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Antifungal activity of endophytic fungus *Piptoporellus soloniensis* L. isolated from *Hopea chinensis* Hand.-Mazz.

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ABSTRACT

Phylogenetic analysis of *Piptoporellus soloniensis* L. and 11 other endophytic fungis isolated from *Hopea chinensis* Hand.-Mazz. was done based on multiple loci, including ITS, nSSU, mtSSU, rbp2 and nLSU. In mycelial growth rate method, the methanol extract of *P. soloniensis* mycelia exhibited potent activity (inhibition rate : 76.79 %) and the acetone extract at 10 mg/mL concentration of this fungi exhibited potent activities against *Colletotrichum musae*, *P. grisea* and *Neoscytalidium dimidiatum* with inhibition rates of 43.95 %, 40.48 % and 35.77 % respectively. The compounds Ergosta - 5,7,22 - trine - 3β - ol (ergosterol) and (22E) - 5α and 8α - epidioxyergosta - 6,22 - dien - 3β - ol were isolated by the filter paper method from the crude mycelial extracts of methanol and acetone, although (22E) - 5α,8α - epidioxyergosta - 6,22 - dien - 3β - ol have no activities to *C. musae*, *N. dimidiatum*, *P. grisea* and *Fusarium oxysporum* race 4 at 5 mg/mL. These data highlight that the crude mycelial extracts of *P. soloniensis* can be used as biogenic fungicides and have great potential in agriculture. The study paves way for the development and chemical characterization of the bioactive compounds.

Keywords: Antifungal activity, *Colletotrichum musae*, endophytic fungi, fungicides, *Fusarium oxysporum*, *Hopea chinensis*, *Neoscytalidium dimidiatum*, Phylogenetic analysis, *Piptoporellus soloniensis*

INTRODUCTION

Plant diseases are the main threat to crop growth, and one of the main factor causing crops damage is fungi, the main fungal diseases affecting crops growth in Guangxi are *F. oxysporum* race 4, *C. musae*, *N. dimidiatum* and *P. grisea*. Bio-control agent is an effective way to prevent and control fungal diseases, such as rice blast the most widespread and serious plant fungal disease (29). Two biocontrol bacterial strains (*Bacillus subtilis* CM-B94, *Bacillus subtilis* BFE 5314) have been isolated which can prevent and control this fungus disease (13). Fungi is a major plant pathogen that causes extensive losses in field and horticulture crops, hence, it is important to explore new and eco-friendly methods to control fungal diseases.

Endophytic fungi are important microbial resources that have the potential to be used to develop bio-pesticides. Many secondary metabolites of endophytic fungi exhibit antifungal activity used as pesticides in the field, demonstrating a symbiotic relationship between endophytic fungi and host plants. Endophytic fungi produce bioactive metabolites that are similar to and have the same activity as those from host plants (16,17). Isolation of endophytic fungi from plants with biological activity and medicinal value is an important

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way to find bioactive (1,3,14). Furthermore, isolation and characterization of bioactive secondary metabolites from these fungi with potent biological activities (antifungal, antibacterial, antitumor etc.) is an important way to explore the biological pesticides and reduce the use of chemical pollutants (34).

The endophytic fungi are not only involved in the synthesis and transformation of plant metabolites but can also produce new bioactive substances under different environmental conditions. Wani *et al.* (35) reported the isolation of the anticancer drug Taxol from the bark of *Taxus brevifolia* Nutt. Similarly, Taxol was isolated from *Taxomyces andreanae*, an endophytic fungus of *Taxus breviscapus* (27). Later, research on medicinal plants resulted in the isolation of 18 endophytic fungi from *Catharanthus roseus* (L.), these 2-fungi (*Alternaria* and *Mycelia sterilia*) could produce vincristine analogues (4). These endophytic fungi also possessed active ingredients in the absence of plants, providing a novel approach for natural drug screening. Xiao-ming (31) isolated and screened a variety of endophytic fungi from tissues of *Dysosma versipellis* Hance, these had good antimicrobial and antineoplastic activities, hence, these active strains can be used to develop new natural antimicrobial agents or anticancer agents. The endophytic fungi may be a potential source of novel bioactive compounds. Thus, the study of endophytic fungi from plants has gradually attracted attention and is a hotspot of current research.

Hopea chinensis Hand.-Mass (Fig.1), (family *Dipterocarpaceae*) is a protected plant in China. It is cultivated in the Shiwan and Daqingshan mountains in Guangxi and is a precious specie in tropical rainforest of China (7). At present, 15 compounds have been isolated from ethyl acetate extracts of its branches and leaves. Among them, morolic acid acetate is antifungal against *Penicillium italicum* Weh and *Alternaria musae* Bour. et Bat, while, friedelin, bergrnin and methylparaben are antifungal against *P. italicum*, *B. oryza*,

Figure 1. The twigs and leaves of *Hopea chinenisis*

P. nicotianae and *A. musae* (37). However, information regarding the endophytic fungi from *H. chinensis* is lacking. Hence, this aimed (i). to isolate and screen endophytic fungi with potent antifungal activity from *H. chinensis* with pharmacological activity, (ii). to explore their antifungal active components and (iii). to determine that antibacterial activity of the chemical components of the secondary metabolites of endophytic fungi. The results of this study will increase the development of biogenic pesticides.

