Diversity, Chemical Constituents, and Biological Activities of Endophytic Fungi Isolated From *Ligusticum chuanxiong* Hort

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The objective of this study was to evaluate the diversity of endophytic fungi of different parts of *Ligusticum chuanxiong* Hort (CX) and further characterize their biological activities and identify chemical compounds produced by these endophytic fungi. A total of 21 endophytic fungi were isolated and identified from CX. *Penicillium oxalicum*, *Simplicillium* sp., and *Colletotrichum* sp. were identified as promising strains by the color reaction. Comparing different organic extracts of the three strains, it was observed that the ethyl acetate extract of *Penicillium oxalicum* and *Simplicillium* sp. and the *n*-butanol extract of *Colletotrichum* sp. showed significant antioxidant and antibacterial activities. The ethyl acetate extracts of *Penicillium oxalicum* had outstanding antioxidant and antibacterial effects, and its radical scavenging rates for ABTS and DPPH were $98.43 \pm 0.006\%$ and $90.11 \pm 0.032\%$, respectively. At the same time, their IC_{50} values were only 0.18 ± 0.02 mg/mL and 0.04 ± 0.003 mg/mL. The ethyl acetate extract of *Penicillium oxalicum* showed MIC value of only 0.5 mg/mL against *Escherichia coli* and *Staphylococcus aureus*. By liquid chromatography-mass spectrometry (LC-MS), we found that *Penicillium oxalicum* could produce many high-value polyphenols, such as hesperidin (36.06 μmol/g), ferulic acid (1.17 μmol/g), and alternariol (12.64 μmol/g), which can be a potential resource for the pharmaceutical industry. In conclusion, these results increase the diversity of CX endophytic fungi and the antioxidant and antibacterial activities of their secondary metabolites.

Keywords: chuanxiong, phenolic compounds, endophytic fungi, antioxidant activity, antimicrobial activity

INTRODUCTION

The medicinal plant *Ligusticum chuanxiong* Hort (CX) belongs to the Umbelliferae family and is commonly cultivated in Sichuan Province, China. It has been used in the treatment of gynecological diseases, headache, rheumatism arthralgia, and hemiplegia (Chen et al., 2018). The major chemical constituents of CX are phthalides, terpenes, polysaccharides, alkaloids, and organic acids

(Ran et al., 2011). Moreover, the young leaf and stem of CX are widely used as edible food materials in tossed salad and fried cuisines (Yuan et al., 2020a).

Endophytic fungi are a valuable resource for the detection of new natural products and have been widely used for medicinal purposes (Tanvir et al., 2017). Endophytic fungi that live with plant tissues without causing harm to hosts can produce different secondary metabolites including alkaloids, steroids, terpenoids, flavonoids, aliphatic compounds, and phenols (Aly et al., 2011). These products have various biological activities, such as antioxidant, anti-diabetic (Sharma and Sharma, 2016), anti-obesity (Noinart et al., 2017), and antimicrobial activities (Padhi et al., 2020). Moreover, plant endophytic fungi have a high species diversity, which is strongly associated with environmental variations and the taxonomy of host plants (Wu et al., 2013). The diversity of endophytic fungi will determine the production of diverse secondary metabolites promoted by endophytic fungi, which can be used as drugs for medical treatment (Jia et al., 2016). Furthermore, when the endophytic fungi was isolated *in vitro* and cultivated in suitable conditions, the number of fungal cells and the production of secondary metabolites would be multiplied far beyond their normal concentration in plants (Wu et al., 2018). Therefore, plant endophytic fungi are not only a source for the discovery of biologically active substances, but also a reactor for the production of drugs more easily and economically.

Currently, oxidative stress is gaining more attention. Reactive oxygen species (ROS) are continuously produced as a by-product of normal basal metabolism in humans (Valko et al., 2006). ROS is essential and beneficial to living systems, and plays a role as signaling molecules in the regulation of many biological processes (Apel and Hirt, 2004). However, oxidative stress is the result of excess unneutralized active substances in the body, which can damage cellular lipids, proteins, cells, tissues, and organs, and inhibit the normal function of DNA (Valko et al., 2007; Desai et al., 2009), and it has been reported as one of the important factors in several types of diseases, such as diabetes and even some types of cancer (Marseglia et al., 2015; Zimta et al., 2019). Although many potent antioxidants have been artificially synthesized, such as BHA and BHT, they are not safe as some natural antioxidants (Kahl and Kappus, 1993). Thus, there is a need to find more natural antioxidants to meet the demands of the food and pharmaceutical industries.

In addition, the quest for new antimicrobial drugs has always been crucial throughout human history. Bacterial infections are the result of pathogenic bacteria entering and multiplying in the human body; these are a major deleterious factor in some infections (Anderson and Kendall, 2017; Martin and Bachman, 2018). For example, the gram-positive bacterium *Staphylococcus aureus* is a human pathogen (Kimmig et al., 2021) that is constantly a source of skin infections, lung infections, gastroenteritis, and urinary tract infections (Thomer et al., 2016; Del Giudice, 2020). Over the years, researchers have observed that antibacterial substances can be isolated from endophytic fungi of various medicinal plants (Pinheiro et al., 2017; Nischitha and Shivanna, 2021). Hence, it is possible to discover new and effective antibacterial substances from endophytic fungi.

Medicinal plants and their endophytes are important resources for the discovery and acquisition of natural products (Huang et al., 2007). The antioxidant (Ge et al., 2018) and antimicrobial (Sim and Shin, 2008) activities of CX have been described using different pharmacological models, and CX is also well documented for production of various bioactive metabolites (Yuan et al., 2020b; Zhang et al., 2020). However, there are few studies on endophytic fungi of CX at present, and endophytic fungi resources should be further developed and utilized. In this context, the present study aimed to identify the diversity of endophytic fungi in CX and the antioxidant and antibacterial activities as well as the chemical compounds of endophytic fungus extracts.

MATERIALS AND METHODS

Experimental Materials

Seedlings of CX were collected from the farm of Sichuan Agricultural University (Ya'an City, Sichuan Province, China) in October 2020 and the lingzi (nodes of the lower part of the stems) were obtained from the local market in Ya'an. The plants were identified by Dr. Hui Chen. Voucher specimens were deposited in the Herbarium "Fermentation Engineering Laboratory" with the identification numbers FEL125 and EFL126. All samples were placed in the boxes and immediately sent to the laboratory for study.

Isolation of Endophytic Fungi

The lingzi and seedlings of CX were washed thoroughly with water, and the roots, stems, and leaves systems were separated from the plant. The collected samples were cut into small 5 mm cubes and soaked in 70% ethanol for 1–2 min, followed by 5% sodium hypochlorite for 2–5 min, and then in 70% ethanol for 0.5–1 min (adapted from Zhao et al., 2012). In addition, the samples were dabbed with sterile filter paper. The surface-sterilized explant fragments were placed in Petri dishes containing PDA which was amended with ampicillin and kanamycin sulfate at a concentration of 50 µg/L to prevent bacterial contamination. The fungal strains in PDA plates were incubated at 28°C for 7–10 days and purified promising fungi were stored in the Laboratory of Molecular Biology and Biochemistry, College of Life Sciences, Sichuan Agricultural University.

Identification and Phylogenetic Analysis of the Endophytic Fungi

The endophytic fungi were identified by their morphology and DNA sequence data. Cellular morphological features including their mycelium color, pigmentation, and spore morphology, were described and investigated using a CX21 FS1 microscope (Olympus, Tokyo, Japan). Genomic DNA was isolated using the CTAB method and the ITS region of the fungal genome was amplified by PCR using the following primers: ITS1-forward primer (TCCGTAGGTGAACCTGCGG) and ITS4-reverse primer

(TCCTCCGCTTATTGATATGC). The reaction mixture and amplification conditions were the same as previously described (Shah et al., 2019). The PCR products were directly sequenced by the TSINGKE Biological Technology Corporation (Shandong, China). The sequences of the ITS regions were compared with the sequences of existing species sequence in GenBank using BLASTN software. A phylogenetic tree was constructed by the neighbor joining method using MEGA 7.0 software.

