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Metabolomics-guided identification of compounds with antibacterial and antioxidant activities from *Polygonatum sibiricum*-derived endophytic fungi

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Abstract

Background Infections caused by multidrug resistant bacterial pathogens have been recognized as major global healthcare threat to medicinal, agricultural and pharmaceutical industries by World Health Organization. In this regard, the present study was aimed to isolate endophytes from medicinal plant *Polygonatum sibiricum* (*P. sibiricum*) and to investigate their antibacterial efficacy, radical scavenging ability and chemical fingerprinting using Gas Chromatography–Mass Spectrum (GC–MS) analysis.

Results Two endophytic fungi *Talaromyces assiutensis* HJ.14 (*T. assiutensis*) and *Fusarium oxysporum* HJ.15 (*F. oxysporum*) were isolated and identified from the rhizomes of *P. sibiricum*. Among the extracts screened, ethyl acetate extract of *F. oxysporum* HJ.15 showed maximum antibacterial activity with the zones of inhibition ranging from 10.98 ± 0.19 to 15.66 ± 1.49 mm and the MIC values ranging from 0.24 to 1.88 $\mu\text{g}/\text{mL}$ against the tested bacterial pathogens. In addition, it showed significant antioxidant activity with EC_{50} values of 6.21–17.97 $\mu\text{g}/\text{mL}$. Further, GC–MS analysis revealed the presence of propanoic acid ethyl ester, hexadecanoic acid methyl ester, hexadecanoic acid ethyl ester, 9-Octadecenoic acid (Z)-methyl ester, 1-Octanol, 2-Undecenal, butanoic acid, 3-hydroxy- and hexanoic acid were the most abundant compounds in the active crude extract which was responsible for the significant antibacterial and antioxidant properties.

Conclusions In summary, our results clearly suggest that the *F. oxysporum* HJ.15 will be a promising starting point for the isolation of active antibacterial compounds with antioxidant properties.

Keywords *Polygonatum sibiricum*, Endophytic fungi, Secondary metabolite, Antibacterial activity, Antioxidant activity

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1 Background

Globally, severe infections caused by drug-resistant bacterial pathogens are the biggest healthcare threats to medical and pharmaceutical industries. According to global health report around 700,000 people die of infections that are caused by antibiotic-resistant bacteria in 2020 [1]. Therefore, maintenance of a healthy antioxidant status is essential for cellular homeostasis. Oxidative stress (OxS) is defined as the condition under which the generation of reactive oxygen species (ROS) exceeds the capacity of antioxidants to detoxify which is related to the pathogenesis of several chronic diseases including types 1 and 2 diabetes, cancer, heart disease, schizophrenia, parkinson's disease and alzheimer's disease [2, 3].

Fortunately, endophytic fungi provide a huge potential as a unique resource for the development of novel antibiotics which live in plant tissues at some or all stages of their life cycle and involve in protecting the host against pathogens [4]. Modern studies have shown that endophytic fungi from medicinal plants can produce secondary metabolites that are the same or similar to the host and are proved to possess antibacterial [5, 6], antioxidant [7], antitumor [8] activities (Table 1) [4, 9–14].

The edible rhizome of *Polygonatum sibiricum* (*P. sibiricum*) is an important medicinal plant used in strengthening the muscle and bone marrow in China [15]. The main active components of *P. sibiricum* are polysaccharides [16], flavonoids [17], steroidal-saponins and polyphenols [18] that are scientifically proved to possess various biological activities. Therefore, symbionts from medicinal plant are known to be an important resource of innumerable classes of bioactive compounds including anticancer drug, antibacterials and antioxidants [19–21]. More recently, Song et al. [22] and Wang et al. [23] have reported that endophytic fungi isolated from *P. sibiricum* were found to possess various biological activities. Hence, we aimed to isolate and identify endophytic fungi associated with the rhizome of medicinal plant *P. sibiricum* and to evaluate their antibacterial and antioxidant properties. In addition, the active components of the

extract were identified by Gas Chromatography–Mass Spectrum (GC–MS) analysis.

2 Methods

2.1 Plant collection and isolation of endophytic fungi

The uninfected rhizome of *Polygonatum sibiricum* (*P. sibiricum*) was procured from the alpine area of Sancha Township (Enshi, China, 109°45'24"E, 30°10'51"N) and the plant material was identified by Dr Meijun He, College of Biological and Food Engineering, Hubei MINZU University (Additional file 1: Fig. S1). The isolation and purification of endophytes were carried out by following the method Li et al. [10]. Briefly, the rhizome of healthy plant was washed under running tap water. Under the aseptic condition, the washed young shoots were surface-sterilized in 5 mL of 70% EtOH for 2 min and then soaked in 5 mL of 4% sodium hypochlorite for 5 min and finally washed with 5 mL of sterile water to remove the traces of sodium hypochlorite and ethanol. The dried shoots were then ground and inoculated onto potato dextrose agar (PDA) medium containing kanamycin with a final concentration of 50 µg/mL. The inoculated plates were incubated at 25 °C for 7 days were observed daily for visible fungal growth. The fungi were later transferred to the freshly prepared PDA for purification. Pure isolates were identified by observing the color, colony morphology, growth pattern and sequencing. The pure fungal isolates were maintained in PDA slants and kept at 4 °C for further studies.

