Secondary metabolites and bioactivity of two fungal strains

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Purpose
The investigation of two fungal strains isolated from Egyptian habitats, namely, the endophytic *Fusarium poae* FUN1 and the terrestrial *Penicillium italicum* FUN2 to illustrate their chemical constituents and their bioactivities.

Materials and methods
See General instrumental procedures.

Results
Linoleic acid (1), indole-3-acetic acid methyl ester (2) and Nb-acetyltryptamine (3) were produced by *F. poae* FUN1, whereas *P. italicum* FUN2 also delivered linoleic acid (1) in addition to cis-cyclo-(prolyl,valyl) (4). The structures of compounds (1)–(4) were elucidated by 1D and 2D NMR, MS data and through comparison with literature reports. In this article, the taxonomical characterization of both fungal strains, their upscale fermentation and the antimicrobial and cytotoxic activities tested have been described.

Conclusion
Two different fungal strains, endophytic *F. poae* FUN1 and terrestrial *P. italicum* FUN2, were intensively studied biologically and chemically. Four bioactive compounds (1)–(4) were isolated, and structurally confirmed by intensive studies of NMR and MS. The antimicrobial and cytotoxic activities of the fungal extracts and their delivered compounds were studied. This might be helpful for the cure of recent diseases, and drug-resistant phenomena as well as in the development of pharmaceutical, agrochemical and biochemical agents and their lead compounds.

Keywords:
bioactive metabolites, biological activities, endophytic, terrestrial fungi

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Introduction

In recent years, numerous metabolites with uncommon structures and potent bioactivity have been isolated from fungal strains, collected from diverse environments [1,2]. Since the discovery of penicillin G from *Penicillium notatum* (1928), fungi have become a hunting ground for novel drug leads [3,4], including antibiotics [5,6], antimycotics [7,8], antivirals [9], anticancers [10] and pharmacologically active agents [11]. During the last decade, the biology of herbs and trees endophytes has become an area of intensive study; however, the chemistry of these microorganisms still needs to be thoroughly examined [12–14]. Literature reports confirmed that endophytic fungi are remarkable producers of bioactive metabolites that combat a number of hazardous diseases significantly [15–17]. Thus, research in this field may offer great opportunities for innovation in the discovery of both drugs and agrochemicals.

In the present study, two fungal strains, the endophytic *Fusarium poae* FUN1 and the terrestrial *Penicillium italicum* FUN2, were subjected to chemical and biological examinations. Biologically, both strains exhibited promising antimicrobial activity against selected microorganisms and cytotoxic potential (brine shrimp lethality assay). Chemical screening using thin-layer chromatography (TLC) of the strains extracts showed the productivity of diverse types of metabolic constituents. Thus, large-scale fermentation was performed; working up, followed by chromatographic fractionation and purification yielded the detected metabolites. In this respect, linoleic acid (**1**), indole-3-acetic acid methyl ester (**2**) and N_{β}-acetyltryptamine (**3**) were obtained from *F. poae* FUN1. Meanwhile, the terrestrial fungal strain *P. italicum* FUN2 yielded linoleic acid (**1**) and the cyclic dipeptide, *cis*-cyclo-(prolyl,valyl) (**4**). The chemical structures of compounds (**1**)–(**4**) were established through their NMR (1D and 2D) and mass (EI, ESI, HRESIMS) spectra, and further confirmed by matching with published data. Finally, the antimicrobial and cytotoxic activities of the strain extracts as well as the metabolites obtained thereof were evaluated.