Screening of Polyphenol-Producing Endophytic Fungi

Endophytic fungi were inoculated into fresh PDB and were allowed to grow for 7 days (160 rpm/min and 28°C). The culture was filtered through four layers of cheesecloth to obtain culture filtrate. Polyphenol-producing endophytic fungi were preliminarily screened by the color reaction. In brief, fermentation broth was mixed with chromogenic reagent (0.1% $FeCl_3$:0.1% $K_3[Fe(CN)_6]$ = 1:1) in a tube. If the color turns blue, it indicates that the fermentation broth contains polyphenols.

Preparation of Extracts of Fermentation Broth of Endophytic Fungi

Expanded fermentation of polyphenol-producing endophytic fungi was carried out in the same culture conditions as in Section "Screening of Polyphenol-Producing Endophytic Fungi." The culture filtrate was extracted three times with ethyl acetate, *n*-butanol, petroleum ether, and chloroform (2 × 200 mL) in a separatory funnel, respectively. The crude extracts were concentrated using a lyophilize under a vacuum at –40°C and dissolved in dimethyl sulfoxide (DMSO) to evaluate their biological activities.

Determination of Total Phenolic Content

Total Phenolic Content (TPC) of the fungal extracts was determined using Folin–Ciocalteu (FC) assay as described by Minussi et al. (2003). In brief, after mixing deionized water (1 mL) and extract solution (1 mL) in a test tube, FC reagent (0.5 mL) was added to the mixture and allowed to react for 4 min. There was 20% Na_2CO_3 (1 mL) added thereafter and the mixture was diluted to 10 mL with distilled water. The color was developed in 2 h, and the absorbance was determined at 760 nm (A_{760}) using a multiskan sky (Thermos Scientific, Singapore). TPC were expressed in mg equivalent in gallic acid and obtained from the regression equation: $y = 0.0064x + 0.063$ with $R^2 = 0.9979$. All analyses were performed in triplicate.

Antioxidant Activity

The above-mentioned fungi extracts were adjusted to six concentrations (0.2, 0.4, 0.6, 0.8, 1.0, and 3 mg/mL) for four antioxidant activity assays [2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging, 2,20-azino-bis radical (ABTS+·) scavenging, hydroxyl radical (·OH) scavenging, and superoxide anion radical (·O_2^-) scavenging]. Ascorbic acid (Vc) was used as the positive control.

2,2-Diphenyl-1-Picrylhydrazyl Radical Scavenging Activity Assay

The DPPH assay was carried out using the method of Nuerxiati et al. (2019). Briefly, in each well of a 96-well microplate, test samples of different concentrations (100 μL) were mixed with DPPH solution (100 μL, 0.2 mM) in the dark for 30 min at room temperature. The absorbance of each well was recorded at 517 nm (A_{517}). The DPPH radical scavenging activity (scavenging rate) was calculated by

$$Scavengingrate(\%) = [1 - (A_{517} - A_0)/A_{max}] \times 100\%$$

where A_{max} is the absorbance of 100 μL of DPPH solution in 100 μL of anhydrous ethanol at 517 nm; A_{517} is the absorbance of 100 μL of DPPH solution mixed with 100 μL of gradient sample solution; A_0 is the absorbance of 100 μL of gradient sample solution in 100 μL of anhydrous ethanol.

Hydroxyl Radical Scavenging Activity Assay

The hydroxyl radical scavenging activity of the crude extracts was measured according to Smirnoff and Cumbes (1989) with some modifications. Different concentrations of the tested sample (50 μL) were added into an enzyme label plate containing a reaction solution of 50 μL of 6.0 mM $FeSO_4$, 50 μL of 6.0 mM salicylic acid-ethanol, and 50 μL of 0.1% H_2O_2. The reaction solution was cultured in a 37°C thermostat for 30 min. The absorbance of the mixture was read at 510 nm (A_{510}). The hydroxyl radical scavenging rate was calculated using the following formula:

$$Scavengingrate(\%) = [1 - (A_{510} - A_0)/A_{max}] \times 100\%$$

where A_{510} is the absorbance of a reaction solution containing the sample solution and H_2O_2 at 510 nm; A_{max} is the absorbance of a blank solution with anhydrous ethanol instead of the sample solution; A_0 is the absorbance of a solution with distilled water instead of H_2O_2.

Superoxide Anion Radical Scavenging Assay

The scavenging activity of superoxide anion radical was estimated by pyrogallol autoxidation as described by Wang et al. (2009) with some modifications. Briefly, 0.05 mol/L Tris-HCl buffer (pH 8.2, 1.5 mL) and test sample with different concentration (0.5 mL) were mixed and incubated in a water bath at 25°C for 25 min. Afterward, 25 mM pyrogallol (200 μL) at 25°C was added. After 4 min of reaction, 8 mol/L HCl (0.25 mL) was added to terminate the reaction. The absorbance of the sample was measured at 325 nm (A_{325}). The scavenging activity of superoxide anion radical scavenging rate was measured using the following equation:

$$Scavengingrate(\%) = [1 - (A_{325} - A_0)/A_{max}] \times 100\%$$

where A_{325} is the absorbance of a reaction solution containing the sample solution and pyrogallol at 325 nm; A_{max} is the absorbance of a blank solution with distilled water; A_0 is the absorbance of a solution with distilled water instead of pyrogallol.

TABLE 1 | The result of separation of endophytic fungi from different parts of CX.

Stage	Parts	Strain no	Total
Lingzi		ZZ2, ZZ3, ZZ5, ZZ9, ZZ10, ZZ11, ZZ12, ZZ13	8
Seedling	Root	YMG1, YMG2	2
	Leaf	YMY3, YMY4, YMY5, YMY6, YMY7	5
	Stem	YMJ8, YMJ9, YMJ10, YMJ11, YMJ12, YMJ13	6

TABLE 2 | The homologous strain of endophyte fungi from CX.

NO	Genus	Most closely related strain	Ident (%)	Accession.
ZZ2	*Rigidoporus* sp.	*Rigidoporus vinctus*	90.14	KF494814.1
ZZ3	*Rigidoporus* sp.	*Rigidoporus vinctus*	99.51	KM277965.1
ZZ5	*Simplicillium* sp.	*Simplicillium* sp.	100	LN808975.1
ZZ9	*Cladosporium* sp.	*Cladosporium cladosporioides*	99.42	HM037955.1
ZZ10	*Alternaria* sp.	*Alternaria alternata*	99.81	MH270549.1
ZZ11	*Alternaria* sp.	*Alternaria alternata*	99.81	MW008961.1
ZZ12	*Fusarium* sp.	*Fusarium fujikuroi*	99.62	MG543727.1
ZZ13	*Fusarium* sp.	*Fusarium tricinctum*	99.62	EF611095.1
YMG1	*Penicillium* sp.	*Penicillium oxalicum*	99.46	MT267816.1
YMG2	*Fusarium* sp.	*Fusarium solani*	99.62	KU321546.1
YMY3	*Alternaria* sp.	*Alternaria* sp.	99.63	MW009028.1
YMY4	*Alternaria* sp.	*Alternaria compacta*	99.81	MW008977.1
YMY5	*Fusarium* sp.	*Fusarium asiaticum*	99.8	KY272822.1
YMY6	*Colletotrichum* sp.	*Colletotrichum camelliae*	99.63	MK041374.1
YMY7	*Nigrospora* sp.	*Nigrospora sphaerica*	99.61	MH790215.1
YMJ8	*Alternaria* sp.	*Alternaria* sp.	99.63	MW009028.1
YMJ9	*Fusarium* sp.	*Fusarium avenaceum*	99.43	KY272780.1
YMJ10	*Sordariomycetes* sp.	*Sordariomycetes* sp.	99.08	MT184111.1
YMJ11	*Fusarium* sp.	*Fusarium proliferatum*	99.05	MH978624.1
YMJ12	*Aporospora* sp.	*Aporospora* sp.	100	MK246947.1
YMJ13	*Colletotrichum* sp.	*Colletotrichum fructicola*	99.08	MK041492.1