MATERIALS AND METHODS

Plant materials

In March 2017, the roots and leaves of the perennial plant *H. chinensis* without pests and diseases, were collected from Shiwan Mountain, Guangxi Province, China, [22. 48° N, 108. 22° E], altitude 79 m, annual rainfall: 1304.2 mm. Maximum and minimum temperature in March 2017 are 16 ℃ and 23 ℃, respectively. An authenticated voucher specimen (No. H2017003) was deposited at the Guangxi Key Laboratory of Agric-Environment and Agric-Product Safety, Agricultural College, Guangxi University, Nanning, China.

ISOLATION OF ENDOPHYTIC FUNGI

(i). Disinfection of plant tissue surface : Firstly, the branches and leaves were disinfected with 75 % ethanol for 2 min (root tissue disinfected for 5 min), washed 2-3 times with sterile water, and then disinfected with 2 % sodium hypochlorite solution for 1 min (root tissue disinfected for 3 min). Finally, rinsed 2-3 times with sterile water, absorbed the water on the surface of tissue with sterilized filter paper, and dried in shade (18,25).

(ii). Culture of plant endophytic fungi : The strain was purified by plate scribing method, when hyphae grew around the sample, a single colony was selected for repeated scribing. After scribing, the plate was inverted and cultured at 28 ℃ in incubator. At the same time, microscopic observations were made until the external morphology of newly grown colonies on the plate were consistent.

(iii). Disinfection detection of plant tissue surface: Firstly, by the rinsing solution test method, absorbed 0.1 - 0.4 mL of the last plant tissue rinsing solution under sterile conditions and applied on the prepared PDA plate to observe, if there was fungal growth. Secondly, the surface of the plant material was contacted with PDA medium by tissue imprinting for 5 min and then removed, observed whether there was fungal growth in the petri dish 4-6 days after culturing. Thirdly, under sterile conditions, the treated plant materials were transferred to the prepared PDA solid medium and cultured in an incubator at 28 ℃ for 4 - 6 days to observe the fungal growth (23).

(iv). Identification of isolated fungal strains : The endophytic fungi were identified using molecular biology methodology. DNA was extracted from a representative strain of each morphological group using an Axygen Biosciences kit according to the manufacturer's recommendations. The ITS1 primer (TCC GTA GGT GAA CCT GCG G) and ITS4 primer (TCC TCC GCT TAT TGA TAT GC) were used to amplify the fungal ITS region. The PCR products were purified (8) and bidirectionally sequenced using the Sanger method.

The sequences were analysed using the BLAST tool (http://www.ncbi.nlm.nih.gov) (2), and the fungal isolates were identified based on their morphological characteristics and the BLAST results. Isolates with sequences exhibiting ≥85 % similarity to those previously deposited in GenBank were analysed in subsequent analyses.

(v). Molecular and phylogenetic analyses : Total genomic DNA was extracted from dried specimens using the CTAB method (5,22) and then used for polymerase chain reaction (PCR) amplification with primers specific to the ITS (36), mtSSU (36), nSSU (36), Rpb2 (20) and nLSU loci (http://www.biology.duke.edu/fungi/mycolab/primers.htm). The PCR thermocycling conditions used for ITS, mtSSU, nLSSU, nSSU and Rpb2 were as per (6). The PCR products were purified and sequenced by the Shanghai Genomics Institute (China).

Ninety-three sequences were generated and deposited in GenBank (Table 1). Additional sequences were downloaded from GenBank (Table 1). The final concatenated sequence alignment was deposited in TreeBase. The simplicity phylogenies were inferred from the combined 5-gene dataset (ITS + nLSU + nSSU + mtSSU + Rpb2), and their congruencies were evaluated using the incongruence length difference (ILD) test (12) implemented in MEGA (version 5.0). Sequences from GenBank were used as outgroups to root trees as described by Han (10). The neighbour-joining method (24) was used to analyse the combined multi-gene datasets, and tree construction was performed in MEGA (version 5.0) (30). All characters were equally weighted, and the phylogenetic trees were visualized using Tree view.

