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# Screening and isolation of antibacterial activities of the fermentative extracts of freshwater fungi from Yunnan Province, China

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**Abstract** - The EtOAc extracts of cultural filtrates of 30 freshwater fungi were assayed for the *in vitro* antibacterial activity against *Bacillus cereus* YMF 3.19, *Brevibacillus laterosporus* YMF 3.08, *Escherichia coli* YMF 3.16, *Staphylococcus aureus* YMF 3.17 using the disc diffusion method. A total of 14 fungal strains displayed active against all of the bacteria tested and the strongest antibacterial activity was recorded in *Camposporium quercicola* YMF1.01300 isolate. Extraction of fermentation broth of *C. quercicola* YMF1.01300 and various separation and purification steps led to isolation of three pure active molecules. The chemical structures of these three compounds, a new diphenyl ether, named as quercilolin (compound **1**), as well as two previously known compounds, tenellic acid A (compound **2**), and 2′,4′-dihydroxyacetophenone (compound **3**), were established by analysis of NMR and MS data, and by comparison with reference data from the literature. These results indicate that some freshwater fungi could be a potential source of antibacterial agents.

**Key words**: *Camposporium quercicola*, freshwater fungi, quercilolin, antibacterial.

## INTRODUCTION

The search for bioactive compounds (for example, to overcome the danger of increasing microbial resistances) is one of the central subjects of industrial and academic natural products chemistry (Zahner and Fiedler, 1995). Various methods to achieve this goal have been described in the literature (such as combinatorial chemistry or high-throughput screening of different biological sources) (Grabley *et al*., 1999; Maier *et al*., 1999; Dolle, 2000). Today, the search for new producers of biologically active compounds is actively underway among fungi growing under extreme conditions, because the synthesis of new secondary metabolites and potential biologically active compounds that help them to survive and adapt to these conditions can be expected in these fungi with the greatest probability (Gloer, 1995, 1997; Grabley *et al*., 1999). Among fungi from the diverse environments, the freshwater aquatic fungi have received attention from the scientific community. Consequently several groups of investigators have identified that freshwater fungi contain some unique biologically active metabolites such as decaspirones (Jiao *et al*., 2006), gliocladines (Dong *et al*., 2005), resorcylides (Dong *et al*., 2007), pseudohalonectrins (Dong *et al*., 2006), massarinolins (Oh *et al*., 1999), ophiocerins (Reátegui *et al*., 2005), massarigenins (Oh *et al*., 2003) etc. There may be many more freshwater fungi, not yet tested, which could prove to be a fruitful source of biologically active secondary metabolites.

In the present work the EtOAc extracts of cultural filtrates of 30 fungi isolated from wood submerged in freshwater habitats of Yunnan Province, China, were selected to screen for their antibacterial activity against four species of bacteria and three antibacterial molecules from *Camposporium quercicola* YMF1.01300, a new diphenyl ether, named as quercilolin (compound **1**), as well as two previously known compounds, tenellic acid A (compound **2**), and 2′,4′-dihydroxyacetophenone (compound **3**), was isolated and elucidated.

## MATERIALS AND METHODS

**Fungal isolate, maintenance and growth conditions.** Thirty freshwater fungal strains investigated in this study were isolated from decaying woods collected from lakes in Yunnan Province, China (Cai *et al*., 2002; Luo *et al*., 2004) and listed in Table 1. These strains were maintained on Potato Dextrose agar (PDA) slants at 4 °C for routine work, and preserved in the Culture Collection of the Laboratory for Conservation and Utilization of Bio-resources, Yunnan University, Yunnan Province, China, in 10% glycerol at -140 °C. Each fungal isolate was grown in submerged culture in 250 mL flasks containing 70 mL Potato Dextrose broth (PDB). A 2 $cm^2$ piece of agar from each ten-day-

