Chemical constituents and biological activities of endophytic fungi from *Fagopyrum dibotrys* were analyzed by LC–MS (#102630)

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Chemical constituents and biological activities of endophytic fungi from *Fagopyrum dibotrys* were analyzed by LC–MS

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Background. *Fagopyrum dibotrys*, a species of buckwheat, serves as a significant wild germplasm resource for food and feed; it has high nutritional and medicinal value and is enriched with natural products, including flavonoids, phenolic acids, coumarins, and alkaloids. Endophytic fungi in *F. dibotrys* have emerged as valuable sources of natural products. However, studies on the biological activity and chemical composition of these endophytic fungi remain limited. **Methods.** In this paper, a new method to obtain natural active ingredients by fermentation of endophytic fungi from medicinal plants was proposed. Then the antioxidant and pathogenic activities of the endophytic fungi extracts were determined in vitro. In addition, secondary metabolites produced by endophytic fungi with medicinal activity were analyzed by high performance liquid chromatography-tandem mass spectrometry (LC-MS). **Results.** Among the 95 endophytic fungal strains in *F. dibotrys*, 4 strains with high phenol yields were selected by reaction: *Alternaria alstroemeriae* (J2), *Fusarium oxysporum* (J15), *Colletotrichum karsti* (J74), and *Colletotrichum boninense* (J61). Compared with those of various extracts, the ethyl acetate fractions of *A. alstroemeriae* (J2), *F. oxysporum* (J15), and *C. boninense* (J61) exhibited superior antioxidant and antibacterial properties. The results indicated that the fungal extract was an excellent natural antioxidant and might be a potential antimicrobial agent. The DPPH free radical clearance of *A. alstroemeriae* was 94.96 ± 0.004%. These findings indicated that *A. alstroemeriae* had strong antioxidant activity. In addition, the extract of *A. alstroemeriae* had good antibacterial activity against *Escherichia coli* and *Staphylococcus aureus*, with MICs of 0.5 and 0.05 mg/mL, respectively. The chemical constituents of the ethyl acetate extract from *A. alstroemeriae* were further analyzed by liquid chromatography–mass spectrometry (LC–MS). We noted that *A. alstroemeriae* can

create a variety of medicinal substances that have high value in medicine, such as caffeic acid (884.75 ng/mL), 3-phenyllactic acid (240.72 ng/mL) and norlichexanthone (74.36 ng/mL).

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Abstract

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Methods. In this paper, a new method to obtain natural active ingredients by fermentation of endophytic fungi from medicinal plants was proposed. Then the antioxidant and pathogenic activities of the endophytic fungi extracts were determined in vitro. In addition, secondary metabolites produced by endophytic fungi with medicinal activity were analyzed by high performance liquid chromatography-tandem mass spectrometry (LC-MS).

Results. Among the 95 endophytic fungal strains in *F. dibotrys*, 4 strains with high phenol yields were selected by reaction: *Alternaria alstroemeriae* (J2), *Fusarium oxysporum* (J15), *Colletotrichum karsti* (J74), and *Colletotrichum boninense* (J61). Compared with those of various extracts, the ethyl acetate fractions of *A. alstroemeriae* (J2), *F. oxysporum* (J15), and *C. boninense* (J61) exhibited superior antioxidant and antibacterial properties. The results indicated that the fungal extract was an excellent natural antioxidant and might be a potential antimicrobial agent. The DPPH free radical clearance of *A. alstroemeriae* was 94.96 ± 0.004%. These findings indicated that *A. alstroemeriae* had strong antioxidant activity. In addition, the extract of *A. alstroemeriae* had good antibacterial activity against *Escherichia coli* and *Staphylococcus aureus*, with MICs of 0.5 and 0.05 mg/mL, respectively. The chemical constituents of the ethyl acetate extract from *A. alstroemeriae* were further analyzed by liquid chromatography–mass spectrometry (LC–MS). We noted that *A. alstroemeriae* can create a variety of

medicinal substances that have high value in medicine, such as caffeic acid (884.75 ng/mL), 3-phenyllactic acid (240.72 ng/mL) and norlichexanthone (74.36 ng/mL).

Discussion. In summary, many valuable active substances and medicinal substances can be obtained through the study of endophytic fungi of *F. dibotrys*.

Key words: endophytic fungi; microbe; antioxidant; antibacterial activity; chemical composition; *F. dibotrys*

Introduction

F. dibotrys, a perennial species within the genus Rhizopyrum, has significant medicinal and economic value. The plant is found mainly in China, India and Nepal and grows in river valleys, swamps and shrubs at altitudes between 250 and 3200 meters. At present, this plant is considered to have anticancer, anti-inflammatory, antioxidant and other properties [1]. *F. dibotrys* contains many flavonoids, terpenes, steroids, organic acids and volatile components, as well as essential amino acids and vitamins [2,3,4].

Endophytic fungi represent crucial resources for the development of new natural products. They provide essential means to discover pharmacodynamic compounds from secondary metabolites and solve the scarcity problem of traditional Chinese medicinal plants [5]. Endophytes, one of the sources of natural products, exhibit significant physiological activities [6]. Their primary components include polyketones, terpenes, steroidal fats, and phenols [7]. Their main effects include antioxidant [8], hypoglycemic [9], anticancer [10], weight loss [11], anti-inflammatory [12], and antimicrobial effects [13,14]. Moreover, both the growth rate and yield of endophytic fungi significantly increase under in vitro conditions [15]. Consequently, active substances can be produced more simply and economically by endophytic fungi in plants. The results of this study lay a foundation for the development of endophytic fungi with high efficiency and low toxicity [16].

Oxidative stress is caused by disturbances in the oxidative stress is gaining more attention. reactive oxygen species (ROS) balance and antioxidant mechanisms produced by the body during metabolism [17]. If antioxidant mechanisms in the body cannot effectively eliminate ROS, an imbalance in cell homeostasis can occur and cause further irreversible damage, such as cell dysfunction, protein damage and DNA damage. Studies have shown that oxidative stress is linked with diseases such as vascular dementia [18], kidney disease [19], diabetes, obesity, cancer, aging and osteoporosis [20]. Currently, the widely used antioxidants include butyl hydroxy anisole, dibutyl hydroxy toluene and tert-butylhydroquinone [21]. Although these substances have strong antioxidant activity, they have highly toxic side effects and are expensive; However, their application in the fields of food and medicine is limited [22]. In recent years, with attention given to food safety and health, natural antioxidants of plant origin have received attention due to their unique properties, such as easy extraction, safety, efficiency, and biological activity [23]. Consequently, natural antioxidants have become a focal point of research in food, medicine, and other fields.

Bacterial infections can cause a range of diseases that can affect multiple tissues and organs, such as the blood, lymphatic system, skin, liver, and heart. *Pseudomonas aeruginosa* is a class of gram-negative opportunistic bacteria. *Pseudomonas aeruginosa* is known for its easy colonization, rapid mutation, and multidrug resistance [25,26]. This bacterium is commonly associated with respiratory infections [27], pulmonary infection [28] and keratitis [29]. Clarifying the characteristics of bacteria, along with their clinical manifestations and treatment options for infections, is crucial for the prevention and control of bacterial diseases.

In recent years, secondary metabolites from endophytic fungi have not only replaced medicinal plants as a substantial resource for screening natural active compounds and lead compounds for new drugs but also have broad application prospects and research value in terms of biological control and other aspects [30]. This development offers a novel approach to discover new antibacterial agents from endophytic fungi. Furthermore, the extraction of endophytic fungal metabolites plays a crucial role in ensuring the rational use of valuable medicinal plant resources in China [31]. *F. dibotrys*, a valuable herb in traditional Chinese medicine, has a wide array of applications [5]. However, studies on endophytic fungi from *F. dibotrys* and their mechanisms of action are still rare and need to be further explored. In this context, the aim of our research is to clarify the antibacterial and antioxidant activities, as well as the chemical constituents, of endophytic fungal extracts from *F. dibotrys*.

Materials & Methods

2.1 Experimental Materials

In 2022, to research the wild germplasm resources of *F. dibotrys*, the biochemistry and molecular biology research group collected plants of 40 strains of *F. dibotrys* from 40 locations at different latitudes and longitudes in Southwest China (Sichuan Meishan city, Ya 'an city, Chengdu city, Deyang city, Leshan city and Liangshan Yi Autonomous Prefecture). To ensure the quality of the experimental materials, after the plants were uprooted, the samples were packed into plastic bags and immediately sent to the laboratory for further study.

2.2 Isolation of endophytic Fungi

The roots, stems and leaves of the plants were cut into small pieces. The samples were washed twice with distilled water, disinfected with 75% ethanol for 2 minutes, soaked with 5% sodium hypochlorite solution for 10 minutes, washed with 75% ethanol for 1 minute and finally washed twice with distilled water. When the samples were dry, they were added to potato dextrose agar medium (100 μg/L ampicillin was added to prevent bacterial contamination). After purification, the mixture was cultured at 28 °C for 5–7 days and preserved at 4 °C [32].

2.3 Screening phenol-producing endophytic fungi

The endophytic fungi were cultured in fresh potato dextrose broth for one week (160 rpm, 28 °C). The filtrate of the culture medium was obtained by filtering the culture medium with sterile gauze. The conditions for the colorimetric reaction were consistent with those described previously [32]. In simple terms, 0.1% $FeCl_3$ and 0.1% K_3 $[Fe(CN)_6]$ were mixed and added to the culture filter. The blue coloration in the final color reaction indicated the production of polyphenols by the endophytic fungal fermentation mixture.