Table 1. Identification of endophytes from *H. chinensis* by the sequences analysis

Isolate strains (GenBank Acc.No.)	Identified species with the highest similarity (GenBank Acc. No.)	Similarity (%)	Place of origin
H1 (MT261862)	*Gliocladiopsis* sp. (KC776122.1)	99	China
H2 (MT261865)	*Coriolopsis* sp. (MH267924.1)	100	China
H3 (MT260802)	*Valsaceae* sp. (AB334109.1)	96	Japan
H4 (MT261908)	*Xylariaceae* sp. (AB741599.1)	99	Japan
H5 (MT259209)	*Phyllosticta* sp. (KP998485.1)	99	Taiwan
H6 (MT259232)	*Lentinus* sp. (KR155105.1)	99	India
H7 (MT259233)	*Phomopsis* sp. (GU066685.1)	99	Malaysia
H8 (MT259289)	*Fusarium* sp. (JN225917.1)	99	India
H9 (MT259782)	*Diaporthales* sp. (JN225917.1)	84	New Zealand
H10 (MT261863)	*Lachnum* sp. (MH578537.1)	91	New Zealand
H11 (MT261864)	*Cercospora* sp. (MK027098.1)	100	India
H12 (MT259221)	*Fomitopsidaceae* sp. (AY336764.1)	98	Taiwan

BIOLOGICAL ACTIVITY

(i). Antifungal activity of endophytic fungi : To qualitatively assess the antifungal activity of the isolated fungal strains and to identify those with potent activity, the activity of the isolates was tested against the plant pathogenic fungal strains *Fusarium oxysporum* f.sp.cubense tropical race 4, *Colletotrichum musae* Berk.et Curt, *Pyricularia grisea* (Cooke) Sacc and *Nescytalidium dimidiatum* (Penz.) Crous &

Slippers The inhibitory activity was evaluated using the plate confrontation method by measuring the resulting diameters of the pathogenic fungi (9).

(ii). Antifungal activity of endophytic fungi extracts : Endophytic strains were grown in potato dextrose broth (PDB)(19) at 28 ℃ in an oscillating shaker set at 120 rpm. After 14 days, the mycelia were used to generate crude extracts with four different polar solvents viz. methanol, ethyl acetate, acetone and petroleum ether in a fast solvent extractor. Subsequently, the crude extracts were used to generate 10 mg/mL solutions. The antifungal activities of four crude extracts were tested against four plant pathogenic fungi (*F. oxysporum* race 4, *N. dimidiatum*, *P. grisea* and *C. musae*) using the mycelial growth rate method (28). The mycelial growth of the control group was recorded when the colony in the control group approached the full culture medium, diameter (mm) of the colony was measured using crossover method, and the bacteriostasis rate was calculated as under :

$$\text{Inhibitory rate (\%)} = [(\text{Control colony diameter} - 6) - (\text{Treatment colony diameter} - 6)] / (\text{Control colony diameter} - 6) \times 100.$$

(iii). Antifungal activity of the endophytic fungal compounds : The antifungal activity of the compounds isolated from extracts of endophytic fungi were assessed against four plant pathogenic fungi (*F. oxysporum* race 4, *N. dimidiatum*, *C. musae* and *P. grisea*) using the filter paper method (26). The pathogenic cultures were inoculated on potato dextrose agar plates, after which paper discs containing 5 mg/mL of compounds were transferred to the petri dishes. After incubating at 28 ℃ for 4-5 days, the antifungal activity was evaluated by the presence of clear zones of inhibition around the paper disks.

Separation of chemical constituents

To separate the extracts into their chemical constituents, silica gel column chromatography, reverse phase silica gel column chromatography, gel column chromatography, thin layer chromatography (TLC) and recrystallization were done to separate and purify the compounds by selecting the suitable mobile phase polarity according to the TLC sample results. The structures of the compounds were analysed by NMR and comparing the spectral data with the relevant literature.

Statistical analysis

Microsoft Excel 2003 and SPSS were used for data processing and data analysis, respectively. A phylogenetic tree of the endophytic fungi was constructed with MEGA (version 5.0) using the neighbour-joining method. DNAMAN software were used for sequence splicing, Blast program were compared with GenBank nucleic acid database, and Sequin software were uploaded to GenBank database to obtain access number. Factor analysis of variance and Duncan’s new complex range method were used for variance analysis.

RESULTS AND DISCUSSION

Endophytic fungal isolation

Twelve endophytic fungi were isolated from the roots, branches and leaves of *H. chinensis* inoculated onto potato dextrose agar (PDA) (Table 1). According to rDNA sequencing results for the ITS region, and the BLAST percentage similarity to sequences from previously identified fungi in the NCBI GenBank database ranged from 85 to 100 %. The 12-identified fungi were : *Gliocladiopsis* sp., *Coriolopsis* sp., *Valsaceae* sp., *Xylariaceae* sp., *Phyllosticta* sp., *Lentinus* sp., *Phomopsis* sp., *Fusarium* sp., *Diaporthales* sp., *Lachnum* sp., *Cercospora* sp., *Fomitopsidaceae* sp. (Fig 2.) and the morphological characteristics given in Table 2.

