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RESEARCH ARTICLES

Phytotoxic naphtho-γ-pyrones from an endophytic fungus *Aspergillus niger* from *Basella alba*

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Abstract

Fermentation of *Aspergillus niger* isolated from the leaves of *Basella alba* in potato dextrose broth medium furnished five naphtho-γ-pyrone type compounds, TMC 256A1 (**1**), rubrofusarin B (**2**), fonsecin B (**3**), aurasperone A (**4**) and fonsecinone A (**5**). Compounds **1**, **2** and **3** showed remarkable phytotoxic activity in lettuce seed germination bioassay with IC_{50} values of 45.4, 49.7 and 47.8 ppm percentage radicle growth inhibition, respectively, while IC_{50} values for percentage hypocotyl growth inhibition were 49.7, 48.7 and 48.2 ppm, respectively. This is the first report on the isolation of *A. niger* from *Basella alba* and the phytotoxic activity of compounds **1**–**3** in lettuce seed germination assay.

Highlights

- Fermented *Aspergillus niger* from *Basella alba* furnished five naphtho-γ-pyrones.
- TMC 256A1 (**1**), rubrofusarin B (**2**), fonsecin B (**3**) showed remarkable phytotoxic activity.
- This is the first report of the isolation of an endophytic fungus *Aspergillus niger* from *Basella alba*.

Keywords *Aspergillus niger* · *Basella alba* · Endophytic fungi · Naphtho-γ-pyrones · Phytotoxicity

Introduction

The overuse of synthetic herbicides has resulted in negative consequences for the environment and human health as well as increased the occurrence of herbicide resistant weeds. Therefore efforts have been made to use natural plant products as eco-friendly weedicides (Suwitchayanon and Kato-Noguchi 2014). We previously reported the isolation and characterization of two phytotoxic azaphilone derivatives from the endophytic fungus *Chaetomium globosum* from *Amaranthus viridis* (Piyasena et al. 2015) together with various biologically active compounds from endophytic fungus on plants in Sri Lanka (Bandara et al. 2015; Dissanayake et al. 2020; Kehelpannala et al. 2018, 2021; Munasinghe et al. 2017, 2021; Padmathilake et al. 2017; Quader et al. 2016, 2017a, b; Rathnayake et al. 2018, 2019; Siriwardane et al. 2015; Sritharan et al. 2019; Thanabalasingam et al. 2015, 2024). *Basella alba*, which belongs to the Basellaceae family, grows in tropical and subtropical regions and is commonly consumed as a leafy vegetable. This plant has traditionally been utilized for the treatments of ulcers, burns, boils, abscesses, and swellings (Jayaweera 1981). Here, we present the isolation and characterization of an endophytic fungus *Aspergillus niger* from *B. alba* leaves, the isolation of compounds TMC 256A1 (**1**), rubrofusarin B (**2**), fonsecin B (**3**), aurasperone A (**4**) and fonsecinone A (**5**) from its culture, and phytotoxicity of compounds **1**, **2** and **3** against lettuce (*Lactuca sativa*) seed germination bioassay.