2.2 Morphological and molecular characterization of endophytic fungus

Colony morphology including color, growth, spore size, shape and spore producing structures were observed by growing the isolates on PDA and incubated for 7–14 days at 28 ± 2 °C [24, 25]. For molecular identification, the isolates were grown on potato dextrose broth (PDB) at 28 ± 2 °C for 3 days on an orbital shaker at 150 rpm. After incubation the biomass was collected and the genomic Deoxyribonucleic acid (DNA) was extracted

Table 1 Secondary metabolites from endophyte fungus and their antibacterial activities reported

Medicinal plants/regions and countries	Endophytic fungi	Active component	Activities	References
<i>Pelargonium sidoides</i> /South Africa	<i>Aspergillus</i> sp.	Extract	Antibacterial activity	[4]
<i>Zingiber officinale</i> /Duesseldorf, Germany	<i>Trichoderma harzianum</i>	Pretrichodermamide A	Antimicrobial activity	[8]
<i>Ampelopsis grossedentata</i> /enshi, china	<i>Alternaria alternata</i>	Extract	Antibacterial activity	[9]
<i>Adiantum capillus</i> /Egypt	<i>Chaetomium globosum</i>	Extract	Antioxidant activity	[10]
<i>Euphorbia hirta</i> /china	<i>Achaetomium</i> sp.	Extract	Antioxidant activity	[11]
<i>Elaeocarpus sylvestris</i> /Guangxi, China	<i>Pseudocercospora</i> sp.	Extract	Antioxidant activity	[12]
<i>Otoba gracilipes</i> /Colombia	<i>Fusarium oxysporum</i>	Extracts	Antioxidant activity	[13]

by cetyltrimethyl ammonium bromide (CTAB) method. Polymerase Chain Reaction-mediated amplification of the Internal transcribed spacer (ITS) gene was carried out using the fungal universal primers and the PCR products were sequenced (Wuhan Aoke Dingsheng). The sequencing results were analyzed by Basic Local Alignment Search Tool (BLAST) in GenBank (<https://blast.ncbi.nlm.nih.gov/Blast.cgi>) to obtain the ITS sequences homologous to the strains. The phylogenetic tree was constructed using Neighbour Joining method with aid of MEGA-X software version 10.2.2 (<https://www.megasoftware.net>).

2.3 Fermentation and extraction of secondary metabolites

The fully-grown spores of endophytic fungi were inoculated into 50 mL of PDB for bioactive secondary metabolites production. The flasks were incubated for 14 days at 28 ± 2 °C on a rotary shaker. At the end of fermentation, the culture broth along with the cells was soaked in equal volume of different organic solvents viz., petroleum ether, ethyl acetate and methanol at room temperature followed by ultra-sonication for 30 min. The organic phase was then evaporated to dryness and stored at 4 °C until use [26].

2.4 Test strains

Eleven strains of bacterial pathogens including *Klebsiella pneumonia* ATCC 13883, *Enterococcus faecalis* ATCC 29212, *Bacillus subtilis* ATCC 19659, *Micrococcus luteus* ATCC 4698, *Bacillus thuringiensis* ATCC 10792, Methicillin resistant *Staphylococcus aureus* ATCC 43300 (MRSA), Multi-drug resistant *Pseudomonas aeruginosa* ATCC 9027, *Escherichia coli* ATCC 25922, *Acinetobacter baumannii* ATCC 19606, Methicillin resistant *Staphylococcus epidermidis* ATCC 12228 (MRSE) and *Staphylococcus aureus* ATCC 29213 were obtained from the Key Laboratory of Tropical Marine Biological Resources and Ecology, Chinese Academy of Sciences.

2.5 Antibacterial assay

The antibacterial activities of the different extracts of the endophytic fungi were assayed by modified filter paper disc method [27]. The sterile filter paper discs were soaked with 20 μ L of crude extract and placed on pre-coated bacterial plates (50 μ L of freshly prepared test cultures, $1 \times 10^{4-6}$ CFU/mL) of Mueller–Hinton agar (MHA). Kanamycin (50 μ g/disc) and Dimethyl sulfoxide (DMSO) (20 μ L/disc) were used as positive and solvent controls, respectively. The plates were first incubated at 4 °C for 10 min to allow proper diffusion of the extract into the medium and then incubated at 37 °C for 24 h.

After incubation period, the inhibition zones were measured and expressed in terms of millimeter (mm). An average inhibition zone was calculated for 5 replicates.

