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Antimicrobial constituents from endophytic fungus *Fusarium* sp.

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PEER REVIEW

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Comments

This is a good manuscript, also well written, in which the authors demonstrate the presence of six compounds with antifungal, antibacterial, and herbicidal activities in endophytic fungus *Fusarium* sp. The results show that these fungi may find applications in various forms.
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ABSTRACT

Objective: To evaluate the antimicrobial potential of fraction of the fungus *Fusarium* sp. and study the tentative identification of their active constituents.
Methods: Six compounds were purified from an fraction of endophytic fungus *Fusarium* sp. using column chromatography and their structures have been confirmed based on ^{1}H and ^{13}C nuclear magnetic resonance spectra, distortionless enhancement by polarization transfer, 2D COSY, heteronuclear multiple quantum correlation and heteronuclear multiple bond correlation experiments. The six isolated compounds were screened for antimicrobial activity using the agar well diffusion method.
Results: Phytochemical investigation of endophytic fungus *Fusarium* sp. lead to the isolation and identification of the following compounds *viz.*, colletorin B, colletochlorin B, LL-Z1272β (llicicolin B), 4,5-dihydroascochlorin, ascochlorin, and 4,5-dihydrodechloroascochlorin. Colletorin B and colletochlorin B displayed moderate herbicidal, antifungal and antibacterial activities towards *Chlorella fusca*, *Ustilago violacea*, *Fusarium oxysporum*, and *Bacillus megaterium*. On the other hand LL-Z1272β (llicicolin B) showed moderate antifungal activity towards *Ustilago violacea* and *Fusarium oxysporum* while 4,5-dihydroascochlorin showed strong antibacterial activity towards *Bacillus megaterium*. Furthermore, 4,5-dihydrodechloroascochlorin showed very strong antifungal activity towards *Eurotium repens*.
Conclusions: Antimicrobial activities demonstrated by five of the six isolated compounds clearly demonstrate that these fungi extracts and active compounds present a great potential for the food, cosmetic and pharmaceutical industries.

KEYWORDS

Natural product, Endrophytic fungi, Antimicrobial activity

1. Introduction

Microorganisms are essential for human life and recently special class of microorganism named endophytes has been isolated. More importantly these endophytes produce number of interesting natural products and

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these secondary metabolites showed interesting biological activities[1]. Endophytes are able to produce a number of anticancer compounds *viz*., taxol (generic name: paclitaxel), camptothecin, anticancer pro-drugs podophyllotoxin and deoxypodophyllotoxin, antidepressants hypericin and emodin, as well as the natural insecticides azadirachtin A and B[1]. Previously our group has reported various natural products from endophytic fungi[2-7], and in this study we reported six compounds, named colletorin B, colletochlorin B, LL-Z1272β (llicicolin B), 4,5-dihydroascochlorin, ascochlorin, and 4,5-dihydrodechloroascochlorin from ethyl acetate extract of endophytic fungus *Fusarium* sp.

2. Materials and methods

2.1. General experimental procedures

Column chromatography was performed using commercial silica gel (Merck, 0.040-0.063 mm) and Sephadex LH-20 (Amersham Biosciences). Analytical and preparative thin-layer chromatography was performed on precoated silica gel plates Merck G60 F-254 or G50 UV-254. Optical rotation was recorded on a Perkin-Elmer 241 MC polarimeter at the sodium D-line. Infrared radiation spectra were recorded on a Nicolet-510P spectrophotometer. ^{1}H and ^{13}C nuclear magnetic resonance (NMR) spectra were recorded on a Bruker Avance 500 (500 MHz for ^{1}H and 125 MHz for ^{13}C) spectrometer. Mass spectra and high resolution mass spectra were recorded in the EI mode on a MAT 8200 and Micromass LCT mass spectrometer. Microbiological methods and culture conditions are as described previously[8,9].