Materials and methods

General instrumental procedures

NMR spectra were measured on a Varian Unity 300 spectrometer. Electron spray ionization mass spectrometry (ESI HRMS): Finnigan LCQ ion trap mass

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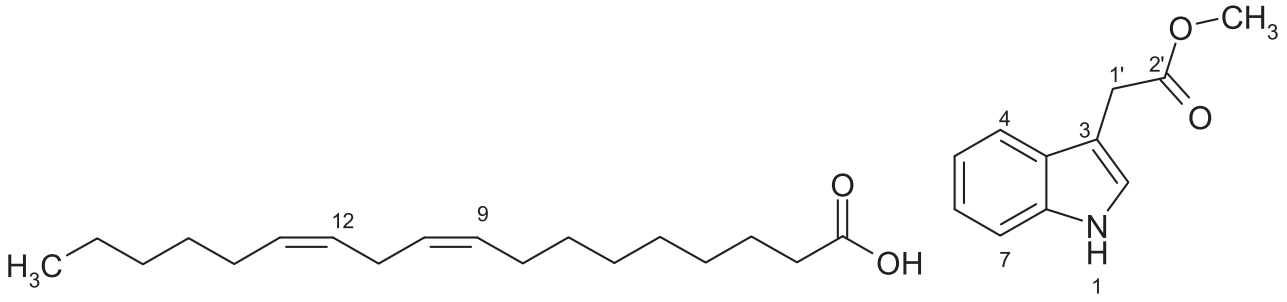
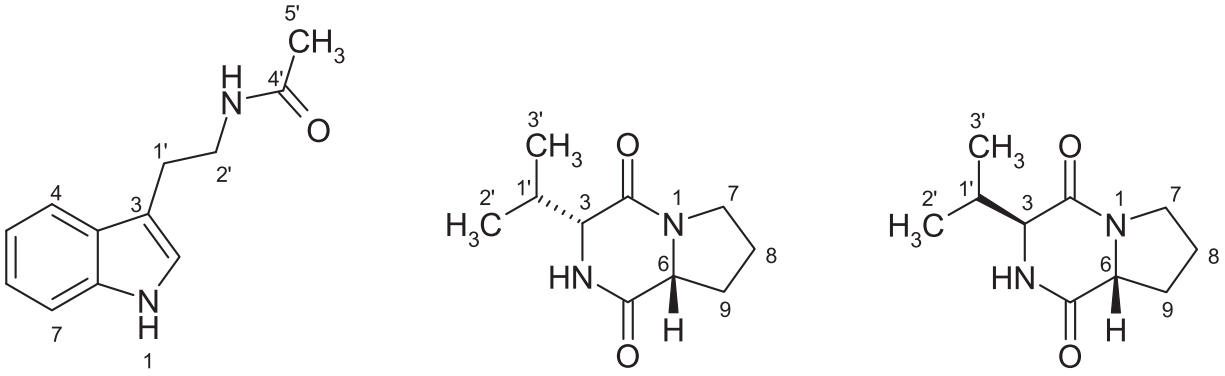


1 **2**



3 **4** **5**

spectrometer coupled with a Flux Instruments (Finnigan Surveyor LC System, Basel, Switzerland) quaternary pump Rheos 4000 and an HP 1100 HPLC (nucleosil column EC 125/2, 100-5, C 18) with an autosampler (Jasco 851-AS; Jasco Inc., Easton, Maryland, USA) and a Diode Array Detector (Finnigan Surveyor LC System) were used. High-resolution mass spectra (HRMS) were recorded by ESI MS on an Apex IV 7 Tesla Fourier-Transform Ion Cyclotron Resonance Mass Spectrometer (Bruker Daltonics, Billerica, Massachusetts, USA). EI MS at 70 eV with Varian MAT 731, Varian 311A and AMD-402, high resolution with perflurokerosine as the standard, were used. R_f values were measured on Polygram SIL F/UV_{254} (pre-coated sheets; Merck). Size exclusion chromatography was performed on Sephadex LH-20 (Pharmacia).

Endophytic *Fusarium poae* FUN1

Isolation

Random samples of the fungus were collected from castor oil plant leaves (*Ricinus communis* Linn., family *Euphorbiaceae*). The samples were thoroughly washed in running tap water and the surfaces were sterilized by submerging in 75% ethanol for 2 min. The samples were further sterilized in 5.3% NaOCl (w/v) for 1 min and thereafter dipped into 75% ethanol for 30 s. After drying under sterile conditions, small discs were cut and placed on potato dextrose agar (PDA) medium amended with 50 mg/l chloramphenicol to suppress bacterial contamination. The Petri dishes were incubated for 25 days at 30°C [18]. Single colonies were picked up and checked for purity. The endophytic fungus isolate FUN1 was found to be related to the genus *Fusarium*, and the species was identified as *F. poae* as described by Barnett [19].