ABTS Radical Scavenging Activity Assay

ABTS assay of all samples was assessed as the method described by Zhao et al. (2005). The ABTS solution was formed by mixing 7 mM ABTS (0.5 mL) with 140 mM $K_2S_2O_8$ (88 μL), and the mixture was kept at room temperature for 12 h. The ABTS radical solution was diluted with distilled water and absorbance was adjusted to 0.70 ± 0.02 at 734 nm. Then, different concentrations of extracts (150 μL) were mixed with 50 μL of ABTS solution in each well of a 96-well microplate for 6 min. The absorbance of the reaction mixture was read at 734 nm (A_{734}). The scavenging activity of ABTS radical was calculated as:

$$Scavenging\,rate(\%) = [1 - (A_{734} - A_0)/A_{max}] \times 100\%$$

where A_{max} is the absorbance of ABTS solution mixed with anhydrous ethanol at 734 nm; A_{734} is the absorbance of ABTS solution mixed with the sample; A_0 is the absorbance of the sample mixed with anhydrous ethanol.

Antibacterial Activity
Determination of the Minimum Inhibitory Concentration

To evaluate the Minimum Inhibitory Concentration (MIC) (Molla et al., 2016), two gram-negative bacteria (*Escherichia coli*: ATCC25922 and *Pseudomonas aeruginosa*: ATCC9027) and two gram-positive bacteria (*Bacillus subtilis*: ATCC6633 and *Staphylococcus aureus*: ATCC6538) were used. Initially, the bacterial strains were inoculated on a sterile nutrient agar plate and incubated at 37°C for 24 h. Subsequently, the fungal extracts (0.2–4 mg/mL) were diluted in nutrient broth using the serial micro-dilution method. Bacterium (10 μL) was inoculated in EP tubes with different concentration samples. There was 0.5% DMSO used as a negative control and chloramphenicol (50 μg/L) as a positive control. Then, all EP tubes were incubated at 37°C for 24 h. To determine the MIC, 0.1 mg/ml MTT (10 μL) was used. Any color change observed from mauve to purple was considered negative for bacterial growth. The lowest concentration of endophytic fungi extracts with no color change occurred was recorded as the MIC value. All the experiments were performed in triplicate for each bacterium.

Determination of Minimum Bactericidal Concentration

To evaluate the Minimum Bactericidal Concentration (MBC) (da Rocha et al., 2020), 10 μL positive wells of bacteria were inoculated on nutrient agar plates at 37°C for 24 h. Then the colonies on the plate were counted, and the result with growth less than 10 colonies was considered to have a bactericidal effect.

Fluorescence Microscopy

Biofilm was visualized using fluorescence microscopy according to Jardak et al. (2017). *E. coli*, *P. aeruginosa,* and *S. aureus* cells were treated with fungal extracts at a concentration of 2MIC. Biofilms were grown on glass slides at 37°C for 48 h. Then, the glass slides were gently washed with physiological saline 2–3 times in order to remove non-adherent bacteria. Then, acridine orange (0.1%, w/v, dissolved in PBS 1X) was used to stain biofilms formed in glass slides. Biofilms were observed under a BX53 fluorescent microscope (Olympus, Tokyo, Japan).

Detection of Bioactive Compounds by Liquid Chromatography-Mass Spectrometry Analysis

Extracts of endophytic fungi were analyzed by UPLC-HRMS (Waters, UPLC; Thermo, Q Exactive). The separation of compounds was achieved on an ACQUITY UPLC HSS T3 column (2.1 × 100 mm i.d) with bead size of 1.8 μm. The mobile phase was 0.05% formic acid in water (A)/acetonitrile (B) with a flow rate of 0.3 mL min^{-1}. The following gradient elution program was used: 0–1 min, 5% B; 5–95% B, 2–13:50 min; 95–5% B, 13:50–16 min. For mass spectrometry (MS) detection, ionization was performed in ESI+ mode (Heater Temp 300°C; Sheath Gas Flow rate, 45arb; Aux Gas Flow Rate, 15arb; Sweep Gas Flow Rate, 1arb; spray voltage, 3.0KV; Capillary Temp, 350°C; S-Lens RF Level, 30%.) and ESI- mode
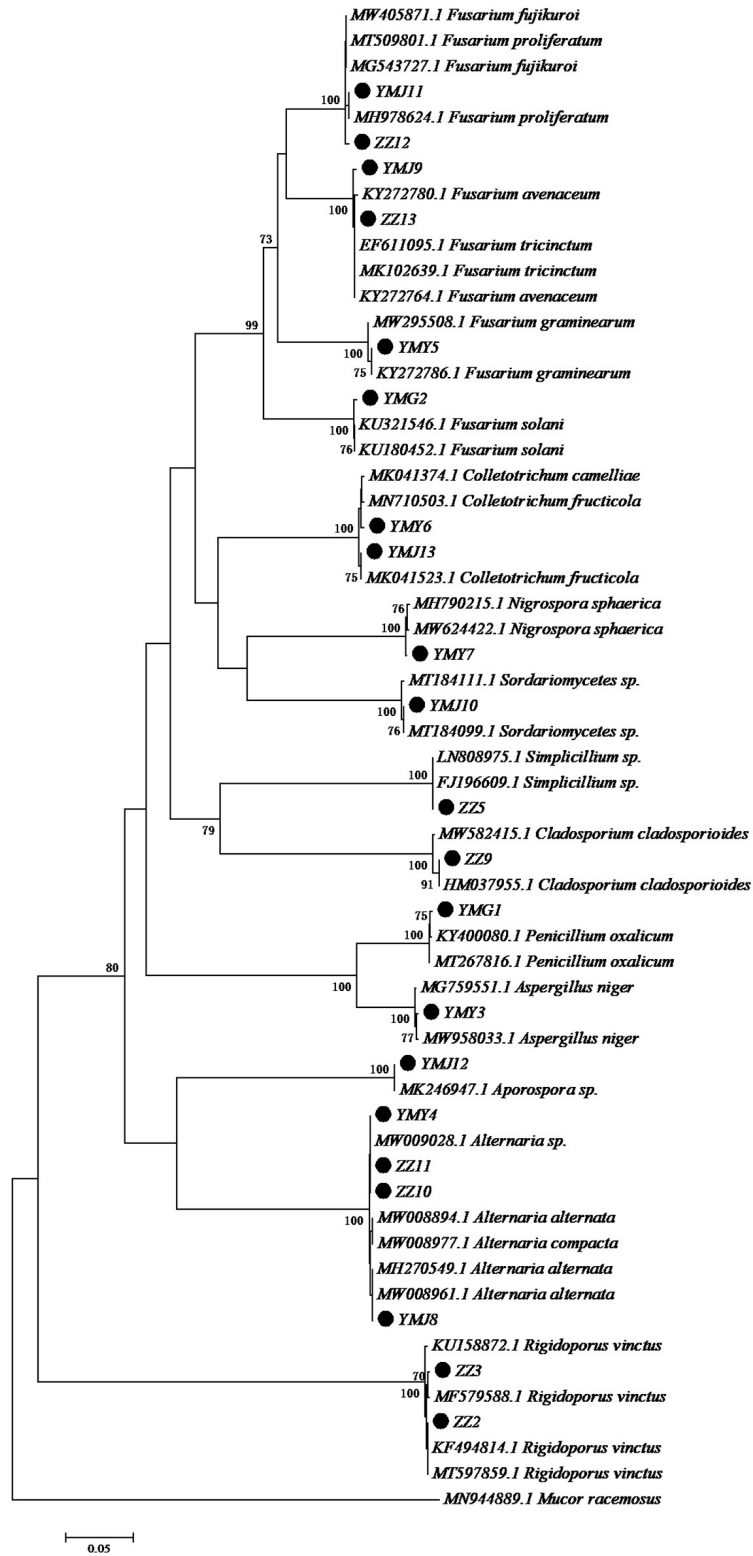
FIGURE 1 | Neighbor-joining tree of the ITS sequences of the endophytic fungi associated with CX. Numbers at nodes are bootstrap scores obtained from 1000 replications. *Mucor racemosus* was used as an out group.