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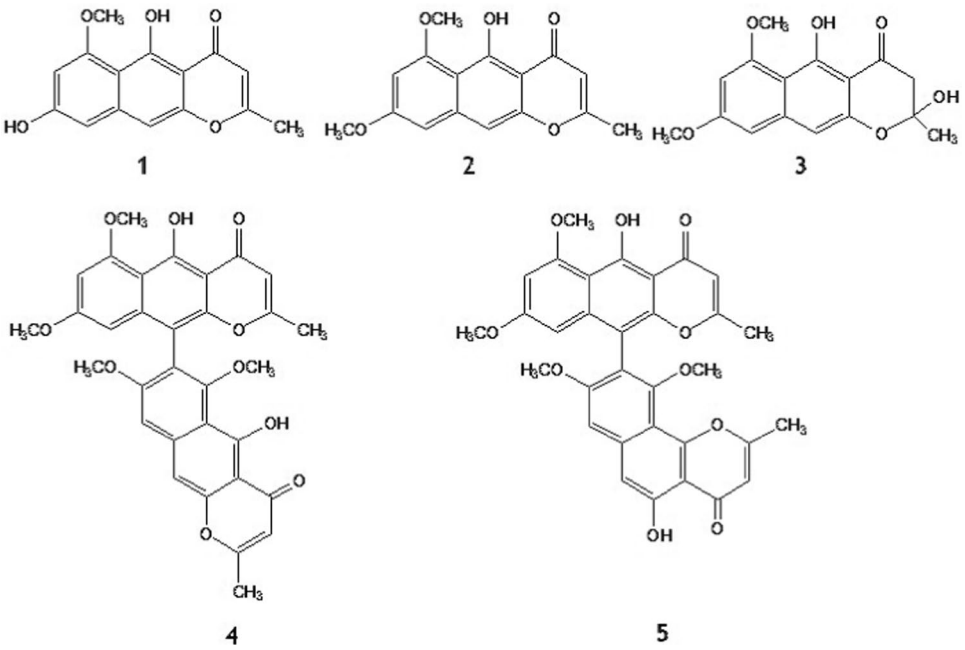

Fig. 1 Chemical structures of compounds **1**–**5** isolated from an endophytic fungus *Aspergillus niger*

Materials and methods

Isolation of endophytic fungus, fermentation and isolation of secondary metabolites

In a 90 mm Petri dish with potato dextrose agar (PDA) medium, a section of the triple-sterilized leaf was incubated at ambient temperature. The emerged fungus was sub-cultured to obtain a pure culture. The endophytic fungus was identified as *Aspergillus niger* performing the sequence of internal transcribed spacer of the rDNA gene of the fungus, which was amplified utilizing primers of ITS1 and ITS4. *A. niger* grown on PDA media was introduced into Erlenmeyer flasks (1 L × 10), each containing 400 mL potato dextrose broth (PDB) medium, which were allowed to grow at room temperature for four weeks. After filtering the resultant culture broth, the mycelium and filtrate were extracted three times separately employing ethyl acetate (EtOAc). The two EtOAc extracts displayed a comparable TLC pattern, therefore, the combined extracts (2.8 g) were used for lettuce seed germination bioassay (Piyasena et al. 2015). The EtOAc extract was subjected to silica gel (Merck 7734) chromatography eluting with dichloromethane-methanol gradient and further purified by preparative TLC and Sephadex LH-20 led to the isolation of TMC 256A1 (**1**, 18 mg), rubrofusarin B (**2**, 15 mg), fonsecin B (**3,** 8 mg), aurasperone A (**4**, 10 mg) and fonsecinone A (**5**, 8 mg) (Fig. 1).

Lettuce seed germination bioassay

After treating lettuce seeds (Rapido 344, Thailand) with a 5% clorox solution for ten minutes, the seeds were thoroughly rinsed with sterile distilled water. Floated immature seeds were discarded. Five lettuce seeds were kept in each Petri dish, which contained 800 µL of EtOAc extract (at 1000 ppm) or the isolated compound dissolved in 1% DMSO in distilled water at concentrations ranging from 200 to 10 ppm. The negative control was 1:99 of DMSO in distilled water while the positive control was 10 ppm of abscisic acid. After placing the seeds on moist filter paper (Whatman No. 4), Petri plates were incubated for a period of five days at 25 °C in the dark. After the incubation period, the length of both the hypocotyl and radicle was measured to the closest millimeter, and the total number of seeds that germinated in each Petri dish was recorded. The lettuce seed germination bioassay was conducted twice, with four replicates utilized for each treatment (Piyasena et al. 2015).

