Antibacterial activities of the chemical constituents of *Schizophyllum commune* MST7-3 collected from coal area

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**ABSTRACT**

Two fusidane-type active compounds (6 and 7) and five new ones (1–5), along with other nine known compounds (8–16) were isolated from the metabolites of *Schizophyllum commune* MST7-3. Their structures were elucidated on the basis of spectroscopic analysis. The absolute configurations of compounds 2 and 3 were established by Mosher’s method and optical rotation. Compounds 6 and 7 showed significant antibacterial activities against *Stenotrophomonas maltophilia* with MIC values of 4 μg/mL and 16 μg/mL, respectively.

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1. Introduction

The Gram-negative bacteria has become an issue of great concern worldwide since the ‘SKAPE’ has been listed as the prime class of opportunistic pathogens to human-kind (Tacconelli et al. 2018). This imminent health threat has put humanity in a bind again especially only one new anti-Gram-negative drug was developed from 2000 to
It signifies that infections caused by pathogens including resistance-bacteria and superbugs would lead to 10 million deaths per annum and would have an impact on the global economy of more than $100 billion by 2050 (Deng and Yu 2018). *Stenotrophomonas maltophilia* is an aerobic, gram-negative bacillus that has emerged as an important opportunistic pathogen to clinic infections including meningitis, septicemia, pneumonia, urinary tract infection, skin and soft-tissue infection (Correia et al. 2014; Furuichi et al. 2016; Saggini et al. 2021). Due to inherent resistance to multiple antibiotics of *S. maltophilia* and lack of antibiotics effective against *S. maltophilia*, *S. maltophilia* infection has posed a therapeutic challenge to infectious disease clinicians. Thus, development of new antibiotics has become a high priority in biomedical research. Fungi collected from extreme environments (e.g. acidic, metal-rich, and ultra-high temperatures) have been proved to be a promising source of novel bioactive compounds (Elleuche et al. 2015; Fellet 2017; Sibanda et al. 2017; Stierle et al. 2017), and *Penicillium* sp. collected from Xiren coal area was demonstrated to produce antibiotic metabolites against *Candida albicans* in our previous study (Xu et al. 2021). As a continuing effort to discover new antibacterial compounds from the secondary metabolites of fungus collected from coal area, a crude extract of solid-cultured fermentation of *Schizophyllum commune* MST7-3 displayed medium antibacterial activity against *Stenotrophomonas maltophilia* (MIC = 2 mg/mL). Two fusidane-type active compounds (6 and 7) and five new ones (1–5, Figure 1), along with other nine known compounds (8–16) were isolated. Here the isolation, structure elucidation, and antibacterial activities of these compounds are described.

### 2. Results and discussion

Compound 1 was obtained as a white amorphous powder. It was determined to have a molecular formula of C$_{12}$H$_{14}$O$_5$ based on the HR ESIMS peak at m/z 237.0768 [M – H]$^-$ (calcd. 237.0763), corresponding to six degrees of unsaturation. The IR spectrum of 1 revealed the presence of hydroxyl (3469 cm$^{-1}$), carbonyl (1691 cm$^{-1}$) and aromatic ring (1617 and 1516 cm$^{-1}$). The $^1$H NMR of 1 revealed the presence of a 1,2,4-trisubstituted phenyl [$\delta_{H}$ 7.45 (1H, d, $J$ = 2.0 Hz), 6.98 (1H, d, $J$ = 8.5 Hz), 7.52 (1H, dd, $J$ = 8.5, 2.0 Hz)], one olefinic proton [$\delta_{H}$ 5.78 (1H, m)], two oxymethylenes [$\delta_{H}$ 4.76 (2H, d, $J$ = 6.4 Hz) and 4.00 (2H, s)], and one methyl [$\delta_{H}$ 1.78 (3H, s)]. $^{13}$C NMR spectrum

![Figure 1. Structures of compounds 1–5.](image-url)
displayed a total of 12 carbon signals for one carboxyl (δC 170.3), six aromatic carbons (δC 152.2, 147.6, 124.9, 123.5, 117.5, and 113.2), two olefinic carbons (δC 141.5 and 120.5), two oxymethylene (δC 67.7 and 66.4), and one methyl (δC 14.0). A 3,4-dioxybenzoic acid fragment was further established by the HMBC correlations from H-2 (δH 7.45) to C-3 (δC 147.6), C-4 (δC 152.2), C-6 (δC 123.5), and C-7 (δC 170.3) (Figures S6 and S43), from H-6 (δH 7.52) to C-2 (δC 117.5), C-4, and C-7, and from H-5 (δH 6.98) to C-1 (δC 124.9), C-3, and C-4. A 4'-hydroxy-3'-methylbut-2'-en-1-yl group was established by HMBC correlations from H2-1' (δH 4.76) to C-2' (δC 120.5), C-3' (δC 141.5), from H2-4' (δH 4.00) to C-3', C-2', and from H3-5' (δH 1.78) to C-2', C-3', and C-4' (δC 67.7). This group was further connected to C-4 via an oxygen-bridge by the HMBC correlations from H2-1 to C-4 (δC 67.7 and 66.4), and one methyl (δC 14.0). The configuration of the δ2' double bond of 1 was shown to be E by the NOESY correlations of H2-1'/H3-5', and H-2'/H2-4' (Figure S7). Based on the foregoing data, compound 1 was finally identified as (E)-4-(4-hydroxy-3-methylbut-2-en-1-yl)oxybenzoic acid.