Table 2. Morphological characters of endophytic fungi from *H. chinensis*

Strains	Colony characteristics	Morphological description
H1	The initial stage of growth is orange, the hyphae are dense, and the culture medium is orange yellow.	Conidia with dark stalk and solitary or clusters of orange to orange conidia at the top.
H2	The initial stage of growth is white, the hyphae are dense, and the culture medium is white.	The conidia stalk is erect, the apical cells are spherical, and the conidia are single spores.
H3	The initial stage of growth is gray black, the hyphae are dense, and the culture medium is gray brown.	Conidia pedicel unbranched, solitary, white.
H4	The initial stage of growth is gray, the hyphae are dense, white spores are produced, and the culture medium is grayish brown.	The conidia stalk is branched and the conidia are strung.
H5	The initial stage of growth, it turns brown and gradually turns black, forming a large number of small granular spores, and the culture medium is brown.	The conidia stalk is erect, the top expands into a sphere, and the conidia are spherical or ovoid.
H6	The initial stage of growth is white, the hyphae are dense, many small granular spores are generated, and the culture substrate is white.	The conidia stalk is upright, the top branches once to many times, forming a broom shape, and the conidia are oval.
H7	The initial stage of growth is light yellow, the hyphae are dense, and the culture medium is light yellow to gray.	Conidia stalk unbranched, solitary, colorless.
H8	The initial stage of growth is gray, the mycelium is thin, and the culture medium is gray.	Conidia stalk unbranched, solitary, colorless.
H9	The initial stage of growth is orange yellow, the mycelium is thin, and the culture medium is orange.	Conidia stalks are usually colorless branches.
H10	The initial stage of growth is light yellow, the mycelium is thin, and the culture medium is light yellow.	Spore stalk erect, colorless branching.
H11	The initial stage of growth is brown, the hyphae are dense, and the culture medium is black.	The conidia stalk is erect, and white spores are solitary or strung at the top.
H12	The initial stage of growth, the hyphae are sparse, and the culture medium is white.	Conidia stalk unbranched, colorless.

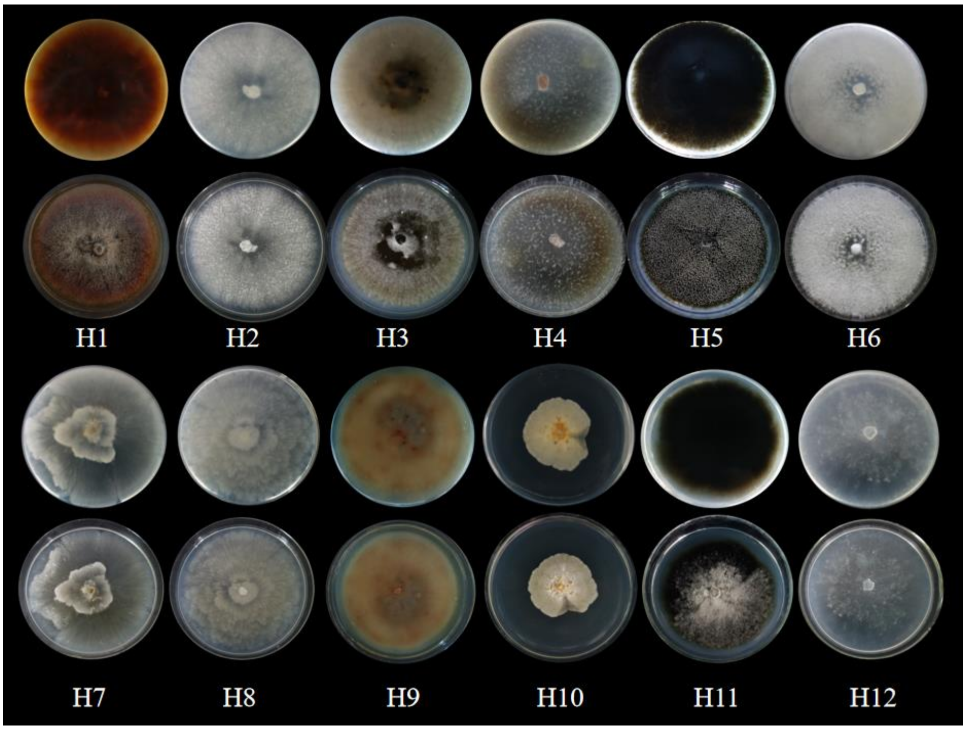


Figure 2. Morphology of 12 endophytic fungi from *H. chinensis*

Molecular phylogeny

The combined use of 5-gene sequences (ITS + nLSU + nSSU + mtSSU + Rpb2) for the MP analysis, according to multiple genes combination sequence, the phylogenetic analysis of H12 strain was done in 40 fungal samples. These data showed that 40 fungal samples represented 21 taxa, the phylogenetic tree of fungi was composed from sequence dataset, which were compared with each other for similarity and the final attribution of fungi was obtained. The result showed that H12 belongs to *Agaricomycotina* (subphylum), *Agaricomycetes* (class), *Polyporales* (order), Fomitopsidaceae (family) *Piptoporellus soloniensis* (genus-species), thus, it was established with strong support in the phylogenetic tree (Fig 3).