Results and discussion

The endophytic fungus isolated from *Basella alba* leaves was identified as *Aspergillus niger* based on the fungal rDNA gene's internal transcribed spacer sequence, which has been amplified employing primers of ITS1 and ITS4. The BLAST search indicated that the sequence matched with that of *Aspergillus niger* (99%, GenBank accession No. KY864240.1). The photographic evidence of the leaves of *B. alba* and fungal strain were deposited at the National Institute of Fundamental Studies. Pure strain of *A. niger* was fermented on potato dextrose medium (PDB). The combined EtOAc extract of the culture broth and mycelium completely inhibited lettuce seed germination at 1000 ppm. Five compounds were isolated by chromatographic separation of

the extract on silica gel, PTLC, and Sephadex LH-20. These compounds were identified as TMC 256A1 (**1**) (Huang et al. 2010), rubrofusarin B (**2**) (Shaaban et al. 2012), foncesin B (**3**) (Shaaban et al. 2012), aurasperone A (**4**) (Campos et al. 2005) and fonsecinone A (**5**) (Campos et al. 2005) by comparison of the ^{1}H & ^{13}CNMR data with the previously reported values. The ^{1}H and ^{13}C NMR spectra of compounds **1**–**5** can be seen in Supplementary material. Compounds **1** and **2** belong to monomeric naphtho-γ-pyrones, compound **3** has a structure of 2,3-dihydronaphtho-γ-pyrones, and compounds **4** and **5** are dimeric naphtho-γ-pyrones. At 100 ppm, compounds **1**, **2** and **3** completely inhibited the germination of lettuce seeds. Furthermore, compounds **1**, **2** and **3** inhibited radicle elongation with IC_{50} values of 45.4, 49.7, and 47.8 ppm, respectively, and inhibited hypocotyl elongation with IC_{50} values of 49.7, 48.7 and 48.2 ppm, respectively. *Aspergillus niger* strains have been isolated from various sources, mostly as an endophytic fungus. It has been documented that *A. niger* produces a wide range of bioactive secondary metabolites including naphtho-γ-pyrones (Yu et al. 2021). Compounds **1**–**5** were previously reported as secondary metabolites of *A. niger* (Leutou et al. 2016; Abdelwahab et al. 2021; Shaaban et al. 2012). However, there are limited reports on the phytotoxicity of naphtho-γ-pyrones and related compounds. Macías and coworkers reported on the phytotoxic activity of dihydronaphtho-γ-pyrones and rubrofusarin B (**2**) against radicle elongation of two weed (*Amaranthus hypochondriacus* and *Echinochloa crus-galli*) seedlings (Macías et al. 2000). Phytotoxic activity of dihydronaphtho-γ-pyrones against radicle and hypocotyl elongation in rice and lettuce seed germination was reported (Lai et al. 2019). Inhibitory activities of dimeric naphtho-γ-pyrone type compounds on radicle and germ elongation of rice seeds were reported (Sun et al. 2017).

Conclusion

In this study *A. niger* was isolated from the leaves of *B. alba* for the first time. The endophytic fungus produced TMC 256A1 (**1**) and rubrofusarin B (**2**), fonsecin B (**3**), aurasperone A (**4**) and fonsecinone A (**5**) through fermentation in PDB medium, although these compounds were previously reported as secondary metabolites of *A. niger*. Among these, compounds **1**, **2** and **3** significantly exhibited lettuce seed germination. Furthermore, these compounds inhibited radicle and hypocotyl elongation in lettuce seed germination. The present results, together with previously reported phytotoxic activities of dihydronaphtho-γ-pyrones and dimeric naphtho-γ-pyrones, suggests the possibility of naphtho-γ-pyrone type compounds as a weedicide or herbicide to reduce or displace from the utilization of hazardous synthetic constituents. These findings contribute to the growing body of knowledge regarding fungal secondary metabolites and their bioactivities, opening new avenues for further exploration and potential utilization in various fields.

Supplementary Information The online version contains supplementary material available at https://doi.org/10.1007/s42535-025-01515-4.

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Data availability All data will be made available upon reasonable request.

Declarations

Conflict of interest No potential conflict of interest was reported by the author.