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TABLE 1 - Antibacterial activity of the EtOAc extracts of 30 freshwater fungi

| Freshwater fungi | Bacteria | | | |
|---|---|---|---|---|
| | *Bacillus cereus* YMF 3.19 | *Brevibacillus laterosporus* YMF 3.08 | *Escherichia coli* YMF 3.16 | *Staphylococcus aureus* YMF 3.17 |
| *Camposporium quercicola* YMF 1.01300 | 2.7 | 2.2 | 1.3 | 3.0 |
| *Caryospora callicarpa* YMF 1.01299 | 2.1 | 1.4 | 0 | 0 |
| *Caryospora minima* YMF 1.02118 | 1.7 | 1.5 | 1.1 | 0.9 |
| Coelomycete sp. 1.02105 | 1.7 | 2.2 | 1.8 | 2.1 |
| *Dactylella leptospora* YMF1.01832 | 0.8 | 0.9 | 1.1 | 0.7 |
| *Dictyochaeta plovercovensis* YMF1.02114 | 0.8 | 1.4 | 1.4 | 1.8 |
| *Dictyosporium cocophylum* YMF1.01123 | 0 | 0 | 0 | 0 |
| *Dictyosporium heptasporum* YMF1.01231 | 1.5 | 0.9 | 0.8 | 1.8 |
| *Dictyosporium heptasporum* YMF1.01266 | 0 | 0 | 0 | 0 |
| *Digitodesmium bambusicola* YMF1.01039 | 0 | 0 | 0.8 | 0.8 |
| *Dyrithiopsis lakefuxianensis* YMF1.01286 | 0 | 0 | 0 | 0 |
| *Lasiosphaeria breviseta* YMF 1.00958 | 1.3 | 1.2 | 0.8 | 1.3 |
| *Massarina bipolaris* YMF 1.01191 | 0 | 0 | 0 | 0 |
| *Massarina fronsisubmersa* YMF 1.01028 | 0.9 | 1.3 | 0.7 | 0.9 |
| *Monacrosporium ellipsosporum* YMF 1.01448 | 0.9 | 0 | 0 | 0 |
| *Monacrosporium longiphorum* YMF 1.01402 | 0.8 | 1.5 | 1.1 | 0 |
| *Monacrosporium reticulatum* YMF 1.01820 | 0 | 0 | 0 | 0 |
| *Monacrosporium sphaeroides* YMF 1.01410 | 0 | 0 | 0 | 1.1 |
| *Ophioceras commune* YMF 1.02126 | 1 | 0 | 1.1 | 0 |
| *Ophioceras dolichostomum YMF 1.00988* | 1.0 | 1.1 | 1.6 | 2.1 |
| *Periconia minutissima* YMF 1.00955 | 0 | 0 | 0.9 | 0 |
| *Pseudohalonectria lignicola* YMF 1.01215 | 1.0 | 1.1 | 0 | 1.1 |
| *Pseudohalonectria lignicola* YMF 1.01214 | 0 | 0 | 0 | 0 |
| *Pseudohalonectria lignicola* YMF 1.01213 | 1.7 | 0.8 | 1.0 | 1.1 |
| *Pseudohalonectria lignicola* YMF 1.00947 | 1.3 | 0 | 0 | 1.7 |
| *Rosella gnutella* YMF 1.01179 | 1.1 | 1.1 | 1.3 | 0.9 |
| *Saccardoella minuta* YMF 1.00961 | 0 | 0 | 0 | 0 |
| *Torula graminis* YMF 1.01053 | 1.8 | 1.0 | 0.7 | 0.9 |
| *Torula herbarum* YMF 1.01021 | 1.7 | 2.1 | 1.4 | 2.2 |
| *Xylomyces chlamydosporus* YMF 1.00956 | 1.0 | 0.9 | 0.8 | 1.0 |

old culture grown on PDA was used to inoculate the flasks. These cultures were grown in a rotary shaker at 200 rpm, 28 °C, for 21 days.

The fungal strain *Camposporium quercicola* YMF1.01300 was selected for further scale-up fermentation grown in shake cultures on PDB medium in 200-mL batches in 500-mL flasks for 18 days at 25 °C, extraction with EtOAc, fractionation and structural elucidation of its bioactive secondary metabolites.

**Chemical extraction of fungal cultures.** Crude antimicrobial compound was recovered from the culture filtrate of each isolate of 30 freshwater fungi by solvent extraction with ethyl acetate (EtOAc). EtOAc was added to the filtrate in the ratio 1 : 1 (v/v) and shaken vigorously for 20 min. The organic layers were collected and ethyl acetate was removed at reduced pressure. The residues were weighed and then reconstituted with acetone to the desired concentration.