TABLE 3 | Assessment of TPC and antioxidant activity of *P. oxalicum*, *Simplicillium* sp., and *Colletotrichum* sp. extracts.

Extracts	TPC (mg GAE/g of extract)	IC$_{50}$ (mg/mL)			
		ABTS radical	Hydroxyl radical	DPPH radical	Superoxide anion radical
P. oxalicum					
Vc	–	–	0.29 ± 0.03$_b$	–	0.07 ± 0.01$_b$
Ethyl acetate	94.58 ± 2.701$_a$	0.03 ± 0.003$_c$	0.38 ± 0.04$_b$	0.16 ± 0.002$_c$	1.41 ± 0.22$_a$
n-Butanol	66.46 ± 8.12$_b$	0.21 ± 0.04$_b$	1.22 ± 0.60$_a$	0.50 ± 0.05$_b$	nd
Petroleum ether	21.67 ± 5.91$_c$	nd	1.29 ± 1.05$_a$	nd	nd
Chloroform	52.40 ± 2.71$_d$	0.35 ± 0.01$_a$	1.9 ± 0.94$_a$	1.15 ± 0.15$_a$	nd
***Simplicillium* sp.**					
Vc	–	–	0.29 ± 0.03$_b$	–	0.07 ± 0.01$_b$
Ethyl acetate	58.64 ± 3.61$_a$	0.18 ± 0.02$_c$	3.37 ± 0.38$_a$	0.04 ± 0.003$_d$	2.00 ± 0.11$_a$
n-Butanol	45.62 ± 8.27$_b$	0.24 ± 0.01$_b$	nd	0.07 ± 0.02$_c$	nd
Petroleum ether	12.29 ± 3.24$_c$	1.03 ± 0.07$_a$	nd	0.45 ± 0.02$_a$	nd
Chloroform	16.98 ± 4.77$_c$	0.43 ± 0.01$_b$	nd	0.28 ± 0.01$_b$	nd
***Colletotrichum* sp.**					
Vc	–	–	0.29 ± 0.03$_b$	–	0.07 ± 0.01$_b$
Ethyl acetate	36.75 ± 4.83$_b$	0.59 ± 0.10$_a$	nd	0.97 ± 0.09$_b$	nd
n-Butanol	58.96 ± 4.10$_a$	0.29 ± 0.011$_c$	0.67 ± 0.05$_a$	0.47 ± 0.004$_c$	2.48 ± 0.52$_a$
Petroleum ether	26.14 ± 0.32$_c$	0.43 ± 0.02$_{ab}$	nd	1.65 ± 0.43$_a$	nd
Chloroform	25.04 ± 5.56$_c$	0.47 ± 0.04$_b$	nd	1.54 ± 0.1$_a$	nd

a–d: significant differences between different groups (p < 0.05).
nd, not detected (the result higher 4 mg/mL).

(Heater Temp 300°C, Sheath Gas Flow rate,45arb; Aux Gas Flow Rate, 15arb; Sweep Gas Flow Rate, 1arb; spray voltage, 3.2KV; Capillary Temp, 350°C; S-Lens RF Level, 60%.). Full Scan (MS$_1$) was performed from 70∼1050 m/z with a resolution of 70,000 and data dependent two-stage mass spectrometry (DD-MS$_2$, TOPN = 10) with a resolution of 17,500. The obtained mass spectra were analyzed with Compound Discoverer 2.0 (Thermo Scientific) and the integrated mzcloud, metlin, and hmdb database were used to detect secondary metabolites in an untargeted method.

Data Analysis

All experimental data are expressed as the mean ± SD from three independent observations. The data were analyzed by one-way analysis of variance (ANOVA) followed by Duncan's multiple range test using the SPSS24.0 software. $P < 0.05$ was used to define statistically significant difference between the control group and the experimental group.
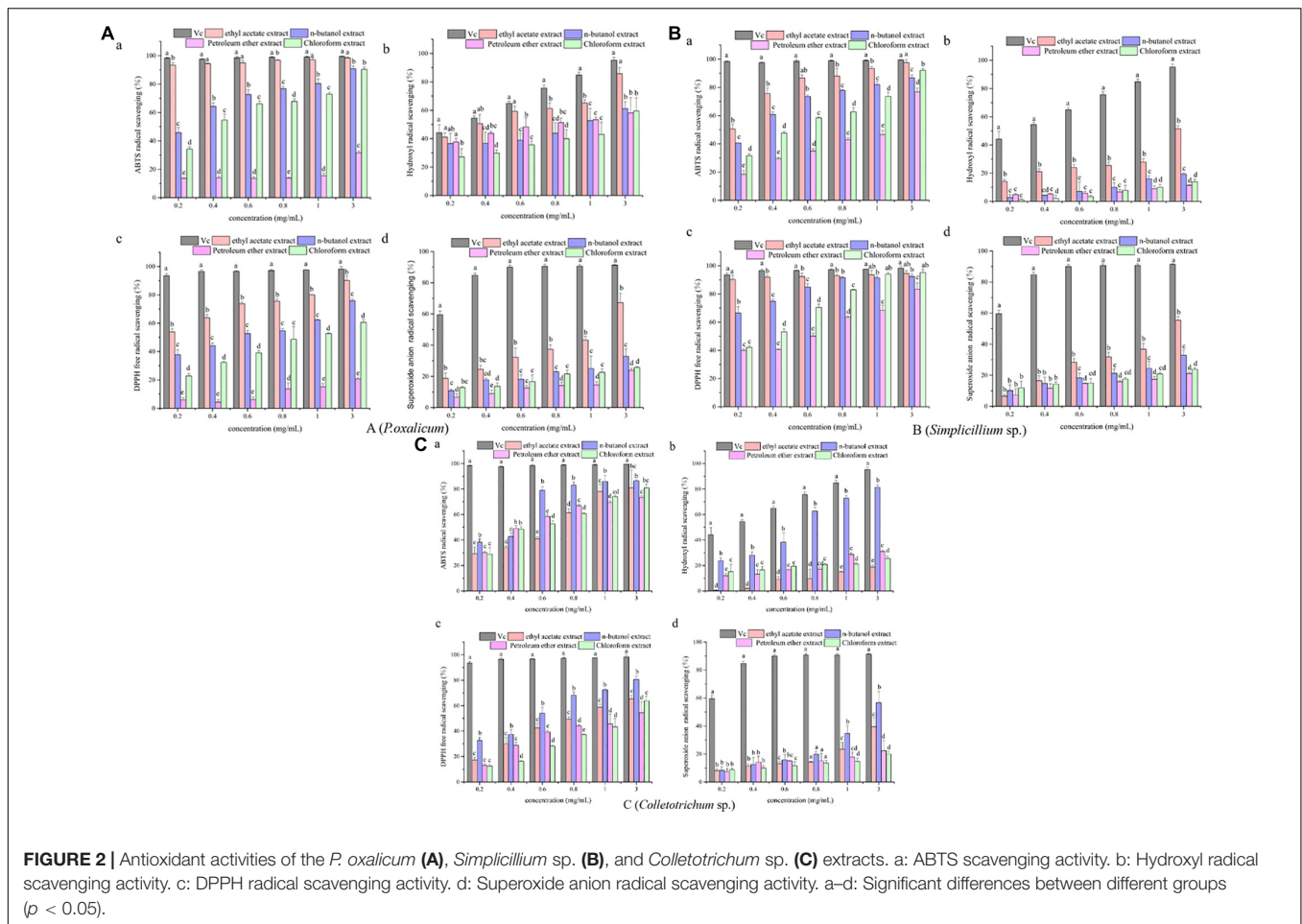
RESULTS