2.6 Determination of minimal inhibitory concentration (MIC) and minimum bactericidal concentration (MBC)

The minimal inhibitory concentration (MIC) values of the active extracts of the endophytic fungi were determined using broth micro dilution assay in sterile 96-well microtiter plate according to the Clinical and Laboratory Standards Institute (CLSI) document M07-A10 and M100-S25 [28, 29]. Briefly, a two-fold dilution of the extract was carried out with double strength Mueller–Hinton broth (MHB). 100 μ L inoculum and extracts were added into each well to a final volume of 200 μ L with the final concentration ranging from 0.1 to 100 μ g/mL. Kanamycin was included as positive antibiotic control. Then, the 96-well microtiter plate was incubated at 37 °C for 24 h. After incubation period, 40 μ L of 0.2 mg/mL iodinitrotetrazolium chloride dissolved in 99.5% ethanol was added into each well and again incubated for 30 min at 4 °C. The color changed from yellow to purple indicating the microbial growth in the well. To determine the minimum bactericidal concentrations (MBC) and to check the viability of the test microorganisms, the mixture in every well was streaked on nutrient agar and again incubated at 37 °C for 24 h [30].

2.7 Antioxidant assay

The antioxidant activities of the extracts of the endophytic fungi were assayed by a modified method of Kumar et al. [31]. Stock solutions of α -diphenyl- β -picrylhydrazyl (DPPH) (200 μ M/L) and extracts of endophytic fungi (50 μ g/mL) were prepared in methanol, respectively. 3 mL extracts of different concentrations added with 1 mL of 200 μ M/L DPPH solution and were wrapped in aluminum foil and kept at 30 °C for 30 min in dark. Meanwhile, 3 mL extracts of different concentrations added with 1 mL of ethanol was set to the blank groups and 3 mL of ethanol added with 1 mL of 200 μ mol/L DPPH solution was labeled as the control groups. All measurements were done under dim light. Spectrophotometric measurements were done at 517 nm using Spectronic Genesys 5 spectrophotometer. The results were expressed as percentage of reduction of the initial DPPH absorption by test samples as follows: DPPH scavenging effect (%) = $[BG - (TG - CG)/BG] \times 100$.

2.8 Identification of secondary metabolites using GC–MS analysis

The active crude extract was analyzed for its chemical constituents with aid of GC–MS (HP6890GC with

5973 MS, Agilent Technologies, Santa Clara, CA, USA) equipped with a HP-5MS column (30 mm × 0.25 mm with film thickness 0.25 μm; Agilent Technologies). Briefly, the sample was dissolved in MeOH (2 μL) and injected with split ratio of 20:1 with anterior column pressure of about 7 psi. Analysis was carried out with oven temperature programmed at 80 °C (hold 3 min) and raised to 280 °C at a rate of 3 °C/min. The injection port temperature was 250 °C, transfer temperature was 250 °C and ion source temperature was 230 °C. Helium was used as carrier gas at a flow rate of 1 mL/min. The instrument was calibrated to a scan range of m/z 35–500. The spectra of known and unknown compounds were identified by comparing their mass spectral fragmentation patterns with the NIST98-MS and the Wiley KnowItAll 2020 Mass Spectral Library (<http://www.knowitall.com>) [32].

2.9 Statistical analysis

All experimental data were statistically analyzed by Statistical Product and Service Solutions (SPSS) 19.0 (IBM, Armonk, NY, USA) and the experimental data were

expressed as mean ± standard deviation ($P < 0.05$), indicating a significant difference ($P < 0.01$), indicating a very significant difference [33].

3 Results

3.1 Identification of endophytes

Two distinct endophytic fungal isolates HJ.14 and HJ.15 were isolated and purified from fresh rhizome of *Polygonatum sibiricum* (*P. sibiricum*). The HJ.14 colonies with radial growth rate of 3.73 ± 0.78 mm/d on potato dextrose agar (PDA) plates are characterized by felt-like, textured, pale-white colonies and white undersides (Fig. 1a–c, Additional file 1: Tables S1, S2). The HJ.15 colonies with radial growth rate of 12.37 ± 0.11 mm/d on PDA plates are characterized by villous colonies, the upper surface is light pink, and the color becomes lighter as the circle spreads to the border, the mycelium is very thin (Fig. 1d–f, Additional file 1: Tables S1, S2). Microscopically, the lower part of the mycelium is thick and erect, rose red and has many diaphragms. The small conidia are mostly unicellular, a few have 1–3 septa; they are elliptical conidia that produce spores from aerial hyphae. The