**Extraction and isolation of secondary metabolites of *Camposporium quercicola* YMF1.01300.** A total of 15 L of cultural filtrates of *C. quercicola* YMF1.01300 grown in PDB were filtered, first through muslin (10 µm) and then a pad of Celite (an inert support) on a filter funnel. The filtrate was concentrated to 3000 mL and extracted three times with equal volumes of EtOAc. The EtOAc layer was dried over anhydrous $MgSO_4$ and evaporated to dryness under reduced pressure to obtain a brown gum (2.3 g) that showed antimicrobial activities. This gum was fractionated by Silica gel CC using petroleum ether (bp 60-90 °C)-EtOAc gradient elution to yield fractions **A1-A5** according to TLC analysis. The active fraction **A3** (84 mg), obtained on elution with petroleum (bp 60-90 °C)/EtOAc 80%, was further purified by a Sephadex LH-20 column with acetone to yield compound **1** (11 mg) and compound **3** (5 mg). Similarly, active fraction **A5** (64 mg), obtained on elution with petroleum (bp 60-90 °C)/EtOAc 25%, was further purified by Sephadex LH-20 column with MeOH to yield compound **2** (6 mg).

**Identification of compounds 1-3.** The structures of compounds **1**-**3** isolated from the cultures of *C. quercicola* YMF1.01300 were determined by spectroscopic analysis. Infrared (IR) spectra were obtained in KBr pellets with a Bio-Rad FTS-135 spectrophotometer (Bio-Rad, Richmond, CA, USA). UV spectra were taken on a Shimadzu double-beam 210A spectrophotometer (Shimadzu, Kyoto, Japan). Optical rotations were measured with a Horiba SEPA-300 polarimeter (Horiba, Tokyo, Japan). MS was performed on an Autospec-3000 spectrometer (VG, Manchester, England). The nuclear magnetic resonance (NMR) spectra were recorded on DRX-500 NMR (Bruker, Karlsruhe, Germany) spectrometers, with TMS as an internal standard and coupling constants were represented in Hertz.

Quercilolin (compound **1**): light yellow powder. $[\alpha]^{18.9D}$+ 0.00° ($CH_3OH$; *c*0.36); UV ($CH_3OH$) $\lambda_{max}$ (log ε) 280.8 (3.38), 205.0 (4.48) nm; IR (film) $\nu_{max}$ 3421, 2953, 2924, 2850, 1619, 1600, 1496, 1467, 1350, 1323, 1218, 1178, 1151, 1124, 1062, 1032, 970, 946, 836, 795, 742, 683 $cm^{-1}$; for NMR data, see Table 1; EI-MS *m/z* (rel. int ) 261 $[M+H]^+$ (22), 260 $[M]^+$ (100),

245 (7), 230 (14), 227 (14), 217 (5), 199 (7), 153 (32), 125 (25), 107 (10), 77 (7); HRMS (ESI-TOF) *m/z*: 261.1134 $[M+H]^+$ (calcd for $C_{15}H_{17}O_4$, 261.1126).

Tenellic acid A (compound **2**): white solid. $[\alpha]^{20.9D}$ – 8.13° ($CH_3OH$, *c*0.21); UV ($CH_3OH$) $\lambda_{max}$ (log ε) 216.3 (4.36), 274.5 (3.64), 324.1 (3.26) nm; IR (film) $\nu_{max}$ 2986, 1690, 1594, 1471, 1320, 1199, 1049 $cm^{-1}$; $^1$H-NMR (500 MHz, $CD_3COCD_3$) 7.33 (1H, d, *J* = 8.5 Hz, H-4), 6.48 (1H, d, *J* = 8.5 Hz, H-5), 4.53 (1H, dd, *J* = 9.0, 4.0 Hz, H-8), 1.60 (1H, ddd, *J* = 14.2, 9.0, 5.4 Hz, H-9), 1.32 (1H, ddd, *J* = 14.2, 8.5, 4.0 Hz, H-9), 1.78 (1H, m, H-10), 0.96 (3H, d, *J* = 6.8 Hz, H-11), 0.92 (3H, d, *J* = 6.8 Hz, H-12), 3.91 (3H, s, H-13), 3.13 (3H, s, H-14), 7.15 (1H, m, H-4′), 7.20 (1H, m, H-6′), 10.2 (1H, s, H-7′), 2.35 (3H, s, H-8′); $^{13}$C-NMR (500 MHz, $CD_3COCD_3$) 119.4 (C-1), 156.6 (C-2), 131.6 (C-3), 129.8 (C-4), 111.5 (C-5), 156.0 (C-6), 167.8 (C-7), 75.8 (C-8), 47.8 (C-9), 25.3 (C-10), 23.6 (C-11), 22.3 (C-12), 63.2 (C-13), 56.6 (C-14), 130.7 (C-1′), 143.1 (C-2′), 151.1 (C-3′), 124.7 (C-4′), 137.6 (C-5′), 120.0 (C-6′), 189.7 (C-7′), 20.9 (C-8′); ESI-MS *m/z* (rel. int ) 425 $[M+Na]^+$ (78).