Fermentation, extraction and isolation

The strain was subjected to cultivation as 10 l using PDA medium (g/l): potato infusion (200), dextrose (20), agar (20) and distilled water (1 l), pH 5.6 ± 0.2, for a 5-day incubation period. After cultivation, the mycelia mats were separated from the culture supernatant by filtration under vacuum, followed by extraction with acetone. The acetone extract was then concentrated *in vacuo*, and the aqueous residue was re-extracted using ethyl acetate (3 × 0.5 l) to yield a dark brown crude extract (0.4 g) after concentration *in vacuo*. The culture filtrate was passed through an Amberlite XAD-16 column, washed with water, and then the adsorbed organic material was eluted by methanol. The aqueous methanol extract obtained was concentrated *in vacuo*, and the remaining aqueous residue was re-extracted with ethyl acetate, followed by concentration *in vacuo* to yield a dark brown crude extract (2.25 g). On the basis of TLC monitoring, the mycelia and filtrate crude extracts showed different metabolic profiles and were thus handled separately.

The mycelia extract (400 mg) was fractionated on a silica gel column; elution with a cyclohexane–CH_2Cl_2–MeOH gradient system yielded fraction FI (200 mg). Column chromatography of this fraction on Sephadex LH-20 (Merck) (CH_2Cl_2/40% MeOH) resulted in the isolation of linoleic acid (**1**, 10 mg) as a colourless oil. The filtrate extract (2.25 g) was subjected to fractionation using a silica gel column, and eluted with CH_2Cl_2–MeOH to

yield two fractions: FI (30 mg) and FII (120 mg). Purification of FI by Sephadex LH-20 (CH_2Cl_2/40% MeOH) yielded indolyl-3-acetic acid methyl ester (**2**, 4 mg) as a colourless solid. An application of FII to Sephadex LH-20 (MeOH) yielded a colourless solid: N_β-acetyltryptamine (**3**, 3 mg).

Linoleic acid (**1**): $C_{18}H_{32}O_2$ (280), a colourless oil, UV nonabsorbing or fluorescent, turned bluish violet on spraying with anisaldehyde/sulphuric acid; $R_f = 0.38$ (CH_2Cl_2/2% MeOH); ^{1}H NMR (300 MHz, $CDCl_3$): $\delta = 10.67$ (brs, 1H, COOH), 5.33 (m, 4H, CH-9,10,12,13), 2.75 (t, $J = 5.9$ Hz, 2H, CH_2-11), 2.32 (t, $J = 7.4$ Hz, 2H, CH_2-2), 2.02 (m, 4H, CH_2-8,14), 1.59 (m, 2H, CH_2-3), 1.29 (m, 14H, CH_2-4,5,6,7,15,16,17), 0.86 (t, $J = 6.1$ Hz, 3H, CH_3-18); EI MS (70 eV): *m/z* (%) = 280.4 (80), 264 (28), 137 (10), 124 (15), 110 (28), 95 (60), 81 (84), 67 (100), 55 (92), 41 (92).

Indole-3-acetic acid methyl ester (**2**): $C_{11}H_{11}NO_2$ (189), a colourless solid, UV absorbing, turned red on spraying with anisaldehyde/sulphuric acid; $R_f = 0.47$ (CH_2Cl_2); ^{1}H NMR (300 MHz, $CDCl_3$): $\delta = 8.07$ (brs, 1H, NH-1), 7.60 (d, $J = 7.9$ Hz, 1H, H-4), 7.34 (d, $J = 7.8$ Hz, 1H, H-7), 7.16 (m, 3H, 2, 5, H-6), 3.78 (s, 2H, CH_2-1′), 3.69 (s, 3H, OCH_3-3′); –(+)ESI MS: *m/z* (%) = 212 ($[M + Na]^+$, 48), 401 ($[2M + Na]^+$, 100); –(–)ESI MS: *m/z* (%) = 188 ($[M - H]^-$, 100).