2.4 Identification of phenol-producing endophytic fungi

The color, humidity, pigmentation and flatness of the fungal strains were observed using a CX21 FS1 microscope (Olympus, Tokyo, Japan). The phenol-producing endophytic fungi were identified by the ITS strain identification method. Fungal DNA was obtained following the directions from the kit. PCR amplification was subsequently performed using an ITS1-forward primer (TCCGTAGGTGAACCTGCGG) and an ITS4-reverse primer (TCCTCCGCTTATTGATATGC) [33]. The PCR products were subsequently sent to Chengdu Qingke Biosequencing Technology Company for sequencing. Next, the ITS region sequences were compared with existing species sequences in the GenBank database. The phylogenetic tree of phenol-producing endophytic fungi was constructed in MEGA 11.0 software.

2.5 Preparation of endophytic fungus fermentation fluid extract

The fermentation mixture was centrifuged at 8000 rpm and 4 °C for 15–20 min. After centrifugation, the precipitate was discarded, and the supernatant was collected. The supernatant was then filtered through a 0.22 μm aqueous filter membrane and set aside for further study [34]. The fermentation mixture was extracted with equal volumes of ethyl acetate, n-butanol, petroleum ether, chloroform and other solvents for 10 minutes for a total of 3 times. The extracted liquid was then transferred to a rotary evaporator, concentrated under reduced pressure, and further processed using a freeze dryer. The final product was dissolved in dimethyl sulfoxide (DMSO) and subjected to testing for biological activity.

2.6 Determination of total phenol content

The gallic acid standard solution was prepared according to a previously described method [35]. Water (1 mL) and sample mixture (1 mL) were added to a beaker. Subsequently, 0.5 mL of Folin–Ciocalteu reagent was added. After 5 min, 1 mL of 20% sodium bicarbonate reagent was added, and then distilled water was added to 10 mL. The absorbance value (A_{760}) was obtained after reacting for 2 hours. A standard curve was drawn with the absorbance as the vertical coordinate and the gallic acid mass concentration as the horizontal coordinate: $y = 0.00302 \times -0.00978$, $R^2 = 0.9905$.

2.7 Antioxidant activity

In this study, we evaluated the antioxidant activities of six different concentrations of the extract (0.2, 0.4, 0.6, 0.8, 1.0, and 2 mg/mL). The assessment methods included DPPH, ABTS, and hydroxyl radical- and superoxide anion-scavenging assays. Ascorbic acid (Vc) was used as a positive control. Three replicates of each analysis were performed to ensure the reliability of the results.

2.7.1 2,2-Diphenyl-1-picrohydrazyl radical-scavenging activity

In accordance with the methods described by Gauchan [36], 150 μL of 0.2 mM DPPH was mixed with a sample mixture with a concentration gradient of equal volume. After 30 min of light-blocking treatment, 300 μL of the mixture was placed on the enzyme label plate, and the absorbance at 517 nm was detected.

The DPPH free radical clearance was calculated as follows:

$$\text{Clearance rate}/\% = [1 - (A_b - A_a)/A_c] \times 100\%$$

where A_c represents the absorbance of 150 μL of DPPH solution and 150 μL of anhydrous ethanol. A_b represents the absorbance of 150 μL of DPPH solution mixed with 150 μL of gradient sample solution. A_a represents the absorbance of 150 μL of gradient sample solution in 150 μL of anhydrous ethanol.

2.7.2 Hydroxyl radical-scavenging activity

In accordance with the methods described by Dhayanithy [37], 150 μL samples with different concentrations were added to 50 μL of 8 mM ferrous sulfate, 50 μL of 8 mM salicylic acid and 50 μL of 8 mM H_2O_2. The mixture was incubated at 37 °C for 20 min. Finally, 300 μL of the mixture was placed on the enzyme-coated plate, and the absorbance at 510 nm was detected.

The scavenging rate of hydroxyl free radicals was calculated as follows:

$$\text{Clearance rate}/\% = [1 - (A_b - A_a)/A_c] \times 100\%,$$

where A_b represents the absorption value of the sample mixture containing 150 μL and the reaction mixture containing 150 μL. A_c represents the absorption value of 150 μL distilled water and 150 μL reaction mixture. A_a represents the light absorption value after the hydrogen peroxide was replaced with distilled water.

2.7.3 ABTS free radical-scavenging activity

In accordance with the methods described by Santos [38], the ABTS mother liquor was obtained by absorbing ABTS (7 mM) and adding 2.45 mM potassium persulfate, and the mixture was incubated at 28 °C for 18 h. The ABTS mother liquor was diluted with phosphoric acid (PBS) solution until the absorption (734 nm) reached 0.7 ± 0.02, and the ABTS working liquor was prepared. For each sample, 150 μL of ABTS working mixture was added. After 30 min of protection from light, 300 μL of the mixture was placed on the enzyme-coated plate, and the absorbance at 734 nm was detected.

The scavenging rate of ABTS free radicals was calculated as follows:

Clearance rate/% = $[1- (A_b-A_a)/A_c] \times 100\%$

where A_c represents the absorbance of 150 μL of ABTS solution and 150 μL of anhydrous ethanol. A_b indicates the absorbance of 150 μL of ABTS solution mixed with 150 μL of sample. A_a represents the absorbance of a 150 μL sample with 150 μL anhydrous ethanol.

2.7.4 Superoxide anion radical-scavenging activity

In accordance with the methods described by Wang [39], 300 μL of Tris-HCl buffer (pH 8.2, 0.05 M) was mixed with 150 μL of sample mixture and incubated at 25 °C for 10 min. Fifty microliters of pyrogallol (25 mM) was quickly added. After 4 min, 50 μL of HCl (8 M) was added to terminate the reaction. The absorption value of the 300 μL mixture was measured at 320 nm.

The superoxide anion radical-scavenging rate was calculated as follows:

Clearance rate/% = $[1- (A_b-A_a)/A_c] \times 100\%$

where A_b represents the absorbance of 150 μL of sample or 150 μL of reaction mixture. A_c represents the absorbance of 150 μL of distilled water or 150 μL of reaction mixture. A_a represents the absorbance of distilled water instead of pyrogallol.

2.8 Antibacterial activity

2.8.1 Minimum inhibitory concentration

The antibacterial activities of the fungal extracts were determined using the Oxford cup method. *E. coli*, *Pseudomonas aeruginosa*, *Bacillus subtilis* and *Staphylococcus aureus* were incubated in sterile nutrient broth at 37 °C for 12 h, and 150 μL of the suspension was uniformly coated on a Petri dish. Once the bacterial mixture was dry, each Petri dish was divided into three parts, and a sterilized Oxford cup was placed in each section. Each fungal extract was diluted with nutrient broth (0.2–3 mg/mL). Then, 150 μL of extract, 0.5% DMSO and 100 μg/L chloramphenicol were added to the Oxford cup for the negative and positive controls, respectively. The culture plate was placed horizontally in a constant-temperature incubator and cultured at 37 °C for 24 h [40]. After incubation, the results were observed, and the diameter of the inhibition zone was recorded. If the diameter was greater than 7.8 mm, the solution in the Oxford cup was considered to have an inhibitory effect.

2.8.2 Minimum bactericidal concentration

A mixture of 100 μL of fungal extract with antibacterial activity (0.2–3 mg/mL) and 100 μL of bacterial suspension was inoculated into aseptic nutrient agar and incubated at 37 °C for 24 h. The number of bacterial colonies on the medium was then counted. If the number of colonies was less than 10, the agent was considered to have bactericidal properties [41].

2.8.3 Fluorescence microscopy

According to Paul, biofilms can be effectively observed by fluorescence microscopy. Four species of bacteria were treated with the endophytic fungal extract at a concentration equivalent to 2 MICs. The treated bacteria were then placed on a slide and incubated at 37 °C. After 48 hours, the slide was rinsed 4 to 5 times with normal saline water to remove any nonadherent

bacteria. The biofilm generated on the slide was stained with 0.1% acridine orange. The formation and characteristics of the biofilms were then examined under a fluorescence microscope (Olympus, Tokyo, Japan).

2.9 Analysis of the bioactive compounds by LC–MS

The compounds were isolated using an ACQUITY UPLC HSST3 column (2.1×100 mm, internal diameter). The mobile phase consisted of 0.05% formic acid (A) and acetonitrile (B) and was mixed at a rate of 0.3 mL per minute.

The gradient elution procedure was as follows: 5% acetonitrile in water at 0 to 1 min. From 2 to 13:50 min, the acetonitrile concentration was increased from 5% to 95%. In the phase from 13:50 to 16 minutes, the acetonitrile concentration was decreased from 95% to 5%. For mass spectrometry (MS) detection, ionization was performed in ESI^+ mode (heater temperature, 300 °C; sheath gas flow rate, 45 arb; aux gas flow rate, 15 arb; sweep gas flow rate, 1arb; spray voltage, 3.0 kV; capillary temperature, 350 °C; S-Lens RF level, 30%) and ESI- mode (heater temperature, 300 °C; sheath gas flow rate, 45 arb; aux gas flow rate, 15 arb; sweep gas flow rate, 1 arb; spray voltage, 3.2 kV; capillary temperature, 350 °C; and S-Lens RF level, 60%). Full Scan (MS1) was performed from 70–1050 m/z with a resolution of 70,000, and data-dependent two-stage mass spectrometry (DD-MS2, TOPN = 10) with a resolution of 17,500 was used. The acquired mass spectra were analyzed using Compound Discoverer 2.0 (Thermo Scientific), and integrated databases, including mzcloud, metlin, and hmdb, were employed for the untargeted detection of secondary metabolites.

