMCC 3.17536^{T}	KP267854	*Camellia sinensis*
D. ganjae	CBS 180.91^{T}	KC343112	*Cannabis sativa*
D. goulteri	BRIP 55657a^{HT}	KJ197290	*Helianthus annuus*
D. longispora	CBS 194.36^{T}	KC343135	*Ribes* sp.
D. malorum	CAA734^{HT}	KY435638	*Malus domestica*
D. mayteni	CBS 133185^{HT}	KC343139	*Maytenus acuminata*
D. neoraonikayaporum	MFLUCC 14-1136^{T}	KU712449	*Tectona grandis*
D. oxe	CBS 133186^{T}	KC343164	*Maytenus ilicifolia*
D. paranensis	CBS 133184^{T}	KC343171	*Maytenus ilicifolia*
D. passifloricola	CBS 141329^{T}	KX228292	*Passiflora foetida*
D. raonikayaporum	CBS 133182^{T}	KC343188	*Spondias mombin*

Table 1. Continued

Species	Isolates	ITS GenBank accession numbers	Sources
DNA sequence accession numbers for phylogenetic analysis of *Diaporthe*			
D. sclerotioides	CBS 296.67[T]	KC343193	*Cucumis sativus*
D. siamensis	MFLUCC 10-0573[aT]	JQ619879	*Dasymaschalon* sp.
D. tectonae	MFLUCC 12-0777[HT]	KU712430	*Tectona grandis*
	IPBCC 11.750	*SUB9431627*	*Leaves of Guazuma ulmifolia*
D. tulliensis	BRIP 62248[aHT]	KR936130	*Theobroma cacao* fruit
D. corylina	CBS 121124	KC343004	*Corylus* sp.
DNA sequence accession numbers for phylogenetic analysis of *Didymella*			
Didymella acetosellae	CBS 179.97	GU237793	*Rumex hydrolapathum*
D. aeria	LC 7441[T]	KY742051	Air
D. arachidicola	CBS 333.75[IT]	GU237833	*Arachis hypogea*
D. chloroguttulata	LC 7435	KY742057	Air
D. coffeae-arabicae	CBS 123380[HT]	FJ426993	*Coffea arabicae*
	IPBCC 13.10895	*SUB9431855*	Leaves *of Hydrocotyle verticillata*
D. dactylidis	CBS 124513[HT]	GU237766	*Dactylis glomerata*
D. exigua	CBS 183.55[NT]	GU237794	*Rumex arifolius*
D. glomerata	CBS 528.66[ET]	FJ427013	*Chrysanthemum* sp.
D. longicolla	CBS 124514[HT]	GU237767	*Opuntia* sp.
D. molleriana	CBS 229.79	GU237802	*Digitalis purpurea*
D. molleriana	CBS 109179	GU237744	*Digitalis* sp.
D. ocimicola	LC 8137	KY7420782	*Ocimum* sp.
D. pinodes	CBS 525.77[ET]	GU237883	*Pisum sativum*
D. protuberans	CBS 381.96	GU237853	*Lycium halifolium*
Didymella pteridis	CBS 379.96	KT389504	*Pteris* sp.
D. rhei	CBS 109177	GU237743	*Rheum rhaponticum*
D. rumicicola	CBS 683.79[IT]	KT389503	*Rumex obtusifolius*
D. sancta	CBS 281.83[HT]	FJ427063	*Ailanthus altissima*
D. senecionicola	CBS 160.78	GU237787	*Senecio jacobaea*
D. suiyangensis	CGMCC 3.18352[T]	NR_158260	Air
Phoma herbarum	CBS 615.75[T]	KF251212	*Rosa multiflora* cv. *cathayensis*
	CBS 377.92	KT389536	Human leg
DNA sequence accession numbers for phylogenetic analysis of *Lasiodiplodia*			
Lasiodiplodia brasiliensis	CMM 4015[HT]	NR_147338	*Mangifera indica*
L. laelio-cattleyae	CBS 167.28[T]	MH854963	*Laelio cattleya*
L. citricola	IRAN1521[CT]	GU945353	*Citrus* sp.
L. crassispora	CBS 118741[T]	DQ103550	*Santalum album*
	CMW 13488	DQ103552	*Eucalyptus urophylla*
L. gilanensis	IRAN 1523[CHT]	GU945351	Unknown
L. jatrophicola	CMM 3610[HT]	NR_147348	*Jatropha curcas*
L. margaritacea	CBS 122519[HT]	EU144050	*Adansonia gibbosa*
	CBS 122065	EU144051	*Adansonia gibbosa*
L. missouriana	CBS 128311[HT]	NR_145222	*Vitis* sp.
L. gonubiensis	CBS 115812[T]	DQ458892	*Syzigium cordatum*
	CBS 116355	AY639594	*Syzigium cordatum*
L. magnoliae	MFLUCC 18-0948	MK499387	*Magnolia candolii*, dead leaves
L. mahajangana	CBS 124925[T]	MH863425	*Terminalia catappa*
	IPBCC 11.751	*SUB 9431869*	Leaves of *Guazuma ulmifolia*
L. parva	CBS 494.78[T]	EF622084	*Cassava-field soil*
L. pseudotheobromae	CBS 116459[T]	EF622077	*Gmelina arborea*
L. theobromae	CBS 164.96[T]	AY640255	Fruit on coral reef coast
L. venezuelensis	WAC12539[HT]	DQ103547	*Acacia mangium*
	WAC12540	DQ103548	*Acacia mangium*
L. viticola	CBS 128313[HT]	MH864855	Wedge-shape canker of grapevine cv. Vignoles
Diplodia mutila	CBS 112553	AY259093	*Vitis vinifera*
D. seriata	CBS 112555[ET]	AY259094	*Vitis vinifera*

Table 1. Continued

Species	Isolates	ITS GenBank accession numbers	Sources
DNA sequence accession numbers for phylogenetic analysis of *Neocosmospora*			
Fusarium brasiliense	NRRL 31757	EF408514	*Glycine max*
F. solani f. sp. *batatas*	NRRL 22400	AF178407	*Ipomoea batatas*
F. solani f pisi	NRRL 22278	DQ094309	*Pisum sativum*
F. solani f. sp. *xanthoxyli*	NRRL 22277	AF178401	*Xanthoxylum* sp.
F. sriatum	NRRL 22101	AF178398	Cotton cloth
Neocosmospora catenata	NRRL 54993^{HT}	KC808256	Zebra shark multiple tissues
N. croci	CBS 142423^{HT}	LT746264	*Citrus sinensis*
N. cyanescens	CBS 518.82^{T}	EU329684	Human foot
N. falciformis	CBS 475.67^{T}	MH859035	Human bronchoalveolar lavage fluid
N. gamsii	NRRL 32323^{HT}	DQ094420	Human bronchoalveolar lavage fluid
N. illudens	NRRL 22090	AF178393	*Beilschmiedia tawa*
N. lichenicola	NRRL 28030	DQ094355	Human
N. macrospora	CBS 142424^{HT}	LT746266	*Citrus sinensis*
	IPBCC 11.756	*SUB9433318*	Leaves of *Centella asiatica*
N. mahasenii	CBS 119594^{HT}	JF433045	Dead branch of live tree
N. petroliphila	NRRL 32315	DQ094412	Human groin ulcer
N. plagianthi	NRRL 22632	AF178417	*Hoheria glabrata*
N. pseudensiforme	CBS 125729^{HT}	KC691584	Unknown dead tree
	IPBCC 11.748	*SUB9431884*	Leaves of *Guazuma ulmifolia*
N. solani	CBS 140079^{ET}	KT313633	*Solanum tuberosum*
N. suttoniana	CBS 143214^{HT}	DQ094617	Human wound
N. vasinfecta	CBS 130182	EF453092	Human
Geejayessia cicatricum	CBS 125552	HQ728145	*Buxus sempervirens*
G. atrofusca	NRRL 22316	AF178423	*Staphylea trifolia*
DNA sequence accession numbers for phylogenetic analysis of *Parengyodontium*			
Akanthomyces arachnophilus	BCC17655	GQ249995	Unknown
A. novoguineensis	BCC22910	GQ250003	Insecta
Beauveria amorpha	ARSEF 2641T	NR_111601	Hymenoptera: Formicidae
B. caledonica	ARSEF 2567T	HQ880817	Soil
B. vermiconia	ARSEF 2922T	HQ880822	Soil
Cordyceps ninchukispora	BCC1422	FJ765278	Insecta
C. pruinose	ARSEF 5413	JN049826	*Iragoides fasciata (*Lepidoptera*)*
C. takaomontana	BCC 1409	EU807995	Pupa
Engyodontium parvisporum	IHEM 22910	LC092896	Indoor contamination
E. rectidentatum	CBS 641.74	LC092895	Buried keratinous substance
Isaria amoenerosea	CBS 107.73	AY624168	Coleopteran pupa
I. cateniobliqua	CBS 153.83T	NR_111170	*Adoxophyes privatana*
I. cicadae	BCC 2574	AY624175	Cicada nymph
I. tenuipes	ARSEF 5135	NR_119512	Lepidopteran pupa
Lecanicillium acerosum	CBS 418.81T	NR_111268	*Crinipellis perniciosa*
L. antillanum	CBS 350.85T	NR_111097	Agaric
L. aphanocladii	CBS 376.77	AJ292431	*Agaricus bitorquis*
L. attenuatum	CBS 170.76T	EF679164	Caterpillar of *Carpocapsa pomonella*
L. dimorphum	CBS 363.86T	NR_111101	*Agaricus bisporus*
L. flavidum	CBS 342.80T	NR_111266	Decaying needle of *Abies alba*
L. fungicola var. aleophilum	CBS 357.80T	NR_111064	*Agaricus bisporus*
L. fungicola var. fungicola	CBS 992.69T	NR_119653	*Agaricus bisporus*
L. indonesiacum	BTCC-F36T	AB378516	Araneae
L. kalimantanense	BTCC-F23T	NR_121200	*Coleoptera in suspended soil*
L. longisporum	IMI 021167T	NR_111095	*Icerya purchasi (*coccidae*)*
L. nodulosum	IMI 338014R	EF513012	Insect coccidae
L. primulinum	JCM 18525T	NR_119418	Soil
L. saksenae	CBS 532.81T	JN049846	Forest soil
L. tenuipes	CBS 658.80	LC092897	Spider
L. wallacei	CBS 101237T	NR_111267	Lepidoptera
Parengyodontium album	CBS 504.83ET	LC092880	Human brain abscess
	IPBCC 11.755	*SUB9446667*	Leaves of *Guazuma ulmifolia*