N_β-acetyltryptamine (**3**): $C_{12}H_{14}N_2O$ (202), a colourless solid, showing UV absorbance, turned orange on spraying with anisaldehyde/sulphuric acid and later to violet; $R_f = 0.41$ (CH_2Cl_2/5% MeOH); ^{1}H NMR ($CDCl_3$, 300 MHz): $\delta = 8.30$ (brs, 1H, NH-1), 7.60 (d, $J = 8.0$ Hz, 1H, H-4), 7.38 (d, $J = 7.8$ Hz, 1H, H-7), 7.26 (t, $J = 7.8$ Hz, 1H, H-6), 7.08 (t, $J = 8.0$, 1H, H-5), 7.03 (d, $J = 1.1$ Hz, 1H, H-2), 5.70 (brs, 1H, NH), 3.58 (q, $J = 6.3$ Hz, 2H, CH_2-2′), 2.98 (t, $J = 6.6$ Hz, 2H, CH_2-1′), 1.93 (s, 3H, CH_3-5′); –(+)ESI MS: *m/z* (%) = 225 ($[M + Na]^+$, 25), 427 ($[2M + Na]^+$, 100); –(–)ESI MS: *m/z* (%) = 201 ($[M - H]^-$).

Terrestrial *Penicillium italicum* FUN2

Isolation

The fungal strain was isolated using the soil dilution plate method [20]. Soil samples (each of 1 g), collected from the El-Mansoura zone (Dekernes, Egypt), were serially diluted in 9 ml of a sterile NaCl aqueous solution (0.85 w/v) to a final concentration of 10^{-3}. The last final concentration (10^{-3}) of soil was added to the soil medium. As soon as the colonies appeared (24–72 h), they were transferred to freshly prepared Petri dishes until pure isolated cultures were obtained. After isolation, whitish single colonies were grown and maintained on PDA medium at 4°C [21]. According to its morphological properties and through detection by light microscope photography and comparison with the literature [19,22], the fungal isolate FUN2 was identified as *P. italicum* FUN2.

Fermentation, extraction and isolation

Well-grown slants of *P. italicum* FUN2 were used to inoculate 40 of 1-l Erlenmeyer conical flasks, each containing 300 ml of GYMP medium. Each flask was inoculated with 1 ml of spore suspension and incubated at 30°C for 10 days. After harvesting, the mycelia mats and filtrate were separated by filtration under vacuum. The mycelia cake was extracted by acetone (3×0.5 l), concentrated *in vacuo* and the residual aqueous solution was re-extracted by ethyl acetate (3×0.5 l) and finally concentrated until dry to yield 0.75 g of a brown crude extract. The culture filtrate was subjected to an extraction process as for *F. poae* FUN1, and yielded 1.05 g of a dark brown crude extract. TLC of the two organic extracts were very similar in composition and were thus mixed. The combined crude extracts (1.8 g) were subjected to fractionation on Sephadex LH-20 (MeOH), yielding three fractions: FI, FII and FIII. Column chromatography of FII (700 mg) on a silica gel and elution with the cyclohexane–CH_2Cl_2–MeOH gradient yielded three subfractions: FII-a (200 mg), FII-b (100 mg) and FII-c (150 mg). Subfraction FII-b was purified on Sephadex LH-20 (CH_2Cl_2/40% MeOH) to yield a colourless oil, linoleic acid (**1**, 11 mg). Purification of FII-c using Sephadex LH-20 (MeOH) resulted in the isolation of *cis*-cyclo-(prolyl,valyl) (**4**, 12 mg) as a colourless solid.