2.10 Data analysis

The data are expressed as the means ± standard deviations from three independent sets of observations. Single-factor variance analysis (ANOVA) and Duncan's multiple range test were performed using SPSS version 26.0. A p value of less than 0.05 ($P < 0.05$) was considered statistically significant for determining differences between groups.

Results

3.1 Screening of endophytic fungi that produce polyphenols

Polyphenol-producing fungi were screened using the Folin–Ciocalteu color rendering test. Polyphenols in fermentation broth can produce a blue color on $FeCl_3$-K_3 $[Fe(CN)_6]$, as shown in Supplementary Figure S1. Among the tested strains, 9 exhibited a blue reaction with the chromogenic agents. Among these, 4 strains presented the deepest coloration, providing preliminary evidence of their high phenol production capabilities. These strains were further identified for their potential in subsequent chemical and pharmacological studies.

3.2 Identification of endophytic fungi that produce polyphenols

Following purification, the morphological characteristics of the endophytic fungi on the agar plates were observed, as detailed in Supplementary Table S1. The selected mycelium samples were then subjected to microscopic observation and identification, as shown in Supplementary Figure S2. The ITS rDNA sequences of the fungi were subsequently identified and matched, and the results are presented in Table 1. The sequence similarity among these identified fungi was found to be at least 99%. The phylogenetic tree of the phenol-producing strains isolated from *F. dibotrys* is depicted in Figure 1. The strains were identified as *A. alstroemeriae* (J2), *F. oxysporum* (J15), *C. karsti* (J74), and *C. boninense* (J61).

3.3 Determination of total phenol content

Extracts of *A. alstroemeriae* (J2), *F. oxysporum* (J15), *C. karsti* (J74) and *C. boninense* (J61) were treated with different solvents. The resulting mixtures contained different total phenols, as detailed in Table S2 and illustrated in Figure 2. The total phenol contents of the fungal extracts ranged from 13.75 ± 5.25 to 135.25 ± 0.33 mg GAE/g. *A. alstroemeriae* had a higher polyphenol content than did *F. oxysporum*, *C. karsti* and *C. boninense*. Among the four solvents, ethyl acetate was the most effective, yielding 135.25 ± 0.33 mg GAE/g for *A. alstroemeriae*, 92.74 ± 4.68 mg GAE/g for *F. oxysporum*, and 129.64 ± 4.28 mg GAE/g for *C. boninense*. For *C. karsti*, the use of n-butanol had greater efficiency; its extraction rate was 97.76 ± 4.31 mg GAE/g. *A. alstroemeriae*, *F. oxysporum*, *C. karsti* and *C. boninense* extracted from petroleum ether had lower polyphenol contents, with values of 26.49 ± 7.25, 53.96 ± 4.31, 13.75 ± 5.25 and 28.96 ± 6.55 mg GAE/g, respectively.

Consequently, polar solvents were more effective at extracting higher quantities of polyphenols from *A. alstroemeriae*, *F. oxysporum*, and *C. boninense*. In contrast, moderately polar solvents were more suitable for extracting polyphenols from *C. karsti*.

3.4 Antioxidant activity

Table S2, Figure 3, Figure 4, Figure 5 and Figure 6 show the antioxidant activities. As shown in Figure 3, the scavenging abilities for ABTS, hydroxyl free radicals, DPPH free radicals and superoxide free radicals increased with increasing extract concentration. At a concentration of 2 mg/mL, the ethyl acetate extracts from *A. alstroemeriae* exhibited more potent antioxidant effects than did the other organic solvents. In the four types of free radical assays, the scavenging capacity of the ethyl acetate extract for DPPH was almost equivalent to that of vitamin C (Vc), reaching the highest level (Figure 3c, $P<0.05$).

Table S2 shows that ethyl acetate had the strongest scavenging ability after extraction of *A. alstroemeriae*, where the $IC_{50\ DPPH}$ was 0.02048 ± 0.009 mg/mL, the $IC_{50\ ABTS}$ was 0.049 ± 0.005 mg/mL, the $IC_{50\ \cdot OH}$ was 0.08 ± 0.001 mg/mL, and the $IC_{50\ \cdot O2^-}$ was 0.28 ± 0.005 mg/mL). For n-butanol extract, the $IC_{50\ ABTS}$ was 0.034 ± 0.002 mg/mL, the $IC_{50\ \cdot OH}$ was 0.96 ± 0.02 mg/mL, the $IC_{50\ DPPH}$ was 0.14368 ± 0.004 mg/mL, and the $IC_{50\ \cdot O2^-}$ was 1.54 ± 0.004 mg/mL.

For *F. oxysporum*, ethyl acetate extraction had the best antioxidant effect and the lowest IC50 value ($P < 0.05$). The $IC_{50\ ABTS}$ was 0.051 ± 0.0002, the $IC_{50\ \cdot OH}$ was 0.25 ± 0.006, the $IC_{50\ DPPH}$ was 0.08 ± 0.001, and the $IC_{50\ \cdot O2^-}$ was 0.65 ± 0.041 (Table S2). For *C. karsti*, n-butanol extraction had the best antioxidant effect and the lowest IC50 ($P < 0.05$). The $IC_{50\ ABTS}$ was 0.098 ± 0.001, the $IC_{50\ \cdot OH}$ was 0.7 ± 0.005, the $IC_{50\ DPPH}$ was 0.08 ± 0.005, and the $IC_{50\ \cdot O2^-}$ was 0.66 ± 0.039 (Table S2). For *C. boninense*, ethyl acetate extraction had the best antioxidant effect and the lowest IC50 value ($P < 0.05$). The $IC_{50\ ABTS}$ was 0.073 ± 0.003, the $IC_{50\ \cdot OH}$ was 0.57 ± 0.005, the $IC_{50\ DPPH}$ was 0.01 ± 0.032, and the $IC_{50\ \cdot O2^-}$ was 0.23 ± 0.006 (Table S2).

Our results showed that ethyl acetate was the most effective extraction agent. The endophytic fungi responded to antioxidant mechanisms by scavenging free radicals. The ability to effectively remove free radicals and protect cells from oxidative damage may be attributed to the different polyphenol contents in the various extracts.

3.5 Antibacterial activity

The inhibitory effects of extracts of *A. alstroemeriae*, *F. oxysporum*, *C. karsti* and *C. boninense* were measured using the four bacteria mentioned in section "3.4 Antioxidant activity". *A. alstroemeriae*, *F. oxysporum*, *C. karsti*, and *C. boninense* had antibacterial effects on all the tested bacteria. However, the antibacterial efficacies varied depending on the solvent used for extraction, as detailed in Tables 2 and 3. Table 2 shows that *A. alstroemeriae* extracted

with ethyl acetate had antibacterial effects on the tested bacteria, with MIC values between 0.5 and 2 mg/mL. Each of the extracts had an inhibitory effect on *E. coli*. Moreover, the n-butanol extract also had a good inhibitory effect on the tested bacteria, except for *P. aeruginosa.* Only *S. aureus* and *E. coli* were strongly inhibited by the use of trichloromethane. The petroleum ether extract had a strong inhibitory effect only on *E. coli*. For *F. oxysporum*, only the ethyl acetate extract had an inhibitory effect on the tested bacteria. For *C. karsti*, only the n-butanol extract inhibited the tested bacteria. The ethyl acetate extract of *C. boninense* showed strong antibacterial activity. The MIC values were between 0.5 and 2 mg/mL. All extracts had inhibitory effects on *S. aureus* and *E. coli*. These results indicated that ethyl acetate could be used to extract *A. alstroemeriae*, *F. oxysporum* and *C. boninense*.

Table 3 shows the MBCs. *A. alstroemeriae*, *F. oxysporum* and *C. boninense* showed good bacteriostatic effects after extraction with ethyl acetate, except for *P. aeruginosa*. The concentrations of MBCs ranged from 1.0–2.0 mg/ml. There was no obvious inhibitory effect on *P. aeruginosa*. Moreover, the four extracts of *C. karsti* showed no bactericidal activity against the four kinds of bacteria, and the four different extracts showed no antibacterial activity.

Acridine orange can bind to nucleic acids to produce green fluorescence. As shown in Figure 7, bacterial cells not treated with the fungal extract remained active and emitted strong green fluorescence (Figure 7A). In contrast, when the bacterial cells were treated with the fungi extracted with ethyl acetate, a reduction in fluorescence was observed. These results indicated that the use of ethyl acetate as the extraction agent achieved antibacterial effects by destroying the bacterial membrane but had no effect on *P. aeruginosa* (Figure 7B–D). These findings implied that ethyl acetate extracts can effectively inhibit cell proliferation and disrupt cellular processes, likely through interactions with the cell membrane structure.