Table 1. Continued

Species	Isolates	ITS GenBank accession numbers	Sources
DNA sequence accession numbers for phylogenetic analysis of *Parengyodontium*			
Simplicillium chinense	LC1345^T	JQ410324	Wood in freshwater
S. cylindrosporum	JCM 18169^T	NR_111023	Soil
S. lamellicola	CBS 116.25^T	NR_111098	*Agaricus bisporus*
S. sympodiophorum	JCM 18184^T	NR_111027	Soil
Torrubiella ratticaudata	ARSEF 1915	JN049837	*Euophrys* sp.
Hypocrea lutea	GJS 89-129	AF275339	Decorticated conifer wood
DNA sequence accession numbers for phylogenetic analysis of *Talaromyces*			
Talaromyces amestolkiae	CBS 132696HT	JX315660	House dust
T. apiculatus	CBS 312.59HT	KF741983	Soil
T. aurantiacus	CBS 314.59HT	JN899380	Soil
T. coalescens	CBS 103.83HT	JN899366	Soil under *Pinus* sp.
T. diversus	CBS 320.48NT	KJ865740	Leather
T. duclauxii	CBS 322.48HT	JN899342	Canvas
T. echinosporus	CBS 293.62HT	JN899363	Wood pulp
T. erythromellis	CBS 644.80HT	JN899383	Soil from creek bank
T. marneffei	CBS 388.87T	JN899344	*Rhizomys sinensis*
T. minioluteus	CBS 642.68HT	JN899346	Unknown
T. muroii	CBS 756.96HT	JN899351	Soil
T. pittii	CBS 139.84NT	JN899325	Clay soil under poplar trees
T. purpureogenus	CBS 286.36HT	JX315671	Parasitic on a culture of *Aspergillus oryzae*
T. rademirici	CBS 140.84NT	JN899386	Air under willow tree
T. ruber	CBS 132704NT	JX315662	Air craft fuel tank
T. trachyspermus	CBS 373.48HT	JN899354	Unknown
	IPBCC 11.753	*SUB9431993*	Leaves of *Guazuma ulmifolia*
T. varians	CBS 386.48HT	JN899368	Cotton yarn
Thermoascus crustaceus	CBS 181.67	FJ389925	*Parthenium argentatum*
*Muyocopron laterale**	IPBCC 13.1097	*SUB9857667*	Leaves of *Hydrocotyle verticillata*
*Speiropsis pedatospora**	IPBCC 11.754	*SUB9861920*	Leaves of *Guazuma ulmifolia*

Accession number of sequences obtained in this study are presented in italic
*Not included in the phylogenetic study

instead of endophytic fungus, while a 5 mm diameter culture plug of pathogenic fungi *F. oxysporum* was placed 1 cm away from the edge of the same Petri dish at the opposite side from the agar disc. All the plates were incubated at room temperature for seven days. The antagonistic activity was checked after incubation by measuring the growth radius of *F. oxysporum* on days 4 and 7 after inoculation. The magnitude of the inhibitory activity was calculated with the formula: PI = (100 × (R1 - R2) / R1), where PI is percentage inhibition of radial growth, R1 is the growth radius of *F. oxysporum* colony in the control plate, and R2 is radial growth of *F. oxysporum* in dual culture with the endophytic fungus. All of the endophytic fungi obtained in this study were tested, and each assay was repeated five times. Statistical analysis was done using the MSTAT program (University of Wisconsin-Madison), and mean values were analyzed by DMRT ($p<0.05$).

3. Results

3.1. Diversity of Endophytic Fungi

A total of 17 isolates of endophytic fungi having different colony characteristics were obtained from the four medicinal plants. Nine isolates of endophytic fungi were obtained from *G. ulmifolia* leaves, three isolates were obtained each from the leaves of *C. asiatica* and *H. verticillata*, and two isolates from the rhizomes of *C. xanthorrhiza*. Based on spore morphological characteristics, twelve of the fungal isolates could be classified into six genera, while the other five isolates were mycelia sterilia without spores. Leaves of *C. asiatica* were inhabited by *Aspergillus, Colletotrichum*, and *Fusarium*. Rhizomes of *C. xanthorrhiza* were inhabited by *Aspergillus* and *Colletotrichum*. Leaves of *G. ulmifolia* were occupied by *Aspergillus, Colletotrichum, Curvularia, Fusarium, Phomopsis, Talaromyces*, and three mycelia

sterilia. Leaves of *H. verticillata* were occupied by *Colletotrichum* and two mycelia sterilia (Table 2).

Identification to species level with sequence analysis of ITS1-5.8S-ITS2 rDNA gave 16 good E value results out of 17 isolates (94.1%), with the one remaining JBd11 isolate having poor quality DNA (Table 3). The JBd11 isolate was identified as *Speiropsis pedatospora* but with a low E value (6e-141). Based

Table 2. The morphological characteristics of the endophytic fungi associated with the medicinal plants

Medicinal plants	Endophytic fungi/IPBCC collection number	Mycelium, conidiophore, and spore characteristics
C. asiatica	*Aspergillus* sp.1 PLd3 (IPBCC 11.760)	Mycelium septate, conidiophores upright, simple, terminating in a globose bearing phialides at the apex, conidia 1 celled, globose, in dry basipetal chains, size 2.9-4.5 x 3.6-4.9 µm
	Colletotrichum sp.1 PLd6 (IPBCC 13.1092)	Mycelium septate, conidiophores simple, elongate, conidia hyaline, 1 celled, ovoid or oblong without appendages, size 17.5-23.2 x 5.9-6.5 µm
	Fusarium sp.1 PLd1 (IPBCC 11.756)	Mycelium septate, conidiophores hyaline, slender and simple cell bearing phialides, conidia hyaline, two kinds, macroconidia several-celled, slightly curved with canoe-shaped, size 43.4-62.4 x 6.5-9.5 µm, microconidia 1-2 celled, ovoid, oblong or slightly curved, size 10.3-16.6 x 3.4-4.6 µm
C. xanthorrhiza	*Aspergillus* sp.2 TLr5 (IPBCC 11.758)	Mycelium septate, conidiophores upright, simple, terminating in a globose bearing phialides at the apex, conidia 1 celled, globose, in dry basipetal chains, size 5.3-6.4 x 4.1-5.5 µm
	Colletotrichum sp.2 TLr2 (IPBCC 11.757)	Mycelium septate, conidiophores simple, elongate, conidia hyaline, 1 celled, ovoid or oblong without appendages, size 5.3-11.4 x 4.1-5.5 µm
G. ulmifolia	*Aspergillus* sp.3 JBd3 (IPBCC 11.749)	Mycelium septate, conidiophores upright, simple, conidia 1 celled, globose, in dry basipetal chains, size 5.3-6.4 x 4.1-5.5 µm
	Colletotrichum sp.3 JBd1 (IPBCC 11.747)	Mycelium septate, conidiophores simple, elongate, conidia hyaline, 1 celled, ovoid or oblong without appendages, size 20.8-29.3 x 6.1-8.3 µm
	Curvularia sp. JBd14 (IPBCC 13.1088)	Mycelium septate, conidiophores brown, simple, bearing spores apically or on new sympodial growing points, conidia dark, end cells lighter, 3-5 celled, one of the central cells enlarged, size 37.4-65.3 x 15.1-18.2 µm
	Fusarium sp.2 JBd2 (IPBCC 11.748)	Mycelium septate, conidiophores hyaline, slender and simple cell bearing phialides, conidia hyaline, two kinds, macroconidia several-celled, slightly curved with canoe-shaped, size 34.0-59.9 x 4.6-6.8 µm, microconidia 1 celled, ovoid, oblong or slightly curved, size 9.2-18.3 x 3.6-5.8 µm
	Mycelia sterilia 1 JBd7 (IPBCC 11.751)	Mycelium septate, no conidia observed
	Mycelia sterilia 2 JBd11 (IPBCC 11.754)	Mycelium septate, no conidia observed
	Mycelia sterilia 3 JBd13 (IPBCC 11.755)	Mycelium septate, no conidia observed
	Phomopsis sp.1 JBd4 (IPBCC 11.750)	Mycelium septate, conidiophores simple, conidia hyaline, 1 celled, size 10.8-15.2 x 4.1-6.7 µm
	Talaromyces sp. JBd10 (IPBCC 11.753)	Mycelium septate, conidiophores arising from the mycelium with verticilate bearing phialide, conidia hyaline, 1 celled, mostly ellipsoidal, size 7.3-8.6 x 6.9-8.3 µm
H. verticillata	*Colletotrichum* sp.4 PBd3 (IPBCC 11.752)	Mycelium septate, conidiophores simple, elongate, conidia hyaline, 1 celled, ovoid or oblong without appendages, size 3.5-6.2 x 8.9-12.5 µm
	Mycelia sterilia 4 PBd2 (IPBCC 13.10895)	Mycelium septate, no conidia observed
	Mycelia sterilia 5 PBd6 (IPBCC 13.1097)	Mycelium septate, no conidia observed

Table 3. The molecular identification and GenBank accession number of the endophytic fungi associated with the medicinal plants