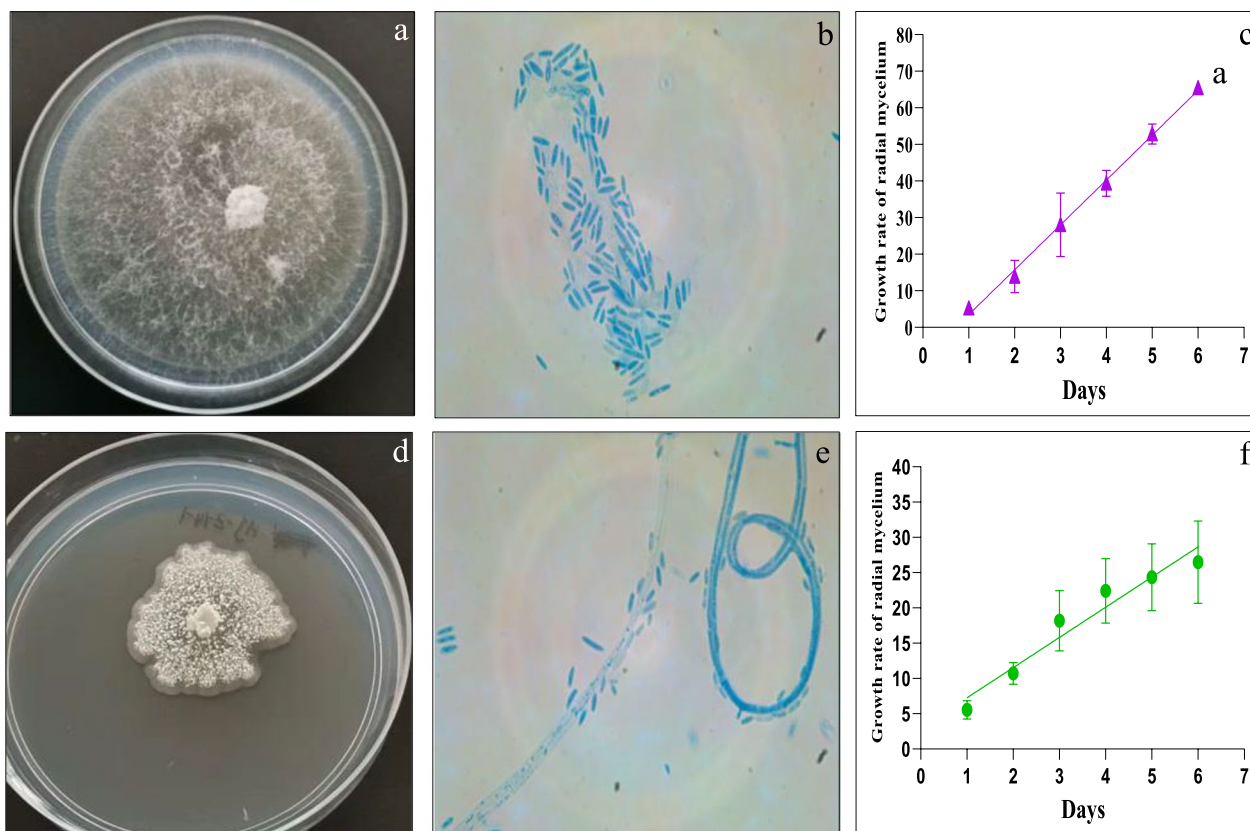


Fig. 1 Morphological characteristics of *P. sibiricum*-derived endophytic fungi (a) Macroscopic image of *F. oxysporum* HJ 15 on Potato Dextrose Agar; (b) Microscopic image (40X) of *F. oxysporum* HJ 15; (c) mycelial growth rate of *F. oxysporum* HJ 15; (d) Macroscopic image of *T. assiutensis* HJ.14 on Potato Dextrose Agar; (e) Microscopic image (40X) of *T. assiutensis* HJ.14; (f) mycelial growth rate of *T. assiutensis* HJ.14

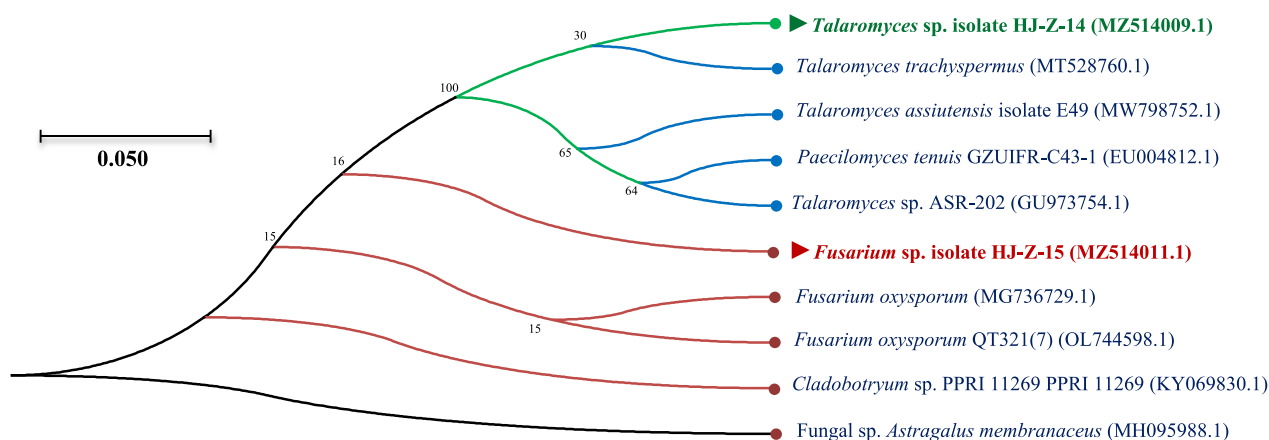


Fig. 2 Phylogenetic relatedness of *T. assiutensis* HJ.14 and *F. oxysporum* HJ.15

macroconidia were sickle-shaped or spindle-shaped, consistent with the morphological characteristics of *Fusarium* [34].