2′,4′-Dihydroxyacetophenone (compound **3**): colourless powder. $[\alpha]^{18.9D}$ 0 ($CHCl_3$, *c*0.16); IR (film) $\nu_{max}$ 3304, 1640, 1610, 1522, 1448. $^1$H-NMR (500 MHz, $CDCl_3$) 6.54 (1H, *s*, H-3), 6.42 (1H, *d*, *J* = 8.6 Hz, H-5), 7.64 (1H, *d*, *J* = 8.6 Hz, H-6), 2.56 (3H, *s*, -CO$\underline{CH_3}$), 12.71 (1H, *s*, OH); $^{13}$C-NMR (400 MHz, $CDCl_3$) 114.5 (C-1), 163.2 (C-2), 103.7 (C-3), 165.4 (C-4), 108.4 (C-5), 133.5 (C-6), 26.4 (-CO$\underline{CH_3}$), 203.4 (-$\underline{CO}CH_3$); ESI-MS *m/z* (rel. int ) 151 $[M - H]^-$.

**Assay of antibacterial activity.** The target strains used for screening antibacterial activity were *Bacillus cereus* YMF 3.19, *Bacillus laterosporus* YMF 3.08, *Escherichia coli* YMF 3.16, *Staphylococcus aureus* YMF 3.17. Cultures were maintained on Nutrient agar (NA) slants at 4 °C for routine work, and preserved in the Culture Collection of the Laboratory for Conservation and Utilization of Bio-resources, Yunnan University, Yunnan Province, China, in 10% glycerol at -140 °C. An inoculum of each bacterial strain was suspended in 5 mL of Muller-Hinton broth (BBL) and incubated overnight at 37 °C.

Antibacterial assays were conducted using the paper disk assay method (El-Masry *et al*., 2000). Whatman No. 1 filter paper disks of 5-mm diameter were sterilised by autoclaving for 15 min at 121 °C. Concentrations of 20 mg/mL of each sample were prepared, the sterile discs were impregnated with 10 µL of each one (final doses per disc: 200 µg). Agar plates were surface inoculated uniformly from the broth culture of the tested microorganisms. In all cases, the concentration was approximately 1.2 x $10^8$ CFU/mL. The impregnated disks were placed on the medium suitably spaced apart and the plates were incubated at 37 °C for 24 h. Discs impregnated with 10 µL of acetone partition, were used as negative controls. Disk of streptomycin (200 µg/mL) was used as a positive control. The diameter (mm) of the growth inhibition halos caused by the ethyl acetate extracts of freshwater fungi was examined. All the assays were carried out in the duplicate.