Fungal identity/GeneBank accession number*	Host	Fungal code/IPBCC collection number	References of GenBank accession number used	Maximum score	Similarity (%)	Query coverage	E value
Aspergillus pseudoterreus (SUB 9428972)	*Curcuma xanthorrhiza*	TLr5 (IPBCC 11.758)	NR_137472.1	1.002	98.76	83	0.0
Aspergillus versicolor (SUB 9427202)	*Guazuma ulmifolia*	JBd3 (IPBCC 11.749)	NR_131277.1	944	95.94	89	0.0
Aspergillus versicolor (SUB 9403371)	*Centella asiatica*	PLd3 (IPBCC 11.760)	NR_131277.1	1.005	98.60	99	0.0
Colletotrichum aeschynomenes (SUB 9432747)	*C. xanthorrhiza*	TLr2 (IPBCC 11.757)	NR_120133.1	1.022	98.95	89	0.0
Colletotrichum siamense (SUB 9446440)	*C. asiatica*	PLd6 (IPBCC 13.1092)	JX010171.1	979	97.89	97	0.0
Colletotrichum tropicale (SUB 9433263)	*G. ulmifolia*	JBd1 (IPBCC 11.747)	MH863435.1	990	99.63	98	0.0
Colletotrichum tropicale (SUB 9446618)	*Hydrocotyle verticillata*	PBd3 (IPBCC 11.752)	MH863435.1	1.027	99.13	99	0.0
Curvularia affinis (SUB 9445050)	*G. ulmifolia*	JBd14 (IPBCC 13.1088)	HG778981.1	1.020	99.12	96	0.0
Diaporthe tectonae (SUB 9431627)	*G. ulmifolia*	JBd4 (IPBCC 11.750)	NR_147590.1	968	99.07	92	0.0
Didymella coffeae-arabicae (SUB 94031855)	*H. verticillata*	PBd2 (IPBCC 13.10895)	MH863293.1	915	97.74	97	0.0
Lasiodiplodia mahajangana (SUB 9431869)	*G. ulmifolia*	JBd7 (IPBCC 11.751)	MH_863425.1	955	99.06	97	0.0
Muyocopron laterale (SUB9857667)	*H. verticillata*	PBd6 (IPBCC 13.1097)	NR_164055.1	1.136	99.84	99	0.0
Neocosmospora macrospora (SUB 9433318)	*C. asiatica*	PLd1 (IPBCC 11.756)	NR_163291.1	848	99.57	88	0.0
Neocosmospora pseudensiforme (SUB 9431884)	*G. ulmifolia*	JBd2 (IPBCC 11.748)	MH863652.1	992	99.63	98	0.0
Parengyodontium album (SUB 9446667)	*G. ulmifolia*	JBd13 (IPBCC 11.755)	LC092881.1	974	100.00	96	0.0
Speiropsis pedatospora (SUB9861920)	*G. ulmifolia*	JBd11 (IPBCC 11.754)	MH857901.1	508	85.52	74	6e-141
Talaromyces trachyspermus (SUB 9431993)	*G. ulmifolia*	JBd10 (IPBCC 11.753)	MH859701.1	850	99.36	99	0.0

*All fungal ITS rDNA sequences were submitted to GenBank (NCBI) to obtain the accession number

on BLAST analysis, the similarity of the isolated fungi to the closest species available in MycoBank and GenBank varied from 85.52% to 100.00%, of which 13 isolates had a similarity value >98%. All of the isolates belong to Phylum Ascomycota in 3 classes, 8 orders, 11 families, and 15 identified species. The species are *Aspergillus pseudoterreus, A. versicolor, Colletotrichum aeschynomenes, C. siamense, C. tropicale, Curvularia affinis, Diaporthe tectonae, Didymella coffeae-arabicae, Neocosmospora pseudensiforme, Lasiodiplodia mahajangana, Muyocopron laterale, Neocosmospora macrospora, Parengyodontium album, Speiropsis pedatospora,* and *Talaromyces trachyspermus* (Table 3).

Fungal identification based on morphological characteristics uses microscopic observation of asexual spores (conidia). However, some isolates could not be differentiated based on morphological identification due to a lack of conidia. Mycelia sterilia 1 JBd7 and mycelia sterilia 3 JBd13 obtained from leaves of *G. ulmifolia* were identified as *Lasiodiplodia mahajangana* with 99.06% similarity and *Parengyodontium album* with 100.00% similarity. Similarly, mycelia sterilia 4 PBd2 derived from leaves of *H. verticillata* were successfully identified as *Didymella coffeae-arabicae* and mycelia sterilia 5 PBd6 was identified as *Muyocopron laterale* with 97.74% and 99.84% similarity to the sequences available in MycoBank, respectively (Table 2 and 3).

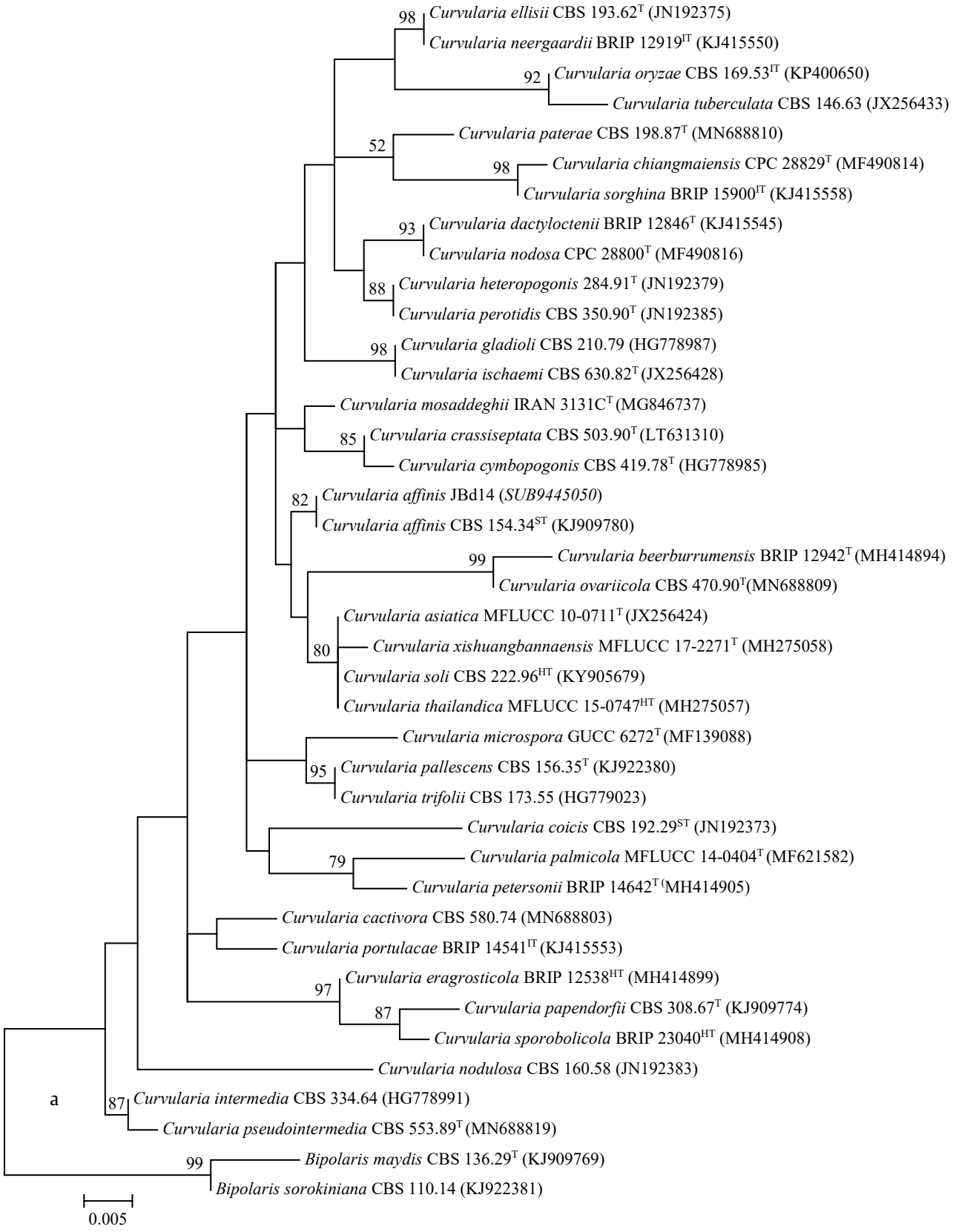
The JBd14 isolate was identified as *Curvularia affinis* with 99.12% similarity, and it was supported by phylogenetic analysis with 82% bootstrap support (Figure 1a). Based on sequence similarity comparison of the ITS region and phylogenetic analysis, PBd2 and JBd7 isolates were identified as *Didymella coffeae-arabicae* and *Lasiodiplodia mahajangana* with 97.74% and 99.06% similarity, respectively. Phylogenetic analysis showed that these isolates were in the same clade as those species with >50% bootstrap support (Figure 1b and c). These three species of fungi belong to the Dothideomycetes class (https://www.mycobank.org).

The TLr5 isolate has sequence similarities of 98.76% with the species of *A. pseudoterreus*, and the isolate is in the same clade as this species with 98% bootstrap support (Figure 2a). The PLd3 isolate had relatively higher sequence similarities (98.6%) with the species *A. versicolor* than with any other sequences, while the JBd3 isolate had sequence similarities at 95.94%. In the phylogenetic tree, the two isolates were in one clade with *A. versicolor* (Figure 2a). Based on sequence similarity comparison of the ITS region and phylogenetic analysis, the JBd10 isolate is identified as *T. trachyspermus* with 99.36% similarity, and the isolate is in the same clade as this species with 100% bootstrap support (Figure 2b). These two species of fungi belong to the Eurotiomycetes class (https://www.mycobank.org).