Compound 2 was obtained as a white amorphous powder. The HR ESIMS and 13C NMR data were consistent with the molecular formula of C13H18O5, corresponding to a molecular ion peak at m/z 230.1. This group was further connected to C-4 via an oxygen-bridge by the HMBC correlations from H2-1 to C-4 (δC 67.7) and 66.4), and one methyl (δC 14.0). The configuration of the δ2' double bond of 1 was shown to be E by the NOESY correlations of H2-1'/H3-5', and H-2'/H2-4' (Figure S7). Based on the foregoing data, compound 1 was finally identified as (E)-4-(4-hydroxy-3-methylbut-2-en-1-yl)oxybenzoic acid.

The 13C NMR data of 3 bore a resemblance to those of 2, the main differences being the replacement of the signal for a methoxyl by signals for an ethyoxyl (δC 60.9 and 14.5) in 3, which suggested 3 to be the ethyl ester analogue of 2. The plane structure of 3 was further confirmed by HMBC correlation analysis (Figures S24 and S43), where correlations from H-1'' (δH 4.35) to C-7 and C-2'' (δC 14.5) confirmed the location of the ethyoxyl group at C-7. Thus, the structure of 3 was established as ethyl 4-(2,3-dihydroxy-3-methylbutoxy)benzoate. The absolute configuration of C-2' in 2 was determined by the Mosher’s method (Tran et al. 2020). Treatment of 2 with (S)-(−)- and (R)-(+) -α-methoxy-α-trifluoromethylphenylacetyl chloride in deuterated pyridine-d5 yielded the (R)- and (S)-MTPA ester derivatives 2a and 2b, respectively. The positive Δδ values [Δδ (in ppm) δS − δR] for H-4' and H-5', and negative Δδ values for H-1', H-2, H-3, H-5, and H-6 (Figures S14–S18 and S43) indicate an S configuration for C-2'. The C-2' of 3 was also assigned as S-configuration due to the similar optical rotation value ([α]D 26.5) of 3 comparison with that of compound 2 ([α]D 29.26.7).