References

Abdelwahab GM, Mira A, Chen YB, Abdelaziz TA, Lahloub MFI, Khalil AT (2021) Acetylcholineesterase inhibitory activity of green synthesized nanosilver by naphthopyrones isolated from marine derived *Aspergillus niger*. PLoS ONE 16(9):e0257071. https://doi.org/10.1371/journal.pone.0257071

Bandara HMSKH, Kumar NS, Jayasinghe L, Masubuti H, Fujimoto Y (2015) A 3-vinyl cephem derivative, useful intermediate in the synthesis of cephem antibiotics, from *Aspergillus awamori* associated with banana fruit. Nat Prod Commun 10(10):1663–1666

Campos FR, Barison A, Daolio C, Ferreira AG, Rodrigues-Fo E (2005) Complete ^{1}H and ^{13}C NMR assignments of aurasperone A and fonsecinone A, two bis-naphthopyrones produced by *Aspergillus aculeatus*. Magn Reson Chem 43(11):962–965

Dissanayake D, Kumar NS, Adikaram NKB, Jayasinghe L, Araya H, Fujimoto Y (2020) Production of small molecules by an endophytic fungus, *Neofusicoccum parvum* from the fruits of *Elaeocarpus serratus*. Asian J Tradit Med 15(2):20–27

Huang HB, Feng XJ, Liu L, Chen B, Lu YJ, Ma L, Lin YC (2010) Three dimeric naphtho-γ-pyrones from the mangrove endophytic fungus *Aspergillus tubingensis* isolated from *Pongamia pinnata*. Planta Med 76(16):1888–1891

Jayaweera DM (1981) Medicinal plants used in Ceylon Part I. The National Science Council of Sri Lanka, Colombo

Kehelpannala CL, Kumar NS, Jayasinghe L, Araya H, Fujimoto Y (2018) Naphthoquinonemetabolites produced by *Monacrosporium ambrosium*, the ectosymbiotic fungus of tea shot-hole borer, *Euwallacea fornicatus*, in stems of tea, *Camellia sinensis*. J Chem Ecol 44(1):95–101

Kehelpannala C, Rathnayake GRN, Dissanayake D, Kanatiwela D, Kumar NS, Adikaram N, Jayasinghe L, Araya H, Fujimoto Y (2021) Determination of the absolute stereochemistry of (+)-solaniol. Chem Pap 75:2233–2235

Lai D, Meng J, Xu D, Zhang X, Liang Y, Han Y, Jiang C, Liu H, Wang C, Zhou L, Xu JR (2019) Determination of the absolute configurations of the stereogenic centers of ustilaginoidins by studying the biosynthetic monomers from a gene knockout mutant of *Villosiclava virens*. Sci Rep 9:1855. https://doi.org/10.1038/s41598-018-37941-5

Leutou AS, Yu K, Son BW (2016) Induced production of 6,9-dibromoflavasperone, a new radical scavenging naphthopyranone in the marine-mudflat-derived fungus *Aspergillus niger*. Arch Pharm Res 39(6):806–810

Macías M, Ulloa M, Gamboa A, Mata R (2000) Phytotoxic compounds from the new coprophilous fungus *Guanomyces polythrix*. J Nat Prod 63(6):757–761

Munasinghe MVK, Kumar NS, Jayasinghe L, Fujimoto Y (2017) Indole-3-acetic acid production by *Colletotrichum siamense*, an endophytic fungus from *Piper nigrum* leaves. J Biol Act Prod Nat 7(6):475–479

Munasinghe MVK, Kumar NS, Adikaram NKB, Jayasinghe L, Araya H, Fujimoto Y (2021) Secondary metabolites from an endophytic fungus *Aspergillus fumigatus* from *Solanum insanum* L. Asian J Tradit Med 16(2):53–59

Padmathilake KGE, Bandara HMSKH, Qader MM, Kumar NS, Jayasinghe L, Masubuti H, Fujimoto Y (2017) Talarofuranone, a new talaroconvolutin analog from the endophytic fungus *Talaromyces purpurogenus* from *Pouteria campechiana* seeds. Nat Prod Commun 12(14):489–490