## RESULTS AND DISCUSSION

### Antibacterial activity of the EtOAc extracts from cultural filtrates of aquatic fungi

Results of the antibacterial activity of the EtOAc extracts from cultural filtrates of 30 freshwater fungi against *B. cereus* YMF 3.19, *B. laterosporus* YMF 3.08, *E. coli* YMF 3.16, and *S. aureus* YMF 3.17 were shown in Table 1. A total of 23 strains representing 77% of all test fungi isolates showed antibacterial activity against at least one of the test bacteria and appeared promising. Out of the 23 isolates, 14 EtOAc extracts from *Camposporium quercicola* YMF 1.01300, *Caryospora callicarpa* YMF 1.01299, *Caryospora minima* YMF 1.02118, *Coelomycete* sp. 1.02105, *Dactylella leptospora* YMF1.01832, *Dictyochaeta plovercovensis* YMF1.02114, *Dictyosporium heptasporum* YMF1.01231, *Lasiosphaeria breviseta* YMF 1.00958, *Massarina fronsisubmersa* YMF 1.01028, *Ophioceras dolichostomum* YMF 1.00988, *Pseudohalonectria lignicola* YMF 1.01213, *Rosella gnutella* YMF 1.01179, *Torula graminis* YMF 1.01053, *Torula herbarum* YMF 1.01021, *Xylomyces chlamydosporus* YMF1.00956 are active against all of the bacteria tested and the strongest antibacterial activity was recorded in *C. quercicola* YMF1.01300 isolate.

It was also observed that antibacterial activity differs significantly among the 30 selected fungi. In addition, the different isolates of the same species had nonconsistent antibacterial inhibition profile, such as *Dictyosporium heptasporum* and *Pseudohalonectria lignicola*. Although the reasons for these inconsistent results are unknown, the similar phenomena have been found in some other papers according to which different isolates of the same species can produce different amounts and types of active metabolites possibly due to the intraspecific differences in these fungal strains (Luckner, 1990; Ghisalberti and Sivasithamparam, 1991; Vizcainot *et al*., 2005)

The antimicrobial activities of *Dictyochaeta*, *Massarina*, *Ophioceras*, and *Torula* species have been previously reported, and the production of secondary metabolites in their cultures has already been investigated, such as L-687781 in *Dictyochaeta simplex* (VanMiddlesworth *et al*., 1991), massarinolins and massaringenins in *Massarina tunicate* (Oh *et al*., 2003), ophiocerin in *Ophioceras venezuelense* (Reátegui *et al*., 2005), and herbarin and dehyroherbarin in *T. herbarum* (Kadkol *et al*., 1971; Narasimhachari and Gopalkrishnan, 1974) etc., which could be responsible for the antibiotic activity shown in *D. plovercovensis* YMF1.02114, *M. fronsisubmersa* YMF 1.01028, *Ophioceras commune* YMF 1.02126, *O. dolichostomum* YMF 1.00988, *T. graminis* YMF 1.01053, *T. herbarum* YMF 1.01021. Likewise, the production of caryosponycins in *Caryospora callicarpa* (Dong *et al*., 2007), and pseudohalonectrins in *Pseudohalonectria adversaria* (Dong *et al*., 2006), which may be also responsible for the announced activity of *C. callicarpa* YMF 1.01299, and *P. lignicola* YMF 1.01213 and *P. lignicola* YMF 1.00947, has already been found in our prior study for nematicidal metabolites although it is not known if these compounds possess any antimicrobial activity. However, further studies are still needed because the chemical constituents of the other active species have not yet been studied, and they include *Camposporium, Dactylella, Dictyosporium, Digitodesmium, Lasiosphaeria, Monacrosporium, Periconia, Rosella*, and *Xylomyces*. Among these active species, we have been specially interested in the active components of *C. quercicola* YMF1.01300 due to its broad spectrum of activity and largest zone of inhibition.

### Identification and antibacterial activity of compounds 1-3 from C*amposporium quercicola* YMF1.01300

The EtOAc extract of the culture broth of *C. quercicola* YMF1.01300 exhibited antifungal activity and was subjected to column chromatography over silica gel, Seqhadex LH-20 to afford one novel diphenyl ether, quercilolin (compound **1**), as well as two known compounds. The structures of the known compounds were established as tenellic acid A (compound **2**) (Oh *et al*., 1999), and 2′,4′-dihydroxy-acetophenone (compound **3**) (Yasuda *et al*.,

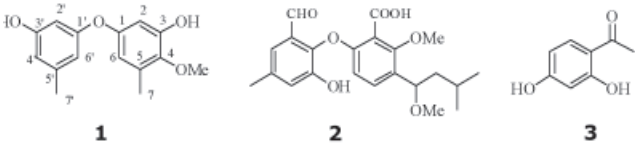

FIG. 1 - Structures of compounds **1-3**.

