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RESEARCH ARTICLES

α-Glucosidase inhibitors from an endophytic fungus *Edenia gomezpompae* from *Costus speciosus*

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Abstract

Costus speciosus is a plant used in traditional medicine. It is widely used as a treatment for diabetes in Sri Lanka, India and neighboring countries. An endophytic fungus, *Edenia gomezpompae,* isolated from the leaves of *Costus speciosus,* was cultured in potato dextrose broth media. Chromatographic separation of the EtOAc extract of the broth and mycelium led to the isolation of rare compounds leptosphaeridione (1) and preussomerin EG_2 (2). This is the first report of the isolation of the endophytic fungus *E. gomezpompae* from *C. speciosus*. Both compounds were screened for their enzyme inhibitory activity toward α-glucosidase, α-amylase, lipase and acetylcholinesterase. Compounds 1 and 2 exhibited significant α-glucosidase inhibitory activity with the IC_{50} values of 2.39 µg/mL and 27.9 µg/mL, respectively. This study contributes to our knowledge on the diversity and chemical potential of fungal endophytes associated with medicinal plants.

Keywords *Edenia gomezpompae* · *Costus speciosus* · Endophyte · Leptosphaeridione · Preussomerin EG_2

Introduction

Endophytic fungi reside within the tissues of their host plants without causing harm. The first discovery of endophytic fungi reported in 1904, but its ability to produce remarkable bioactive compounds were not realized until the recent years. Endophytic fungi are well known to produce novel bioactive secondary metabolites with a wide range of new structures (Strobel et al. 2004). The discovery of penicillin during the World War II was a remarkable event that paved the way for scientist to search for bioactive compounds from fungal flora. Fungi are leading sources to produce a wide variety of secondary metabolites, most of which have potent antimicrobial, antiviral, and anticancer activities. We have recently reported some bioactive compounds from endophytic fungi from Sri Lankan plants (Bandara et al. 2015; Dissanayake et al. 2020; Kehelpannala et al. 2018; Munasinghe et al. 2017 and 2021; Padmathilake et al. 2017; Piyasena et al. 2015; Qader et al. 2016 and 2018; Rathnayake et al. 2018 and 2019; Siriwardane et al. 2015; Sritharan et al. 2019; Thanabalasingam et al. 2015 and 2024), including the isolation of an endophyte *Bipolaris sorokiniana* from *Costus speciosus* (Qader et al. 2017). *Costus speciosus* is used in traditional medicine. It is widely used as a treatment for diabetes in Sri Lanka, India and neighboring countries. We previously reported the isolation of an endophytic fungus *Bipolaris sorokiniana* from *C. speciosus* and its secondary metabolites. We have now isolated another endophytic fungi *Edenia gomezpompae* from the leaves of *C. speciosus*. This paper delves into the secondary metabolites produced by this fungus. Fermentation of the fungus in potato dextrose broth followed by extraction with EtOAc and chromatographic separation yielded two rare natural products, leptosphaeridione (1) and preussomerin EG_2 (2). Compounds 1 and 2 were found to have significant α-glucosidase inhibitory activity (Fig. 1).

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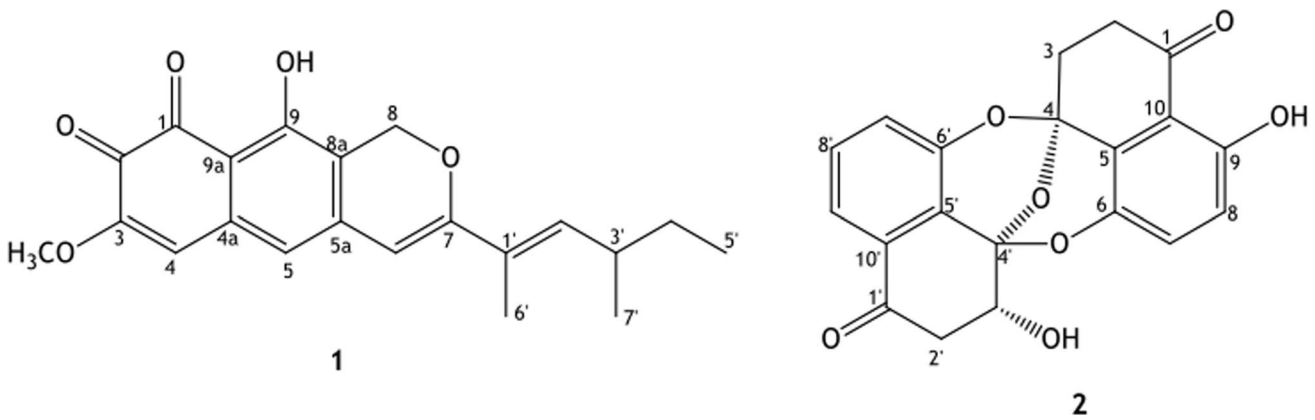