Antifungal activity

The 12 fungal strains obtained from *H. chinensis* were tested for antifungal activities against 4 different plant pathogenic fungi by plate confrontation method (Table 3). The antifungal activity of endophytic fungi showed that H12 (*P. soloniensis*) has the highest antagonistic activity against *F. oxysporum* race 4, *C. musae*, *P. grisea* and *N. dimidiatum* (Figs. 4,5). In this study, the antagonistic activity was taken as the main line for which H12 was preliminarily screened as a strain with significant antifungal activity.

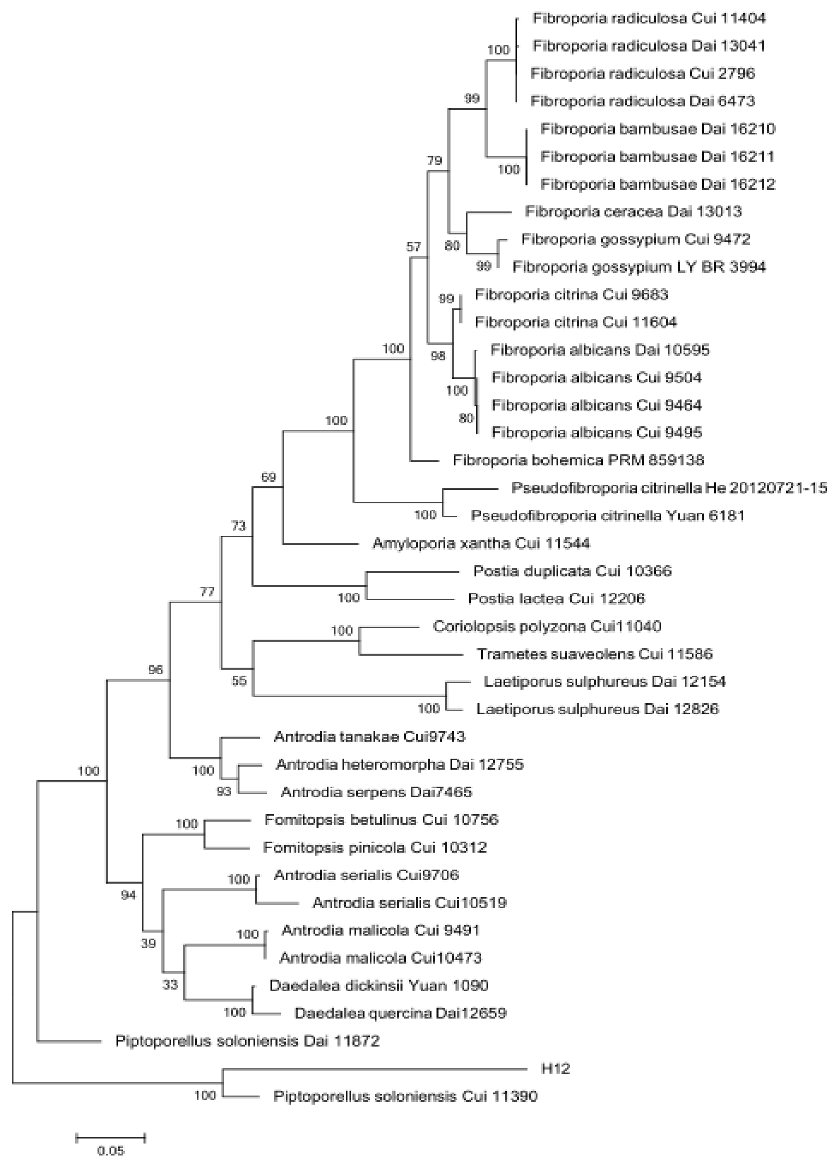


Figure 3. The phylogenetic tree shows the relationship between H12 and its related species based on 5 sequences. Maximum likelihood tree illustrating the phylogeny of *Piptoporellus soloniensis* and its related genera in the antrodia clade based on a combined sequence dataset of ITS + nLSU + nSSU + mtSSU + rpb2. The branches are labelled with maximum likelihood bootstrap values higher than 50 %, parsimony bootstrap proportions higher than 50 % and Bayesian posterior probabilities > 0.95.