Compound 4 was obtained as a white amorphous powder and exhibited a pseudomolecular ion peak at m/z 192.0676 [M − H]− (calcd. 192.0666) in the HR ESI
spectrum, indicating a molecular formula of C\textsubscript{10}H\textsubscript{11}NO\textsubscript{3} with six degrees of unsaturation. The \textsuperscript{1}H NMR data revealed the presence of a 1,2-disubstituted benzene [δ\textsubscript{H} 7.33 (1H, d, J = 7.2 Hz), 7.18 (1H, dd, J = 7.6, 7.5 Hz); 6.93 (1H, dd, J = 7.5, 7.2 Hz); 6.76 (1H, d, J = 7.6 Hz)], and a –CH\textsubscript{3}CH\textsubscript{3} fragment [δ\textsubscript{H} 3.88 (1H, q, J = 6.4 Hz); 0.80 (3H, d, J = 6.4 Hz)]. In the HSQC spectrum, signals at δ\textsubscript{c} 10.16 (1H, s), 5.88 (1H, s), and 4.85 (1H, s) that had no correlating carbons were assigned as exchangeable hydrogens. Its \textsuperscript{13}C NMR spectrum displayed a total of 10 carbon signals for one carbonyl (\textit{J} \textsubscript{C, C} 178.4), six aromatic carbons (\textit{J} \textsubscript{C, C} 142.4, 130.3, 128.6, 125.4, 121.2, and 109.0), one oxygenated quaternary carbon (\textit{J} \textsubscript{C} 78.2), one oxymethine (\textit{J} \textsubscript{C} 70.0), and one methyl (\textit{J} \textsubscript{C} 16.7). These data were very similar to those of 3-hydroxy-3-methylindolin-2-one except for those for a hydroxyethyl group instead of a methyl group in 4 (Nakazaki et al. 2016). A 3-hydroxyindolin-2-one moiety in 4 was further confirmed by HMBC correlations from H-5 (δ\textsubscript{H} 7.33) to C-3 (δ\textsubscript{C} 78.2), C-7 (δ\textsubscript{C} 128.6), and C-9 (δ\textsubscript{C} 142.4) (Figures S30 and S43), from H-6 (δ\textsubscript{H} 6.93) to C-8 (δ\textsubscript{C} 109.0) and C-4 (δ\textsubscript{C} 130.3), from H-7 (δ\textsubscript{H} 7.18) to C-5 (δ\textsubscript{C} 125.4) and C-9, from H-8 (δ\textsubscript{H} 6.76) to C-6 (δ\textsubscript{C} 121.2) and C-4, and from NH (δ\textsubscript{H} 10.16) to C-2 (δ\textsubscript{C} 178.4), C-3, C-4, and C-9 as well as from OH-C\textsubscript{3} (δ\textsubscript{H} 5.88) to C-2, C-3, and C-4. A hydroxyethyl fragment (C\textsubscript{10}–C\textsubscript{11}) was figured out by the coupling pattern (δ\textsubscript{H} 3.88, 1H, q, J = 6.4 Hz; δ\textsubscript{H} 0.80, 3H, d, J = 6.4 Hz) and the HMBC correlation from H-11 (δ\textsubscript{H} 0.80) to C-10 (δ\textsubscript{C} 70.0), and was placed at C-3 by the HMBC correlations from H-10 (δ\textsubscript{H} 3.88) to C-2, C-3, and C-4. The presence of strong NOESY correlation of OH-C\textsubscript{10} (δ\textsubscript{H} 4.85)/OH-C\textsubscript{3} (δ\textsubscript{H} 5.88), and weak NOESY correlations of H-10 (δ\textsubscript{H} 3.88)/CH\textsubscript{3}-11 (δ\textsubscript{H} 0.80) and OH-C\textsubscript{10} and absence of NOESY correlation of OH-C\textsubscript{3}/CH\textsubscript{3}-11 and H-10 indicated that the hydroxyethyl group likely adopted a preferred configuration with the OH-C\textsubscript{3} close to OH-C\textsubscript{10} and far from CH\textsubscript{3}-11 and H-10, suggesting the configurations of C\textsubscript{3} and C\textsubscript{10} to be R and R (or S and S), respectively (Figures S31 and S32). Compound 5 was identified to have the same planar structure as 4 with the aid of 1D and 2D NMR spectra (Figures S36–S40 and S43), yet the configurations of C\textsubscript{3} and C\textsubscript{10} should be S and R (or R and S) due to a weak NOESY correlation of OH-C\textsubscript{3} (δ\textsubscript{H} 5.80)/H-10 (δ\textsubscript{H} 3.88) and the absence of NOESY correlation between OH-C\textsubscript{10} close to OH-C\textsubscript{3} (Figures S40 and S41). Finally, compounds 4 and 5 were determined to be racemates because of their lack of optical activity and have been further confirmed with chiral column analysis (Figures S33 and S42). Based on the above evidence, the structures of 4 and 5 were successively identified as \textit{(R/S)-3-hydroxy-3-((R/S)-1-hydroxyethyl)indolin-2-one} and \textit{(R/S)-3-hydroxy-3-((S/R)-1-hydroxyethyl)indolin-2-one}, respectively.

Eleven known compounds were identified as helvolinic acid (6) (Lv et al. 2017), 3,7-diketo-cephalosporin P\textsubscript{1} (7) (Limbadi et al. 2018), (24R)-6β-hydroxy-24-ethylcholest-4-en-3-one (8) (Huang et al. 2015), dankasterone A (9) (Amagata et al. 2007), (22E,24R)-3β-hydroxyergosta-7,22-diene-6-one (10) (Zang et al. 2013), (3β, 22E)-ergosta-5, 7, 22-trien-3-ol (11) (Choon et al. 2012), juglansnoid B (12) (Cheng et al. 2017), \textit{trans}-ferulic acid (13) (Prachayasittikul et al. 2009), (S)-3-hydroxy-3-(2-oxopropyl)indolin-2-one (14) (Bähn-Caballero et al. 2015), schizostatin (15) (Woo et al. 2019), and (13S)-8-oxo-(9E,11E)-8-oxo-octadeca-9,11-dien-13-olide (16) (Yang et al. 2020) by comparison of their optical rotation, NMR and MS data with those reported.

Since fraction-extract from metabolites of \textit{Schizophyllum commune} MST7-3 displayed significant growth inhibition activity against Gram negative pathogen of
Stenotrophomonas maltophilia ATCC 13637. All isolated compounds (1–16) were tested for their antimicrobial activities against Escherichia coli ATCC 25922, Pseudomonas aeruginosa ATCC9027, Enterobacter cloacae ATCC13047, Stenotrophomonas maltophilia ATCC 13637, Klebsiella pneumonia CGMCC 1.839, Aeromonas hydrophila ATCC 7966, Vibrio vulnificus NH 87-17, Vibrio alginolyticus ATCC17749, and Vibrio parahaemolyticus ATCC 17802. Compounds 6 and 7 showed significant antibacterial activities against S. maltophilia ATCC 13637 with MIC values of 4 µg/mL and 16 µg/mL and MBC values of 16 µg/mL and 64 µg/mL, respectively. However, the rest of the isolated compounds showed no antibacterial activity even at a high concentration of 128 µg/mL. The antibacterial activity of 6 against S. maltophilia ATCC 13637 was further confirmed by bacterial growth curves. According to Figure S44, S. maltophilia in the control and DMSO groups began to grow from 6 h and increased rapidly during 6–14 h. When S. malto-
philia was treated with compound 6 at 1/2 MIC, initiation of the bacterial growth of S. maltophilia was prolonged to 8 h, whereas, when S. maltophilia was treated with com-
 pound 6 at MIC and 2 MIC, bacterial growth was suppressed to 14 and 24 h, respect-
ively. Complete 36 h bacterial growth inhibition was observed at 4 MIC (MBC). Fusidane-type antibacterial compounds (e.g. helvolic acid and fusidic acid) exhibit sig-
nificant antibacterial activity against Gram positive bacteria (e.g. Staphylococcus aureus, Streptococcus agalactiae, Mycobacterium tuberculosis, S. saprophyticus, Enterococcus faecalis, E. faecalis, and Bacillus subtilis), yet they were reported to be active against only several Gram negative bacteria (e.g. Ralstonia solanacearum and Xanthomonas campestris pv. Vescitaria) (Sanmanoch et al. 2016; Kong et al. 2018). The current study showed that helvolic acid analogues helvolinic acid (6) and 3,7-diketo-cephalosporin P1 (7) also possessed significant antibacterial activities against Gram negative bacteria S. maltophilia, which will possibly broaden their application as antibacterial agents.