3.6 Liquid chromatography–mass spectrometry

The chemical constituents of *A. alstroemeriae*, *F. oxysporum*, *C. karsti* and *C. boninense* were determined by LC–MS to explore the functions of their metabolites. The relationships among their metabolites and their antibacterial and antioxidant activities were determined, and the results are presented in Table 4. Chromatograms of the metabolites of the four fungi are shown in Supplementary Figure S3. The compounds detected by LC–MS included phenolic acids, such as caffeic acid, syringic acid, and ferulic acid; hydroxybenzoic acids, such as gallic acid, gentian acid, haematommic acid; flavonoids (quercetin and taxifolin); coumarins (aesculetin); phenylacetic acids (vanillin, homogentisic acid, and homovanillic acid); anthrones (e.g., hematommone and norlichexanthone); and simple phenols (4-methylcatechol and catechol). In addition, organic acids, such as citric acid, azelaic acid, ala-phe, alpha-linolenic acid, and acetic acid; hormones such as epinephrine, (±)-abscisic acid, and indole-3-acetic acid; and simple sugars such as d-fructose and d-ribose were included.

A total of 52 compounds were identified in *A. alstroemeriae*, with caffeic acid, 3-phenyllactic acid, and norlichexanthone being the predominant compounds at concentrations of 884.75 ng/mL, 240.72 ng/mL and 74.36 ng/mL, respectively. A total of 47 compounds were identified from *F. oxysporum*, the main component of which was ferulic acid (44.84 ng/mL). Fifty-one compounds were identified from the *C. karsti* extract, the main component of which was 3-phenyllactic acid (334.27 ng/mL). Fifty-five compounds were identified from *C. boninense* extracts, the main components of which were caffeic acid (36.75 ng/mL), ferulic acid (37.98 ng/mL) and gentisic acid (46.98 ng/mL). In conclusion, the metabolites of these four fungal

strains included primarily phenolic and organic acids, which may contribute to their biological activity.

Discussion

F. dibotrys, a variety of buckwheat, is renowned as both a medicinal and edible plant, exemplifying the concept of food–medicine homology. This perennial herb predominantly grows in hillside grasslands and forest understories in northern China. Characterized by its cold and bitter taste, *F. dibotrys* is believed to influence the stomach and liver channels in traditional practices, offering detoxification benefits, blood pressure reduction, and intestinal health improvements [43]. *F. dibotrys* contains flavonoids, terpenoids and enzymes [44]; the flavonoids include mainly rutin, quercetin, and epicatechin.

Endophytic fungi have the unique ability to survive in plant tissues without causing harm to their host. They can produce a variety of bioactive substances during growth and have the potential for new drug development. Endophytes are also important sources of natural products [45]. Studies have shown that polyphenols have antitumor activity, which provides new ideas for the development of drugs for cancer prevention and treatment [46]. In addition, phenolic substances play important roles in antioxidation, free radical scavenging, and other pharmacological activities [47].

Consequently, the development of endophytic fungi that produce polyphenolic compounds holds considerable potential in the fields of medicine, food, and beyond. For example, Marchut [35] reported that *Bacillus cereus* and *Bacillus mycoides*, which were isolated from Urtica dioica, may be potential sources of biosurfactants and polyphenols. Marsola [48] obtained Phomopsis *archeri* from *Brunfelsia uniflora*, a fungus that produces cellulase and lassase and has antioxidant effects. *Coriolopsis rigida,* a fungus isolated from rice, can yield hydroxyphenylacetamide, which has shown potent antioxidant activity [49].

In this study, we focused on the phenol-producing properties of endophytic fungi from *F. dibotrys*. The endophytic fungi *A. alstroemeriae*, *F. oxysporum*, *C. karsti* and *C. boninense*, which have high phenol yields and are particularly worthy of further investigation, were selected. Our findings indicated that, compared with nonpolar solvents, both small and large polyphenols were more efficiently extracted using polar and medium-polar solvents. This observation was consistent with previous research in the field [50].

The extract of *A. alstroemeriae* presented the highest antioxidant activity, and caffeic acid, a component of this extract, has been recognized for its effectiveness against bacterial, fungal, viral, and other diseases [51]. caffeic acid is not only a potent antioxidant but also has anticancer and anti-inflammatory effects [52]. Compared with that of the conventional antioxidant vitamin C (Vc), the antioxidant capacity of the *A. alstroemeriae* extract was significantly lower. Standard antioxidants are purified small molecules, whereas plant endophytic fungal extracts are mixtures of multiple ingredients. The natural secondary metabolites within these extracts have emerged as significant sources of new antimicrobial agents because of their unique biological activities. For example, the endophytic fungus *Alternaria* sp. of *Salvia miltiorrhiza* had strong antibacterial effects on experimental bacteria, with the lowest inhibitory concentrations ranging from 86.7 to 364.7 μM [53].

Our study revealed that four endophytic fungal strains exhibited inhibitory effects on the tested bacterial strains, although the extent of inhibition varied among the different extracts. In recent years, researchers have reported that the inhibitory effect of endophytic fungi on microorganisms is closely related to the extraction solvent. Debalke [54] reported that the ethyl acetate extract of *Colletotrichum* sp. had inhibitory effects on *Escherichia coli* and

Staphylococcus aureus, and our results were consistent with these findings. Among the endophytes studied, *A. alstroemeriae* presented the best antioxidant and bactericidal activities. This may be related to the presence of organic acids and phenolic compounds in the extracts. Among all the ingredients, 3-phenyllactic acid and norlichexanthone were the principal antibacterial compounds. Norlichexanthone has been shown to have antibacterial benefits; for example, it can inhibit the formation of *Staphylococcus aureus* biofilms and reduce virulence gene expression [55]. Techaoei [56] reported the antibacterial potential of 2-naphthalenemethanol produced by the plant endophytic fungus *Aspergillus cejpii*.

Bacterial cells rely on their membrane structure for proper internal activities. Antimicrobial compounds can kill pathogenic bacteria by destroying cell membranes[57]. Among them, phenolic substances have abundant hydroxyl group types, numbers, substitution positions, and saturated side chains and have good antibacterial performance. Phenolic substances have good lipid solubility and can directly enter bacterial cell membranes and interfere with or even destroy the membrane structure, thus inhibiting the adhesion of pathogenic bacteria to host cells [58,59]. For example, in a study by Naveed [60], phenolic acid (chlorogenic acid) was found to play an important role in human diseases not only as antibacterial agent but also in regulating lipid metabolism, sugars and other pathological processes in hereditary and healthy metabolic diseases. Our findings further confirmed that the extracts from *A. alstroemeriae*, *F. oxysporum*, and *C. boninense* possessed robust antibiofilm capabilities, potentially leading to their complete eradication.

A. alstroemeriae, *F. oxysporum*, *C. karsti*, and *C. boninense* are useful endophytic fungi. Substances produced by endophytic fungi were further analyzed by LC–MS. Notably, some of the substances identified are very difficult to extract directly from the plant. However, endophytic fungi can act as containers to aid in production. Quercetin, a flavonol compound known for its tumor-inhibitory, free radical-fighting, antioxidant, antibacterial, and anti-inflammatory properties [61], is traditionally extracted directly from plants. However, this extraction process is difficult and costly, posing challenges to medical and economic advancements. Our results suggest that endophytic fungi may serve as effective alternatives for the generation of these natural products. Through the fermentation of *A. alstroemeriae*, valuable compounds such as phenolic acids, flavonoids, organic acids, and auxins can be produced.

Phenolic acids are biologically active compounds known for their lack of adverse side effects [62]. Coumarin, owing to its antioxidant properties, can mitigate oxidative damage and influence various physiological and biochemical processes [63]. The hydroxyl group present in hydroxybenzoic acid enhances its antioxidant efficacy [64]. Flavonoids can directly damage the bacterial envelope and can also act on specific molecular targets of these microbes [65]. Plant hormones regulate plant growth, development, and stress response [66].

Based on the above results, *A. alstroemeriae* from *F. dibotrys* was determined to have strong biological activity; thus, this strain might be a promising molecular source for future studies. Notably, secondary metabolites such as paclitaxel, a potent anticancer drug, inhibit mitosis and induce apoptosis, making it one of the most successful natural anticancer agents [67,68]. Quercetin, abscisic acid, and indoleacetic acid may be related to the growth mechanism of host plants[69]. Quercetin regulates IAA oxidation by delaying the dioxygenase activity of auxin oxidation 1 (DAO2) proteins, which belong to the 1-oxyglutaric acid and Fe(II)-dependent oxygenase superfamily, to mediate auxin signaling in plants [70]. Abscisic acid plays important roles in several physiological processes in plants, such as stomatal closure, epidermal wax accumulation, leaf senescence, bud dormancy, seed germination, osmoregulation, and growth

inhibition [66]. Indoleacetic acid is a plant hormone that not only regulates plant growth and development but also plays important roles in microbe–plant interactions and interacts with the metabolites of Xanthomonas aeruginosa [71,72]. These results suggest that *A. alstroemeriae* may have positive effects on the growth quality and yield of *F. dibotrys* through its interaction with specific fungal hosts.

Building on these insights, we propose a novel approach that leverages the inherent advantages of endophytic fungi, namely, their high utilization value, compact size, and rapid anabolic capabilities. Through the optimization of fermentation conditions, we can expedite and increase the production of target metabolites, thereby increasing their biochemical efficacy [73,74]. Therefore, the medicinal value of endophytic fungi of *F. dibotrys* warrants further exploration and study.