Table 3. Antifungal activities of 12 fungal strains isolated from *H. chinensis* against 4 plant pathogenic fungi

Strains	Pathogenic fungi			
	F. oxysporum race 4	*C. musae*	*P. grisea*	*N. dimidiatum*
H1	++	++	++	++
H2	++	++	++++	+++
H3	++	++	++	++
H4	+++	++	+++	+++
H5	++	++	++	++
H6	++	++	+++	+++
H7	+++	++	+++	++
H8	+++	++	+++	++
H9	++	+	++	+
H10	++	++	++	++
H11	++	++	++	++
H12	+++	+++	+++	+++

Data in table are means of 3- replicates average inhibition rates. + : Inhibition rate < 10 %； ++ : Inhibition rate of 10-30 %； +++ : Inhibition rate of 30-50 %； ++++ : Inhibition rate is greater than 50 %.

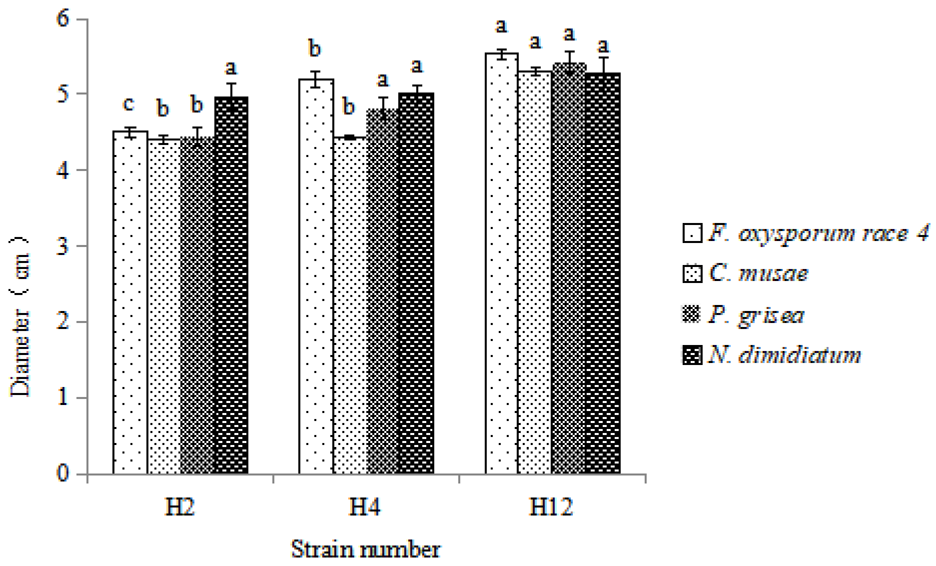


Figure 4. Antifungi activity of H2, H4 and H12 against 4 plant pathogens (*F. oxysporum race* 4, *C. musae*, *P. grisea*, and *N. dimidiatum*)

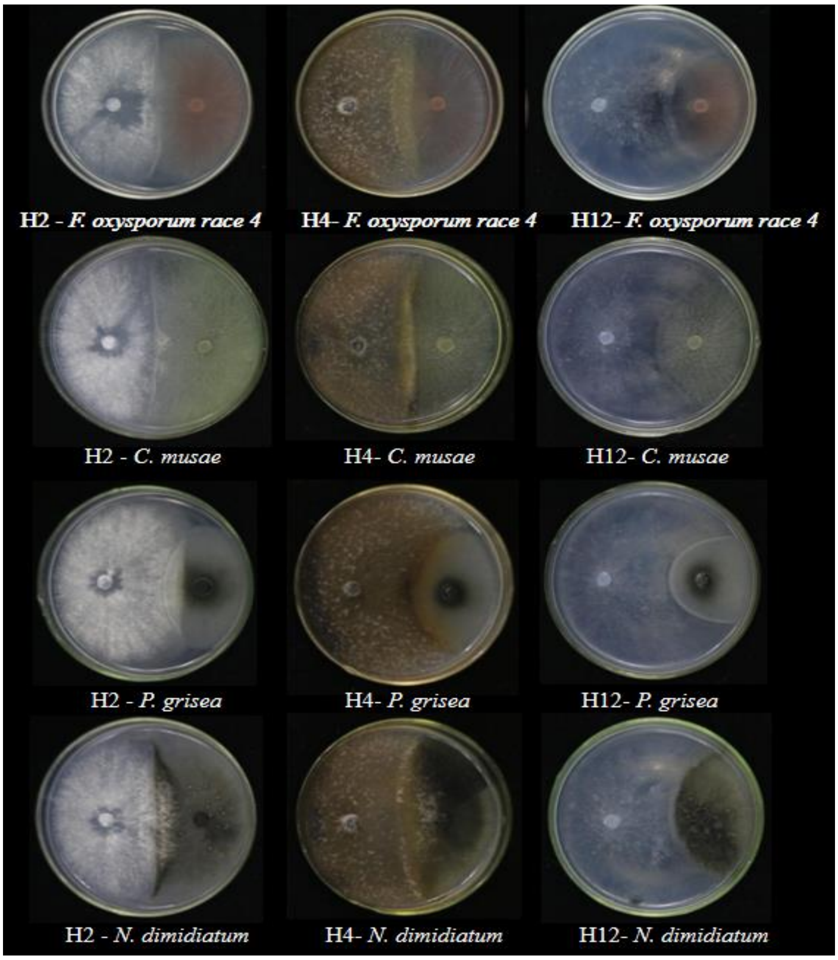


Figure 5. Plate confrontation diagram of H2, H4 and H12 against 4 plant pathogens (*F. oxysporum race* 4, *C. musae*, *P. grisea*, and *N. dimidiatum*)