Isolation and Identification of Endophytic Fungi

A total of 21 strains of endophytic fungi were isolated from different parts of CX for the first time. There were 8, 2, 5, and 6 isolates obtained from lingzi, root, leaf, and stem of CX, respectively (**Table 1**). All these strains were preliminarily identified by their colony morphology and microscopic examination (**Supplementary Figure 1** and **Supplementary Table 1**). Further, their molecular identification

was carried out by ITS-based rDNA sequence analysis and the closest identified matching strains were listed in **Table 2**. All nucleotide sequences of these isolates were no less than 99% similarity to the closest matches in the nucleotide database, except for strain ZZ2, which showed only 90.14% identity with the sequence KM268690.1 retrieved from the NCBI GenBank database (**Table 2**). The most common genera were *Fusarium* sp. and *Alternaria* sp. (seven isolates and four isolates, respectively) (**Table 2**). The phylogenetic tree of the identified endophytic fungi from CX is shown in **Figure 1**. The 21 isolates were assigned into 11 different genus (**Figure 1**), including *Rigidoporus* sp., *Aspergillus* sp., *Simplilium* sp., *Cladosporium* sp., *Alternaria* sp., *Fusarium* sp., *Penicillium* sp., *Colletotrichum* sp., *Nigrospora* sp., *Sordariomycetes* sp., and *Aporospora* sp., showing the rich biodiversity of endophytic fungi from CX. Finally, ZZ2 was characterized as *Rigidoporus* sp. at the genus level based on the observation of colony morphology and phylogenetic analysis (**Supplementary Figure 1**, **Supplementary Table 1**, and **Figure 1**). In addition, since Colletotrichum is a very complex genus and need for identification of it at the species level by further genomics analyses (Gupta et al., 2019), strains YMY6 and YMJ13 also were preliminarily ascribed to *Colletotrichum* sp. at the genus level.

Screening of Polyphenol-Producing Endophytic Fungi

Polyphenol-producing strains were screened by color reaction, as shown in **Supplementary Figure 2**. The fermentation broth containing polyphenols reacted a blue reaction in FeCl$_3$-K$_3$[Fe(CN)$_6$] solution. The color reaction of *Penicillium oxalicum* (YMG1), *Simplilium* sp. (ZZ5), and *Colletotrichum* sp. (YMJ13)

FIGURE 2 | Antioxidant activities of the *P. oxalicum* **(A)**, *Simplicillium* sp. **(B)**, and *Colletotrichum* sp. **(C)** extracts. a: ABTS scavenging activity. b: Hydroxyl radical scavenging activity. c: DPPH radical scavenging activity. d: Superoxide anion radical scavenging activity. a–d: Significant differences between different groups ($p < 0.05$).

were blue, indicating that these strains had the potential to produce polyphenols, and were further selected for chemical and pharmacological studies.

Determination of Total Phenolic Content

Extraction of *Penicillium oxalicum* (*P. oxalicum*), *Simplilium* sp., and *Colletotrichum* sp. using different solvents allowed the collection of mixture with different total phenolic contents (**Table 3**). The TPC of the fungal extracts ranged between 12.29 ± 3.24 and 94.58 ± 2.70 mg GAE/g with *P. oxalicum* possessing higher polyphenol content than *Simplilium* sp. and *Colletotrichum* sp. For *P. oxalicum* and *Simplicillium* sp., ethyl acetate was the most effective solvent for polyphenol extraction, and the recovered polyphenols are 94.58 ± 2.70 and 58.64 ± 3.61 mg GAE/g, respectively. As for *Colletotrichum* sp., *n*-butanol was the effective solvent for the extraction of polyphenols, with 58.96 ± 4.10 GAE/g polyphenols. However, the petroleum ether extract of *P. oxalicum*, *Simplicillium* sp., and *Colletotrichum* sp. contained considerably less polyphenols with 21.67 ± 5.91, 12.29 ± 3.24, and 26.14 ± 0.32 mg GAE/g, respectively. Therefore, for *Colletotrichum* sp., polar solvents allowed the recovery of more polyphenol. For *P. oxalicum* and *Simplicillium* sp., the medium polarity solvents were more suitable for polyphenol extraction.

Antioxidant Activity

To further explore whether the extracts possess antioxidant activity, four different methods, namely ABTS radical scavenging assay, hydroxyl radical scavenging assay, DPPH radical scavenging assay, and superoxide anion radical scavenging assay, were used to evaluate the antioxidant activities of the ethyl acetate fractions, *n*-butanol fractions, petroleum ether fractions, and chloroform fractions extracted from the *P. oxalicum*, *Simplicillium* sp., and *Colletotrichum* sp. The different extracts of the fermentation broth of the three strains showed antioxidant activity to different degrees, and the antioxidant activities seemed to be correlated with their TPC. In addition, the ethyl acetate extract of *P. oxalicum* and *Simplicillium* sp., and the n-butanol extract of *Colletotrichum* sp. had the highest antioxidant activity.

The results of antioxidant activity are shown in **Figure 2** and **Table 3**. As seen in **Figure 2A**, all samples exhibited scavenging activity of ABTS, hydroxyl radical DPPH, and superoxide anion free radical at all concentrations in a concentration-dependent pattern. At 0.6 mg/mL, the scavenging rate of ABTS and hydroxyl radical of the *P. oxalicum* ethyl acetate extracts was similar to Vc and significantly higher than other extracts (**Figures 2Aa,b**; $P < 0.05$). As shown in **Table 3**, the ethyl acetate extracts of *P. oxalicum* exhibited the strongest scavenging activity (IC$_{50}$ $_{ABTS+.}$ = 0.03 ± 0.003 mg/mL; IC$_{50}$

$._{OH}$ = 3.38 ± 0.04 mg/mL; IC_{50} $_{DPPH}$ = 0.16 ± 0.002 mg/mL; IC_{50} $._{O_2^-}$ = 1.41 ± 0.22 mg/mL) followed by the *n*-butanol extracts (IC_{50} $_{ABTS+.}$ = 0.21 ± 0.04 mg/mL; IC_{50} $._{OH}$ = 1.22 ± 0.60 mg/mL; IC_{50} $_{DPPH}$ = 0.50 ± 0.05 mg/mL) ($p < 0.05$). Regarding the *Simplicillium* sp. extract, the IC_{50} of the ethyl acetate extracts in the four tests were significantly lower ($P < 0.05$) compared with the other three extracts, with IC_{50} of 0.18 ± 0.002, 3.37 ± 0.38, 0.04 ± 0.003, and 2.00 ± 0.11 for ABTS radical, hydroxyl radical, DPPH radical, and superoxide anion radical, respectively (**Table 3**). Moreover, the DPPH radical scavenging rate of the ethyl acetate extracts of *Simplicillium* sp. was close to Vc at the same concentration, especially at 0.2, 1, and 3 mg/mL (**Figure 2Bc**; $p < 0.05$). The results suggest that ethyl acetate extracts of *P. oxalicum* and *Simplicillium* sp. have the strongest antioxidant activity. However, at 3 mg/mL, only the *n*-butanol extracts from *Colletotrichum* sp. showed higher 50% scavenging rate of ABTS, hydroxyl radical, DPPH, and superoxide anion radical, and the best antioxidant activity was obtained with *n*-butanol, which IC_{50} was 0.29 ± 0.011, 0.67 ± 0.05, 0.67 ± 0.05, and 2.48 ± 0.52 mg/mL, respectively (**Figure 2C** and **Table 3**). The differences observed in the radical scavenging effects among the extracts could be attributed to the differences in their polyphenol content.

Antibacterial Activity

Antimicrobial activities of the extracts of *P. oxalicum*, *Simplicillium* sp., and *Colletotrichum* sp. extracts were tested against gram-positive and gram-negative bacteria (**Tables 4**, **5**).