Fig. 1 Chemical structures of compounds 1 and 2

Methods

General

The TLC was performed using pre-coated aluminum sheets with silica gel 60F254 (Merck 1.05554). For column chromatography, silica gel (Merck 1.07734) and Sephadex LH20 (Fluka, 84952) were used. NMR spectra were recorded on a JEOL JNM-ECP500 spectrometer in $CDCl_3$ solution. UV absorbance values were taken using the multimode reader (BioTek Synergy HTX). AChE enzyme (from Electrophorus electrical), α-amylase and α-glucosidase (from Saccharomyces cerevisiae) were purchased from Sigma Aldrich. All the other chemicals used were of the analytical grade unless otherwise specified.

Isolation & identification of the endophytic fungus

Plant leaves of *Costus speciosus* were collected from the home garden in Kandy, Sri Lanka and identified by Prof. Nimal Adikaram, Professor of Botany, National Institute of Fundamental Studies, Kandy, Sri Lanka. The leaves of the plant were washed, and triple sterilized with 90% ethanol, 2.5% NaOCl, 90% ethanol and distilled water. Leaf segments (≥ 5 mm^2) were placed on potato dextrose agar medium and incubated at room temperature (32 °C) for 7 days. The emerging fungus was carefully sub-cultured to obtain its pure cultures. The external characteristics and the spores from the pure cultures were microscopically observed. Molecular level identification was carried out by amplifying the Internal Transcribed Spacers (ITS) of rDNA gene using universal eukaryotic primers, ITS 1-F- 5′ CTT GGT CAT TTA GAG GAA GTA A 3′ (Gardes and Bruns 1993) and ITS 4—5′ TCC TCC GCT TAT TGA TAT GC 3′ (White et al. 1990). The resulting sequence was compared for similarities by performing nucleotide BLAST. The amplified gene sequence had 99.48% similarity with *Edenia gomezpompae* (GenBank accession number: NR 156217.1). The pure culture of the fungus and the photographic evidences were deposited at the Natural Products laboratory of the National Institute of Fundamental Studies (NIFS).

Fermentation & isolation of compounds

Large scale fermentation of the fungus was done in potato dextrose broth for 4 weeks in twenty 1 L-flasks each containing 400 mL medium. Extraction of the broth with EtOAc followed by removal of the solvent in vacuo gave a light brown solid while extraction of the mycelium with EtOAc gave a dark brown solid. The two EtOAc extracts showed a similar TLC pattern and thus they were combined and separated by chromatography over silica gel to give compounds 1 (deep purple solid, 8 mg) and 2 (pale yellow solid, 7 mg). The structures of compounds 1 and 2 were determined by spectral means including 2D-NMR.

Enzyme inhibitory assays

Two compounds were tested for enzyme inhibitory activity for α-glucosidase (Sathya et al. 2020), α-amylase (Alakolanga et al. 2015), acetylcholinesterase activity (Sathya et al. 2020) and lipase enzyme inhibitory activity (Fernando et al. 2019).

α-Glucosidase inhibitory assay The samples were dissolved in either phosphate buffer (pH 6.9) or distilled water. To the test wells of 96-well plate, 100 µL of buffer followed by 25 µL sample and 25 µL enzyme were added. The plate was incubated for 10 min at 37 °C. 50 µL of 4-nitrophenyl α-D-glucopyranoside (pNPG) was added and incubated for 30 min at 37 °C. The blanks and the controls were carried out in the

same procedure. For the test-blanks, 25 µL of buffer was added instead of the enzyme. 25 µL from the solvent to the control wells were added instead of the sample. The absorbance was measured at 410 nm. Acarbose was used as the positive control. The IC_{50} value of the sample was calculated by using the log dose-inhibitory curve. The percentage of α-glucosidase inhibitory activity was calculated (Sathya et al. 2020).

α-Amylase inhibitory assay The samples and the enzyme 100 µL were mixed and incubated at room temperature for 30 min. After that, 100 µL of starch was added and the mixture was re-incubated for 10 min at room temperature. Then, 100 µL of DNSA was added and the mixture was re-incubated in a water bath at 85.5 °C. After 15 min, samples were taken out and diluted with 900 µL of distilled water. 200 µL of the diluted solution from each sample was taken and added to a 96-micowell plate and absorbance values were measured at 540 nm using a microplate reader (Alakolanga et al. 2015). Acarbose was used as the positive control.