JBd1 and TLr2 isolates were identified as *C. tropicale* with a relatively high homology value of >98%, while PLd6 and PBd3 were identified as *C. siamense* and *C. aeschynomenes*, respectively. Further analysis by phylogenetic tree showed that these isolates formed a sister clade with 88% bootstrap support (Figure 3a). The JBd4 isolate was closer to *D. tectonae* with 99.07% similarity. The result was supported by phylogenetic analysis with 97% bootstrap support (Figure 3b). The ITS sequence of the JBd2 isolate had a high similarity (>99%) to *N. pseudensiforme* CBS 125729. The result was supported by phylogenetic analysis with 78% bootstrap support (Figure 3c). The PLd1 isolate is closer to *N. macrospora* species with a relatively higher similarity value of >99%, and phylogenetic analysis shows that the isolate belongs to the *N. macrospora* CBS 142424 clade with 97% bootstrap support (Figure 3c). Based on sequence similarity comparison of the ITS region and phylogenetic analysis, the JBD13 isolate was identified as *P. album* and phylogenetic analysis showed that this isolate was in the *P. album* CBS 504.83 clade with 99% bootstrap support (Figure 3d). All four endophytic fungal genera belong to the Sordariomycetes class (https://www.mycobank.org).

3.2. Antifungal Activity of Endophytic Fungi

All isolated endophytic fungi showed inhibition activity against *F. oxysporum*. The percentage of inhibition varied from 6.0 to 78.9%, and the differences are statistically significant. The inhibition values of endophytic fungi derived from leaves of *C. asiatica, G. ulmifolia, H. verticillata*, and rhizomes of *C. xanthorhiza* against *F. oxysporum* ranged 28.3-40.2%, 6.0-78.9%, 22.9-36.1%, and 41.0-44.8%, respectively. The endophytic fungi *T. trachyspermus* and *Curvularia affinis* derived from leaves of *G. ulmifolia* showed the highest biocontrol activities against *F. oxysporum* with values of 78.9% and 60% inhibition, respectively (Table 4).


98 Curvularia ellisii CBS 193.62T (JN192375)
Curvularia neergaardii BRIP 12919IT (KJ415550)
92 Curvularia oryzae CBS 169.53IT (KP400650)
Curvularia tuberculata CBS 146.63 (JX256433)
Curvularia paterae CBS 198.87T (MN688810)
52
98 Curvularia chiangmaiensis CPC 28829T (MF490814)
Curvularia sorghina BRIP 15900IT (KJ415558)
93 Curvularia dactyloctenii BRIP 12846T (KJ415545)
Curvularia nodosa CPC 28800T (MF490816)
88 Curvularia heteropogonis 284.91T (JN192379)
Curvularia perotidis CBS 350.90T (JN192385)
98 Curvularia gladioli CBS 210.79 (HG778987)
Curvularia ischaemi CBS 630.82T (JX256428)
Curvularia mosaddeghii IRAN 3131CT (MG846737)
85 Curvularia crassiseptata CBS 503.90T (LT631310)
Curvularia cymbopogonis CBS 419.78T (HG778985)
82 Curvularia affinis JBd14 (SUB9445050)
Curvularia affinis CBS 154.34ST (KJ909780)
99 Curvularia beerburrumensis BRIP 12942T (MH414894)
Curvularia ovariicola CBS 470.90T(MN688809)
Curvularia asiatica MFLUCC 10-0711T (JX256424)
80 Curvularia xishuangbannaensis MFLUCC 17-2271T (MH275058)
Curvularia soli CBS 222.96HT (KY905679)
Curvularia thailandica MFLUCC 15-0747HT (MH275057)
Curvularia microspora GUCC 6272T (MF139088)
95 Curvularia pallescens CBS 156.35T (KJ922380)
Curvularia trifolii CBS 173.55 (HG779023)
Curvularia coicis CBS 192.29ST (JN192373)
79 Curvularia palmicola MFLUCC 14-0404T (MF621582)
Curvularia petersonii BRIP 14642T (MH414905)
Curvularia cactivora CBS 580.74 (MN688803)
Curvularia portulacae BRIP 14541IT (KJ415553)
97 Curvularia eragrosticola BRIP 12538HT (MH414899)
87 Curvularia papendorfii CBS 308.67T (KJ909774)
Curvularia sporobolicola BRIP 23040HT (MH414908)
Curvularia nodulosa CBS 160.58 (JN192383)
a
87 Curvularia intermedia CBS 334.64 (HG778991)
Curvularia pseudointermedia CBS 553.89T (MN688819)
99 Bipolaris maydis CBS 136.29T (KJ909769)
Bipolaris sorokiniana CBS 110.14 (KJ922381)
0.005

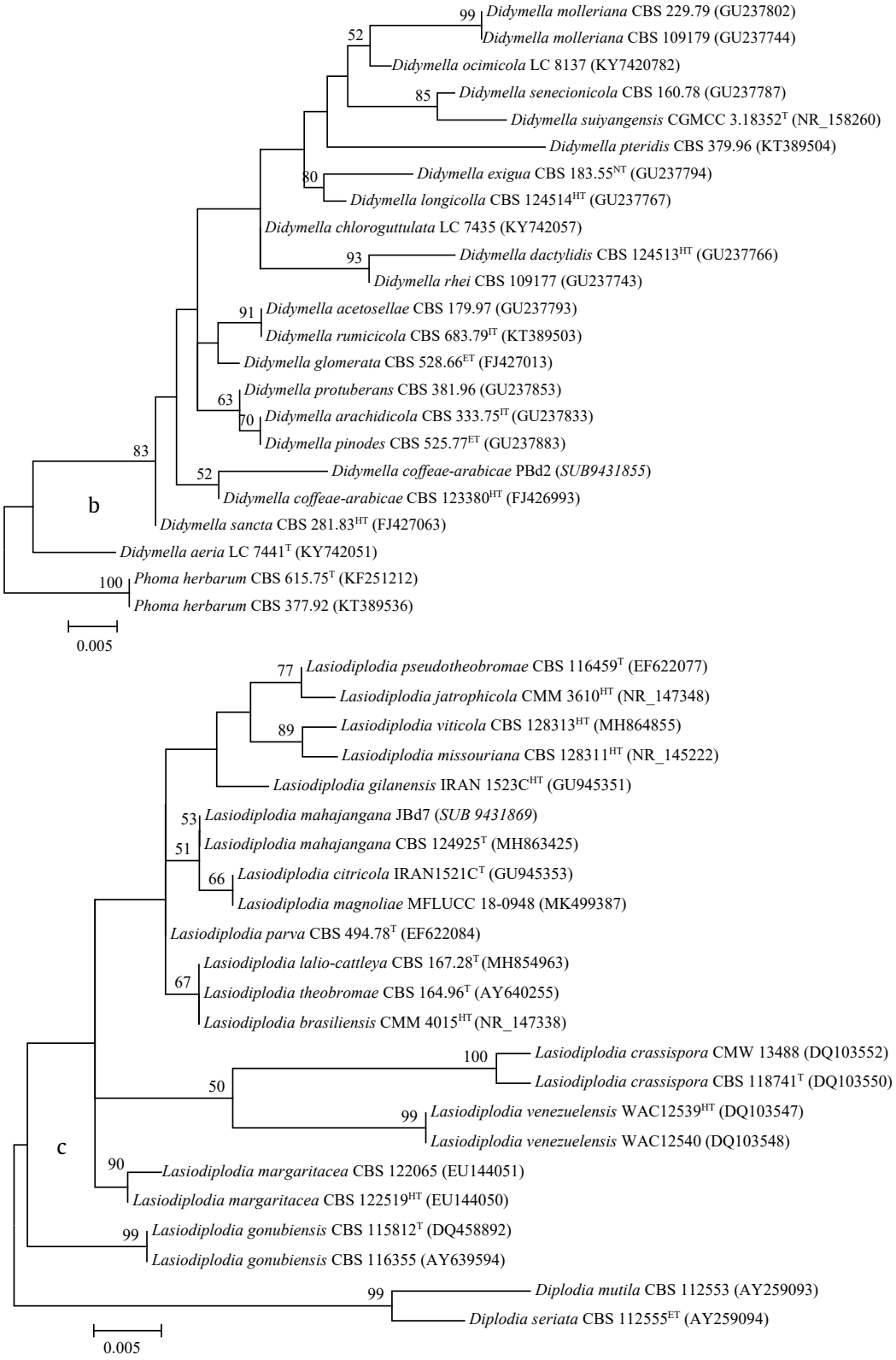


Figure 1. Maximum Likelihood phylogenetic analysis of ITS rDNA sequences of the Dothideomycetes endophytic fungi isolated from *Guazuma ulmifolia* and *Hydrocotyle verticillata* belonging to the genera (a) *Curvularia*, (b) *Didymella*, and (c) *Lasiodiplodia*. A phylogenetic tree was constructed using the MEGA ver. 6 program. Sequences obtained in the study are shown in bold. The marks ET, HT, IT, NT, T, and ST indicate epitype, holotype, isotype, neotype, type, and syntype strain, respectively. Numerical values (>50) on branches are the bootstrap values as a percentage of bootstrap replication from a 1,000 replicate analysis