The MIC of the samples was determined using the broth micro-dilution method. *P. oxalicum*, *Simplicillium* sp., and *Colletotrichum* sp. showed antibacterial effects against all the tested bacteria (**Table 4**). However, different extracts of the three fungi showed varying degrees of antibacterial and bactericidal activity depending on the solvent used (**Table 4**). As shown in **Table 4**, the ethyl acetate extracts of *P. oxalicum* had antibacterial effects against all the tested bacteria with a MIC between 0.5 and 2 mg/mL. However, the *n*-butanol, petroleum ether, and chloroform extracts of *P. oxalicum* had antibacterial activity only against *E. coli* and *S. aureus*, but not against antibacterial effect of *B. subtilis* and *P. aeruginosa*. Similarly, only the ethyl acetate extracts of *Simplicillium* sp. showed antibacterial effect against *E. coli*, *B. subtilis*, *P. aeruginosa,* and *S. aureus* with MIC of 0.5, 1, 2, and 1 mg/mL, respectively (**Table 4**). Regarding *Colletotrichum* sp., *n*-butanol and ethyl acetate extracts also inhibited all tested bacteria with MICs between 0.5 and 2 mg/mL (**Table 4**). The above results indicated that *P. oxalicum*, *Simplicillium* sp., and *Colletotrichum* sp. have a certain degree of antibacterial ability and that ethyl acetate can effectively extract bioactive compounds of *P. oxalicum*, *Simplicillium* sp., and *Colletotrichum* sp.

The MBCs of the fungal extracts are summarized in **Table 5**. Against *S. aureus*, *E. coli,* and *P. aeruginosa*, ethyl acetate extracts of the three strains had bactericidal activity, with MBCs between 1.0 and 2.0 mg/mL. In contrast, none of the extracts exhibited bactericidal activity against *B. subtilis*.

Acridine orange can freely penetrate the cell membrane of bacterial cells, bind to nucleic acids, and emit green fluorescence. As shown in **Figure 3**, untreated bacterial cell maintained

TABLE 4 | Minimum inhibitory concentration (MIC) (mg/mL) of the *P. oxalicum*, *Simplicillium* sp., and *Colletotrichum* sp. extracts.

Extracts	Gram-positive bacteria		Gram-negative bacteria	
	S. aureus	*B. subtilis*	*E. coli*	*P. aeruginosa*
P. oxalicum				
Ethyl acetate	0.5	2	0.5	1
n-Butanol	1	nd	0.5	2
Petroleum ether	2	nd	1	nd
Chloroform	2	nd	0.5	nd
***Simplicillium* sp.**				
Ethyl acetate	1	1	0.5	2
n-Butanol	Nd	1	1	nd
Petroleum ether	Nd	1	2	nd
Chloroform	Nd	nd	nd	nd
***Colletotrichum* sp.**				
Ethyl acetate	0.5	2	1	1
n-Butanol	1	2	0.5	0.5
Petroleum ether	2	nd	1	2
Chloroform	2	nd	2	2

nd, not detected (result higher 3.00 mg/mL).

TABLE 5 | Minimum bactericidal concentration (MBC) (mg/mL) of the *P. oxalicum*, *Simplicillium* sp., and *Colletotrichum* sp. extracts.

Extracts	Gram-positive bacteria		Gram-negative bacteria	
	S. aureus	*B. subtilis*	*E. coli*	*P. aeruginosa*
P. oxalicum				
Ethyl acetate	2	nd	2	2
n-butanol	nd	nd	2	nd
Petroleum ether	nd	nd	nd	nd
Chloroform	nd	nd	nd	nd
***Simplicillium* sp.**				
Ethyl acetate	2	nd	2	nd
n-Butanol	nd	nd	2	nd
Petroleum ether	nd	nd	nd	nd
Chloroform	nd	nd	nd	nd
***Colletotrichum* sp.**				
Ethyl acetate	2	nd	1	1
n-Butanol	2	nd	1	2
Petroleum ether	nd	nd	nd	nd
Chloroform	nd	nd	nd	nd

nd, not detected (result higher 3.00 mg/mL).

intact cell membrane and emitted intense green fluorescence (**Figure 3A**). In contrast, the green fluorescence significantly decreased after treatment with ethyl acetate extracts at a concentration of 2MIC, which indicated that the cell membranes of pathogenic bacteria (*S. aureus*, *E. coli,* and *P. aeruginosa*) induced by ethyl acetate extracts were damaged (**Figures 3B–D**). The above results suggested that ethyl acetate extracts can potently inhibit cell growth and differentiation by disrupting cell membranes.
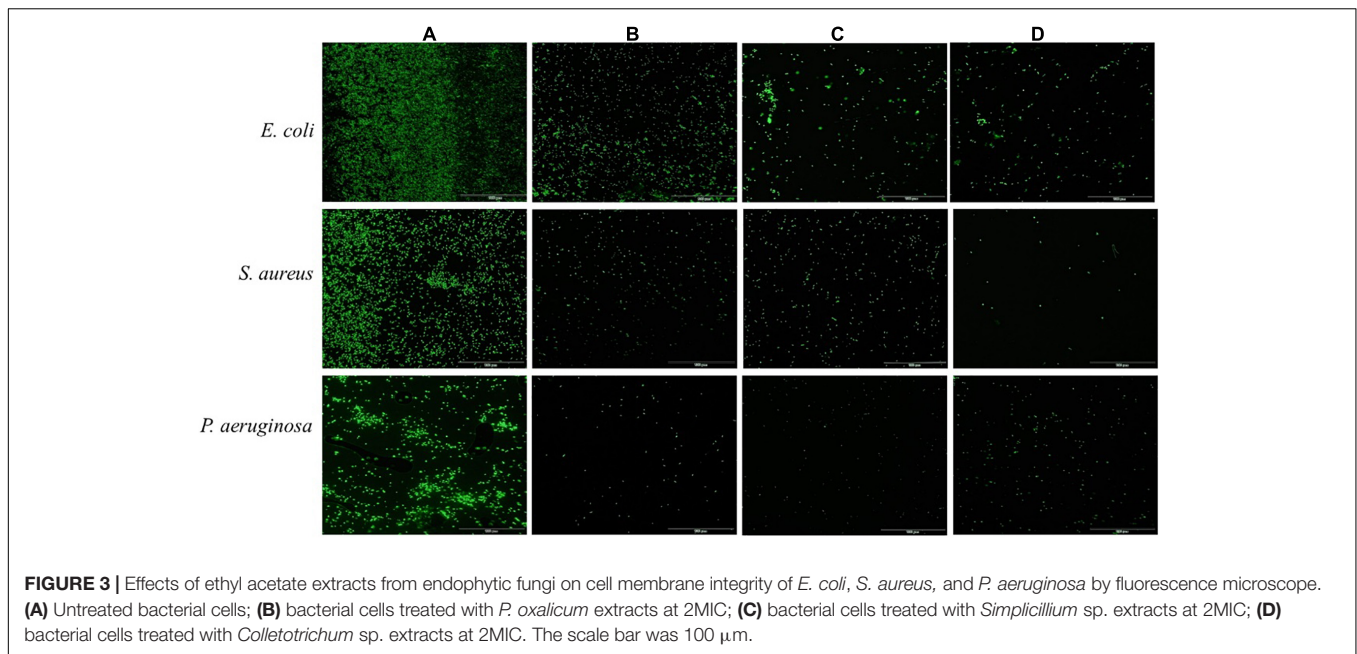
FIGURE 3 | Effects of ethyl acetate extracts from endophytic fungi on cell membrane integrity of *E. coli*, *S. aureus,* and *P. aeruginosa* by fluorescence microscope. **(A)** Untreated bacterial cells; **(B)** bacterial cells treated with *P. oxalicum* extracts at 2MIC; **(C)** bacterial cells treated with *Simplicillium* sp. extracts at 2MIC; **(D)** bacterial cells treated with *Colletotrichum* sp. extracts at 2MIC. The scale bar was 100 μm.