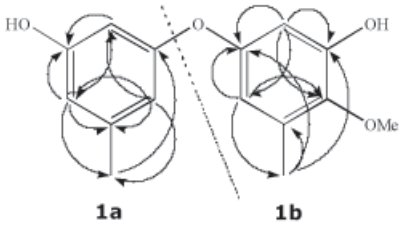

FIG. 2 - Fragment structures and key HBMC correlations of compound **1**.

1999) by comparing their spectroscopic data with those in the literature Fig. 1).

The EIMS of quercilolin (compound **1**) showed a molecular ion peak at *m/z* 260 $[M]^+$ (100), and the molecular formula $C_{15}H_{17}O_4$ was established by HRMS (ESI-TOF) [*m/z* 261.1134 $[M+H]^+$, Δ +8 mmu]. The UV spectrum of compound **1** displayed absorption maxima at 280.5 and 205.0 nm, indicating the presence of conjugated or aromatic systems. The IR spectrum showed the absorptions for hydroxyl (3421 $cm^{-1}$) and aromatic rings (1619, 1600, 1496, 1467 $cm^{-1}$). The $^1H$ NMR (Table 2) displayed two aromatic methyls at $\delta_H$ 2.04 (3H, s, $H_3$-7′) and 1.86 (3H, s, $H_3$-7), a methoxy at $\delta_H$ 3.65 (3H, s, $OCH_3$-4) and five aromatic protons at $\delta_H$ 6.28 (1H, d, *J* = 1.80 Hz, H-2), 6.21 (1H, d, *J* = 2.00 Hz, H-6), 6.16 (1H, s, H-6′), 6.00 (1H, s, H-4′), and 5.90 (1H, s, H-2′). The $^{13}C$ NMR spectrum (Table 2) exhibited 15 carbon resonances due to three methyls ($\delta_C$ 16.5, 21.6 and 55.6), five aromatic methines ($\delta_C$ 100.2, 101.1, 107.7, 107.8 and 110.3) and seven aromatic quaternary carbons ($\delta_C$ 160.5, 159.3, 158.2, 151.6, 140.9, 134.8 and 133.4). The HMQC data allowed the assignment of all the protons to their bonding carbons. Inspection of the $^1H$-$^1H$ COSY spectrum indicated that the molecule contains few spin systems, all being restricted to few resonances, and therefore providing scarce information about the carbon framework of compound **1**. However, extensive analysis of the HMBC data in acetone-$d_6$ led to two quite informative partial structures (Fragment 1a and 1b, Fig. 2). The partial structure of the 1,3,5- trisubstituted aromatic ring (Fig. 2, 1a) was established by the HMBC correlations from H-2′ to C-3′, C-4′, and C-6′, H-4′ to C-7′, C-6′, C-3′, and C-2′, H-6′ to C-2′, C-4′, C-5′ and C-7′, and H-7′ to C-1′, C-4′, and C-6′. Similarly, the presence of the 1,3,4,5- tetrasubstituted aromatic ring (Fig. 2, 1b) can also be supported by the detected correlations from H-2 to C-1, C-3, C-4 and C-6, H-6 to C-2, C-4 and C-7, H-7 to C-1, C-3, C-5 and C-6, and MeO-4 to C-4 in HMBC spectrum. Finally, according to the constraints of the molecular formula and chemical shift values of C-1′ and C-1, the C-1′ position of fragment 1a could be linked to the C-1 position of fragment 1b through an oxygen bridge, leading to the completion of the chemical skeleton of compound **1** (Fig. 2). Thus, compound **1** was concluded to be a new diphenyl ether. We named this compound quercilolin.

The antibacterial activities of compounds **1**-**3** are shown in Table 3. As shown in Table 3, Results demonstrated that compounds **1**-**3** were able to inhibit *B. cereus* YMF 3.19, *B. laterosporus* YMF 3.08, and *S. aureus* YMF 3.17 but were not effective against *E. coli* YMF 3.16 in standard disk assays at 200 μg/disk.