2.2. Culture, extraction and isolation

The culture filtrate from *Fusarium* sp. (internal strain 3042) was exhaustively extracted with ethyl acetate and which was concentrated in vacuo to afford 8.0 g of extract. The crude extract was separated into different fractions by column chromatography on silica gel, using gradients of dichloromethane/ethyl acetate (85:15, 50:50, 0:100). The colletorin B, colletochlorin B, LL-Z1272β, 4,5-dihydroascochlorin, ascochlorin, and 4,5-dihydrodechloroascochlorin were isolated from subfractions by using high performance liquid chromatography and middle pressure liquid chromatography separation techniques using dichloromethane-methanol (97:3) as eluent.

2.3. Agar diffusion test for biological activity

Tests for antifungal, antibacterial, and herbicidal activities were performed as previously described[9]. Compounds 1-6 were dissolved in acetone at a concentration of 1 mg/mL. Fifty microliters of the solutions was pipetted onto a sterile filter disk (Schleicher & Schuell, 9 mm), which was placed onto an appropriate agar growth medium for the respective test organism and subsequently sprayed with a suspension of the test organism[9]. The test organisms were *Chlorella fusca* (*C. fusca*), *Ustilago violacea* (*U. violacea*), *Eurotium repens* (*E. repens*), *Fusarium oxysporum* (*F. oxysporum*), *Mycotypha microspora* (*M. microspora*), *Escherichia coli* (*E. coli*) and *Bacillus megaterium* (*B. megaterium*).

Colletorin B (1): m.p.: 124 °C; ^{1}H-NMR (CD_2Cl_2, 200 MHz): δ=12.75 (s, 1H, 2-OH), 10.07 (s, 1H, 8-H), 6.20 (s, 1H, 5-H), 5.25 (t, *J*=7.1 Hz, 1H, 2′-H), 5.04 (t, *J*=6.6 Hz, 1H, 6′-H), 3.39 (d, *J*=7.2 Hz, 2H, 1′-H), 2.48 (s, 3H, 7-H), 2.16-2.04 (m, 4H, 4′-H, 5′-H), 1.80 (s, 3H, 8′-H), 1.67 (s, 3H, 9-H), 1.58 (s, 3H, 10′-H); ^{13}C-NMR (CD_2Cl_2, 50 MHz): δ=193.4 (d, C-8), 164.0 (s, C-2), 163.1 (s, C-4), 142.4 (s, C-6), 139.9 (s, C-3′), 132.5 (s, C-7′), 124.1 (d, C-6′), 121.4 (d, C-2′), 113.9 (s, C-1), 112.1 (s, C-3), 111.3 (d, C-5), 40.0 (t, C-4′), 26.7 (t, C-5′), 26.1 (q, C-8′), 21.6 (t, C-1′), 18.4 (q, C-6), 18.1 (q, C-9′), 16.6 (q, C-10′); HREIMS: m/z 288.1717 [Calcd. 288.1725 for ($C_{18}H_{24}O_3$)].

Colletochlorin B (2): m.p.: 88 °C; ^{1}H-NMR (CD_2Cl_2, 200 MHz): δ=12.72 (s, 1H, 2-OH), 10.17 (s, 1H, 8-H), 6.40 (s, 1H, 4-OH), 5.25 (t, *J*=7.3 Hz, 1H, 2′-H), 5.09 (m, 1H, 6′-H), 3.43 (d, *J*=7.3 Hz, 2H, 1′-H), 2.63 (s, 3H, 7-H), 2.16-1.94 (m, 4H, 4′-H, 5′-H), 1.82 (s, 3H, 8′-H), 1.68 (s, 3H, 9-H), 1.60 (s, 3H, 10′-H); ^{13}C-NMR (CD_2Cl_2, 50 MHz): δ=193.6 (d, C-8), 162.6 (s, C-2), 156.9 (s, C-4), 138.0 (s, C-6), 137.4 (s, C-3′), 131.9 (s, C-7′), 124.5 (d, C-6′), 121.1 (d, C-2′), 114.7 (s, C-1), 113.9 (s, C-3), 113.7 (d, C-5), 40.2 (t, C-4′), 27.0 (t, C-5′), 26.1 (q, C-8′), 22.4 (t, C-1′), 18.1 (q, C-6), 16.6.1 (q, C-9′), 14.9 (q, C-10′); HREIMS: m/z 322.1329 [Calcd. 322.1335 for ($C_{18}H_{23}O_3Cl$)].