Cis-cyclo-(prolyl,valyl) (**4**): $C_{10}H_{16}N_2O_2$ (196), a colourless solid, UV nonabsorbing, turned violet with anisaldehyde/sulphuric acid; $R_f = 0.12$ (CH_2Cl_2/3% MeOH); ^{1}H NMR (300 MHz, $CDCl_3$): $\delta = 6.71$ (s, 1H, NH-4), 4.04 (t, $J = 7.0$ Hz, H-6), 3.58 (s, 1H, H-3), 3.52 (m, 1H, H_a-9), 3.49 (m, 1H, H_b-9), 2.56 (m, 1H, H-1′), 2.30 (m, 1H, H_a-7), 1.98 (m, 2H, H_b-7, H_a-8), 1.84 (m, 1H, H_b-8), 1.04 (d, $J = 7.2$ Hz, 3H, CH_3-2′), 0.87 (d, $J = 6.8$ Hz, 3H, CH_3-3′); ^{13}C NMR (75 MHz, $CDCl_3$): $\delta = 170.3$ (C_q-2), 165.0 (C_q-5), 60.4 (CH-3), 58.7 (CH-6), 45.0 (CH_2-9), 28.4 (CH_2-7), 28.3 (CH-1′), 22.3 (CH_2-8), 19.0 (CH_3-2′), 18.0 (CH_3-3′); –(+)ESI MS: *m/z* (%) = 219 ($[M + Na]^+$, 80), 415 ($[2M + Na]^+$, 100); –(–)ESI MS: *m/z* (%) = 389 ($[2M - 3H]^-$).

Biological activity

Antimicrobial assays were carried out using the disc-agar method [23] against diverse sets of microorganisms. Extracts were dissolved in CH_2Cl_2/10% MeOH at a concentration of 1 mg/ml. Aliquots of 40 µl were soaked on filter paper discs (Ø9 mm, no. 2668; Schleicher & Schüll, Germany) and dried for 1 h at room temperature under sterilized conditions. The paper discs were placed on inoculated agar plats and incubated for 24 h at 37°C for the bacterial isolates and 48 h (30°C) for the fungal isolates, whereas the algal test strains were incubated at ~22°C in day light for 8–10 days. The pure compounds were examined against the test microorganisms shown in Table 1. The cytotoxic assay was performed according to the screening method of Sajid *et al.* [24].

Results and discussion

Metabolites from *Fusarium poae* FUN1

The endophytic fungal species *F. poae* FUN1 was isolated from the leaves of *R. communis* Linn., family *Euphorbiaceae* [18]. The fungus, grown on PDA medium, showed an

Table 1 Antimicrobial activity of the fungal extracts [100 μg/disc (∅9 mm (mm))] and compounds (*1*)–(*4*) [40 μg/disc (∅9 mm (mm))]

Extract/compound	BS	EC	SA	CA	MM	RS	PU	AC
Fusarium poae	17	0	18	11	15	0	0	0
Penicillium italicum	0	0	12	7	0	0	0	24
Linoleic acid (**1**)	0	0	0	0	0	0	0	0
Indole-3-acetic acid methyl ester (**2**)	0	0	0	0	0	0	0	0
N^{β}-acetyltryptamine (**3**)	0	0	0	0	0	0	0	0
Cis-cyclo-(prolyl,valyl) (**4**)	0	0	0	0	0	0	0	0

AC, *Aphanomyces cochlioides*; BS, *Bacillus subtilis*; CA, *Candida albicans*; EC, *Escherichia coli*; MM, *Mucor miehei* (Tü 284); PU, *Pythium ultimum*; RS, *Rhizoctonia solani*; SA, *Staphylococcus aureus*.

extensive cottony pinkish mycelium. The conidiophores are slender and simple or conidia hyaline, microconidia one-celled, ovoid or oblong, born singly or in chains. These features allowed the identification of the fungal isolate as *F. poae* FUN1. This was further confirmed according to Barnett [19].