3. Experimental

3.1. General experimental

High resolution electrospray ionization mass spectra (HR ESIMS) were recorded on a Shimadzu LC MS-IT-TOF mass spectrometer equipped with an ESI interface. Optical rotations were measured on an Autopol I automatic polarimeter. UV spectra were obtained on a Shimadzu UV-2700 spectrometer. IR spectra were recorded by the FT-IR-650 spectrometer (as KBr disk; in cm⁻¹). NMR spectra were recorded on a Bruker AVANCE NEO 600 M NMR spectrometer (¹H: 600 MHz; ¹³C: 150 MHz). Chemical shifts are expressed in δ (ppm) referring to the residual solvent peak. Semi-preparative HPLC isolation was performed on a LC 3000 system equipped with UV detector and a Kromasil C₁₈ column (10 mm × 250 mm, 5 µm) with flow rate of 3 mL/min. Sample analysis was executed on LC-10Atvp system equipped with SPD-10Avp detector and CHIRALPAK® IA chiral column (Lot No. IA00CE-UFO73, 4.6 mm × 250 mm, 5 µm). Sephadex LH-20 was purchased from GE healthcare company. Silica gel (200–300 mesh) for column chromatography (CC) and silica gel GF₂₅₄ (10–40 µm) for TLC and preparative TLC were obtained from Qingdao Haiyang Chemical Co. Ltd., China. All solvents were of analytical grade.
3.2. Fungal material

_Schizophyllum commune_ MST7-3 was isolated from the soil collected in Xinren coal area of Guizhou province in China, in May 2020, and identified on the basis of the sequence analysis of the ITS region of the rDNA (see Supplementary material S1). The fungus was deposited at the Microbiological Collection Center of Guizhou Medical University (GMU-2020-MST 7-3).

3.3. Extraction and isolation

The rice medium (8 kg) cultured _Schizophyllum commune_ MST7-3 was extracted with ethyl acetate (30 L × 2, 2 days for each time) at room temperature. The solvent was evaporated under reduced pressure to afford a residue (46.0 g). The residue was subjected over a silica gel column (Φ 60 mm × 500 mm) and eluted with petroleum ether-acetone (10:1, 5:1, 3:1, 1:1, 0:1, each 3000 mL) to give four fractions (Fr. A–D). Fraction B (1.3 g) showing strong antibacterial activity against _S. maltophilia_ was further separated over a Sephadex LH-20 column eluted with CHCl3-MeOH (3:2, V:V) to yield Fr. B1 (210 mg), B2 (480 mg), B3 (116 mg), B4 (210 mg) and B5 (110 mg). Compound 11 (7 mg) was obtained in methanol as a crystal from fraction B1, the rest of B1 was subsequently purified by semi-preparative HPLC gradient elution with MeOH-H2O [85:15→93:7 (V:V)] to produce compounds 15 (5 mg, tR = 16 min), 9 (10 mg, tR = 21 min) and a subfraction B1a which was further isolated to provide 8 (3 mg) by preparative TLC developed with CH2Cl2-MeOH (V:V = 30:1). Fraction B2 was chromatographed over a silica gel column (2.0 × 45 cm) eluted with petroleum ether-EtOAe (10:1, 7:1, 5:1, 3:1, each 700 mL) to afford subfractions B2a–B2d. Fraction B2b was subjected to semi-preparative HPLC eluted with MeOH-H2O (72:28, V:V) to provide 6 (16.0 mg, tR = 20 min) and 7 (7.0 mg, tR = 22 min). 10 (4.0 mg, tR = 48 min) was obtained from B2c by HPLC eluted with MeOH-H2O (80:20, V:V). Fraction B2d was subjected to semi-preparative HPLC eluted with MeOH-H2O (66:34, V:V) to afford 12 (2.0 mg, tR = 28 min). Compounds 2 (6.2 mg, tR = 15 min) and 3 (3.7 mg, tR = 10 min) were obtained from fraction B3 by HPLC using MeOH-H2O (55:45, V:V). Fraction B4 was subjected to semi-preparative HPLC eluted with MeOH-H2O (28:72→40:60, V:V) to afford 13 (2.0 mg, tR = 30 min) and 16 (0.6 mg, tR = 42 min) . Fraction B5 was subsequently purified by semi-preparative HPLC using eluent of MeOH-H2O (38:62→45:55, V:V) to afford compound 1 (2.2 mg, tR = 20 min) and a mixture, which was further subjected to HPLC using MeOH-H2O (15:85, V:V) as mobile phase to produce compounds 4 (3.7 mg, tR = 19 min), 5 (1.6 mg, tR = 22 min), and 14 (0.7 mg, tR = 32 min).