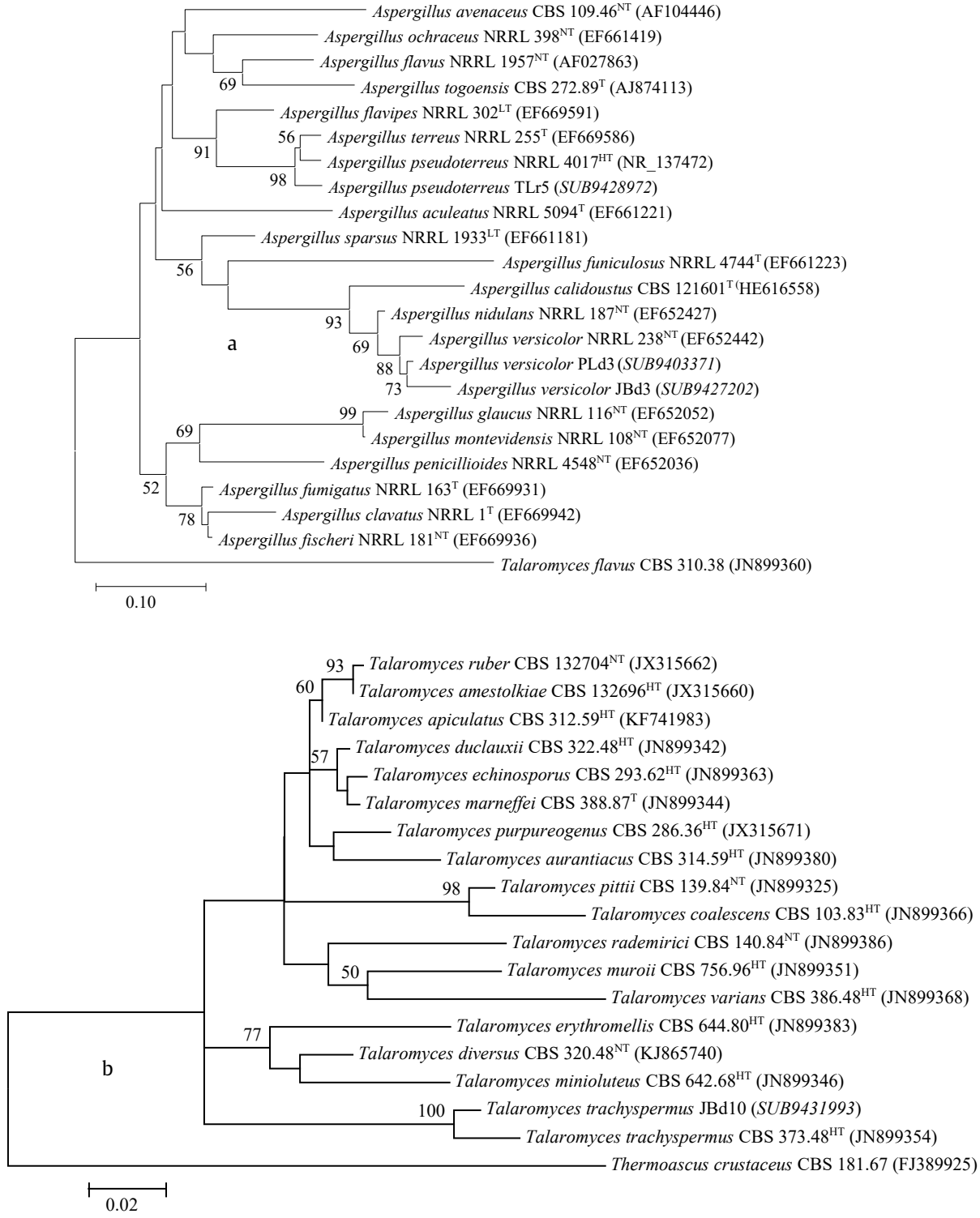
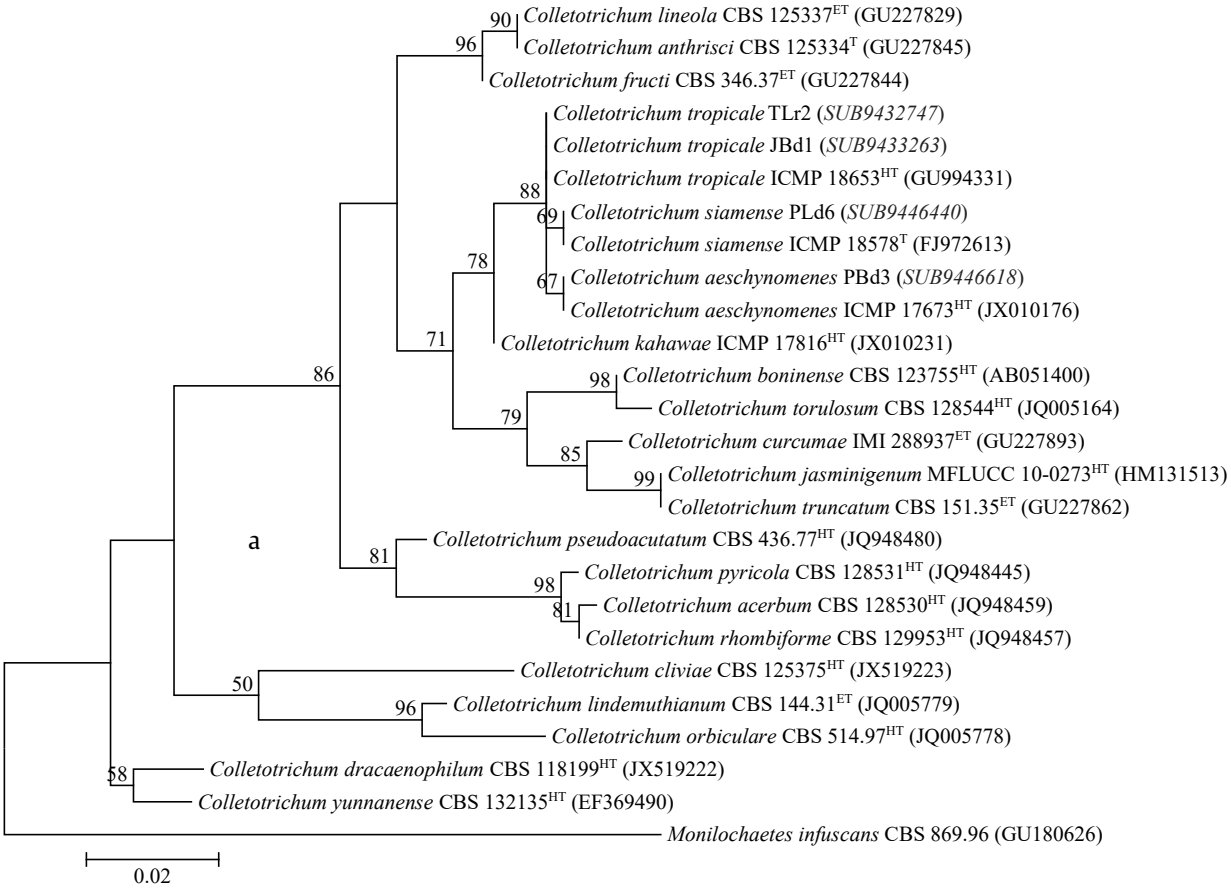
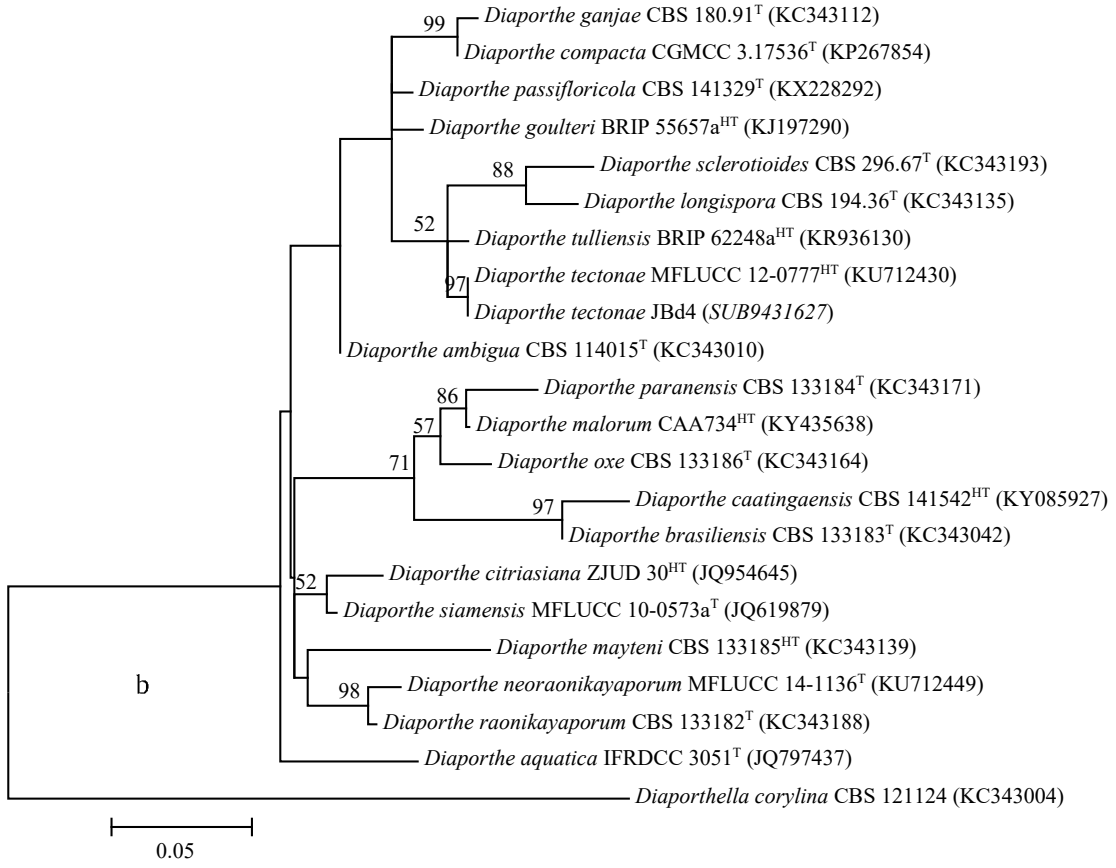
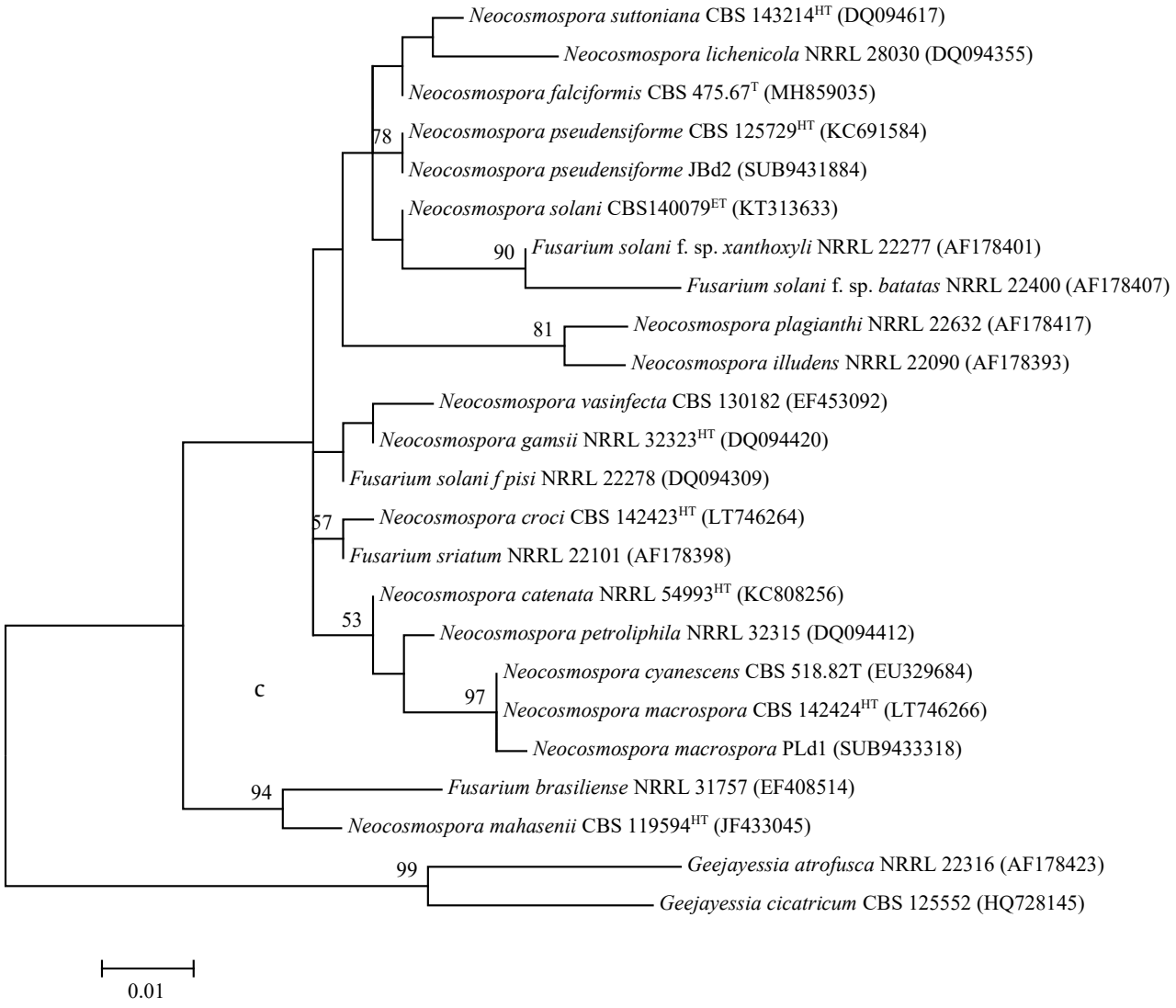