The strain was upscale fermented on a rotary shaker using 40 of 1-l Erlenmeyer flasks, each containing 250 ml of PDA medium. After harvesting, working up and TLC screening, the brown crude extracts of each of the mycelium and filtrate were fractionated separately. Repeated column chromatography of the mycelial extract using a silica gel, followed by Sephadex LH-20 resulted in the isolation of linoleic acid [(9*Z*,12*Z*)-9,12-octadecanoic acid] (**1**). However, when the filtrate extract was applied to a silica gel column, three fractions were obtained (FI, FII and FIII). Rechromatography of fraction FI on a Sephadex LH-20 column yielded indole-3-acetic acid methyl ester (**2**); similarly, N_{β}-acetyltryptamine (**3**) was isolated from FIII.

Linoleic acid

Compound (**1**) was obtained as a colourless oil; it was $UV_{254\,nm}$ nonabsorbing and stained violet, turning to blue on visualization with anisaldehyde/sulphuric acid. The molecular weight of (**1**) was determined by EI MS as 280 Da corresponding to the molecular formula $C_{18}H_{32}O_2$. The ^{1}H NMR spectrum exhibited a 1H broad singlet at δ 10.67 attributed to a free OH of aliphatic carboxylic acid group, and a 4H multiplet signal at δ 5.33, which could be assigned as two olefinic double bonds. Furthermore, two triplet protons representing two methylene groups, most likely adjacent to sp^2 carbons, were found at δ 2.75 and 2.32. A multiplet of 4H visible at δ 2.02 indicated the presence of two additional methylene groups attached to the sp^2 system. A further multiplet of 2H methylene (δ 1.59) was observed, along with a broad singlet of 14 protons (δ 1.42–1.23), representing a side chain of seven methylene groups. Finally, a triplet methyl signal was evident at δ 0.86.

The chromatographic pattern, spectroscopic analysis and search in AntiBase [2] allowed the establishment of the structure of (**1**) as linoleic acid. It was further confirmed by matching with authentic spectra and published data [25]. Linoleic acid (**1**) is a well-known component of most vegetable and animal fats and is biosynthetically involved in the production of prostaglandin [26]. Linoleic acid was frequently isolated from marine brown algae and from a *Micrococcus* spp. as well [27].

Indole-3-acetic acid methyl ester

Compound (**2**) was obtained as a middle polar colourless solid; it was $UV_{254\,nm}$-absorbing and stained red with anisaldehyde/sulphuric acid. The molecular weight of (**2**) was determined to be 189 Da from its ESI MS spectrum, corresponding to the molecular formula $C_{11}H_{11}NO_2$. The ^{1}H NMR spectrum of (**2**) showed a broad singlet at δ 8.07 of the NH proton. Two aromatic doublets protons (δ 7.60 and 7.34) together with a multiplet signal of three protons (δ 7.1–7.23) were observed, representing a further 3-susbtituted indolyl system. In the aliphatic region, a singlet 2H of the methylene group (δ 3.78) was visible, flanked by two sp^2 systems; a singlet 3H of a methoxy group was also found at δ 3.69.

The structural formula of compound (**2**) was established as indole-3-acetic acid methyl ester, on the basis of its chromatographic and spectral characteristics as well as a search in AntiBase [2]. This was confirmed by comparison with a reference sample and a literature report [25]. Indole-3-acetic acid methyl ester (**2**) was formerly isolated from *Pseudomonas amygdali* [28]. Indole acetic acid, the parent compound, is a well-known auxin that plays an important role in plant growth [28,29]; in addition, it has strong antibacterial and antifungal properties as well [30].