**Compound 1**: white amorphous powder; UV (MeOH) λmax (log ε): 204 (4.40), 218 (4.28), 257 (4.00), 294 (3.71) nm; IR (KBr) νmax: 3469, 2936, 2862, 1691, 1617, 1516, 1426, 1369, 1278, 1221, 1137, 1063, 983, and 768 cm⁻¹; HR ESIMS (neg.) m/z 237.0768 [M – H]⁻ (calcd. 237.0763). ¹H NMR (600 MHz, CD3OD): δ 7.52 (1H, dd, J = 8.5, 2.0 Hz, H-6), 7.45 (1H, d, J = 2.0 Hz, H-2), 6.98 (1H, d, J = 8.5 Hz, H-5), 5.78 (1H, m, H-2), 4.76 (2H, dd, J = 6.4 Hz, H-1'), 4.00 (2H, s, H-4'), 1.78 (3H, s, H-5'), 13C NMR (150 MHz, CD3OD) δ 170.3 (C-7), 152.2 (C-4), 147.6 (C-3), 141.5 (C-3'), 124.9 (C-1), 123.5 (C-6), 120.5 (C-2'), 117.5 (C-2), 113.2 (C-5), 67.7 (C-4'), 66.4 (C-1'), 14.0 (C-5').
Compound 2: white amorphous powder; [δ]D0 -26.7 (c 0.15, CH3OH); UV (MeOH) λmax (log e): 207 (3.98), 256 (4.02) nm; IR (KBr) νmax: 3426, 2928, 2855, 1715, 1607, 1514, 1438, 1383, 1287, 1259, 1171, 1108, 1027, 851, and 771 cm\(^{-1}\); HR ESIMS (pos.) m/z 277.1025 [M + Na]+ (calcd. 277.1046). \(^1\)H NMR (600 MHz, CDCl3) δ 8.00 (2H, d, J = 8.3 Hz, H-2, 6), 6.94 (2H, d, J = 8.3 Hz, H-3, 5), 4.20 (1H, d, J = 8.5 Hz, H-1'a), 4.07 (1H, dd, J = 8.4, 7.7 Hz, H-1'b), 3.84 (1H, d, J = 7.7 Hz, H-2'), 1.34 (3H, s, H-5'), 1.29 (3H, s, H-5''), 3.89 (3H, s, H-1''). \(^13\)C NMR (150 MHz, CDCl3) δ 166.9 (C-7), 162.3 (C-4), 131.8 (C-2, 6), 123.4 (C-1), 114.3 (C-3, 5), 69.4 (C-1'), 75.9 (C-2'), 71.8 (C-3'), 52.1 (C-1''), 26.8 (C-4'), 25.2 (C-5').

Compound 3: white amorphous powder; [δ]D0 -26.5 (c 0.15, CH3OH); UV (MeOH) λmax (log e): 207 (3.97), 256 (4.04) nm; IR (KBr) νmax: 3442, 2979, 2933, 1705, 1607, 1513, 1466, 1369, 1285, 1255, 1174, 1104, 1030, and 775 cm\(^{-1}\); HR ESIMS (pos.) m/z 291.1200 [M + Na]+ (calcd. 291.1203). \(^1\)H NMR (600 MHz, CDCl3) δ 8.00 (2H, d, J = 8.9 Hz, H-2, 6), 6.94 (2H, d, J = 8.9 Hz, H-3, 5), 4.35 (2H, q, J = 7.1 Hz, H-1''), 4.20 (1H, dd, J = 9.5, 2.9 Hz, H-1'a), 4.07 (1H, dd, J = 9.5, 7.8 Hz, H-1'b), 3.84 (1H, dd, J = 8.7, 2.9 Hz, H-2'), 1.38 (3H, t, J = 7.1 Hz, H-2''), 1.34 (3H, s, H-4'), 1.29 (3H, s, H-5'). \(^13\)C NMR (150 MHz, CDCl3) δ 166.2 (C-7), 162.2 (C-4), 131.8 (C-2, 6), 123.8 (C-1), 114.3 (C-3, 5), 75.8 (C-2'), 71.8 (C-3'), 69.4 (C-1''), 60.9 (C-1''), 26.8 (C-4'), 25.2 (C-5''), 14.5 (C-2'').

