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.

Details on Page 189

**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 1H and 13C 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 (*Illicicolin* B), 4,5-dihydroascoclorin, ascochlorin, and 4,5-dihydrodechloroascoclorin. 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 (*Illicolin* B) showed moderate antifungal activity towards *Ustilago violacea* and *Fusarium oxysporum* while 4,5-dihydroascoclorin showed strong antibacterial activity towards *Bacillus megaterium*. Furthermore, 4,5-dihydrodechloroascoclorin 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, Endophytic fungi, Antimicrobial activity

**1. Introduction**

Microorganisms are essential for human life and recently a special class of microorganism named endophytes has been isolated. More importantly these endophytes produce number of interesting natural products and...
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-Z127β (Llicicinol B), 4,5-dihydroascochlorin, ascochlorin, and 4,5-dihydrodechloroascoclorin 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 G60 F-254 or G50 UV-254]. Optical rotation was measured in an automatic polarimeter (Perkin Elmer 241). The test organisms were Colletorin B (1): m.p.: 124°C, 1H-NMR (CDCl3, 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). 13C-NMR (CDCl3, 50 MHz): δ=193.4 (d, 8-C), 164.0 (s, 6-C), 136.1 (s, C-3), 134.2 (d, C-5), 121.1 (d, C-6'), 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 (q, C-9'); HREIMS: m/z 322.1329 [Caled. 322.1335 for (C10H16O3)].

Colletorin B (2): m.p.: 88°C; 1H-NMR (CDCl3, 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). 13C-NMR (CDCl3, 50 MHz): δ=193.6 (d, 8-C), 162.6 (s, 2-C), 156.9 (s, C-4), 138.0 (s, C-6), 137.4 (s, C-4'), 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 (q, C-9'); HREIMS: m/z 322.1329 [Caled. 322.1335 for (C10H16O3)].

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-Z127β, 4,5-dihydroascochlorin, ascochlorin, and 4,5-dihydrodechloroascoclorin 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), Mycopathya microspora (M. microspora), Escherichia coli (E. coli) and Bacillus megaterium (B. megaterium).

Colletorin B (1): m.p.: 124°C; 1H-NMR (CDCl3, 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). 13C-NMR (CDCl3, 50 MHz): δ=193.4 (d, 8-C), 164.0 (s, 6-C), 136.1 (s, C-3), 134.2 (d, C-5), 121.1 (d, C-6'), 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 (q, C-9'); HREIMS: m/z 322.1329 [Caled. 322.1335 for (C10H16O3)].

Ascochlorin (5): m.p.: 168°C; 1H-NMR (CDCl3, 200 MHz): δ=12.72 (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, 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=6.5 Hz, 12'-H), 0.49 (s, 3H, 6-H); 13C-NMR (CDCl3, 50 MHz): δ=215.1 (s, 8'), 194.2 (d, C-8), 163.2 (s, 2-C), 157.3 (s, 4-C), 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-1'), 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') 15.8 (q, C-14'), 8.57 (q, C-7); HREIMS: m/z 422.2223 [Caled. 422.2223 for (C14H16O3)].

4,5-Dihydrodechloroascoclorin (6): m.p.: 180°C; 1H-NMR (CDCl3, 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, 4'-H, 5'-H),
organisms in agar diffusion assay, Ellestad et al.

megaterium, and compounds 1-6 were tested towards Hosono et al.

Biological activities of pure metabolites 1-6 against microbial test (6) are compiled in Table 1. The antimicrobial potential of (4), ascochlorin (5), and 4,5-dihydrodechloroascochlorin B (2), LL-Z1272 The antibacterial, fungicidal, and herbicidal properties of six pure compounds viz., colletorin B (1), colletochlorin B (2), LL-Z1272β (illicicolin B) (3), 4,5-dihydroascochlorin (4), ascochlorin (5), and 4,5-dihydrodichloroascochlorin (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.](image)

The antibacterial, fungicidal, and herbicidal properties of six pure compounds viz., colletorin B (1), colletochlorin B (2), LL-Z1272β (illicicolin B) (3), 4,5-dihydroascochlorin (4), ascochlorin (5), and 4,5-dihydrodichloroascochlorin (6) are summarized in Figure 2. 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.

![Figure 2. Pathway for the biosynthesis of compounds 1-6.](image)

### Table 1

| 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  | 0 |
| 5        | 0  | 0  | 0  | 0  | 0  | 0  | 0 |
| 6        | 0  | 3  | 15 | 0  | 0  | 0  | 0 |

*: 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-Z1272a (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 farnesy1 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.

### 3. Results

Colletorin B (1), colletochlorin B (2), LL-Z1272β (illicicolin B) (3), 4,5-dihydroascochlorin (4), ascochlorin (5), and 4,5-dihydrodichloroascochlorin (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.

### 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 (illicicolin 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-dihydrodichloroascochlorin (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 illicicol[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 (illicicolin 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-dihydrodichloroascochlorin (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 orgamisms.

The antimicrobial properties of five pure compounds viz., colletorin B (1), colletochlorin B (2), LL-Z1272β (illicicolin B) (3), 4,5-dihydroascochlorin (4), and 4,5-dihydrodichloroascochlorin (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, 1,1–Z 1272β, 4,5–dihydroascoclorin, ascoclorin, and 4,5–dihydrodechloroascoclorin. Colletorin B and colletochlorin B showed moderate hericidal, antifungal and antibacterial activities towards C. fusa, U. violacea, F. oxysporum, and B. megaterium. Moreover 1,1–Z 1272β demonstrated moderate antifungal activity towards U. violacea and F. oxysporum while 4,5–dihydroascoclorin showed strong antibacterial activity towards B. megaterium. Furthermore 4,5–dihydrodechloroascoclorin 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 hericidal activities in endophytic fungus Fusarium sp. The results show that these fungi may find applications in various forms.

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