N_{β}-acetyltryptamine

Compound (**3**) was obtained as a colourless solid; it was characterized by being $UV_{254\,nm}$-absorbing and stained orange, turning to violet on spraying with anisaldehyde/sulphuric acid, and acquired a pink colour with Ehrlich's reagent. The molecular weight of (**3**) as established by ESI MS was 202 Da, corresponding to the molecular formula of $C_{12}H_{14}N_2O$. The ^{1}H NMR spectrum of (**3**) showed a broad singlet at δ 8.30 of the NH proton, five aromatic signals as well as three signals in the aliphatic region. In the aromatic region, two doublet protons (δ 7.60 and 7.38) as well as two overlapped triplet protons appeared (δ 7.26–7.08), indicating an 1,2-disubstituted benzene ring. A further doublet 1H evident at δ 7.03 was attributed to either an *m*-coupled proton or to being attached to an amino group. Thus, a 3-substituted indolyl system was deduced. In the aliphatic zone, a broad singlet (δ 5.70) of an additional NH group together with a quartet methylene (δ 3.58) was found; the latter methylene transformed into a triplet after H/D exchange, thus confirming its linkage to the NH group. Furthermore, the triplet observed at δ 2.98 of methylene protons indicated an ethandiyl group. Finally, a singlet of a methyl group, typical for an acetyl group, was visible at δ 1.93. According to the aforementioned physic-chemical and spectral characteristics, as well as search in AntiBase and published data [25], the structural formula of compound (**3**) was confirmed to be N_{β}-acetyltryptamine [25,31]. N_{β}-aceyltryptamines have been isolated from different

plants [32,33] and bacterial [34] species; they are known to possess antifungal properties [34].

Metabolites from *Penicillium italicum* FUN2

The fungus FUN2, isolated from a soil sample, showed bright dark green colonies on PDA medium. When examined under light microscope, the colonies showed conidiophores that are arising from the mycelium singly or less often in synnemata. These synnemata are branched near the apex to form a brush-like. Furthermore, the conidia-bearing apparatus ended in phialides that pinched off conidia in dry chains. Conidia, hyaline or brightly coloured in mass, one-celled, mostly globose or ovoid, produced basipetally. On the basis of these morphological and microscopic features, and according to Barnett's [19] and Pitt's [22] taxonomic keys, the fungal strain isolate FUN2 was characterized as *P. italicum*.

P. italicum FUN2 was cultivated on GYMP medium for 10 days using a rotary shaker. After harvesting, working up and TLC monitoring mycelia and culture filtrate extracts were combined and concentrated, and yielded a brown residue. Fractionation of the extract using several chromatographic techniques led to the isolation of linoleic acid (**1**) and *cis*-cyclo-(prolyl,valyl) (**4**).

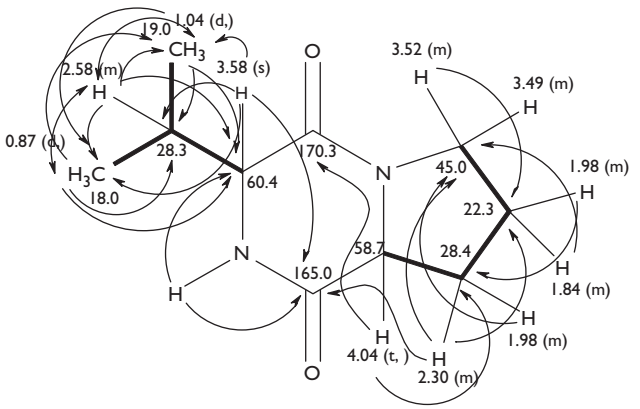
Cis-cyclo-(prolyl,valyl)

Compound (**4**) was obtained as a low polar colourless solid, quenching $UV_{254\,nm}$ absorbance, and stained violet on spraying with anisaldehyde/sulphuric acid. The molecular weight of compound (**4**) was determined to be 196 Da according to both (+) and (–) modes of ESI MS, with a corresponding molecular formula of $C_{10}H_{16}N_2O_2$. The ^{1}H NMR spectrum of **4** showed no aromatic property, except that a sole 1H singlet at δ 6.71, attributed to an amide (NH–C = O) group, was observed. Two methine protons were found at δ 4.04 (t) and 3.58 (s), representing most likely amide-bounded methines of an amino acid system (N–CH–C = O). Two 1H multiplets were visible at δ 3.52 and 3.49, which might be attributed to an amide-bounded methylene protons (–O = C–N–CH_2). In addition, a multiplet 1H signal was evident at δ 2.56 together with further three multiplets at δ 2.30 (1H), δ 1.98 (2H) and δ 1.84 (1H). Finally, two doublet methyls were observed at δ 1.04 and 0.87, corresponding to an isopropyl system.