LL-Z1272β (Llicicolin B) (3): m.p.: 96 °C; ^{1}H-NMR (CD_2Cl_2, 200 MHz): δ=12.79 (s, 1H, 2-OH), 10.10 (s, 1H, 8-H), 6.20 (s, 1H, 5-H), 5.30 (t, *J*=7.1 Hz, 1 H, 2′-H), 5.10 (m, 2H, 6′-H, 10′-H), 3.43 (d, *J*=7.3 Hz, 2H, 1′-H), 2.52 (s, 3H, 7-H), 2.16-2.04 (m, 6H, 4′-H, 5′-H, 8′-H, 9′H), 1.80 (s, 3H, 15′-H), 1.67 (s, 3H, 14′-H), 1.58 (s, 6H, 13′-H, 12′-H); ^{13}C-NMR (CD_2Cl_2, 50 MHz): δ=193.4 (d, C-8), 164.0 (s, C-2), 163.0 (s, C-4), 142.4 (s, C-6), 140.3 (s, C-3′), 136.1 (s, C-7′), 132.5 (s, C-10′), 124.7 (d, C-6′), 123.9 (d, C-9′), 121.4 (d, C-2′), 113.6 (s, C-1), 111.9 (s, C-3), 111.3 (d, C-5), 40.1 (t, C-1′), 26.7 (t, C-5′), 26.1 (q, C-8′), 21.6 (t, C-1′), 18.4 (q, C-6), 18.1 (q, C-9′), 16.6 (q, C-10′); HREIMS: m/z 356.2347 [Calcd. 356.2351 for ($C_{23}H_{32}O_3$)].

4,5-Dihydroascochlorin (4): m.p.: 130 °C; ^{1}H-NMR (CD_2Cl_2, 200 MHz): δ=12.60 (s, 1H, 2-OH), 10.07 (s, 1H, 8-H), 6.40 (s, 1H, 4-OH), 5.82 (t, *J*=7.2 Hz, 1H, 2′-H), 3.32 (d, *J*=7.2 Hz, 2H, 1′-H), 2.53 (s, 3H, 7-H), 2.4-2.19 (m, 4H, 9′-H, 10′-H), 2.03-1.74 (m, 4H, 4′-H, 5′H), 1.74 (s, 3H, 15′-H), 1.65-1.49 (m, 2H, 11′-H, 7′-H), 0.83 (d, 3H, *J*=6.5 Hz, 14′-H), 0.80 (d, 3H, *J*=6.8 Hz, 12′-H), 0.49 (s, 3H, 6-H); ^{13}C-NMR (CD_2Cl_2, 50 MHz): δ=215.1 (s, 8′), 194.2 (d, C-8), 163.2 (s, C-2), 157.3 (s, C-4), 138.6 (s, C-6), 137.6 (d, C-2′), 121.9 (d, C-4′), 115.3 (s, C-1), 114.6 (s, C-3), 114.2 (d, C-5), 51.7 (d, C-4), 44.2 (t, C-5), 42.6 (d, C-4′), 37.0 (t, C-5′), 36.6 (t, C-8′), 23.1 (t, C-1′), 17.4 (q, C-6), 16.3 (q, C-9′), 16.1 (q, C-10′) 15.5(q, C-14′), 8.57 (q, C-7); HREIMS: m/z 422.2223 [Calcd. 422.2223 for ($C_{24}H_{35}O_4Cl$)].