In the primary screening, the EtOAc extract from cultural filtrates of *C. quercicola* YMF 1.01300 isolate showed noticeable activity against all tested bacteria, but none of the three isolated compounds showed any activity against the strain of *E. coli* YMF 3.16 at the same level. Therefore, we consider that the YMF 1.01300 strain produces not only the three active molecules described in this work but also at least one other active compound. Moreover, the active compounds showed weaker activity than the crude extract, implying that some other active compound had still not been isolated, and maybe a synergistic action existed in the crude extract. The results indicated that the antibacterial components and antagonistic effects of *C. quercicola* YMF 1.01300 were complex, and will require further study.

TABLE 2 - The NMR data of compound **1** in $CD_3COCD_3$ (δ ppm, *J* Hz)

| Position | $\delta_H$ (mult., *J*, Hz) | $\delta_C$ (mult.) | Position | $\delta_H$ (mult., *J*, Hz) | $\delta_C$ (mult.) |
|---|---|---|---|---|---|
| 1 | | 134.8 (s) | 1′ | | 140.9 (s) |
| 2 | 6.28 (d, 1.80) | 101.1 (d) | 2′ | 5.90 (s) | 100.2 (d) |
| 3 | | 151.6 (s) | 3′ | | 160.5 (s) |
| 4 | | 158.2 (s) | 4′ | 6.00 (s) | 107.8 (d) |
| 5 | | 133.4 (s) | 5′ | | 159.3 (s) |
| 6 | 6.21 (d, 2.00) | 107.7 (d) | 6′ | 6.16 (s) | 110.3 (d) |
| 7 | 1.86 (s) | 16.5 (q) | 7′ | 2.04 (s) | 21.6 (q) |

TABLE 3 - Antibacterial activities of compounds **1**-**3** and streptomycin (200 μg/disk, diameter of inhibition zones in mm)

| Test bacteria | Diameter of inhibition zones (mm) | | | |
|---|---|---|---|---|
| | Compound **1** | Compound **2** | Compound **3** | Streptomycin |
| *Bacillus cereus* YMF 3.19 | 16 | 18 | 15 | 35 |
| *Bacillus laterosporus* YMF 3.08 | 15 | 14 | 17 | 30 |
| *Escherichia coli* YMF 3.16 | - | - | - | 15 |
| *Staphylococcus aureus* YMF 3.17 | 19 | 16 | 13 | 18 |

The present paper demonstrated that occurrence in cultures of the freshwater fungus *C. quercicola* YMF1.01300 of three antibacterial compounds, quercilolin (compound **1**), tenellic acid A (compound **2**), and 2′,4′-dihydroxyacetophenone (compound **3**). Among them, the new compound, quercilolin (compound **1**) possessed the diphenyl ether structure. The compounds of this type were often found in marine organisms, which were reported to have a wide range of biological activities such as antibacterial and antifungal activities (Sharma and Vig, 1972; Kurata and Amiya, 1980; Salva and Faulkner, 1990), antimicroalgal activity against *Prorocentrum micans* and *Brachiomonas submaria* (Hattori *et al*., 2001), toxicity to brine shrimp (Handayani *et al*., 1997), anti-inflammatory activity (Wiemer *et al*., 1991), and inhibitory activity to several enzymes including inosine monophosphate dehydrogenase, guanosine monophosphate synthetase, and 15-lipoxygenase (Fu *et al*., 1995) etc. And it was interesting to found that marine organisms produced polyhalogenated diphenyl ethers while the diphenyl ethers from the aquatic fungi have no halogenated moieties so far. Tenellic acid A (compound **2**) was previously obtained from cultures of another freshwater fungus *Dendrospora tenella* and proven to possess active against the Gram-positive bacterium *Bacillus subtilis* ATCC 6051 (Oh *et al*., 1999). 2′,4′-Dihydroxyacetophenone (compound **3**) was commonly found in plant before, which showed antimicrobial activites (Ibewuike *et al*., 1997; Yasuda *et al*., 1999). Recently, compound **3** was also isolated from the cultures of an endophytic fungus *Geotrichum* sp. AL4 collected in plant *Azadirachta indica* as a nematicidal antibiotic (Li *et al*., 2007). However, compounds **1**-**3** were not reported to occur in cultures of *C. quercicola* YMF1.01300. In addition, they appear to be the first natural products described from any member of the genus of *Camposporium*.

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