The ^{13}C NMR/HMQC spectra of compound (**4**) showed 10 carbon signals, among which were two amide carbonyls (δ 170.3 and 164.9), two methines for α-amino methine carbons (δ 61.0 and 58.8) and three methylenes (δ 45.9, 28.4, 22.3). A third methine carbon (δ 28.3), along with two methyls (δ 23.3 and 21.2), confirmed the aforementioned isopropyl group.

Structure of **4** was, thus, subjected to interpretation on the bases of ^{1}H–^{1}H COSY and HMBC experiments (Fig. 1), confirming the structure of **4** as *cis*-cyclo-(prolyl,valyl) [35]. A search in AntiBase [2] revealed two possible stereo isomers: *cis*-cyclo-(prolyl,valyl) (**4**) and *trans*-cyclo-(prolyl,valyl) (**5**). However, a comparison of the results of spectroscopic analysis of the compound with those published [31] confirmed its structure to be *cis*-cyclo-(prolyl,valyl) (**4**). Diketopiperazines are very frequently isolated as secondary metabolites from microorganisms, for example, *Penicillium piscarium* [36] and other *Penicillium* spp.[37]. Diketopiperazines are the smallest known cyclic dipeptides [38,39]. They have a wide spectrum of biological activities including antitumour, antiviral, antifungal, antibacterial, antihyperglycaemic and others [40,41].

Figure 1



^{1}H–^{1}H COSY (⟷) and HMBC (⟶) correlations of *cis*-cyclo-(prolyl,valyl) (**4**).

Table 2 Brine shrimp cytotoxicity against the fungal extracts and pure compounds (*1*)–(*4*)

Extract/compound	Brine shrimp (100 μg/ml, 24 h) (%)
Fusarium poae	83
Penicillium italicum	0
Linoleic acid (**1**)	0
Indole-3-acetic acid methyl ester (**2**)	0
$N_β$-acetyltryptamine (**3**)	0
Cis-cyclo-(prolyl,valyl) (**4**)	0

Evaluation of biological activities

Antimicrobial activity

Extracts of the selected fungal strains, namely, the endophytic *F. poae* FUN1, and the terrestrial *P. italicum* FUN2 and their respective isolated metabolites (**1**)–(**4**) were evaluated against a set of pathogenic microorganisms at a concentration of 40 μg/disc (Table 1). The extract of *F. poae* showed high activity against Gram-positive bacteria: *Bacillus subtilis* (17 mm) and *Staphylococcus aureus* (18 mm). The strain showed further moderate antiyeast (*Candida albicans*, 11 mm) and high antifungal [*Mucor miehei* (Tü 284), 15 mm] activities. However, the extract of *P. italicum* showed high activity against the phytofungal strain, *Aphanomyces cochlioides* (24 mm), whereas it showed moderate and weak activities against *S. aureus* (12 mm) and *C. albicans* (7 mm). In contrast, none of the isolated compounds (**1**)–(**4**) showed any activity against the pathogenic microorganisms tested.

Brine shrimp cytotoxicity

The two fungal extracts and the isolated metabolites (**1**)–(**4**) were subjected to a brine shrimp lethality assay for the evaluation of their cytotoxicity. The results of this study (Table 2) showed that the extract of *P. italicum* FUN2 and all isolates [compounds (**1**)–(**4**)] showed no activity, whereas the extract of endophytic fungi *F. poae* FUN1 showed high cytotoxicity (83%).

Conclusion

Two different fungal strains, endophytic *F. poae* FUN1 and terrestrial *P. italicum* FUN2, were intensively studied biologically and chemically. Four bioactive compounds (**1**)–(**4**) were isolated, and structurally confirmed by intensive studies of NMR and MS. The antimicrobial and cytotoxic activities of the fungal extracts and their delivered compounds were studied. This might be helpful for the cure of recent diseases, and drug-resistant phenomena as well as in the development of pharmaceutical, agrochemical and biochemical agents and their lead compounds.

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Conflicts of interest

There are no conflicts of interest.

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