Conclusions

This study revealed that the endophytic fungus *F. dibotrys* has potent antioxidant and antibacterial properties. Specifically, the phenolic compounds in *A. alstroemeriae* extracts contribute to these antioxidant and antibacterial activities, whereas flavonoids, including compounds such as paclitaxel, offer anticancer benefits. In summary, our findings suggest a novel approach to harness *F. dibotrys* for the extraction of innovative medicinal substances. In particular, *A. alstroemeriae* within *F. dibotrys* has emerged as a promising candidate for natural antioxidant and antibacterial applications. Nonetheless, the broader application of endophytic fungi in medicine warrants additional, in-depth research.

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Table 1(on next page)

Table 1 Study on endophytic strains of phenol-producing fungi from *F. dibotrys*.

Table 1 Study on endophytic strains of phenol-producing fungi from *F. dibotrys*.

NO	Genus	Most closely related strain	Ident (%)	Accession.
J2	*Alternaria sp.*	*A. alstroemeriae*	100.00 %	OP482339.1
J15	*Fusarium sp.*	*F. oxysporum*	100.00 %	OP714469.1
J74	*Colletotrichum sp.*	*C. karsti*	99.65 %	OQ652534.1
J61	*Colletotrichum sp.*	*C. boninense*	100.00 %	MF062469.1

Table 2(on next page)

Table 2 Minimum inhibitory concentration (MIC) (mg/mL) of the J2□J15□J74 and J61 extracts.

Table 2 Minimum inhibitory concentration (MIC) (mg/mL) of the J2、J15、J74 and J61 extracts.

Extracts	Gram-positive bacteria		Gram-negative bacteria	
	S.aureus	*B. subtills*	*E. coli*	*P. aeruginosa*
J2				
Ethyl acetate	0.5	2	0.5	1
n-Butanol	2	Nd	1	2
Chlorom	2	Nd	1	Nd
Petroleum ether	Nd	Nd	1	Nd
J15				
Ethyl acetate	2	2	1	2
n-Butanol	2	2	2	Nd
Chloro	Nd	Nd	2	2
Petroleum ether	Nd	Nd	Nd	Nd
J74				
n-Butanol	2	2	2	2
Ethyl acetate	Nd	Nd	2	Nd
Chlorom	Nd	Nd	Nd	Nd
Petroleum ether	Nd	Nd	Nd	Nd
J61				
Ethyl acetate	0.5	1	0.5	0.5
n-Butanol	1	2	1	Nd
Chlorom	1	Nd	0.5	2
Petroleum ether	2	Nd	2	2

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

Table 3(on next page)

Minimum bactericidal concentration (MBC) (mg/mL) of the J2、J15、J74 and J61 extracts.

Table 3 Minimum bactericidal concentration (MBC) (mg/mL) of the J2、J15、J74 and J61 extracts.

Extracts	Gram-positive bacteria		Gram-negative bacteria	
	S.aureus	*B. subtills*	*E. coli*	*P. aeruginosa*
J2				
Ethyl acetate	2	Nd	2	Nd
n-Butanol	Nd	Nd	Nd	Nd
Chlorom	Nd	Nd	Nd	Nd
Petroleum ether	Nd	Nd	Nd	Nd
J15				
Ethyl acetate	2	Nd	Nd	Nd
n-Butanol	Nd	Nd	Nd	Nd
Chlorom	Nd	Nd	Nd	Nd
Petroleum ether	Nd	Nd	Nd	Nd
J74				
n-Butanol	Nd	Nd	Nd	Nd
Ethyl acetate	Nd	Nd	Nd	Nd
Chlorom	Nd	Nd	Nd	Nd
Petroleum ether	Nd	Nd	Nd	Nd
J61				
Ethyl acetate	2	2	2	Nd
n-Butanol	2	1	Nd	Nd
Chlorom	2	Nd	Nd	Nd
Petroleum ether	Nd	Nd	Nd	Nd

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

Table 4(on next page)

Table 4 The identification of the chemical composition of endophytic fungi extracts by LC-MS analysis.

Table 4 The identification of the chemical composition of endophytic fungi extracts by LC-MS analysis.

NO	Name of identified compound	RT(min)	Formula	m/z	Addduction	Endophytic fungi (ng/ml)			
						J61	J15	J74	J2
1	4-Methylcatechol	2.9	$C_7H_8O_2$	123.044	[M-H]	6.04	1.48	1.32	nd
2	Aesculetin	4.4	$C_9H_6O_4$	177.019	[M-H]	2.76	2.77	12.44	1.52
3	Aesculetin	4.2	$C_9H_6O_4$	177.019	[M-H]	12.00	1.77	6.09	1.20
4	Caffeic acid	4.4	C9H8O4	179.034	[M-H]	19.86	nd	nd	9.61
5	Caffeic acid	4.2	$C_9H_8O_4$	179.034	[M-H]	36.75	11.93	9.34	884.75
6	Caffeic acid	4.0	$C_9H_8O_4$	179.035	[M-H]	nd	0.59	0.56	7.26
7	Catechol	4.0	$C_6H_6O_2$	109.028	[M-H]	5.69	1.01	9.62	2.62
8	Divaricatinic acid	5.8	$C_{11}H_{14}O_4$	209.082	[M-H]	7.90	1.03	4.32	nd
9	Ferulic acid	5.0	$C_{10}H_{10}O_4$	193.050	[M-H]	37.98	24.01	44.84	nd
10	Ferulic acid	5.2	$C_{10}H_{10}O_4$	193.050	[M-H]	6.20	2.72	2.63	nd
11	Gallic acid	4.2	$C_7H_6O_5$	169.014	[M-H]	nd	nd	1.19	2.65
12	Gentisic acid	4.2	$C_7H_6O_4$	153.019	[M-H]	3.36	nd	3.90	11.98
13	Gentisic acid	3.8	$C_7H_6O_4$	153.019	[M-H]	46.98	5.10	8.11	8.77
14	Haematommic acid	3.3	$C_9H_8O_5$	195.030	[M-H]	32.79	1.20	1.69	0.73
15	Haematommone	7.1	$C_{16}H_{10}O_7$	313.036	[M-H]	8.31	nd	nd	nd
16	Haematommone	7.2	$C_{16}H_{10}O_7$	313.036	[M-H]	7.10	nd	nd	nd
17	Homogentisic acid	5.0	$C_8H_8O_4$	167.034	[M-H]	2.44	14.83	11.89	3.57
18	Homogentisic acid	5.4	$C_8H_8O_4$	167.034	[M-H]	2.87	1.23	nd	0.91
19	Homovanillic acid	7.0	$C_9H_{10}O_4$	181.050	[M-H]	2.80	1.71	nd	0.81
20	Isovanillic acid	4.5	$C_8H_8O_4$	167.034	[M-H]	2.48	1.29	3.36	1.31
21	Norepinephrine	4.7	$C_8H_{11}NO_3$	168.066	[M-H]	nd	nd	nd	16.67
22	norlichexanthone	7.1	$C_{14}H_{10}O_5$	257.046	[M-H]	nd	nd	nd	74.36
23	Quercetin	6.2	$C_{15}H_{10}O_7$	301.036	[M-H]	11.03	1.29	3.39	0.83
24	Quercetin	6.3	$C_{15}H_{10}O_7$	301.036	[M-H]	6.74	0.61	2.13	0.83
25	Syringic acid	4.8	$C_9H_{10}O_5$	197.045	[M-H]	2.21	nd	1.14	nd
26	Syringic acid	4.3	$C_9H_{10}O_5$	197.045	[M-H]	2.63	nd	1.16	0.37
27	Taxifolin	5.1	$C_{15}H_{12}O_7$	303.052	[M-H]	2.21	nd	nd	1.16
28	Vanillin	4.4	$C_8H_8O_3$	151.039	[M-H]	6.09	1.93	3.52	4.15
29	(±)-Abscisic acid	6.1	$C_{15}H_{20}O_4$	263.129	[M-H]	30.79	24.21	24.73	34.28
30	(±)-Abscisic acid	5.8	$C_{15}H_{20}O_4$	263.129	[M-H]	4.93	3.75	5.26	6.31