Further, the isolates were confirmed by molecular identification in which highly conserved Internal transcribed spacer (ITS) gene sequences of the fungus were amplified using ITS universal primers (ITS1 and ITS4) by PCR and sequenced. Approximately 571 and 524 bps of two sequences (HJ.14 and HJ.15) were subjected to Basic Local Alignment Search Tool (BLAST) analysis. HJ.14 showed a high homology (100%) with the previously submitted sequences of *Talaromyces assiutensis* (GenBank accession number MW798752.1); HJ.15 revealed maximum homology (100%) with *Fusarium oxysporum* (GenBank accession number MN749138.1). Furthermore, the phylogenetic tree for the HJ.14 and HJ.15 isolates constructed using Mega X 10.2.2 software as depicted in Fig. 2 confirmed that they belonged to *T. assiutensis* and *F. oxysporum*, respectively. These sequences were further deposited in the GenBank of NCBI under the accession numbers MZ514009.1 (HJ.14), MZ514011.1 (HJ.15) (Additional file 1: Table S3).

3.2 Preliminary antibacterial activity

As shown in Fig. 3 and Additional file 1: Table S4, among the extracts screened the ethyl acetate extract of *F. oxysporum* HJ.15 showed significant broad spectrum of antibacterial activity against all the 11 bacterial strains tested with the zones of inhibition ranging from 10.98 ± 0.19 to 15.66 ± 1.49 mm. Interestingly, it showed highest antibacterial activity against the multidrug-resistant bacteria such as *B. thuringiensis*, *E. faecalis*, *S. aureus* and *P. aeruginosa* with the zones of inhibition ranging from 13.19 ± 0.19 to 15.66 ± 1.49 mm. On the

other hand, methanol and petroleum ether extracts showed moderate and weak activities as compared to kanamycin (Additional file 1: Fig. S1).

3.3 Determination of MIC and MBC

Based on the preliminary antibacterial activities, the ethyl acetate extract of *F. oxysporum* HJ.15 was alone individually tested against selected pathogenic strains to determine their MIC and MBC values. The resulting MICs and MBCs of ethyl acetate extract against the tested pathogens is described in Table 2 and Additional file 1: Fig. S2. Interestingly, the ethyl acetate extract of *F. oxysporum* HJ.15 showed potential antibacterial activity with MICs ranging from 0.24 to 1.88 $\mu\text{g}/\text{mL}$. MIC testing indicated that the volume of ethyl acetate extract required to prevent the growth of the test microorganisms was found to be as follows: 1.88 $\mu\text{g}/\text{mL}$ for *S. aureus* (ATCC 29213), *B. thuringiensis* (ATCC 10792), *K. pneumoniae* (ATCC 13883), *P. aeruginosa* (ATCC 9027) and *A. bammannii* (ATCC 19606) 0.94 $\mu\text{g}/\text{mL}$ for *M. luteus* (ATCC 4698), 0.24 $\mu\text{g}/\text{mL}$ for *E. faecalis* (ATCC 29212). The MBC of ethyl acetate extract ranged from 0.94 to 1.88 $\mu\text{g}/\text{mL}$.

3.4 Antioxidant activity

The ability of three different extracts (methanol, ethyl acetate extract and petroleum ether) of *F. oxysporum* HJ.15 to scavenge DPPH radicals were shown in Fig. 4. The results clearly indicated that the ethyl acetate extract induced 45.41 ± 0.30 – 94.47 ± 0.94 % of radicals scavenging rate followed by methanol extract 34.82 ± 4.66 – 62.91 ± 0.53 % and petroleum ether 24.83 ± 0.04 – 47.59 ± 0.16 % from 6 to 16 $\mu\text{g}/\text{mL}$ with the EC_{50} value of 6.21 $\mu\text{g}/\text{mL}$, 11.70 $\mu\text{g}/\text{mL}$ and 17.97 $\mu\text{g}/\text{mL}$, respectively. The results were compared with that of well-known antioxidant standard drug ascorbic acid (Additional file 1: Fig. S3).