For biomass production of *P. soloniensis*, it was cultured in 600 L culture medium and collected by filteration. After thorough washing the mycelium was dried and 406 g dry mycelial was obtained. Subsequently, the mycelium was extracted thrice using a rapid solvent extractor and obtained 17.94 g crude methanol extract, 10.23 g crude ethyl acetate extract, 5.62 g crude petroleum ether extract and 13.47 g crude of acetone extract.

The four extracts of *P. soloniensis* obtained using solvents with different polarities were tested for antifungal activities against four different plant pathogenic fungi, and the inhibition rate were calculated by measuring the colony diameter of the plant pathogenic fungi treated with the crude extracts at 10 mg/mL concentrations. The inhibition rates of methanol crude extract on *C. musae*, *P. grisea* and *N. dimidiatum* were 28.40 %, 76.79 % and 30.83 %, respectively. The inhibition rates of ethyl acetate crude extract on *C. musae*, *P. grisea* and *N. dimidiatum* were 32.84 %, 22.62 % and 22.13 %, respectively. The inhibition rates of acetone crude extract on *C. musae*, *P. grisea* and *N. dimidiatum* were 43.95 %, 40.48 % and 35.77 %, respectively. The inhibition rates of petroleum ether crude extract on *C. musae*, *P. grisea* and *N. dimidiatum* were 17.04 %, 18.15 % and 12.06 %, respectively (Table 4). However, the different extracts did not show any activity against *Fusarium oxysporum* race 4. The results showed that methanol and acetone extracts should be further studied.

Table 4. Antifungal activities of extracts from *Piptoporellus soloniensis* mycelia against plant pathogenic fungi at 10 mg/mL concentration

Plant pathogen species	Crude methanol extract	Crude ethyl acetate extract	Crude acetone extract	Crude petroleum ether extract
F. oxysporum race 4	NA[1)]	NA	NA	NA
C. musae	28.40± 3.16 b[2)]	32.84 ±1.66 b	43.95±2.71 a	17.04±2.98 c
P. grisea	76.79±2.61 a	22.62±0.77 c	40.48±1.22 b	18.15±0.08 c
N. dimidiatum	30.83±2.27 a	22.13±1.32 b	35.77±2.41 a	12.06±1.36 c

NA: Not active. Data represent the means ± S.E. of 3-separate experiments; Data within a line followed by the same letter were non- significant at $P < 0.05$ based on Duncan's multiple range test (DMRT).

Separation of chemical constituents

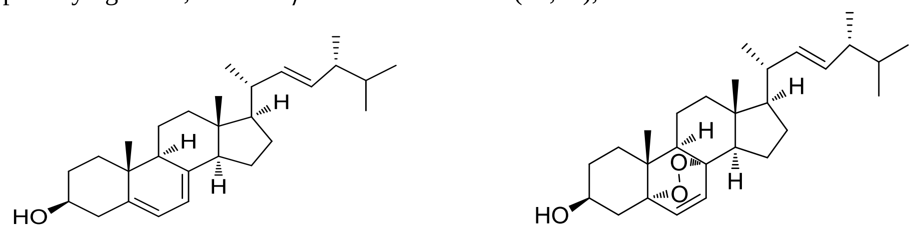
The methanol and acetone extracts of *P. soloniensis* mycelia were separated by silica gel and Sephadex LH-20 chromatography, and obtained compounds **1** (6.5 mg) and **2** (12.6 mg) from the methanol and acetone extracts, respectively, the compounds were assayed by ^{1}H and ^{13}C NMR, the data for which was as under:

Compound 1: ^{1}H NMR (600 MHz, $CDCl_3$) δ 0.65 (s, Me(18)); 0.85 (d, *J* = 6.6 Hz, Me(26)); 0.94 (d, *J* = 6.8 Hz, Me(27)); 0.97 (s, Me(19)); 1.06 (d, *J* = 6.6 Hz, Me(28)); 2.05 (m, H - C(14)); 2.39 - 2.24 (bt, H - C(9)); 2.49 (bd, 2H - C(4)); 3.66 (m, OH - C(3)); 5.40 (m, H - C(7)); 5.59 (dd, *J* = 5.6, 2.4 Hz, H - C(6));^{13}C NMR (150 MHz, $CDCl_3$): δ 12.1 (C(18)); 16.3 (C(19)); 17.6 (C(25)); 19.7 (C(27); 20.0 (C(28)); 21.1 (C(11)); 21.1 (C(21)); 23.0 (C(15)); 28.3 (C(12)); 31.5 (C(2)); 32.0 (C(26)); 37.0 (C(10)); 38.4 (C(1)); 39.1 (C(16)); 40.4 (C(4)); 40.8 (C(20)); 42.8 (C(13)); 42.8 (C(24)); 46.3 (C(9)); 54.6 (C(14)); 55.7 (C(17)); 70.5 (C(3)); 116.3 (C(7)); 119.6 (C(6)); 132.0 (C(22)); 135.6 (C(23)); 139.8 (C(8)); 141.4 (C(5)).

The NMR data were reported and compound was named ergosta-5,7,22-trine-3β-ol (ergosterol) in the literature (15,32,33), its structure was shown in Fig 6.

Compound 2: ^{1}H NMR (600 MHz, $CDCl_3$): δ 0.83 (d, *J* = 7.3 Hz, Me(26)); 0.84 (s, Me(18)); 0.84 (d, *J* = 3.0 Hz, Me(27)); 0.90 (s, Me(19)); 0.93 (d, *J* = 6.8 Hz, Me(28)); 1.02 (d, *J* = 6.6 Hz, Me(21)); 3.95 - 4.01 (m, H - C(3)); 5.16 (dd, *J* = 15.4, 8.4 Hz, H - C(22)); 5.24 (dd, *J* = 15.2, 7.7 Hz, H - C(23)); 6.26 (d, *J* = 8.5 Hz, H - C(6)); 6.52 (d, *J* = 8.5 Hz, H - C(7); ^{13}C NMR (150 MHz, $CDCl_3$): δ 12.9 (C(18)); 17.6 (C(28)); 18.2 (C(19)); 19.6 (C(26)); 20.0 (C(27)); 20.6 (C(15)); 20.9 (C(21)); 23.4 (C(11)); 28.6 (C(16)); 30.1 (C(2)); 33.1 (C(25)); 34.7 (C(1)); 36.9 (C(10)); 37.0 (C(4)); 39.3 (C(12)); 42.8 (C(24)); 44.6 (C(13)); 51.1 (C(9)); 51.7 (C(14)); 66.5 (C(3)); 56.2 (C(17)); 79.4 (C(8)); 82.2 (C(5)); 130.7 (C(7)); 132.3 (C(23)); 135.2 (C(22)); 135.4 (C(6)).

The NMR data were reported, and the compound was named (22E)-5α,8α-epidioxyergosta-6,22-dien-3β-ol in the literature (11,21), its structure was shown in Fig 6.



Ergosta-5,7,22-trine-3β-ol (**1**) (22E) - 5α, 8α - epidioxyergosta - 6, 22 - dien - 3β – ol (**2**)

Figure 6. Structures of purified compounds from *P. soloniensis*.

The antifungal activity of two isolated compounds was determined by filter paper method, but the results showed that the compounds had no activity against *C. musae*, *P. grisea* and *N. dimidiatum*. The loss of antifungal activity of the two purified compounds indicates that either the activity is due to some other component in the crude extract or some helper molecule is lost during the purification procedure. Therefore, the secondary metabolites of the strain with antifungal activity need to be further separated and analysed.

CONCLUSIONS

In this study, 12 strains of endophytic fungus were isolated and purified from the medicinal plant *H.chinensis* by tissue culture, its sequencing and NCBI gene sequence comparison. After screening, a strain H12 (*P. soloniensis*) with high antifungal activities was obtained. The strain had potent antifungal effects on 4-plant pathogenic fungi (*F. oxysporum* race 4, *C. musae*, *P. grisea* and *N. dimidiatum*) and the inhibition rate exceeded 30 %. The methanol crude extraction of the *P. soloniensis* strain had potent antifungal effect on *P. grisea*, and its inhibition rate was 76.79 %, while the acetone crude had potent antifungal effect on *C. musae* and *P. grisea* with the inhibition rate of 43.95 % and 40.48 %, respectively. It showed that endophytic fungi in *H. chinensis* are rich in antifungal strains and potential antifungal substances, which had good application prospects.

ACKNOWLEDGEMENTS

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DECLARATION

We declare that all authors of this Ms. have made substantial contributions. We did not exclude any author who substantially contributed to this Ms. We have followed our ethical norms established by our respective institutions.

CONFLICT OF INTEREST

The authors announce that they have no conflict of interest.

ETHICAL APPROVAL

The authors declare that the study was carried out following scientific ethics and conduct. However, this study did not involve any use of animals, hence no ethical approval has been obtained from the concerned committee.

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