Ascochlorin (5): m.p.: 168 °C; ^{1}H-NMR (CD_2Cl_2, 200 MHz): δ=12.72 (s, 1H, 2-OH), 10.14 (s, 1H, 8-H), 6.47 (s, 1H, 4-OH), 5.88 (d, 1H, *J*=16 Hz, 4′-H), 5.46 (t, *J*=7.2 Hz, 1H, 2′-H), 5.30 (d, *J*=16 Hz, 1H, 5′-H), 3.47 (d, *J*=7.4 Hz, 2H, 1′-H), 2.54 (s, 3H, 7-H), 2.40-2.19 (m, 4H, 9′-H, 10′-H), 1.82 (s, 3H, 15′-H), 1.65-1.49 (m, 2H, 11′-H, 7′-H), 0.77 (d, 3H, *J*=6.9 Hz, 14′-H), 0.74 (d, 3H, *J*=6.5 Hz, 12′-H), 0.63 (s, 3H, 6-H); ^{13}C-NMR (CD_2Cl_2, 50 MHz): δ=213.8 (s, 8′), 194.2 (d, C-8), 163.2 (s, C-2), 157.2 (s, C-4), 138.8 (s, C-6), 136.6 (d, C-5′), 135.1 (s, C-3′), 134.2 (d, C-2′), 128.5 (d, C-4′), 114.8 (s, C-1), 114.7 (s, C-3), 114.2 (d, C-5), 40.2 (t, C-4′), 27.0 (t, C-5′), 26.1 (q, C-8′), 22.4 (t, C-1′), 18.1 (q, C-6), 16.6.1 (q, C-9′), 14.9 (q, C-10′) 8.4 (q, C-7); HREIMS: m/z 420.2066 [Calcd. 420.2067 for ($C_{24}H_{35}O_4Cl$)].

4,5-Dihydrodechloroascochlorin (6): m.p.: 171 °C; ^{1}H-NMR (CD_2Cl_2, 200 MHz): δ=12.74 (s, 1H, 2-OH), 10.09 (s, 1H, 8-H), 6.26 (s, 1H, 5-H), 5.30 (t, *J*=7.2 Hz, 1H, 2′-H), 3.39 (d, *J*=7.2 Hz, 2H, 1′-H), 2.52 (s, 3H, 7-H), 2.4-2.19 (m, 4H, 9′-H, 10′-H),

2.03–1.74 (m, 4H, 4′–H, 5′H), 1.85 (s, 3H, 15′–H), 1.65–1.49 (m, 2H, 11′–H, 7′–H), 0.93 (d, 3H, *J*=6.5 Hz, 14′–H), 0.91 (d, 3H, *J*=6.3 Hz, 12′–H), 0.59 (s, 3H, 6–H); ^{13}C–NMR (CD_2Cl_2, 50 MHz): δ=215.1 (s, 8′), 193.4 (d, C–8), 164.1 (s, C–2), 162.7 (s, C–4), 142.3 (s, C–6), 138.6 (d, C–2′), 121.8 (d, C–4′), 113.6 (s, C–1), 112.4 (s, C–3), 111.0 (d, C–5), 50.9 (d, C–4), 43.9 (t, C–5), 42.0 (d, C–4′), 36.5 (t, C–5′), 36.0 (t, C–8′), 21.6 (t, C–1′), 16.9 (q, C–6), 15.7 (q, C–9′), 15.5 (q, C–10′) 15.4(q, C–14′), 8.0 (q, C–7); HREIMS: m/z 388.2614 [Calcd. 388.2613 for ($C_{24}H_{36}O_4$)].

3. Results

Colletorin B (1), colletochlorin B (2), LL–Z1272β (llicicolin B) (3), 4,5–dihydroascochlorin (4), ascochlorin (5), and 4,5–dihydrodechloroascochlorin (6) (Figure 1) were isolated from *Fusarium* sp.[10–15], and the structures of compounds 1–6 were identified by comparison with published spectral data.