31	(Z)-9,12,13-trihydroxyoctadec-15-enoic acid	7.6	$C_{18}H_{34}O_5$	329.234	[M-H]	21.06	21.16	47.1	25.59
32	1,5,6,7-TETRAHYDRO-4H-INDOL-4-ONE	4.1	C_8H_9NO	134.060	[M-H]	2.75	nd	nd	1.43
33	12-Hydroxyoctadecanoic acid	11.7	$C_{18}H_{36}O_3$	299.260	[M-H]	278.40	360.43	232.45	138.16
34	13(S)-HOTrE	9.7	$C_{18}H_{30}O_3$	293.213	[M-H]	2.34	3.64	3.30	6.42
35	2,6-Dihydroxy-4-Methoxytoluene	4.8	$C_8H_{10}O_3$	153.055	[M-H]	278.40	360.43	232.45	138.16
36	2-Hydroxybenzyl alcohol	3.5	C7 H8 O2	123.044	[M-H]	89.03	nd	23.00	15.96
37	2-Hydroxycinnamic acid	5.5	C9 H_8O_3	163.039	[M-H]	nd	nd	2.54	0.73
38	2-Methoxycinnamic acid	4.9	$C_{10}H_{10}O_3$	177.055	[M-H]	3.10	1.82	1.44	0.62
39	2-Methylglutaric acid	2.0	$C_6H_{10}O_4$	145.050	[M-H]	46.37	14.08	19.08	62.31
40	2-Oxobutyric acid	0.8	$C_4H_6O_3$	101.023	[M-H]	12.89	18.79	8.08	42.68
41	3-Hydroxyphenylacetic acid	4.7	$C_8H_8O_3$	151.039	[M-H]	862.25	7.66	140.82	9.78
42	3-Hydroxypicolinic acid	1.8	$C_6H_5NO_3$	138.019	[M-H]	27.36	27.13	28.06	32.08
43	3-Phenyllactic acid	4.9	$C_9H_{10}O_3$	165.055	[M-H]	142.81	334.27	81.61	240.72
44	3-Phosphoglyceric acid	14.1	$C_3H_7O_7P$	184.984	[M-H]	12.46	10.57	12.18	12.08
45	4-Acetamidobutanoic acid	1.8	$C_6H_{11}NO_3$	144.066	[M-H]	5.67	1.85	1.90	79.83
46	4-Dodecylbenzenesulfonic acid	14.3	$C_{18}H_{30}O_3S$	325.185	[M-H]	110.56	138.27	107.13	72.28

47	4-methyl-2-oxopentanoic acid	3.9	$C_6H_{10}O_3$	129.055	[M-H]	14.20	121.83	4.86	13.83
48	4-Oxoproline	0.9	$C_5H_7NO_3$	128.034	[M-H]	5.46	50.36	8.56	307.42
49	6-Hydroxycaproic acid	4.4	$C_6H_{12}O_3$	131.070	[M-H]	32.57	43.55	17.25	72.32
50	7-Methylxanthine	0.8	$C_6H_6N_4O_2$	165.040	[M-H]	4.74	9.05	2.96	38.49
51	9-HpODE	8.5	$C_{18}H_{32}O_4$	311.223	[M-H]	20.97	49.99	74.16	19.92
52	Ala-Phe	7.2	$C_{12}H_{16}N_2O_3$	235.109	[M-H]	273.59	nd	98.17	nd
53	alpha-Hydroxyhippuric acid	3.9	$C_9H_9NO_4$	194.046	[M-H]	7.27	11.68	5.49	51.88
54	alpha-Linolenic acid	12.4	$C_{18}H_{30}O_2$	277.218	[M-H]	6.58	9.25	22.26	4.94
55	Anthranilic acid	5.0	$C_7H_7NO_2$	136.040	[M-H]	113.21	2.04	189.24	2.00
56	Azelaic acid	5.5	$C_9H_{16}O_4$	187.097	[M-H]	304.69	110.84	27.58	66.58
57	Benzoic acid	4.6	$C_7H_6O_2$	121.029	[M-H]	81.58	53.66	120.92	82.07
58	Citric acid	1.3	$C_6H_8O_7$	191.019	[M-H]	12.86	3.57	6.54	111.38
59	D-Fructose	0.8	$C_6H_{12}O_6$	179.056	[M-H]	6.61	98.86	3.84	298.18
60	Indole-3-acetic acid	5.8	$C_{10}H_9NO_2$	174.055	[M-H]	150.03	3.86	134.91	18.18

2 nd, not detect.

Figure 1

Fig. 1 Adjacent tree of ITS sequence of phenol-producing endophytic fungi of *F. dibotrys*. The number on the node is the boot score obtained from 1,000 replicates. *Mucor racemose* was selected as the outer group.

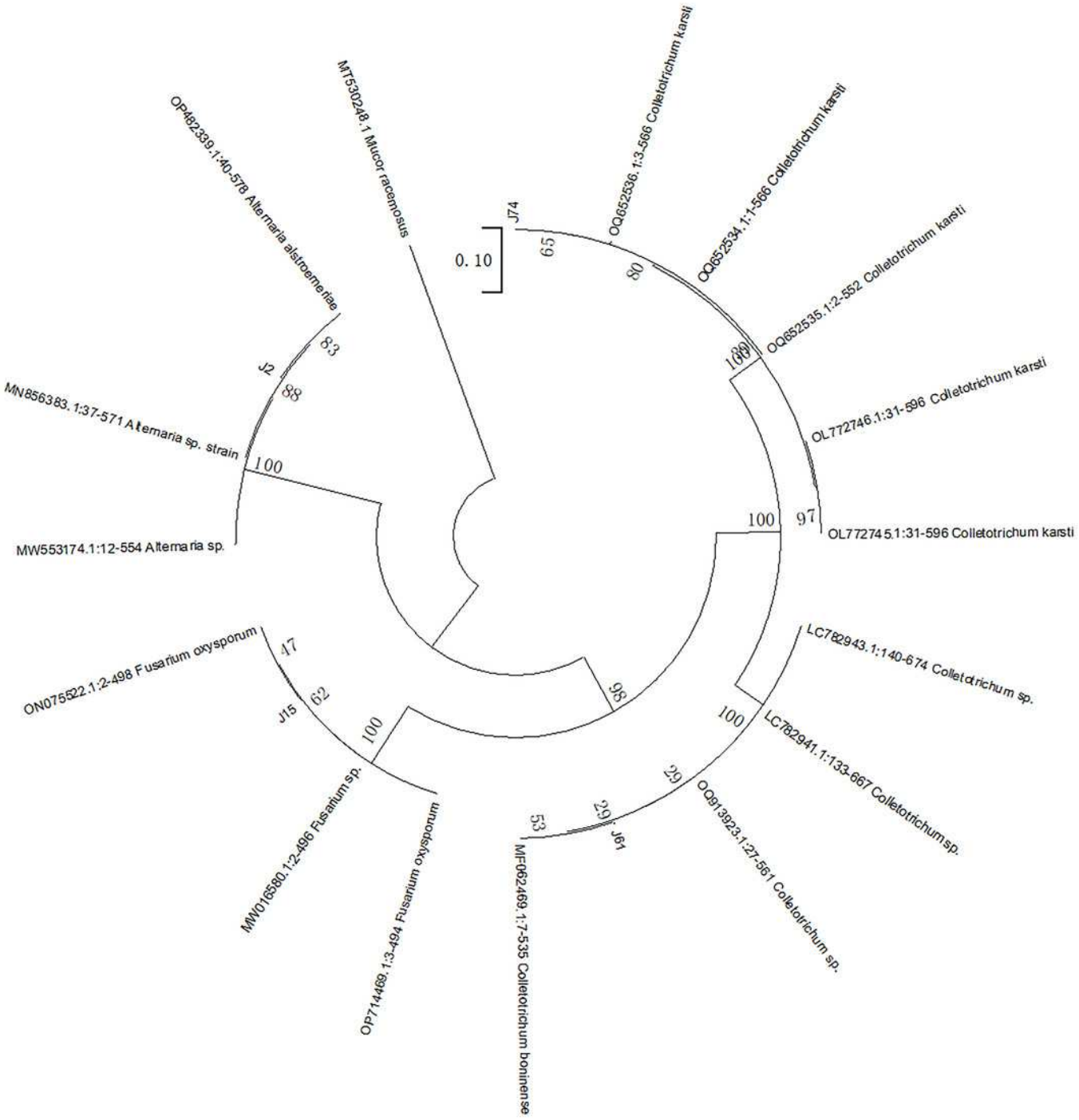
0.10
MT530248.1 Mucor racemosus
OP482339.1:40-578 Alternaria alstroemeriae
MN856383.1:37-571 Alternaria sp. strain
MW553174.1:12-554 Alternaria sp.
ON075522.1:2-498 Fusarium oxysporum
MW016580.1:2-496 Fusarium sp.
OP714469.1:3-494 Fusarium oxysporum
MF062469.1:7-535 Colletotrichum boninense
OQ913923.1:27-561 Colletotrichum sp.
LC782941.1:133-667 Colletotrichum sp.
LC782943.1:140-674 Colletotrichum sp.
OL772745.1:31-596 Colletotrichum karsti
OL772746.1:31-596 Colletotrichum karsti
OQ652535.1:2-552 Colletotrichum karsti
OQ652534.1:1-566 Colletotrichum karsti
OQ652536.1:3-566 Colletotrichum karsti
J74
J2
J15
J61
65
80
100
83
88
100
47
62
100
53
29
29
100
98
100
97

Figure 2

Fig. 2 Total Phenol Content of J2, J15, J61, J74 extracts. a-d and A–D: significant differences between different groups ($p < 0.05$).