Figure 2. Maximum Likelihood phylogenetic analysis of ITS rDNA sequences of the Eurotiomycetes endophytic fungi isolated from *Centella asiatica*, *Curcuma xanthorrhiza*, and *Guazuma ulmifolia* that belonging to the genera (a) *Aspergillus* and (b) *Talaromyces*. A phylogenetic tree was constructed using the MEGA ver. 6 program. Sequences obtained in the study are shown in bold. The marks HT, LT, NT, and T indicate holotype, lectotype, neotype, and type strain, respectively. Numerical values (>50) on branches are the bootstrap values as a percentage of bootstrap replication from a 1,000 replicate analysis


90
96
Colletotrichum lineola CBS 125337ET (GU227829)
Colletotrichum anthrisci CBS 125334T (GU227845)
Colletotrichum fructi CBS 346.37ET (GU227844)
Colletotrichum tropicale TLr2 (SUB9432747)
Colletotrichum tropicale JBd1 (SUB9433263)
88
Colletotrichum tropicale ICMP 18653HT (GU994331)
69
Colletotrichum siamense PLd6 (SUB9446440)
Colletotrichum siamense ICMP 18578T (FJ972613)
78
67
Colletotrichum aeschynomenes PBd3 (SUB9446618)
Colletotrichum aeschynomenes ICMP 17673HT (JX010176)
71
Colletotrichum kahawae ICMP 17816HT (JX010231)
86
98
Colletotrichum boninense CBS 123755HT (AB051400)
79
Colletotrichum torulosum CBS 128544HT (JQ005164)
85
Colletotrichum curcumae IMI 288937ET (GU227893)
99
Colletotrichum jasminigenum MFLUCC 10-0273HT (HM131513)
Colletotrichum truncatum CBS 151.35ET (GU227862)
a
81
Colletotrichum pseudoacutatum CBS 436.77HT (JQ948480)
98
Colletotrichum pyricola CBS 128531HT (JQ948445)
81
Colletotrichum acerbum CBS 128530HT (JQ948459)
Colletotrichum rhombiforme CBS 129953HT (JQ948457)
Colletotrichum cliviae CBS 125375HT (JX519223)
50
96
Colletotrichum lindemuthianum CBS 144.31ET (JQ005779)
Colletotrichum orbiculare CBS 514.97HT (JQ005778)
58
Colletotrichum dracaenophilum CBS 118199HT (JX519222)
Colletotrichum yunnanense CBS 132135HT (EF369490)
Monilochaetes infuscans CBS 869.96 (GU180626)
0.02


99
Diaporthe ganjae CBS 180.91T (KC343112)
Diaporthe compacta CGMCC 3.17536T (KP267854)
Diaporthe passifloricola CBS 141329T (KX228292)
Diaporthe goulteri BRIP 55657aHT (KJ197290)
88
Diaporthe sclerotioides CBS 296.67T (KC343193)
Diaporthe longispora CBS 194.36T (KC343135)
52
Diaporthe tulliensis BRIP 62248aHT (KR936130)
97
Diaporthe tectonae MFLUCC 12-0777HT (KU712430)
Diaporthe tectonae JBd4 (SUB9431627)
Diaporthe ambigua CBS 114015T (KC343010)
86
Diaporthe paranensis CBS 133184T (KC343171)
57
Diaporthe malorum CAA734HT (KY435638)
71
Diaporthe oxe CBS 133186T (KC343164)
97
Diaporthe caatingaensis CBS 141542HT (KY085927)
Diaporthe brasiliensis CBS 133183T (KC343042)
52
Diaporthe citriasiana ZJUD 30HT (JQ954645)
Diaporthe siamensis MFLUCC 10-0573aT (JQ619879)
Diaporthe mayteni CBS 133185HT (KC343139)
b
98
Diaporthe neoraonikayaporum MFLUCC 14-1136T (KU712449)
Diaporthe raonikayaporum CBS 133182T (KC343188)
Diaporthe aquatica IFRDCC 3051T (JQ797437)
Diaporthella corylina CBS 121124 (KC343004)
0.05


Neocosmospora suttoniana CBS 143214HT (DQ094617)
Neocosmospora lichenicola NRRL 28030 (DQ094355)
Neocosmospora falciformis CBS 475.67T (MH859035)
78
Neocosmospora pseudensiforme CBS 125729HT (KC691584)
Neocosmospora pseudensiforme JBd2 (SUB9431884)
Neocosmospora solani CBS140079ET (KT313633)
90
Fusarium solani f. sp. xanthoxyli NRRL 22277 (AF178401)
Fusarium solani f. sp. batatas NRRL 22400 (AF178407)
81
Neocosmospora plagianthi NRRL 22632 (AF178417)
Neocosmospora illudens NRRL 22090 (AF178393)
Neocosmospora vasinfecta CBS 130182 (EF453092)
Neocosmospora gamsii NRRL 32323HT (DQ094420)
Fusarium solani f pisi NRRL 22278 (DQ094309)
57
Neocosmospora croci CBS 142423HT (LT746264)
Fusarium sriatum NRRL 22101 (AF178398)
Neocosmospora catenata NRRL 54993HT (KC808256)
53
Neocosmospora petroliphila NRRL 32315 (DQ094412)
Neocosmospora cyanescens CBS 518.82T (EU329684)
c
97
Neocosmospora macrospora CBS 142424HT (LT746266)
Neocosmospora macrospora PLd1 (SUB9433318)
94
Fusarium brasiliense NRRL 31757 (EF408514)
Neocosmospora mahasenii CBS 119594HT (JF433045)
99
Geejayessia atrofusca NRRL 22316 (AF178423)
Geejayessia cicatricum CBS 125552 (HQ728145)
0.01