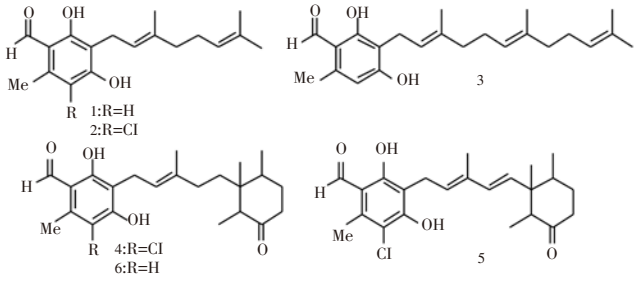

Figure 1. Structures of compounds 1–6 isolated from *Fusarium* sp.

The antibacterial, fungicidal, and herbicidal properties of six pure compounds *viz.*, colletorin B (1), colletochlorin B (2), LL–Z1272β (llicicolin B) (3), 4,5–dihydroascochlorin (4), ascochlorin (5), and 4,5–dihydrodechloroascochlorin (6) are compiled in Table 1. The antimicrobial potential of compounds 1–6 were tested towards *C. fusca, U. violacea, E. repens, M. microspora, F. oxysporum, E. coli*, and *B. megaterium*.

Table 1

Biological activities of pure metabolites 1–6 against microbial test organisms in agar diffusion assay[a].

Compound	Herbicidal	Antifungal				Antibacterial	
	Chl	Ust	Eur	Mm	Fo	Ec	Bm
1	4	6	0	0	6	0	5
2	4	5	0	0	5	0	1
3	0	4	0	0	5	0	1
4	0	1	0	0	0	0	10
5	0	0	0	0	0	0	0
6	0	3	15	0	0	0	0

[a]: 10 mg/mL of extracts and compounds 1–6 were tested for inhibitions. Chl: *C. fusca*; Ust: *U. violacea*; Eur: *E. repens*; Mm: *M. microspora*; Fo: *F. oxysporum*; Ec: *E. coli*; Bm: *B. megaterium*; The radius of zone of inhibition was measured in mm.

Hosono *et al.* reported the isolation of LL–Z1272α (7) and/or LL–Z1272α epoxide (8)[16], and it has been suggested that compounds 7 and 8 may be precursor of ascochlorin (5) (Figure 2). It is interesting to note that both compounds 5 and 7 have been reported together from *Fusarium* species, *Nectria coccinea* and *Verticillium* sp[16]. In an earlier study, Ellestad *et al.* postulated that the farnesyl chain of compound 7 was epoxidized at the terminal olefin[14], then cyclized to a cyclohexanone ring with migration of a methyl group and converted to ascochlorin (5) (Figure 2). Later the report of compound 8 further supports the Ellestad *et al.* hypothesis[14]. The biosynthesis of compounds 1–6 is summarized in Figure 2.

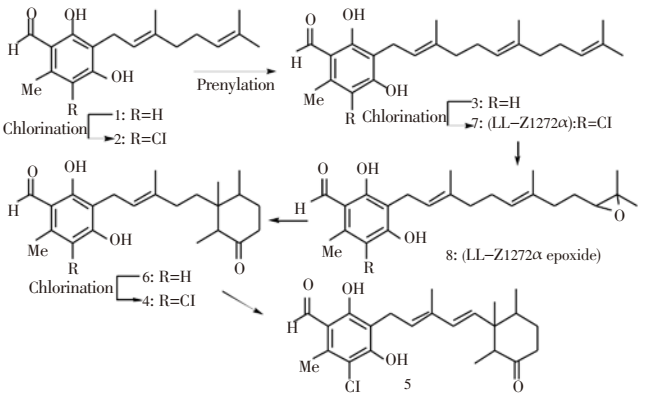

Figure 2. Pathway for the biosynthesis of compounds 1–6.