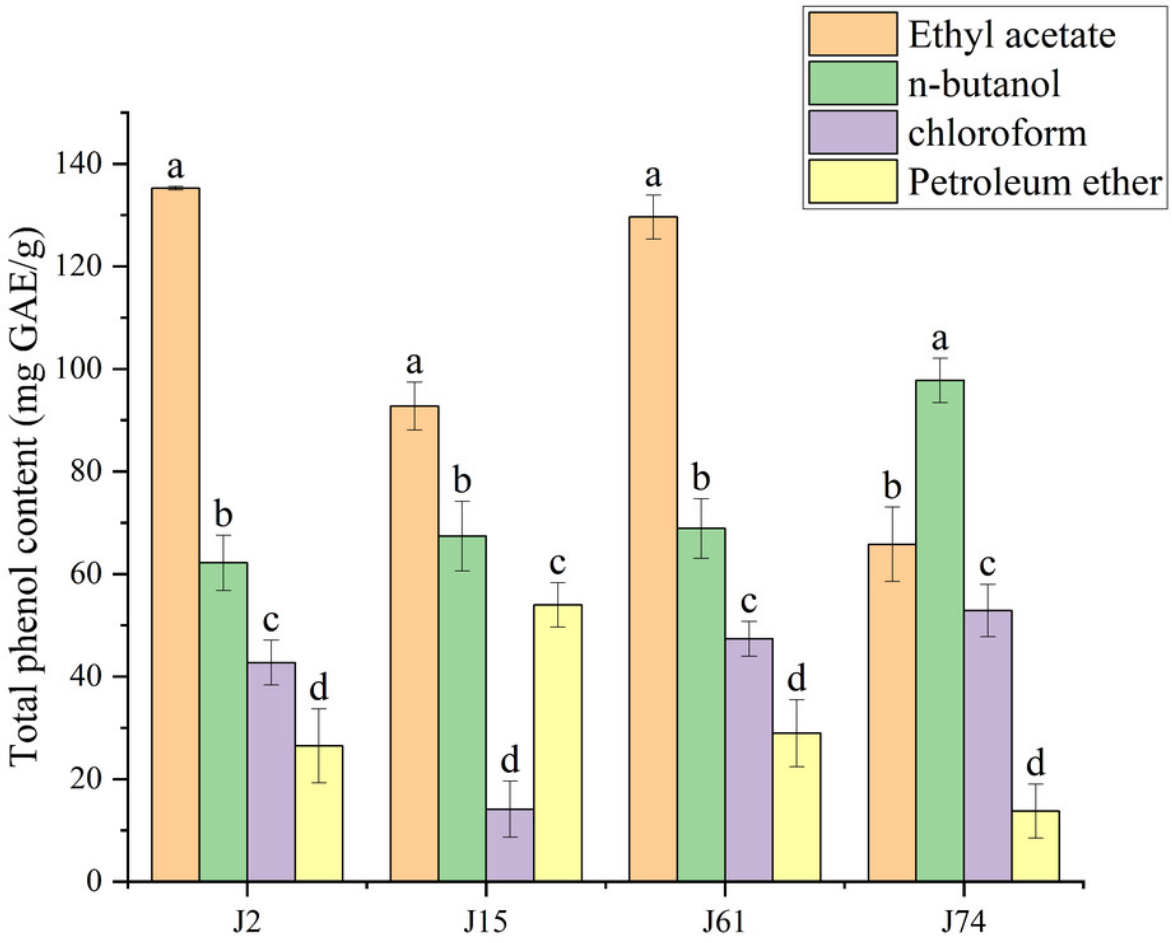

Figure 3

Fig. 3 Antioxidant activities of the J2 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 gr

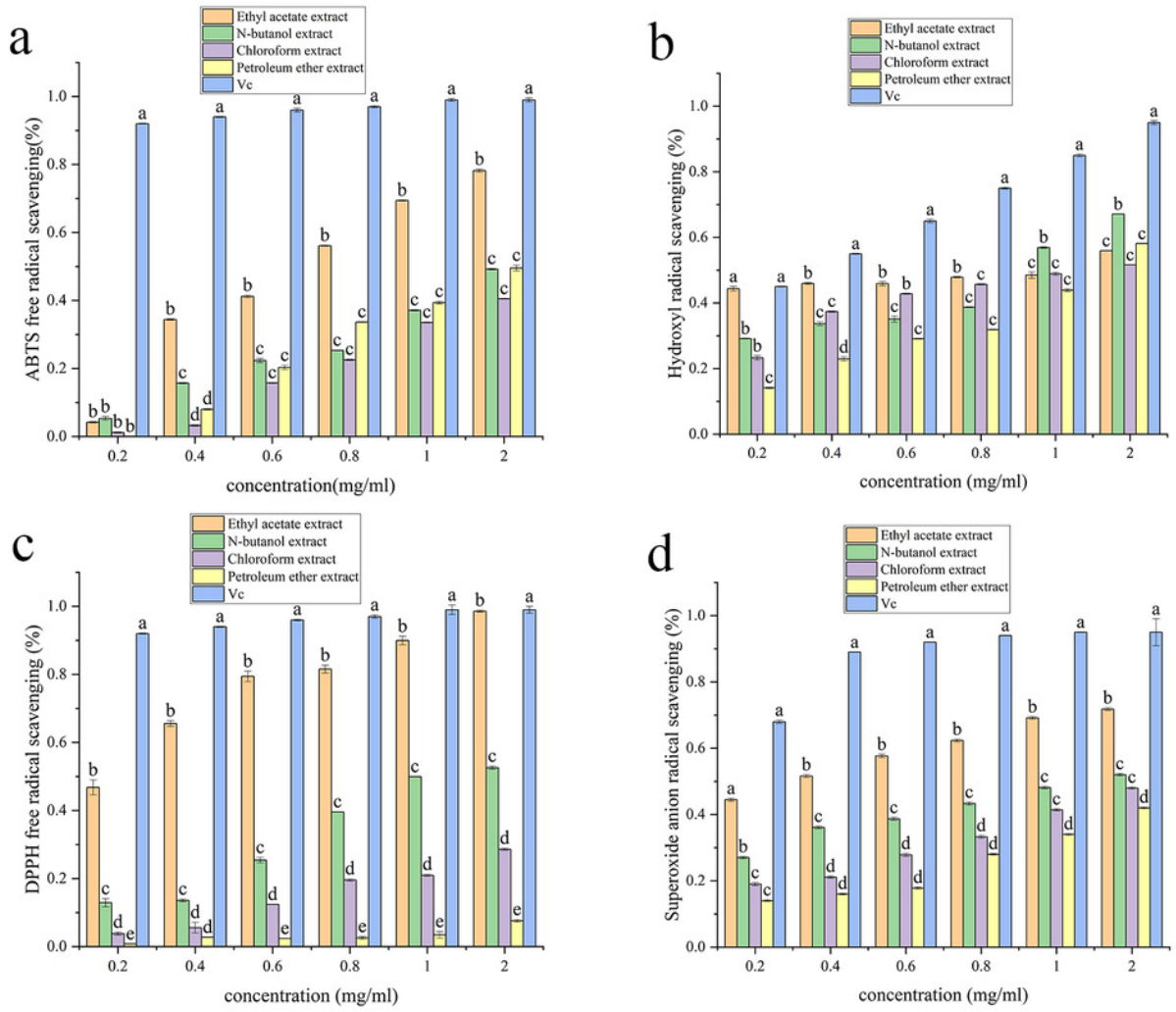

Figure 4

Fig. 4 Antioxidant activities of the J15 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 g

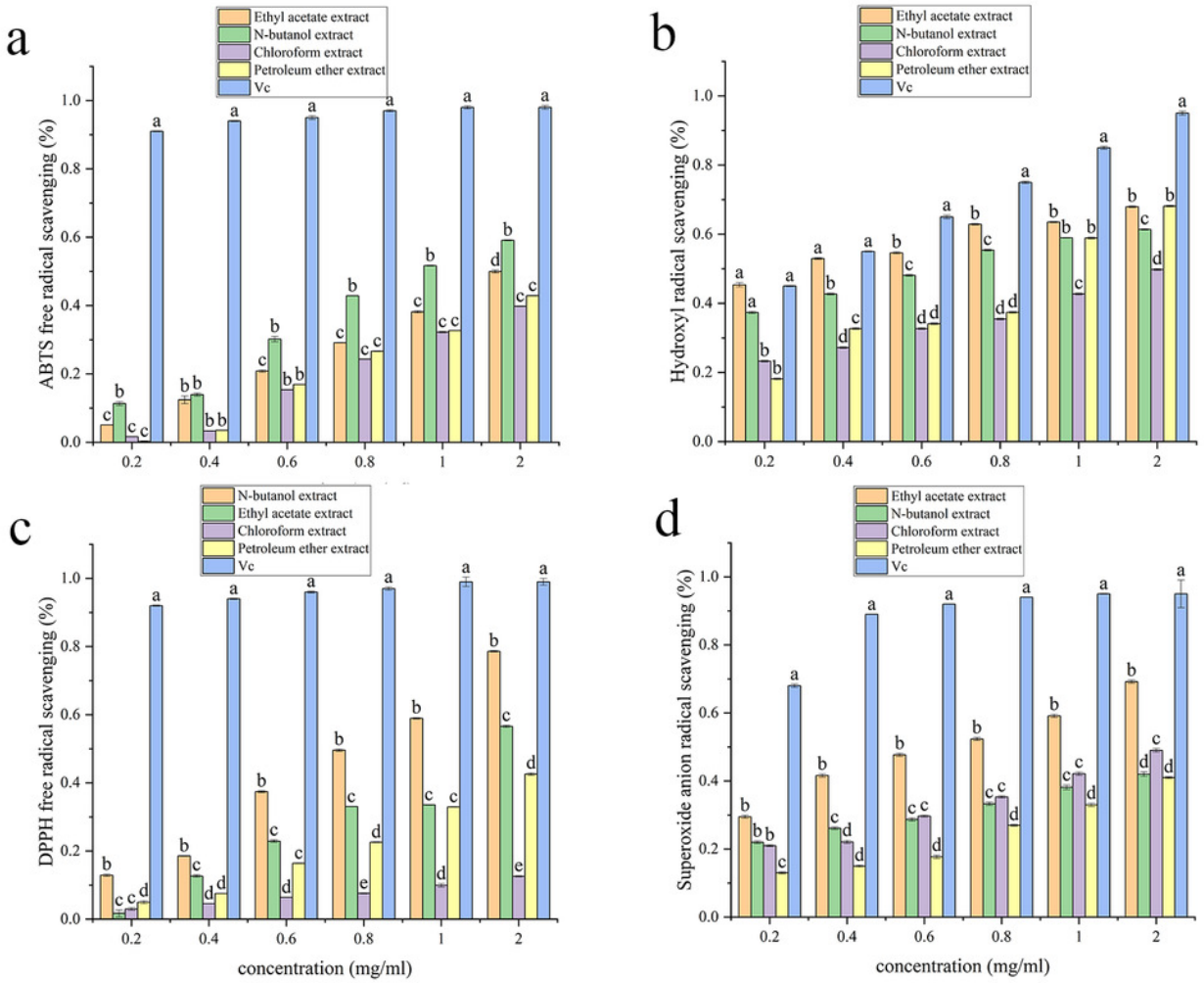

Figure 5

Fig. 5 Antioxidant activities of the J61 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 g