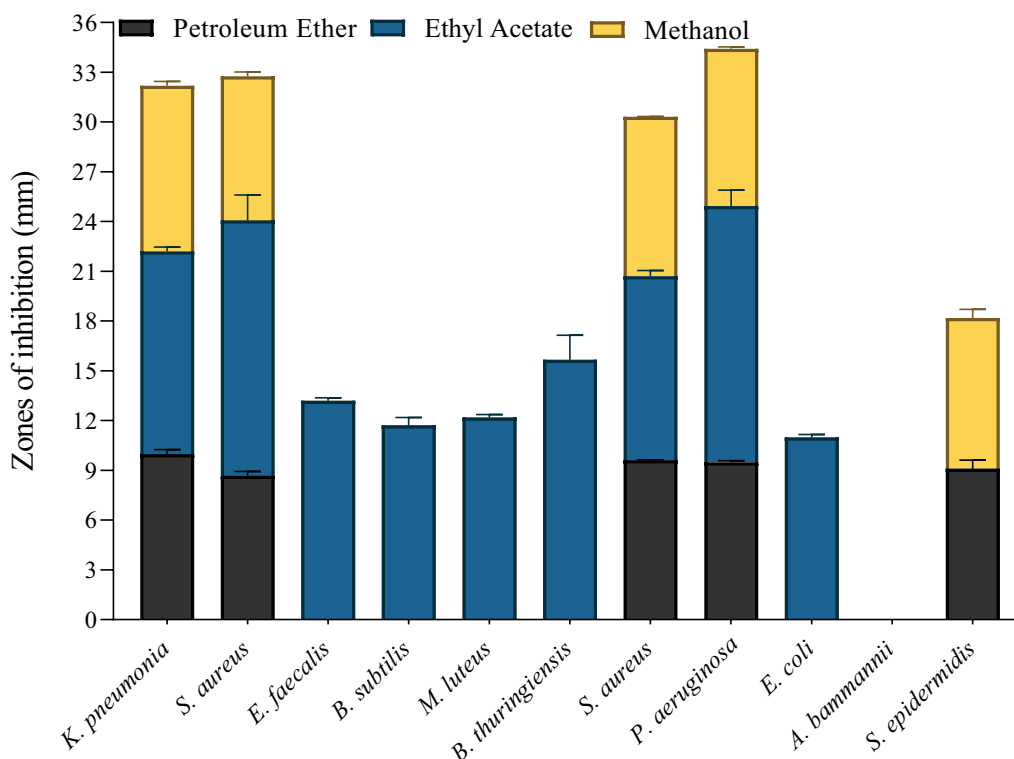


Fig. 3 Antibacterial activity of extracts from endophytic fungi

Table 2 Minimum Inhibitory Concentrations (MIC) and Minimum Bactericidal Concentrations (MBC) of *Fusarium oxysporum* HJ.15 extracts

Test organisms	<i>F. oxysporum</i> HJ.15 (µg/mL)		Kanamycin (µg/mL)	
	MIC	MBC	MIC	MBC
Gram-positive				
<i>B. subtilis</i>	0	0	0.016	0.031
<i>B. thuringiensis</i>	1.88	1.88	0.063	0.125
<i>E. faecalis</i>	0.24	0.94	6.250	6.250
<i>M. luteus</i>	0.94	0.94	0.002	0.002
MRSA	1.88	0	0.024	6.250
<i>S. aureus</i>	0	0	3.125	3.125
<i>S. epidermidis</i>	0	0	0.008	0.008
Gram-negative				
<i>E. coli</i>	0	0	0.031	0.031
<i>K. pneumonia</i>	1.88	0	0.031	0.063
<i>P. aeruginosa</i>	1.88	1.88	0.063	0.063
<i>A. bammannii</i>	1.88	0	0.008	0.016

3.5 GC-MS analyses of active crude extract

The GC-MS spectra of the active ethyl acetate extract of *F. oxysporum* HJ.15 and its chemical constituents are depicted in Additional file 1: Table S5 and Figs. S4-S28. It

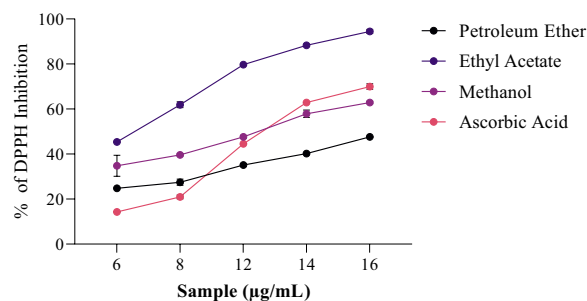


Fig. 4 Antioxidant activity of extracts from endophytic fungi *F. oxysporum* HJ.15

can be seen from Additional file 1: Table S5 that there are 25 volatile compounds detected from the crude extract of *F. oxysporum* HJ.15, including 9 esters (13.91%), 3 alcohols (3.04%), 3 aldehydes (2.18%), 6 carboxylic acids (12.56%), and 4 other types (3.53%). Of the 25 compounds identified, the most important compounds are propanoic acid ethyl ester, hexadecanoic acid methyl ester, hexadecanoic acid ethyl ester, 9-Octadecenoic acid (Z)-methyl ester,

Table 3 Metabolites with antibacterial and antioxidant activities identified by GC–MS of highly potent extracts from *Fusarium oxysporum* HJ.15

Peak	Active metabolites	RT (min)	Mol.For	Mol.Wt	Similarity (%)	Content (%)	Antibacterial activity	Antioxidant activity	References
1	Propanoic acid, ethyl ester	2.875	C ₅ H ₁₀ O ₂	102	97.3	5.184	✓	×	[28]
2	Hexadecanoic acid, methyl ester	51.281	C ₁₇ H ₃₄ O ₂	270	94	0.769	✓	×	[27]
3	Hexadecanoic acid, ethyl ester	53.584	C ₁₈ H ₃₆ O ₂	254	90.1	1.709	×	✓	[39]
4	9-Octadecenoic acid (Z)-, methyl ester	57.008	C ₁₉ H ₃₆ O ₂	296	85.4	0.480	✓	×	[26]
5	1-Octanol	15.178	C ₈ H ₁₈ O	130	87.7	0.459	×	✓	[24]
6	2-Undecenal	29.175	C ₁₁ H ₂₀ O	168	87.9	0.829	✓	×	[25]
7	Butanoic acid, 3-hydroxy	10.135	C ₄ H ₈ O ₃	104	89.8	10.115	✓	×	[29]
8	Hexanoic acid	11.722	C ₆ H ₁₂ O ₂	116	91.8	0.080	✓	×	[30]