Liquid Chromatography-Mass Spectrometry

The ethyl acetate extracts of *P. oxalicum* and *Simplicillium* sp. and the *n*-butanol extracts of *Colletotrichum* sp. exhibited effective anti-microbial and antioxidant activity with high polyphenol content and were selected for compound analysis by liquid chromatography-mass spectrometry (LC-MS) analytical technique. **Table 6** shows the retention time, molecular weight, molecular formula, M/Z, and content of the identified chemical constituents presented in the extracts. The chromatograms are shown in **Supplementary Figure 3**. The identified compounds included phenolic acids (caffeic acid, chlorogenic acid, ferulic acid, etc.); flavonoids (zpigenin, phloretin, luteolin, hesperetin, etc.); fatty acids (palmitic acid, oleic acid, linoleic acid); organic acid (succinic acid, citric acid, etc.); and monosaccharide [sucrose, d-(−)-fructose, and d-(+)-glucose] (**Table 6**). As shown in **Table 6**, a total of 36 compounds were identified in *P. oxalicum*, of which hesperetin was the major compound with a concentration of 36.06 μmol/g. A total of 28 compounds were identified in *Colletotrichum* sp., and its major compound was succinic acid (8.11 μmol/g). This was followed by *Simplicillium* sp. extracts with 34 identified compounds. 4-hydroxyphenylpyruvic acid (17.23 μmol/g) and d (+)-phenylactic acid (4.01 μmol/g) were major compounds in *Simplicillium* sp. extracts. All in all, phenolic and organic acids were the most abundant compounds in the extracts of the three endophytic fungi, which may be closely related to the biological activity of the secondary metabolites of endophytic fungi.

DISCUSSION

The CX is a well-known Chinese medicinal plant with diverse phytochemicals such as ferulic acid, ligustrazine, and ligustilide,

which contribute to its medicinal properties (Yuan et al., 2020a). Endophytic fungi are not only one of the most important elements in plant micro-ecosystems but also a storehouse for natural products, including antibiotics, antioxidants, and anticancer agents, which could be an alternative source of secondary metabolites for medical and pharmacological applications (Strobel et al., 2002). A previous research showed that the different ages and tissues of host plants may influence the species composition of endophytic fungus community (Sieber, 2007). In this study, a total of 21 endophytic fungi belonging to 11 different genera were isolated from different parts of CX. This is similar to what was observed in CX by Wang et al. (2008), who also found *Penicillium*, *Fusarium*, *Aspergillus*, *Alternaria*, etc. Some species, such as *Nigrospora sphaerica* and *Sordariomycetes* sp., are exclusive to different plant parts. The tissue specificity of a particular endophyte suggests that certain species perpetuate within the specific chemistry or texture of a particular tissue due to the differences in anatomical structure and physiological conditions (Park et al., 2012). Only *Fusarium* sp. was present in the four sites studied. The pleiotropic colonization of *Fusarium* sp. may be related to its secreted metabolites (Pappas et al., 2018), which can protect host plants from biotic or abiotic stresses. The *Aporospora* sp. and *Sordariomycetes* sp. isolated from CX in this study have not been reported previously (Hu et al., 2016). These results increase the diversity of endophytic fungi in CX.

Polyphenols have hydroxyl groups and play a vital role in anti-oxidation, scavenging free radicals and other pharmacological activities (Gangwar et al., 2014). Therefore, the discovery of polyphenol-producing endophytic fungi could help meet the needs of the pharmaceutical and food industry. For instance, Govindappa et al. (2013) reported that organic extracts of *Penicillium* sp. present a large amount of phenolic compounds that act as antioxidants and could be used for medicinal or food preservation. Fill et al. (2010) discovered an effective antioxidant

TABLE 6 | The identification of the chemical composition of endophytic fungal extracts by LC-MS analysis.

S/N	Name of identified compound	Adducts	Molecular formula	RT (min)		Endophytic fungi (μmol/g)		
					M/S	P. oxalicum	Colletotrichum sp.	Simplicillium sp.
1	4-Methylcatechol	[M-H]⁻	$C_7H_8O_2$	2.671	123.044	1.35	0.41	0.10
2	Gentisic acid	[M-H]⁻	$C_7H_6O_4$	3.084	153.018	8.10	0.33	1.15
3	3,4-Dihydroxyphenylacetic acid	[M-H]⁻	$C_8H_8O_4$	2.673	167.034	5.73	1.73	0.39
4	Caffeic acid	[M-H]⁻	$C_9H_8O_4$	4.018	179.034	3.01	0.52	0.12
5	Alternariol	[M-H]⁻	$C_{14}H_{10}O_5$	5.978	257.045	12.64	0.05	0.57
6	Isorhapontigenin	[M-H]⁻	$C_{15}H_{14}O_4$	8.46	257.082	0.39	nd	nd
7	Apigenin	[M-H]⁻	$C_{15}H_{10}O_5$	9.207	269.045	4.56	nd	0.06
8	Genistein	[M-H]⁻	$C_{15}H_{10}O_5$	6.784	269.045	0.35	nd	0.01
9	Phloretin	[M-H]⁻	$C_{15}H_{14}O_5$	4.751	273.076	1.86	nd	0.02
10	Luteolin	[M-H]⁻	$C_{15}H_{10}O_6$	6.875	285.040	0.56	nd	0.01
11	Eriodictyol	[M-H]⁻	$C_{15}H_{12}O_6$	7.423	287.056	0.57	nd	nd
12	Hesperetin	[M-H]⁻	$C_{15}H_{10}O_7$	6.789	301.071	36.06	0.06	0.87
13	Neochlorogenic acid	[M-H]⁻	$C_{16}H_{18}O_9$	3.167	353.087	1.14	0.14	0.34
14	Cryptochlorogenic acid	[M-H]⁻	$C_{16}H_{18}O_9$	3.671	353.087	0.93	0.21	1.21
15	Chlorogenic acid	[M-H]⁻	$C_{16}H_{18}O_9$	3.94	353.087	0.11	0.04	0.12
16	Pyruvic acid	[M-H]⁻	$C_3H_4O_3$	0.917	87.007	1.06	2.25	0.69
17	Succinic acid	[M-H]⁻	$C_4H_6O_4$	1.412	117.018	16.22	8.11	3.47
18	Benzoic acid	[M-H]⁻	$C_7H_6O_2$	4.41	121.028	1.19	0.92	0.46
19	Itaconic acid	[M-H]⁻	$C_5H_6O_4$	1.192	129.018	1.19	0.50	0.36
20	2,4-Dihydroxybenzoic acid	[M-H]⁻	$C_7H_6O_4$	4.05	153.018	0.83	0.19	0.03
21	D(+)-Phenyllactic acid	[M-H]⁻	$C_9H_{10}O_3$	4.775	165.055	1.98	2.27	4.01
22	Theanine	[M-H]⁻	$C_7H_{14}N_2O_3$	3.898	173.092	1.10	nd	0.29
23	Indoleacetic acid	[M-H]⁻	$C_{10}H_9NO_2$	5.582	175.023	0.22	0.12	0.01
24	2-Isopropylmalic acid	[M-H]⁻	$C_7H_{12}O_5$	3.623	175.060	5.95	0.85	1.29
25	Esculetin	[M-H]⁻	$C_9H_6O_4$	5.524	177.018	0.01	0.02	2.94
26	D-(-)-Fructose	[M-H]⁻	$C_6H_{12}O_6$	0.874	179.055	1.53	0.36	1.91
27	D-(+)-Glucose	[M-H]⁻	$C_6H_{12}O_6$	1.099	179.055	1.67	0.45	2.84
28	Citric acid	[M-H]⁻	$C_6H_8O_7$	0.918	191.019	18.49	5.56	1.97
29	Ferulic acid	[M-H]⁻	$C_{10}H_{10}O_4$	4.757	193.050	1.17	0.11	1.10
30	Palmitic acid	[M-H]⁻	$C_{16}H_{32}O_2$	13.345	255.232	0.93	0.13	0.23
31	Alpha-linolenic acid	[M-H]⁻	$C_{18}H_{30}O_2$	12.177	277.217	1.57	0.03	0.08
32	Linoleic acid	[M-H]⁻	$C_{18}H_{32}O_2$	12.795	279.232	4.70	0.28	0.70
33	Oleic acid	[M-H]⁻	$C_{18}H_{34}O_2$	13.512	253.217	0.99	0.20	0.33
34	Maleic acid	[M-H]⁻	$C_4H_4O_4$	1.329	115.002	7.81	0.14	0.13
35	Juzirine	[M-H]⁻	$C_8H_{11}N_9O_3$	3.112	280.093	4.69	nd	0.02
36	4-Hydroxyphenylpyruvic acid	[M-H]⁻	$C_9H_8O_4$	4.364	179.034	0.25	1.20	17.23

nd, not detect.