Compound 4: white amorphous powder; [δ]D0 0 (c 0.15, CH3OH); UV (MeOH) λmax (log e): 209 (3.21), 248 (2.59), 290 (1.99) nm; IR (KBr) νmax: 3386, 2973, 2928, 1715, 1624, 1469, 1324, 1263, 1195, 1056, and 755 cm\(^{-1}\); HR ESIMS (neg.) m/z 192.0676 [M – H]⁻ (calcd. 192.0666). \(^1\)H NMR (600 MHz, DMSO-d6): δ 10.16 (1H, s, -NH), 7.33 (1H, d, J = 7.2 Hz, H-5), 7.18 (1H, dd, J = 7.6, 7.5 Hz, H-7), 6.93 (1H, dd, J = 7.5, 7.2 Hz, H-6), 6.76 (1H, d, J = 7.6 Hz, H-8), 5.88 (1H, s, OH-C3), 4.85 (1H, s, OH-C10), 3.88 (1H, q, J = 6.4 Hz, H-10), 0.80 (3H, d, J = 6.4 Hz, CH3-11). \(^13\)C NMR (150 MHz, DMSO-d6): δ 178.4 (C-2), 142.4 (C-9), 130.3 (C-4), 128.6 (C-7), 125.4 (C-5), 121.2 (C-6), 109.0 (C-8), 78.2 (C-3), 70.0 (C-10), 16.7 (C-11).

Compound 5: white amorphous powder; [δ]D0 0 (c 0.15, CH3OH); UV (MeOH) λmax (log e): 209 (3.16), 248 (2.52), 290 (1.99) nm; IR (KBr) νmax: 3398, 2962, 2925, 1713, 1623, 1475, 1276, 1200, 1099, 1062, and 802 cm\(^{-1}\); HR ESIMS (pos.) m/z 216.0620 [M + Na]+ (calcd. 216.0631). \(^1\)H NMR (600 MHz, DMSO-d6): δ 10.07 (1H, s, -NH), 7.24 (1H, d, J = 7.3 Hz, H-5), 7.18 (1H, dd, J = 7.6, 7.6 Hz, H-7), 6.92 (1H, dd, J = 7.6, 7.3 Hz, H-6), 6.75 (1H, d, J = 7.6 Hz, H-8), 5.80 (1H, s, OH-C3), 4.64 (1H, d, J = 5.7 Hz, OH-C10), 3.88 (1H, q, J = 6.4 Hz, H-10), 1.22 (3H, d, J = 6.4 Hz, H-11). \(^13\)C NMR (150 MHz, DMSO-d6): δ 179.7 (C-2), 143.2 (C-9), 130.0 (C-4), 128.6 (C-7), 125.4 (C-5), 120.8 (C-6), 109.1 (C-8), 78.1 (C-3), 69.8 (C-10), 17.1 (C-11).

\((R\))-MTPA ester (2a): HR ESIMS (pos.) m/z 493.1439 [M + Na]+ (calcd. 493.1445). \(^1\)H NMR (600 MHz, pyridine-d5): 8.18 (2H, d, J = 8.8 Hz, H-2, 6), 7.92 (2H, m, MTPA-Ar-H), 7.40 (3H, m, MTPA-Ar-H), 7.16 (2H, d, J = 8.8 Hz, H-3, 5), 4.90 (1H, d, J = 10.7 Hz, H-1'), 4.60 (1H, dd, J = 10.7, 9.0 Hz, H-1''), 6.00 (1H, dd, J = 9.0, 2.0 Hz, H-2'), 3.81 (3H, s, -OCH3), 3.73 (3H, s, MTPA-OCH3), 1.48 (3H, s, CH3-4'), 1.43 (3H, s, CH3-5').

\((S\))-MTPA ester (2b): HR ESIMS (pos.) m/z 493.1441 [M + Na]+ (calcd. 493.1445). \(^1\)H NMR (600 MHz, pyridine-d5): 8.16 (2H, d, J = 8.8 Hz, H-2, 6), 7.96 (2H, m, MTPA-Ar-H), 7.40 (3H, m, MTPA-Ar-H), 7.10 (2H, d, J = 8.8 Hz, H-3, 5), 4.73 (1H, d, J = 10.5 Hz, H-1'), 4.40 (1H, dd, J = 10.5, 8.9 Hz, H-1''), 6.02 (1H, dd, J = 8.9, 2.0 Hz, H-2'), 3.81 (3H, s, -OCH3), 3.75 (3H, s, MTPA-OCH3), 1.53 (6H, s, CH3-4, 5).
3.4. Antibacterial assay