RT Retention time, Mol.Wt. Molecular weight, Mol.For. Molecular formula, ✓, having biological activity; ×, without biological activity

1-Octanol, 2-Undecenal, butanoic acid, 3-hydroxy and hexanoic acid which are crucial for its antibacterial and antioxidant activity (Table 3, Fig. 5).

4 Discussion

The use of life-saving antibiotics has long been plagued by the ability of pathogenic bacteria to acquire and develop an array of antibiotic resistance mechanisms, the sum of which is a formidable threat to antibiotic discovery, development and use [35]. The emergence and spread of antibiotic resistance have coincided with slow progress in the development of new antibiotics which further complicates the situation. Consequently, many common and once easily treated infectious diseases are becoming more challenging to treat [36] and the investigation for novel antibiotics against these bacteria is urgent [37].

For the past two decades the endophytic fungi have been explored as bio-factories of novel bioactive molecules. The extracts and pure compounds obtained from the culture broths or fungal biomass have exerted significant antibacterial activity when tested on the bacterial strains resistant to the antibiotics currently in use [38]. In the present study, we isolated two endophytic fungi *T. assiutensis* HJ.14 and *F. oxysporum* HJ.15 from the rhizomes of *P. sibiricum* and tested for their antibacterial efficacy. Among the extracts of endophytes screened, the ethyl acetate extracts of *F. oxysporum* HJ.15 exhibited significant antibacterial activity against all the tested type strains including multidrug resistant bacterial pathogens. Contrarily petroleum ether and methanol extracts showed weak antibacterial activity. In addition, the ethyl acetate extract exerted good antioxidant activity. Further chemical analyses of the active ethyl acetate extract of *F. oxysporum* HJ.15 revealed the presences of propanoic acid ethyl ester, hexadecanoic acid methyl ester, hexadecanoic acid ethyl ester, 9-Octadecenoic acid (Z)-methyl

ester, 1-Octanol, 2-undecenal, butanoic acid, 3-hydroxy and hexanoic acid. These compounds might be responsible for the antibacterial activity of the extract as previously reported elsewhere. Similarly, Naoko Togashi et al. [39] reported that the antimicrobial effect of long-chain fatty alcohol 1-Octanol on the growth of *S. aureus* with the MIC value of 256 µg/mL. On the other hand, Villa-Ruano et al. [40] reported that the compound 2-undecenal exhibited a moderate growth inhibitory activity against *H. pylori* at 94.7–110.4 µg/mL. More recently, Zahara et al. [41] and Shaaban et al. [42] reported that 9-Octadecenoic acid (Z)-, methyl ester, Hexadecanoic acid methyl ester were effective against *S. aureus*, *P. aeruginosa*, *K.pneumoniae*, and *K. pneumoniae* with very low MIC values. Similarly, other studies conducted by Nayak et al. [43], Ma et al. [44] and Yuan et al. [45] the compounds propanoic acid ethyl ester, butanoic acid, 3-hydroxy- and hexanoic acid displayed a strong antimicrobial activity against *S. aureus*, *K. pneumoniae* and *C. albicans*. By correlating the previous reports, these compounds might be responsible for the significant antibacterial activity of the extract of *F. oxysporum* against tested bacteria.

5 Conclusions

Two endophytic fungi were isolated from rhizomes of *P. sibiricum*, which were identified as *T. assiutensis* HJ.14 and *F. oxysporum* HJ.15 based on ITS sequence analysis. The ethyl acetate extracts of *F. oxysporum* HJ.15 exhibited significant antibacterial activities with very low MICs and MBCs values against the tested bacterial pathogens. In addition, it showed significant DPPH radical scavenging activity with very low EC₅₀ values. Further, GC–MS analysis showed a few crucial chemical constituents for its antibacterial and antioxidant activities were propanoic acid ethyl ester, hexadecanoic acid methyl ester, hexadecanoic