compound, namely, phenylpropanoid amide, isolated from the endophytic fungus *Penicillium brasilianum*. Similarly, Tian et al. (2020) found that *Simplicillium* sp. ethyl acetate extracts have excellent antioxidant activity, which contains various chemical components. In the present study, *P. oxalicum*, *Simplicillium* sp., and *Colletotrichum* sp. isolated from CX were polyphenol-producing strains, which deserve further study.

Different solvents can recover compounds of different contents and components in the current study, we found polar and medium polarity solvents are more favorable to the extraction of low and high molecular weight polyphenols in endophytic fungus fermentation broth compared to those of non-polar solvent. This finding is consistent with a previous study (Scholz and Rimpler, 1989). The highest antioxidant activity

was observed in the *P. oxalicum* extracts, which may be linked to the concentrations of the chemical compounds and the compositions identified in the fungal extracts. Hesperidin was the main phenolic compound in the extracts of *P. oxalicum*, which has powerful radical scavenging activity, and it augmented the antioxidant cellular defenses *via* the ERK/Nrf2 signaling pathway as well (Parhiz et al., 2015). However, compared to the potency of the standard antioxidants Vc, the extracts of the endophytic fungi showed lower antioxidant activity. Moreover, the antioxidants used as standards are purified molecules, while the endophytic fungi extracts represent a group of mixtures containing different concentrations of substances.

Endophytic fungi have also been recognized as producers of antimicrobial substances, such as Simplicildone A and

Botryorhodine C isolated from the fungus *Simpilcillium* sp. PSU-H41 displayed antibacterial against *S. aureus* (Saetang et al., 2017). In our study, we observed that three isolated endophytic fungi showed bacteriostatic activity against the test strain, whereas different extract parts of endophytic fungi showed varying degrees of antibacterial effect. Data from the literature demonstrating that the antibacterial effects of endophytic fungi extracts are related to the extraction solvent (Mefteh et al., 2018), which corroborates our results. Among the endophytic fungi studied, *P. oxalicum* stood out for the best antibacterial and bactericidal activity, which could be attributed to organic acids and phenolic compounds. Among the compounds identified in the extracts of *P. oxalicum*, citric acid and hesperidin were the major compounds, followed by alternariol, succinic acid, and gentisic acid, which were higher than those of *Simplicillium* sp. and *Colletotrichum* sp. Gu (2009) have reported that alternariol from endophytic fungus *Alternaria brassicicola* possesses antibacterial properties. The antibacterial activity of citric acid against bacteria was attributed to its physical and chemical properties, reduced production of extracellular aggregates, and hydrophobicity of the cell surface (Zhang et al., 2019).

Antibacterial compounds can act on bacterial cell walls, plasma membranes, protein synthesis, nucleic acid metabolism, and DNA, thereby killing pathogenic bacteria (Li et al., 2015). Among the numerous antibacterial compounds, we have emphasized phenolic compounds, which exhibit great structural diversity, such as the presence, number and substitution position of hydroxyl groups, and the length of saturated side chains, which confer antibacterial activity to these compounds (Cushnie and Lamb, 2011; Rempe et al., 2017). For example, Rasch (2002) found that phenolic acid inhibited the activity of ribonucleic acid reductase, a key enzyme required for DNA synthesis, and that bacterial DNA synthesis was blocked. Our findings provided further evidence that extracts of *P. oxalicum*, *Simplicillium* sp., and *Colletotrichum* sp. displayed anti-biofilm activity against the tested strains with an almost complete destruction of the biofilm matrix.

The *P. oxalicum*, *Simplicillium* sp., and *Colletotrichum* sp. isolated from CX are promising endophytic fungi, and the chemical composition of endophytic fungus extracts have been identified by LC-MS. Some of the identified chemical components have been reported to have significant antimicrobial, antioxidant, and other biological activities, while others have not been reported, such as juzirine. In addition, some of these compounds with biological activity were isolated and identified from plants rather than microorganisms. For example, hesperidin, as an anti-inflammatory and antioxidant drug (Li and Schluesener, 2017), is traditionally extracted from abundant natural citrus fruits using large amounts of ethanol or methanol solvents, which is not conducive to sustainable development in the pharmaceutical industry. In our study, *P. oxalicum* can produce abundant secondary metabolites including phenolic acids, flavonoids, and fatty acids. Phenolic acids and flavonoids are important secondary metabolites that exert therapeutic effects (Winter et al., 2017; Adamczak et al., 2019). Fatty acids are able to reduce and resist the oxidative stress of free radicals through a series of physiological and biochemical reactions (Gülçin, 2012; Mocking et al., 2018). Previous studies have reported the isolation and characterization of Penicilisorin (Arunpanichlert et al., 2010) and *P*-hydroxybenzaldehyde (Schmeda-Hirschmann et al., 2005), an antibacterial compound from the endophytic fungi *Penicillium sclerotiorum* PSU-A13 and *Penicillium janczewskii*, respectively. Similarly, a coumarone antioxidant, pestacin and isopestacin, were isolated from the endophytic fungus *Pestalotiopsis microspora* (Strobel et al., 2002). These results suggested that *P. oxalicum* from CX containing many active compounds makes it a promising source of molecules for future studies. Interestingly, we also found that *P. oxalicum* strain can produce ferulic acid, chlorogenic acid, and caffeic acid, just like their host plant, indicating that *P. oxalicum* may play an important role in affecting the quality and quantity of the CX through a specific fungus-host interaction (Ding et al., 2018). In the future, because of the fast growth rate of endophytic fungi and the advantage of easy operation during fermentation (Ludwig-Müller, 2015), these active substances can be obtained by fermentation rather than from the plant tissues itself. Moreover, the production capacity of secondary metabolites of *P. oxalicum* and their biological effects could be increased by optimizing cultivation conditions or genetic engineering. In conclusion, in order to use the endophytic fungus of CX in medicine, more detailed studies are needed in future research.

CONCLUSION

In conclusion, the present study expanded the diversity of endophytic fungal species in CX and confirmed antioxidant and antimicrobial activities of several endophytic fungi extracts, among which the ethyl acetate extract of the endophytic fungus *P. oxalicum* stood out. The antioxidant and antibacterial activities of the *P. oxalicum* extracts are attributed to the phenolic compounds. Together, this study suggests that *P. oxalicum* from CX is a promising source for detection of natural antioxidants and antibacterial compounds. However, in order to use this endophytic fungus in medicine, more detailed studies need to be conducted.

DATA AVAILABILITY STATEMENT

The original contributions presented in the study are included in the article/**Supplementary Material**, further inquiries can be directed to the corresponding author/s.

AUTHOR CONTRIBUTIONS

ZT and YQ conceived and designed the experiments, performed the experiments, and prepared figures and/or tables. WC, ZZ, WL, QL, HY, YL, and YW performed the experiments, prepared the figures and/or tables. HuC conceived and designed the experiments, drafted the work, and approved the final draft. YX and YC analyzed the data and drafted the work. TB and

HoC analyzed the data and revised it critically for important content. All authors contributed to the article and approved the submitted version.

SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fmicb.2021.771000/full#supplementary-material

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