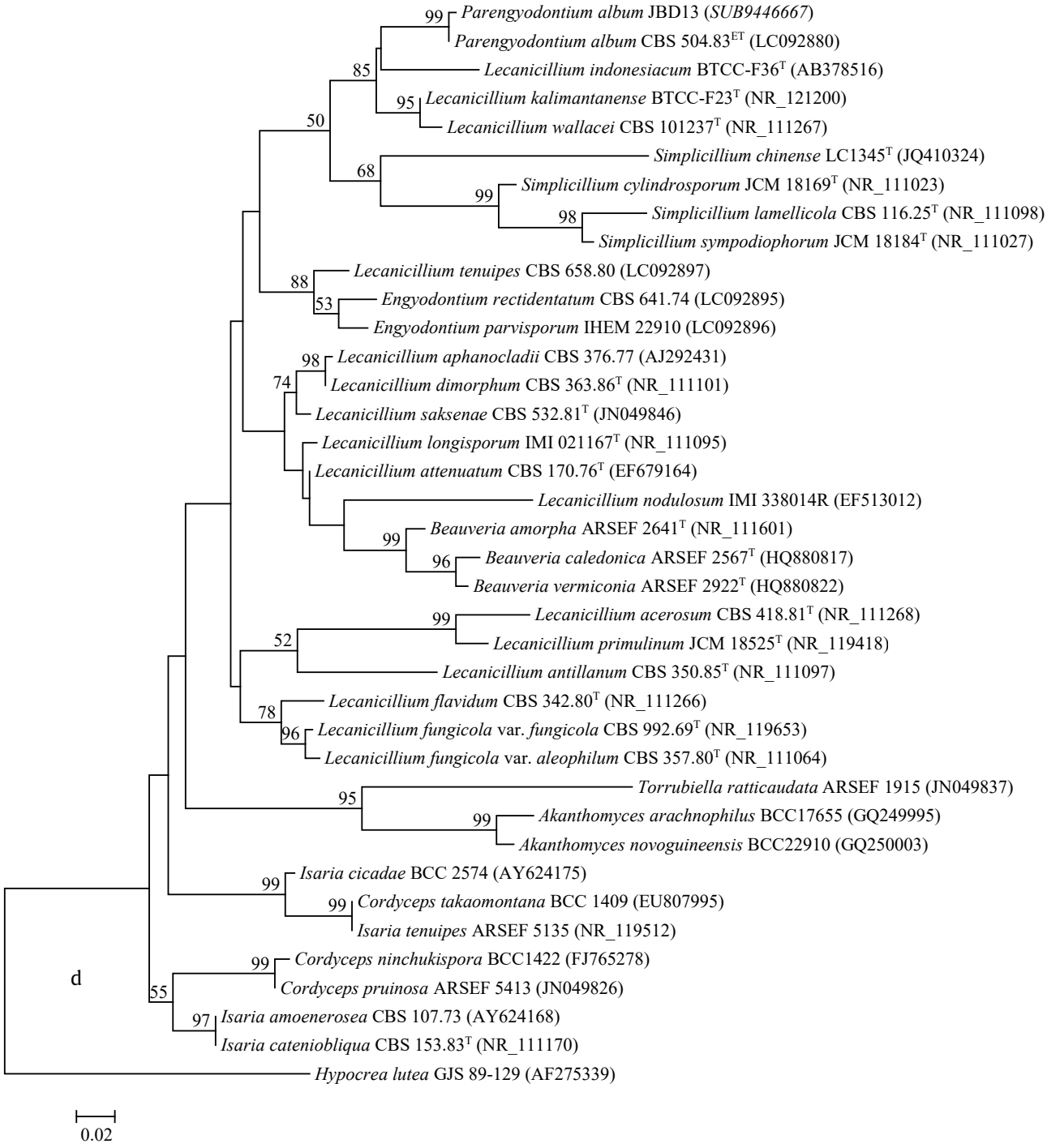


Figure 3. Maximum Likelihood phylogenetic analysis of ITS rDNA sequences of the Sordariomycetes endophytic fungi isolated from *Centella asiatica, Curcuma xanthorrhiza, Guazuma ulmifolia*, and *Hydrocotyle verticillata* belonging to the genera (a) *Colletotrichum*, (b) *Diaporthe*, (c) *Neocosmospora*, and (d) *Parengyodontium*. A phylogenetic tree was constructed using the MEGA ver. 6 program. Sequences obtained in the study are shown in bold. The marks ET, HT, and T indicate epitype, holotype, and type strain, respectively. Numerical values (>50) on branches are the bootstrap values as a percentage of bootstrap replication from a 1,000 replicate analysis

Table 4. Inhibition activity of fungal endophytes derived from the medicinal plants against *Fusarium oxysporum*

Endophytic fungi	Fungal code/IPBCC collection number	Host	% Inhibition[a]
Aspergillus pseudoterreus	TLr5 (IPBCC 11.758)	*Curcuma xanthorrhiza*	44.8 e
Aspergillus versicolor	PLd3 (IPBCC 11.760)	*Centella asiatica*	28.3 hi
Aspergillus versicolor	JBd3 (IPBCC 11.749)	*Guazuma ulmifolia*	41.2 f
Colletotrichum aeschynomenes	TLr2 (IPBCC 11.757)	*C. xanthorrhiza*	41.0 f
Colletotrichum siamense	PLd6 (IPBCC 13.1092)	*C. asiatica*	30.5 h
Colletotrichum tropicale	JBd1 (IPBCC 11.747)	*G. ulmifolia*	49.1 c
Colletotrichum tropicale	PBd3 (IPBCC 11.752)	*Hydrocotyle verticillata*	36.1 g
Curvularia affinis	JBd14 (IPBCC 13.1088)	*G. ulmifolia*	60.0 b
Diaporthe tectonae	JBd4 (IPBCC 11.750)	*G. ulmifolia*	45.8 de
Didymella coffeae-arabicae	PBd2 (IPBCC 13.10895)	*H. verticillata*	25.9 ij
Lasiodiplodia cit mahajangana	JBd7 (IPBCC 11.751)	*G. ulmifolia*	48.1 cd
Muyocopron laterale	PBd6 (IPBCC 13.1097)	*H. verticillata*	22.9 k
Neocosmospora macrospora	PLd1 (IPBCC 11.756)	*C. asiatica*	40.2 f
Neocosmospora pseudensiforme	JBd2 (IPBCC 11.748)	*G. ulmifolia*	25.0 jk
Parengyodontium album	JBd13 (IPBCC 11.755)	*G. ulmifolia*	6.0 l
Speiropsis pedatospora	JBd11 (IPBCC 11.754)	*G. ulmifolia*	40.3 f
Talaromyces trachyspermus	JBd10 (IPBCC 11.753)	*G. ulmifolia*	78.9 a

[a]Values of % inhibition are means from 5 replications, means followed by the same letter are not significantly different in DMRT ($p<0.05$)

4. Discussion

4.1. Diversity of Endophytic Fungi

All medicinally used organs of the plants studied were associated with endophytic fungi. The genus *Colletotrichum* was the most frequent endophyte found in this study and occupied all of the plants. The species *C. aeschynomenes* was found in leaves of *C. xanthorrhiza, C. siamense* was found in leaves of *C. asiatica*, and *C. tropicale* was found in leaves of *G. ulmifolia* and *H. verticillata*. The genus *Colletotrichum* is a ubiquitous endophyte and was isolated from roots, stems, branches, petioles, leaves, flowers, veins, bark, twig bark, twig xylem, intervein, and phloem of 73 medicinal plants (Rai *et al.* 2014). The genus *Colletotrichum* is reported as the dominant fungal endophyte in 16 out of 29 traditional Chinese medicinal plants (Huang *et al.* 2008) and *Zingiber officinale* (Ginting *et al.* 2013). The genus *Aspergillus occupied* all plants studied except *H. verticillata*. The fungus *A. pseudoterreus* was found to be associated with *C. xanthorrhiza*, whereas *A. versicolor* was associated with *C. asiatica* and *G. ulmifolia* in this study. The fungus *Neocosmospora* was found to be associated with *G. ulmifolia* and *C. asiatica* with different species for each plant. The species *N. pseudensiforme* was associated with *G. ulmifolia*, while *N. macrospora* was associated with *C. asiatica* in this study.

Some endophytic fungi are associated with specific hosts, and some species are associated with more than one host (Suryanarayanan *et al.* 2002). In this study, *C. affinis, D. tectonae, L. mahajangana, P. album, S. pedatospora*, and *T. trachyspermus* were found only in *G. ulmifolia*; while *D. coffeae-arabicae* and *M. laterale* were found in *H. verticillata* (Table 3). Host-specificity, host-recurrence, host selectivity, or host-preference is the relationship of fungal endophytes with single or multiple plant hosts (Cohen 2006). Some endophytic fungi are even tissued specific, fungi of different species occupy different tissues of a single plant (Ganley and Newcombe 2006).

4.2. Antifungal Activity of Endophytic Fungi

The inhibition activity of endophytic fungi against *F. oxysporum* can be grouped into low, medium, and high inhibition activity. Low inhibition activity was represented by the inhibition activity of <30%, the moderate inhibition activity by 30% to 59%, and the high inhibition by >60% (Table 4). From these isolates, five isolates (29.4%) showed low, 10 isolates (58.8%) showed moderate, and two isolates (11.8%) showed high inhibition activity against *F. oxysporum*. The endophytic fungi obtained from *G. ulmifolia* ranged from low to high inhibition activity. The isolates derived from rhizomes of *C. xanthorrhiza* showed moderate inhibition activity,

and the isolates from leaves of *C. asiatica* and *H. verticillata* showed low to moderate activity against *F. oxysporum*. The endophytic fungi *T. trachyspermus* JBd10 and *C. affinis* JBd14 derived from leaves of *G. ulmifolia* showed high inhibition activity. Strobel *et al.* (2007) reported that *Muscodor albus* isolated from *G. ulmifolia* produced unusual biochemical and biological properties.