Acetylcholinesterase inhibitory assay The samples were dissolved in 1% DMSO or distilled water. 100 µL of phosphate buffer (pH 8), 25 µL of sample and 50 µL of enzyme were added to the wells of a 96-microwell plate. After 10 min 50 µL of 5,5′ -dithiobis(2-nitrobenzoic acid) (DTNB) and 25 µL of substrate (acetylthiocholine iodide) were added and incubated for 10 min at room temperature. The same procedure was followed for the controls and blanks. For the test-blanks 50 µL of buffer was added instead of the enzyme. 25 µL of solvent was added to the controls instead of the sample. Absorbance was measured at 412 nm. Donepezil was used as the positive control. Percentage inhibition of AChE was calculated. The IC_{50} value of the sample was calculated by the using log dose-inhibitory curve. (Sathya et al. 2020).

Lipase inhibitory assay The experiment was carried out in a 96-microwell plate. In the initial step, 100 µL of buffer solution was mixed with 25 µL of the enzyme and 25 µL of the sample, followed by an incubation period at 15 min at 37 °C.

Afterward, 25 µL of PNPB solution was added and the mixture was re-incubated for 30 min at 37 °C. Absorbance was measured at 400 nm using a microplate reader (Fernando et al. 2019). Orlistat was used as the positive control.

$$\textit{Enzyme inhibitory activity}\,(\%) = (\delta A\ \textit{control} - \delta A\ \textit{sample})/(\delta A\ \textit{control}) \times 100$$

Statistical analysis

All measurements in this study were obtained by analysis in triplicate (n = 3). The IC_{50} value of the sample was determined by using the log dose-inhibitory curve.

Results and discussion

The isolated fungus from the leaves of *Costus speciosus* was identified as *Edenia gomezpompae* by molecular means (the sequence of the ITS region showed 99.48% similarity (GenBank Accession No. NR 156217.1)). Fermentation of the fungus in potato dextrose broth followed by extraction with EtOAc and chromatographic separation yielded two rare natural products, leptosphaeridione (1) and preussomerin EG_2 (2). The structure of compound 1 was elucidated by detailed NMR analysis including 2D-NMR and differential NOE measurements, since this compound had been previously reported but no NMR data was found. Compound 2 was identified αs preussomerin EG_2 by comparison with the reported NMR data. The 2D-NMR spectra for compounds 1 and 2 are provided in supplementary material. The two compounds were screened for their enzyme inhibitory activity toward α-glucosidase, α-amylase, lipase and acetylcholinesterase. Results of the enzyme inhibitory activity are as follows. The α-glucosidase inhibitory activity of compounds 1 and 2 was observed for 99% and 91% inhibition at 100 µg/mL and the IC50 values were determined as 2.39 and 27.9 µg/mL, respectively. The percentage lipase inhibitory activity of compound 1 and 2 at 100 µg/mL was found to be 36.1% and 29.8% and IC_{50} values were 508 and 657 µg/mL, which were more potent than the positive control acarbose. The acetylcholinesterase inhibitory activity of compounds 1 and 2 at 100 µg/mL was weak (34% and 32% inhibition, respectively). It is important to note that these two compounds showed very low α-amylase inhibitory activity.

The polyketide leptosphaeridione (1) was reported from the marine ascomycete *Leptosphaeria oraemaris* (Guerriero et al. 1991) and subsequently from *Stagonospora* sp. (Ahonsi et al. 2005). This is the first report of the strong α-glucosidase inhibitory activity of compound 1. Phytotoxic activity of compound 1 against (*Convolvulus arvensis*) and tomato (*Lycopersicon esculentum*) plants was reported (Nicolet 1999). Structurally close polyketides, obionin A (C-1′-C-2′-double bond of 1 is saturated) from the marine fungus *Leptosphaeria obiones* (Poch and Gloer 1989) and laccaridiones A and B (C-6 of 1 was substituted with a methoxy or ethoxy group, respectively) from the terrestrial basidiomycete *Laccaria amethystea* (Berg et al. 2000) have been isolated. Preussomerin EG_2 (2) belongs to the naphthoquinone spiroketal class of natural products and was first isolated as a secondary metabolite of the endophytic fungus

Edenia gomezpompae (Macías-Rubalcava et al. 2008). Some of these naphthoquinone spiroketal-type compounds are reported to show phytotoxic activity against several plants, antimicrobial activity against economically important phytopathogenic microorganisms (Macias-Rubalcava et al. 2008, 2014) and anti-inflammatory activity (Tan et al. 2020). The potent α-Glucosidase inhibitory activity of compound 2 was reported for the first time in this paper.

Conclusion

α-Glucosidase is a key enzyme that catalyzes the final step in the carbohydrate digestion process. Therefore, α-glucosidase inhibitors can reduce postprandial blood glucose levels and suppress postprandial hyperglycemia by inhibiting the release of glucose from dietary complex carbohydrates and delaying glucose absorption. In recent years, many efforts have been made to identify effective α-glucosidase inhibitors from natural sources, with the aim of developing lead compounds for diabetes treatment (Kumar et al. 2011). In this study, two rare compounds, leptosphaeridione (1) and preussomerin EG_2 (2), which have potent α-glucosidase inhibitory activity, were identified as secondary metabolites of *E. gomezpompae*, which was isolated from *C. speciosus*. Continuing research in this area, especially on secondary metabolites of endophytic fungi will not only enhance our understanding of fungal biochemistry but also contribute to the development of new pharmacological agents with potential applications in medicine and agriculture.