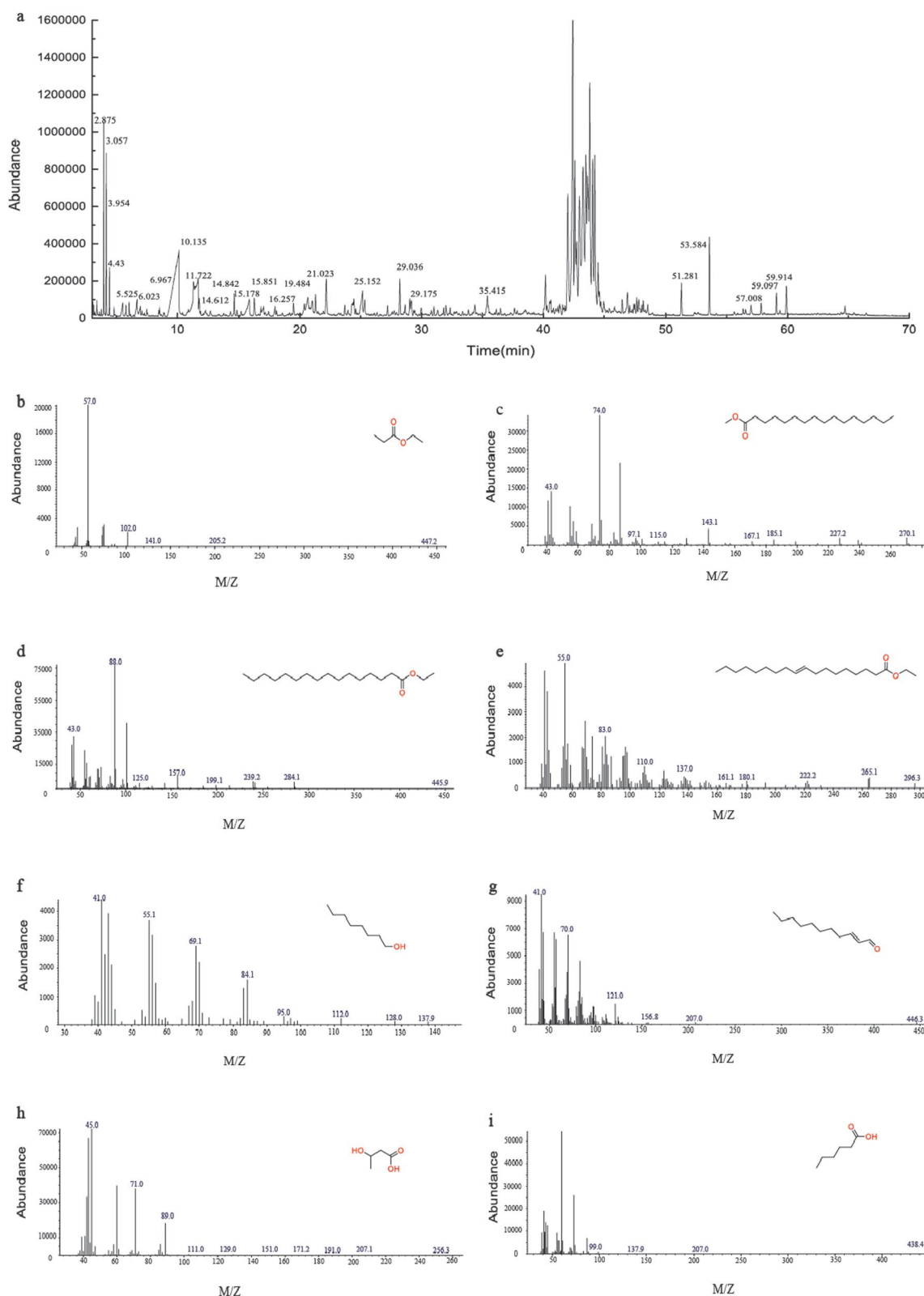


Fig. 5 GC–MS analysis of the active extract from *F. oxysporum* HJ 15 showing major volatile compounds; **a** Chromatogram of the EtOAc extract from *F. oxysporum* HJ 15; **b** mass spectrum of propanoic acid, ethyl ester; **c** mass spectrum of hexadecanoic acid, methyl ester; **d** mass spectrum of hexadecanoic acid, ethyl ester; **e** mass spectrum of 9-Octadecenoic acid (Z)-, methyl ester; **f** mass spectrum of 1-Octanol; **g** mass spectrum of 2-Undecenal; **h** mass spectrum of butanoic acid, 3-hydroxy; **i** hexanoic acid

acid ethyl ester, 9-Octadecenoic acid (Z)-methyl ester, 1-Octanol, 2-Undecenal, butanoic acid, 3-hydroxy and hexanoic acid. Overall, our investigation demonstrated the antibacterial and antioxidant capacity of endophytes isolated from *P. sibiricum* rhizomes and provided a good starting point for the identification of powerful antibacterial and antioxidant compounds with novel mechanisms of action.

Supplementary Information

The online version contains supplementary material available at <https://doi.org/10.1186/s43088-023-00392-7>.

Additional file 1. Supplementary tables and figures.

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Author contributions

Conceptualization: YL and YLR; Methodology: LYZ and YL; Software and Validation: HRS, YXF and PSK; Formal analysis, investigation and resources: XHT, YQ and PSK; Data curation: YL and MJH; Writing-original draft preparation and writing-review and editing: YL, PSK and LYZ; Visualization: KL; Supervision: YL and PSK; Project administration and funding acquisition: MJH. All authors read and approved the final manuscript

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Availability of data and materials

The supplementary data of the findings of this study are available from the corresponding author upon request.

Declarations

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper. Therefore, the authors declare no conflict of interest and approved the final manuscript.

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