The endophytic fungi *T. trachyspermus* and *C. affinis* derived from leaves of *G. ulmifolia* showed the highest biocontrol activities against *F. oxysporum*. Chomcheon *et al.* (2010) reported that *Talaromyces* sp. derived from mangrove could produce antimicrobial metabolites (7-epiaustdiol, stemphyperylenol, and secalonic acid A) to control *Pseudomonas aeruginosa*. *Talaromyces* is a teleomorph stage of *Penicillium*. Devi *et al.* (2012) isolated endophytic *Penicillium* sp. from *C. asiatica*, and it produced alkaloids, phenols, flavonoids, tannin, and glycosides. Furthermore, Shiozawa *et al.* (1994) reported that the species *T. trachyspermus* SANK 12191 produced trachyspic acid, a new metabolite that inhibited tumor cell's heparanase. *Curvularia affinis* isolated from the stem of *Zingiber officinale* had high antagonistic activity against *F. oxysporum* with a percentage inhibition value of 68.8% (Ginting *et al.* 2013). *Curvularia affinis* isolated from soil could produce the secondary metabolites pyrenocine J, pyrenochaetic acid D, pyrenocine A, and pyrenochaetic acid A. The metabolite pyrenocine J showed cytotoxic activity against human hepatic cancer (Zhang *et al.* 2012). In symbiotic interactions inside the host plants, the role of endophytic fungi is to protect the host plants from fungal pathogenic attack by direct and indirect mechanisms. The direct mechanism occurs through interaction between endophytes with fungal pathogens occupying the ecological niche, while the indirect mechanism is by inducing plant resistance. In the direct mechanism, endophytic fungi produce antibiotics and lytic enzymes that suppress the growth or kill pathogens. Various reports have documented that endophytic fungi grown in synthetic medium produce secondary metabolites that are powerful against pathogenic bacteria and fungi, including plant fungal pathogens (Gunatilaka 2006). These endophytic fungi are potential sources of antifungal compounds, particularly for controlling *F. oxysporum*.

5. Conclusion

There were 9 isolates of endophytic fungi obtained from the leaves of *G. ulmifolia*, 3 isolates each from the leaves of *C. asiatica* and *H. verticillata*, and 2 isolates from the rhizomes of *C. xanthorrhiza*. The genus *Colletotrichum occupied* all of the plants studied. The fungi *C. affinis, D. tectonae, L. mahajangana, P. album, T. trachyspermus*, and *S. pedatospora* were found only in *G. ulmifolia*; while *D. coffeae-arabicae* and *M. laterale* were found only in *H. verticillata*. Endophytic fungi derived from medicinally used organs of *G. ulmifolia, C. asiatica, H. verticillata*, and *C. xanthorrhiza* had inhibition activity against *F. oxysporum* with inhibition values ranging 6.0-78.9%. *T. trachyspermus* JBd10 and *C. affinis* JBd14 had the highest inhibition values.

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References

Abdollahzadeh J *et al.* 2010. Phylogeny and morphology of four new species of *Lasiodiplodia* from Iran. *Persoonia* 25:1-10.

Adhikari M *et al.* 2015. Discovery of two new *Talaromyces* species from crop field soil in Korea. *Mycobiology* 43:402-407.

Barnett HL, Hunter BB. 1998. *Illustrated Genera of Imperfect Fungi.* 4th ed. USA: Prentice-Hall Inc.

Cannon PF *et al.* 2012. *Colletotrichum*-current status and future directions. *Stud Mycol* 73:181-213.

Chen Q *et al.* 2017. Didymellaceae revisited. *Stud Mycol* 87:105-159.

Chomcheon P *et al.* 2010. Curvularides A-E: antifungal hybrid peptide-polyketides from the endophytic fungus *Curvularia geniculata. Chem Eur J* 16:11178-11185.
Cohen SD. 2006. Host selectivity and genetic variation of *Discula umbrinella* isolates from two oak species: analyses of intergenic spacer region sequences of ribosomal DNA. *Microb Ecol* 52:463-469.
Cook RK. 1993. Making greater use of introduced microorganisms for biological control of plant pathogens. *Annu Rev Phytopathol* 31:53-80.
Devi NN *et al.* 2012. Phytochemical analysis and enzyme analysis of endophytic fungi from *Centella asiatica. Asian Pac J Trop Biomed* 2:1280-1284.
Dissanayake AJ *et al.* 2017. The current status of species in *Diaporthe. Mycosphere* 8:1106–1156.
Ganley RJ, Newcombe G. 2006. Fungal endophytes in seeds and needles of *Pinus monticola. Mycol Res* 110:318-327.
Ginting RCB *et al.* 2013. Diversity of endophytic fungi from red ginger (*Zingiber officinale* Rosc.) plant and their inhibitory effect to *Fusarium oxysporum* plant pathogenic fungi. *Hayati J Biosci* 20:127-137.
Gunatilaka AAL. 2006. Natural products from plant-associated microorganisms: distribution, structural diversity, bioactivity, and implications of their occurrence. *J Nat Prod* 69:509-526.
Hall TA. 1999. BioEdit: a user-friendly biological sequence analysis program for windows 95/98/NT. *Nucleic Acids Sym Ser* 41:95-98.
Hallmann J *et al.* 2007. Isolation procedures for endophytic microorganisms. In: Schulz B, Boyle C, Sieber TN (Eds.). *Soil biology Vol. 9*. New York: Springer-Verlag Berlin Heidelberg. pp. 299-319.
Hammerschmidt L *et al.* 2015. Two new metabolites from the endophytic fungus *Xylaria* sp. isolated from the medicinal plant *Curcuma xanthorrhiza. Tetrahedron Lett* 56:1193-1197.
Hardoim PR *et al.* 2015. The hidden world within plants: ecological and evolutionary considerations for defining functioning of microbial endophytes. *Microbiol Mol Biol Rev* 79:293-320.
Huang WY *et al.* 2008. Biodiversity of endophyte fungi associated with 29 traditional Chinese medicinal plants. *Fungal Divers* 33:61-75.
Kusari P *et al.* 2013. Endophytic fungi harbored in *Cannabis sativa* L.: diversity and potential as biocontrol agents against host plant-specific phytopathogens. *Fungal Divers* 60:137-151.
Marin-Felix Y *et al.* 2020. Multi-locus phylogeny of the genus *Curvularia* and description of ten new species. *Mycol Progress* 19:559-588.
Mycobank database. 2021. Fungal Databases, Nomenclature and Species Banks. Available at https://www.mycobank.org. [Date accessed: 5 March 2021]
Morton DT, Stroube NH. 1955. Antagonistic and stimulatory effects of microorganisms upon *Sclerotium rolfsii. Phytopathology* 45:417-420.
Nei M, Kumar S. 2000. *Molecular evolution and phylogenetics*. New York: Oxford University Press.
Rai M *et al.* 2014. Multiple applications of endophytic *Colletotrichum* species occurring in medicinal plants. In: Gurib-Fakim A (Eds.). *Novel plant bioresources: applications in food, medicine, and cosmetics.* Chichester: Wiley. pp. 227-236.
Rakotoniriana EF *et al.* 2008. Endophytic fungi from leaves of *Centella asiatica*: occurrence and potential interactions within leaves. *Antonie van Leeuwenhoek* 93:27-36.
Russell JR *et al.* 2011. Biodegradation of polyester polyurethane by endophytic fungi. *Appl Environ Microbiol* 77:6076-6084.
Sambrook J, Russel DW. 2000. *Molecular Cloning: A Laboratory Manual*. 3rd ed. Vol 3. Cold Spring Harbor:Laboratory Press.
Samson RA *et al.* 2014. Phylogeny, identification, and nomenclature of the genus *Aspergillus. Stud Mycol* 78:141-173.
Sandoval-Denis M, Crous PW. 2018. Removing chaos from confusion: assigning names to common human and animal pathogens in *Neocosmospora. Persoonia* 41:109-129.
Scarpari M *et al.* 2020. *Didymella corylicola* sp. nov., a new fungus associated with hazelnut fruit development in Italy. *Mycol Prog* 19:317-328.
Septiana E *et al.* 2017. Endophytic fungi associated with turmeric (*Curcuma longa* L.) can inhibit histamine-forming bacteria in fish. *Hayati J Biosci* 24:46-52.
Shaw JJ *et al.* 2015. Biosynthesis and genomic analysis of medium-chain hydrocarbon production by the endophytic fungal isolate *Nigrograna mackinnonii* E5202H. *Appl Microbiol Biotechnol* 99:3715-3728.
Shiozawa H *et al.* 1994. Trachyspic acid, a new metabolite produced by *Talaromyces trachyspermus* that inhibits tumor cell heparanase: taxonomy of the producing strain, fermentation, isolation, structural elucidation, and biological activity. *J Antibiot* 48:357-362.
Strobel GA *et al.* 2007. *Muscodor albus* E-6, an endophyte of *Guazuma ulmifolia* making volatile antibiotics: isolation, characterization, and experimental establishment in the host plant. *Microbiology* 153:2613-2620.
Strobel GA, Daisy B. 2003. Bioprospecting for microbial endophytes and their natural products. *Microbiol Mol Biol Rev* 67:491-502.
Suryanarayanan TS *et al.* 2002. Occurrence and distribution of fungal endophytes in tropical forests across a rainfall gradient. *Can J Bot* 80:818-826.
Tamura K *et al.* 2013. Mega6: molecular evolutionary genetics analysis version 6.0. *Mol Biol Evol* 30:2725-2729.
Thompson JD *et al.* 1994. Clustal W: improving the sensitivity of progressive multiple sequence alignment through sequence weighting, position-specific gap penalties, and weight matrix choice. *Nucleic Acids Res* 22:4673-4680.
Tsang *et al.* 2016. Cutaneous hyalohyphomycosis due to *Parengodontium album* gen. et comb. nov. *Med Mycol* 54:699-713.
White TJ *et al.* 1990. Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. In: Innis MA, Gelfand DH, Sninsky JS, White TJ. (Eds). *PCR Protocols: a Guide to Methods and Applications*. New York: USA, Academic Press. pp. 315-322.
Zhang H *et al.* 2012. Two new metabolites from a soil fungus *Curvularia affinis* strain HS-FG-196. *J Asian Nat Prod Res* 14:1078-1083.