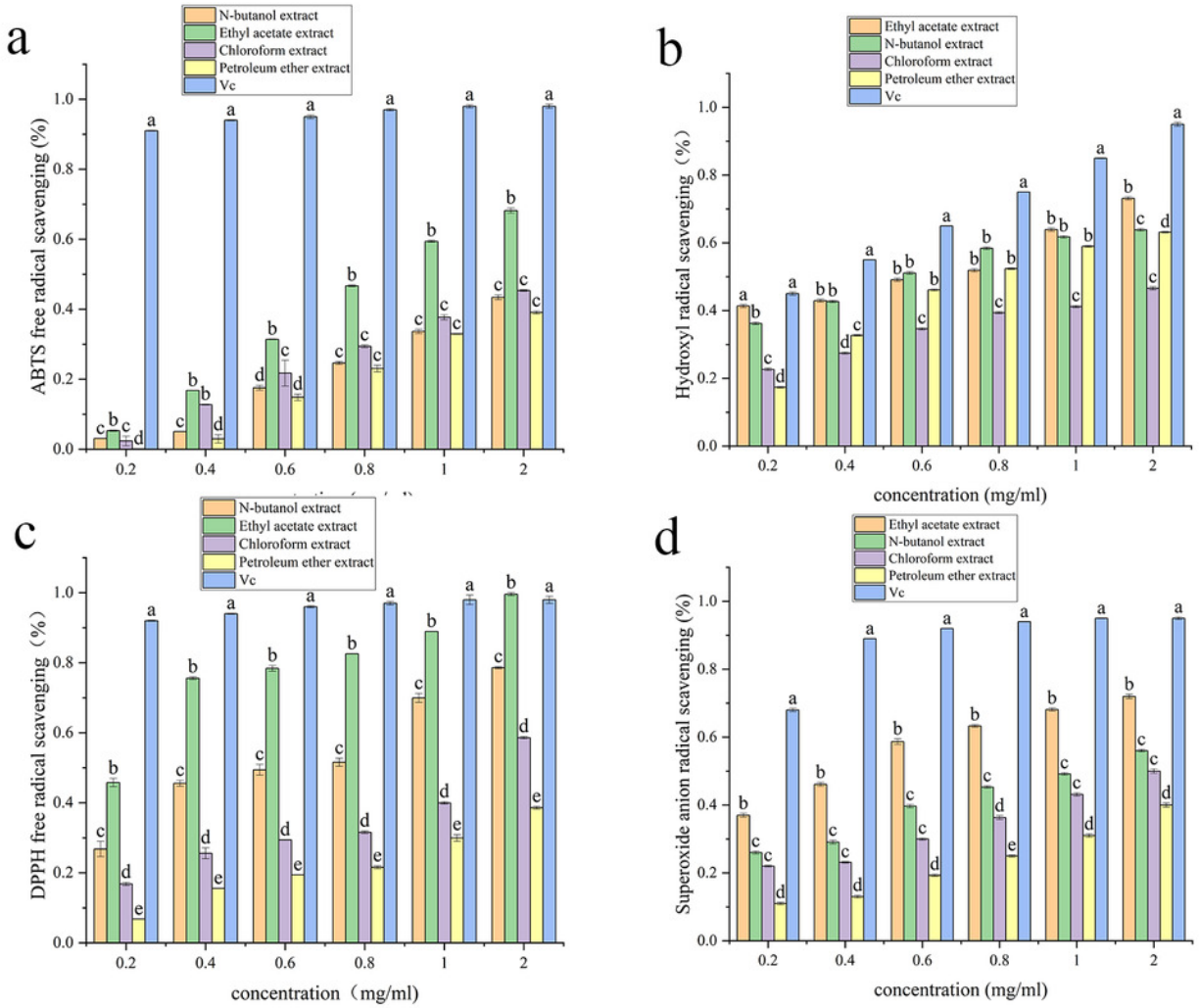

Figure 6

Fig. 6 Antioxidant activities of the J74 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 g

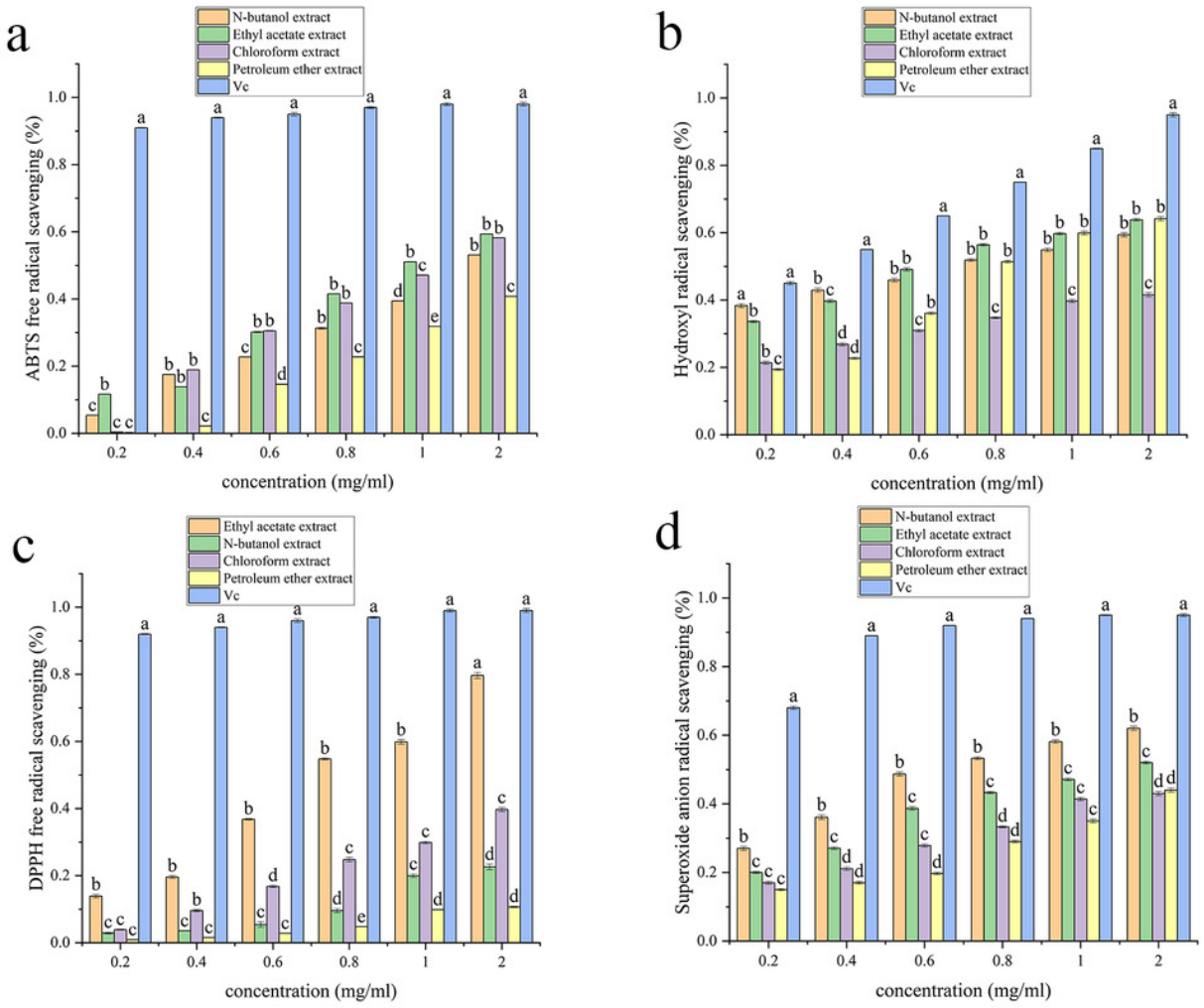

Figure 7

Fig. 7 Effects of ethyl acetate extracts from endophytic fungi on cell membrane integrity of *E. coli*, *S. aureus,* and *B. subtills* by fluorescence microscope. (A) Untreated bacterial cells; (B) bacterial cells treated with J2 extracts at 2