4. Discussion

4.1. Chemotaxonomic significance

Colletorin B (1) and colletochlorin B (2) was previously reported from the fungi *Cephalosporium diospyri* (*C. diospyri*) and *Nectria galligena* while LL–Z1272β (llicicolin B) (3) was reported previously from *Nectria coccinea* (*N. coccinea*), *Nectria lucida* (*N. lucida*) and *Fusarium* sp[16,17]. Furthermore, 4,5–dihydroascochlorin (4) and ascochlorin (5) were isolated from fungi *N. coccinea*, *N. lucida* and *C. diospyri*[17]. Similarly 4,5–dihydrodechloroascochlorin (6) was isolated from fungi *N. lucida, N. coccinea, C. diospyri, Verticillium* sp., *Cylindrocarpon lucidum* and *Fusarium* sp[17].

Ascochlorin (5) was initially reported from broth of *Ascochyta viciae* Libert[18], and later various ascochlorin (5) derivatives have been reported from various fungi *viz.*, *Fusarium* sp., *Cylindrocladium* sp., and *Cylindrocladium ilicicola*[16].

4.2. Biological activity

Colletorin B (1) and colletochlorin B (2) showed moderate herbicidal, antifungal and antibacterial activities towards *C. fusca, U. violacea, F. oxysporum*, and *B. megaterium*. On the other hand LL–Z1272β (llicicolin B) (3) showed moderate antifungal activity towards *U. violacea* and *F. oxysporum*, while 4,5–dihydroascochlorin (4) showed strong antibacterial activity towards *B. megaterium*. Furthermore 4,5–dihydrodechloroascochlorin (6) showed very strong antifungal activity towards *E. repens* while compounds 1–5 were not active against *E. repens*. It is important to note that none of these compounds were active against *M. microspora* and *E. coli*. Compounds 1–3 were active against *F. oxysporum* while compounds 1 and 2 were active towards *C. fusca*. Ascochlorin (5) was not active against all tested oragisms.

The antimicrobial properties of five pure compounds *viz.*, colletorin B (1), colletochlorin B (2), LL–Z1272β (llicicolin B) (3), 4,5–dihydroascochlorin (4), and 4,5–dihydrodechloroascochlorin (6) confirms their potential use in the food and pharmaceutical industries. Similarly, the

antimicrobial potential of the extracts and compounds 1–4 and 6 also confirms their potential use in the preservation of foodstuffs against bacteria and fungi and that these compounds may also be valuable for extending the shelf life of foodstuffs. It is of interest to note that the introduction of an additional double bond in the side chain of 4 to give 5 negates its antimicrobial activity.

Conflict of interest statement

We declare that we have no conflict of interest.

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Comments

Background

Medicinal plants have been known for centuries and are a valuable source of endophytes. Bioactive metabolites produced by endophytic fungi represent a chemical reservoir for new physiologically active compounds.

Research frontiers

Although six known compounds were found, antimicrobial activities demonstrated by six compounds isolated from them show potential for the food, cosmetic and pharmaceutical industries.

Related reports

The authors' own previous reports are mentioned as references 2–8. Scant reference is made to other authors' work.

Innovations & breakthroughs

Phytochemical investigation of the endophytic fungus *Fusarium* sp. lead to the isolation and identification of six compounds *viz*., colletorin B, colletochlorin B, LL-Z 1272β, 4,5-dihydroascochlorin, ascochlorin, and 4,5-dihydrodechloroascochlorin. Colletorin B and colletochlorin B showed moderate herbicidal, antifungal and antibacterial activities towards *C. fusca*, *U. violacea*, *F. oxysporum*, and *B. megaterium*. Moreover LL-Z1272β demonstrated moderate antifungal activity towards *U. violacea* and *F. oxysporum* while 4,5-dihydroascochlorin showed strong antibacterial activity towards *B. megaterium*. Furthermore 4,5-dihydrodechloroascochlorin showed very strong antifungal activity towards *E. repens*.

Applications

The paper shows that these fungal extracts and active compounds have great potential for application in the food, cosmetic and pharmaceutical industries.

Peer review

This is a good manuscript, also well written, in which the authors demonstrate the presence of six compounds with antifungal, antibacterial, and herbicidal activities in endophytic fungus *Fusarium* sp. The results show that these fungi may find applications in various forms.

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