Piyasena KGNP, Wickramarachchi WART, Kumar NS, Jayasinghe L, Fujimoto Y (2015) Two phytotoxic azaphilone derivatives from *Chaetomium globosum*, a fungal endophyte isolated from *Amaranthus viridis* leaves. Mycology 6(3–4):158–160

Qader MM, Kumar NS, Jayasinghe L, Fujimoto Y (2016) Production of antitumor antibiotic GKK1032B by *Penicillium citrinum*, an endophytic fungus isolated from *Garcinia mangostana* fruits. Med Aromatic Plant 5(1):225

Qader MM, Kumar NS, Jayasinghe L, Araya H, Fujimoto Y (2017a) Bioactive sesquiterpenes from an endophytic fungus *Bipolaris sorokiniana* isolated from a popular medicinal plant *Costus speciosus*. Mycology 8(1):17–20

Qader MM, Kumar NS, Jayasinghe L, Fujimoto Y (2017b) Shikimic acid production by *Fusarium decemcellulare*, an endophytic fungus isolated from *Flacourtia inermis* fruits. J Biol Active Prod Nat 8(1):43–50

Rathnayake GRN, Kumar NS, Jayasinghe L, Araya H, Fujimoto Y (2018) Chemical investigation of metabolites produced by an endophytic fungi *Phialemonium curvatum* from the leaves of *Passiflora edulis*. Nat Prod Res 32(20):2483–2486

Rathnayake GRN, Kumar NS, Jayasinghe L, Araya H, Fujimoto Y (2019) Secondary metabolites produced by an endophytic fungus *Pestalotiopsis microspora*. Nat Prod Bioprospect 9(6):411–417

Shaaban M, Shaaban KA, Abdel-Aziz MS (2012) Seven naphtho-γ-pyrones from the marine-derived fungus *Alternaria alternata*: structure elucidation and biological properties. Org Med Chem Lett 2:6. https://doi.org/10.1186/2191-2858-2-6

Siriwardane AMDA, Kumar NS, Jayasinghe L, Fujimoto Y (2015) Chemical investigation of metabolites produced by an endophytic *Aspergillus* sp. isolated from *Limonia acidissima*. Nat Prod Res 29(14):1384–1387

Sritharan T, Kumar NS, Jayasinghe L, Araya H, Fujimoto Y (2019) Isocoumarins and dihydroisocoumarins from the endophytic fungus *Biscogniauxia capnodes* isolated from the fruits of *Averrhoa carambola*. Nat Prod Commun 14(5):1934578X19851969

Sun W, Wang A, Xu D, Wang W, Meng J, Dai J, Liu Y, Lai D, Zhou L (2017) New ustilaginoidinsfrom rice false smut balls caused by *Villosiclava virens* and their phytotoxic and cytotoxic activities. J Agric Food Chem 65(25):5151–5160

Suwitchayanon P, Kato-Noguchi H (2014) Allelopathic activity of leaves, stalks and roots of *Cymbopogon nardus*. Emir J Food Agric 26(5):436–443

Thanabalasingam D, Kumar NS, Jayasinghe L, Fujimoto Y (2015) Endophytic fungus *Nigrospora oryzae* from a medicinal plant *Coccinia grandis*, a high yielding new source of phenazine-1-carboxamide. Nat Prod Commun 10(10):1659–1660

Thanabalasingam D, Dissanayake D, Samarakoon K, Kumar NS, Adikaram N, Jayasinghe L, Araya H, Fujimoto Y (2024) Oryzanigral: a new polyketide from an endophytic fungus *Nigrospora oryzae* isolated from *Coccinia grandis*. Nat Prod Res 38(15):2703–2710

Yu R, Liu J, Wang Y, Wang H, Zhang H (2021) *Aspergillus niger* as a secondary metabolite factory. Front Chem 9:701022