The antibacterial activity was evaluated according to the reported procedure (Liu et al. 2020) with minor modification. All test microorganisms were prepared using a final concentration of $5 \times 10^5$ CFU/mL for antibacterial assay. Specifically, strains of *E. coli* ATCC 25922, *P. aeruginosa* ATCC9027 and *E. cloacae* ATCC13047 were incubated in Mueller-Hinton Broth (Lot: 401E031, Solarbio) at 37°C, *S. maltophilia* ATCC 13637, *K. pneumonia* CGMCC 1.839 and *A. hydrophila* ATCC 7966 were cultured on 0002 medium (1% peptone, 0.3% beef extract, 0.5% NaCl in distilled water with pH 7.0) at 30°C, *V. vulnificus* NH 87-17 and *V. alginolyticus* ATCC17749 were grew on 2216E Liquid Medium (Lot: 20210529, Hopebio) at 30°C and 37°C, respectively. *V. parahaemolyticus* ATCC 17802 was incubated in 0232 medium (1% peptone, 0.3% beef extract, 3% NaCl in distilled water with pH 7.0) at 37°C. Then two-fold dilution of samples (1–16) were made in 96-well plates (128 µg/mL and 2 µg/mL for the starting and the end concentrations, respectively, 100 µL in each well), and 100 µL of bacterial suspension was transferred to the wells to incubate for 16 h at their optimum culture temperatures. The final concentration of 0.25% TTC (2,3,5-triphenyl tetrazolium chloride, Sigma) in wells as microorganism growth indictor was added and the microorganism was continuously incubated for 3 h before MIC reading. Equivalent amounts of DMSO (2.5%) and medium were successively used as negative controls and blank control. Trimethoprim and levofloxacin were used as positive controls. All experiments were performed in three replicates. The growth curve of *S. maltophilia* was determined according to literature (Chen et al. 2020). Briefly, 100 µL of bacterial suspension ($5 \times 10^5$ CFU/mL) and samples (blank, DMSO, and compound 6) were successively added into MH culture solution and incubated at 37°C, and the growth was tested by measuring OD$_{600nm}$ using microplate reader at intervals from 0 to 36h. All experiments were performed in triplicate and each with 4 parallels. The data were expressed as the mean ± SD and analyzed through ANOVA by using Graphpad prism 9.0.

4. Conclusion

In summary, sixteen compounds (1–16) were isolated from the ethyl acetate extract of the solid-state fermented culture of *S. commune* MST7-3, including five new compounds named as (E)-4-((4-hydroxy-3-methylbut-2-en-1-yl)oxy) benzoic acid (1), methyl (S) 4-(2,3-dihydroxy-3-methylbutoxy)benzoate (2), ethyl (S) 4-(2,3-dihydroxy-3-methylbutoxy)benzoate (3), (R/S)-3-hydroxy-3-((R/S)-1-hydroxyethyl)indolin-2-one (4), and (R/S)-3-hydroxy-3-((S/R)-1-hydroxyethyl)indolin-2-one (5). Compounds 6 and 7 showed significant antibacterial activity against *S. maltophilia* ATCC 13637 with MIC values of 4 µg/mL and 16 µg/mL.

Disclosure statement

No potential conflict of interest was reported by the authors.
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References

Amagata T, Tanaka M, Yamada T, Doi M, Minoura K, Ohishi H, Yamori T, Numata A. 2007. Variation in cytostatic constituents of a sponge-derived Gymnascella dankaliensis by manipulating the carbon source. J Nat Prod. 70(11):1731–1740.

Bañín-Caballero A, Flores-Ferrándiz J, Guillena G, Nájera C. 2015. Enantioselective solvent-free synthesis of 3-alkyl-3-hydroxy-2-oxoindoles catalyzed by binam-prolinamides. Molecules. 20(7):12901–12912.

Butler MS, Blaskovich MAT, Cooper MA. 2017. Antibiotics in the clinical pipeline at the end of 2015. J Antibiot (Tokyo). 70(1):3–24.

Chen H, Tang Y, Weir MD, Gao J, Imazato S, Oates TW, Lei L, Wang S, Hu T, Xu HHK. 2020. Effects of S. mutans gene-modification and antibacterial monomer dimethylaminohexadecyl methacrylate on biofilm growth and acid production. Dent Mater. 36(2):296–309.

Cheng ZY, Yao GD, Guo R, Huang XX, Song SJ. 2017. Phenylpropanoids from Juglans mandshurica exhibit cytotoxicities on liver cancer cell lines through apoptosis induction. Bioorg Med Chem Lett. 27(3):597–601.

Choon RLT, Sariah M, Mariam MNS. 2012. Ergosterol from the soilborne fungus Ganoderma boninense. J Basic Microbiol. 52(5):608–612.

Correia CR, Ferreira ST, Nunes P. 2014. Stenotrophomonas maltophilia: rare cause of meningitis. Pediatr Int. 56(4):e21–e22.

Deng H, Yu H. 2018. New antibiotics: where are they?. Biomed J Sci Tech Res. 10(1):1–3.

Elleuche S, Schafers C, Blank S, Schröder C, Antranikian G. 2015. Exploration of extremophiles for high temperature biotechnological processes. Curr Opin Microbiol. 25:113–119.

Fell E. 2017. Fungal duo from toxic lake produces novel antibiotic. C&EN Glob Enterp. 95(17):3.

Furuichi M, Ito K, Miyairi I. 2016. Characteristics of Stenotrophomonas maltophilia bacteremia in children. Pediatr Int. 58(2):113–118.

Huang R, Ma KX, Xie XS, Wang T, Wu SH. 2015. Secondary metabolites of an endophytic fungus Phomopsis sp. Chem Nat Compd. 51(2):392–394.

Kong F-D, Huang X-L, Ma Q-Y, Xie Q-Y, Wang P, Chen P-W, Zhou L-M, Yuan J-Z, Dai H-F, Luo D-Q, et al. 2018. Helvolic acid derivatives with antibacterial activities against Streptococcus agalactiae from the marine-derived fungus Aspergillus fumigatus HNMF0047. J Nat Prod. 81(8):1869–1876.

Limbadri S, Luo X, Lin X, Liao S, Wang J, Zhou X, Yang B, Liu Y. 2018. Bioactive novel indole alkaloids and steroids from deep sea-derived fungus Aspergillus fumigatus SCSIO 41012. Molecules. 23(9):2379.

Liu X, Cai J, Chen H, Zhong Q, Hou Y, Chen W, Chen W. 2020. Antibacterial activity and mechanism of linalool against Pseudomonas aeruginosa. Microb Pathog. 141:103980.

Lv JM, Hu D, Gao H, Kushiro T, Awakawa T, Chen GD, Wang CX, Abe I, Yao XS. 2017. Biosynthesis of helvolic acid and identification of an unusual C-4-demethylation process distinct from sterol biosynthesis. Nat Commun. 8(1):1644.

Nakazaki A, Mori A, Kobayashi S, Nishikawa T. 2016. A divergent approach to the diastereoselective synthesis of 3,3-disubstituted oxindoles from atropisomeric N-aryl oxindole derivatives. Chem Asian J. 11(22):3267–3274.
Nguyen MTT, Dang PH, Nguyen TN, Bui LTH, Nguyen HX, Le TH, Do TNV, Nguyen NT. 2018. Paratrimerins G and H, two prenylated phenolic compounds from the stems of Paramignya trimera. Phytochem Lett. 23:78–82.

Prachayasitikul S, Suphapong S, Worachartcheewan A, Lawung R, Ruchirawat S, Prachayasitikul V. 2009. Bioactive metabolites from Spilanthes acmella Murr. Molecules. 14(2):850–867.

Saggini A, Gorkiewicz G, Cerroni L. 2021. Cutaneous lymphohistiocytic infiltrates with foamy macrophages: a novel histopathological clue to Stenotrophomonas maltophilia septicemia. J Cutan Pathol. 48(1):160–164.

Sanmanoch W, Mongkolthanaruk W, Kanokmedhakul S, Aimi T, Boonlue S. 2016. Helvolic acid, a secondary metabolite produced by Neosartorya spinosa KKU-1NK1 and its biological activities. Chiang Mai J Sci. 43(3):483–493.

Sibanda T, Selvarajan R, Tekere M. 2017. Synthetic extreme environments: overlooked sources of potential biotechnologically relevant microorganisms. Microb Biotechnol. 10(3):570–585.

Stierle AA, Stierle DB, Decato D, Priestley ND, Alverson JB, Hoody J, McGrath K, Klepacki D. 2017. The berkeleylactones, antibiotic macrolides from fungal coculture. J Nat Prod. 80(4):1150–1160.

Tacconelli E, Carrara E, Savoldi A, Harbarth S, Mendelson M, Monnet DL, Pulcini C, Kahlmeter G, Kluytmans J, Carmeli Y, et al. 2018. Discovery, research, and development of new antibiotics: the WHO priority list of antibiotic-resistant bacteria and tuberculosis. Lancet Infect Dis. 18(3):318–327.

Tran TD, Wilson BAP, Henrich CJ, Wendt KL, King J, Cichewicz RH, Stchigel AM, Miller AN, O’Keefe BR, Gustafson KR. 2020. Structure elucidation and absolute configuration of metabolites from the soil-derived fungus Dictyosporium digitatum using spectroscopic and computational methods. Phytochemistry. 173:112278.

Woo EE, Kim JY, Kim JS, Kwon SW, Lee IK, Yun BS. 2019. Mannonerolidol, a new nerolidol mannioside from culture broth of Schizophyllum commune. J Antibiot. 72(3):178–180.

Xu GB, Yang FY, Wu XY, Li R, Zhou M, Wang B, Yang XS, Zhang TT, Liao SG. 2021. Two new dihydroisocoumarins with antimicrobial activities from the fungus Penicillium sp. XR046 collected from Xinren coal area. Nat Prod Res. 35(9):1445–1451.

Yang H, Gan C, Guo Y, Qu L, Ma S, Ren Y, Wang X, Wang L, Huang J, Wang J. 2020. Two novel compounds from green walnut husks (Juglans mandshurica Maxim.). Nat Prod Res.:1–9. doi:10.1080/14786419.2020.1860976.

Zang Y, Xiong J, Zhai WZ, Cao L, Zhang SP, Tang Y, Wang J, Su J, Yang GX, Zhao Y, et al. 2013. Fomentarols A-D, sterols from the polypore macrofungus Fomes fomentarius. Phytochemistry. 92(4):137–145.