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Data availability Data sharing is not applicable to this article.

Declarations

Conflict of interest The authors declare no conflict of interest.

References

Ahonsi MO, Maurhofer M, Boss D, Défago G (2005) Relationship between aggressiveness of *Stagonospora* sp. isolates on field and hedge bindweeds, and in vitro production of fungal metabolites cercosporin, elsinochrome A and leptosphaerodione. Eur J Plant Pathol 111(3):203–215

Alakolanga AGAW, Kumar NS, Jayasinghe L, Fujimoto Y (2015) Antioxidant property and α-glucosidase, α-amylase and lipase inhibiting activities of *Flacourtia inermis* fruits: characterization of malic acid as an inhibitor of the enzymes. J Food Sci Technol 52(12):8383–8388

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

Berg A, Reiber K, Dorfelt H, Walther G, Schlegel B, Gräfe U (2000) Laccaridiones A and B, new protease inhibitors from *Laccaria amethystea*. J Antibiotics 53(11):1313–1316

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

Fernando WIT, Attanayake AMKC, Perera HKI, Sivakanesan R, Jayasinghe L, Araya H, Fujimoto Y (2019) Isolation, identification and characterization of pancreatic lipase inhibitors from Trigonella foenum-graecum seeds. S Afr J Bot 121:418–421

Gardes M, Bruns TD (1993) ITS primers with enhanced specificity for basidiomycetes-application to the identification of mycorrhizae and rusts. Mol Ecol 2(2):113–118

Guerriero A, D'Ambrosio M, Cuomo V, Pietra F (1991) A novel degraded polyketidic lactone, leptosphaerolide, and its likely diketone precursor, leptosphaerodione. Isolation from cultures of the marine ascomycete Leptosphaeria oraemaris (Linder). Helv Chim Acta 74(7):1445–1450

Kehelpannala CL, Kumar NS, Jayasinghe L, Araya H, Fujimoto Y (2018) Naphthoquinone metabolites 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

Kumar S, Narwal S, Kumar V, Prakash O (2011) α-Glucosidase inhibitors from plants: a natural approach to treat diabetes. Pharmacognosy Rev 5(9):19-29

Macías-Rubalcava ML, Hernández-Bautista BE, Jiménez-Estrada M, González MC, Glenn AE, Hanlin RT, Hernández-Ortega S, Saucedo-García A, Muria-González JM, Anaya AL (2008) Naphthoquinone spiroketal with allelochemical activity from the newly discovered endophytic fungus *Edenia gomezpompae*. Phytochemistry 69(5):1185–1196

Macías-Rubalcava M, Ruiz-Velesco Sobrino ME, Meléndez-González C, Hernandez-Ortega S (2014) Naphthquinone spiroketals and organic extracts from the endophytic fungus *Edenia gomezpompae* as potentiall herbicides. J Agric Food Chem 62(16):3553–3562

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

Nicolet B. 1999. Isolement et identification de métabolites secondaires de deux souches de champignon *Stagonospora* sp., agent pathogène du liseron des haies (*Calystegia sepium*) et du liseron des champs (*Convolvulus arvensis*). Travail de thèse, Faculté des Sciences, Université de Neuchâtel.

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(4):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

Poch GK, Gloer JB (1989) Obionin A: A new polyketide metabolite from the marine fungus *Leptosphaeria obiones*. Tetrahedron Lett 30(27):3483–3486

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, ArayaFujimoto HY (2017) 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 (2018) Shikimic acid production by *Fusarium decemcellulare,* an endophytic fungus isolated from *Flacourtia inermis* fruits. J Biol Active Prod Nature 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*. Natural Products and Bioprospecting 9(6):411–417

Sathya S, Amarasinghe NR, Jayasinghe L, Araya H, Fujimoto Y (2020) Enzyme inhibitors from the aril of *Myristica fragrans*. South Afr J Botany 130(1):172–176

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

Strobel G, Daisy B, Castillo U, Harper J (2004) Natural products from endophytic microorganisms. J Nat Prod 67(2):257–268

Tan Y, Guo Z, Zhu M, Shi J, Li W, Jiao R, Tan R, Ge H (2020) Anti-inflammatory spirobisnaphthalene natural products from a plant-derived endophytic fungus *Edenia gomezpompae*. Chin Chem Lett 31(6):1406–1409

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

White TJ, Bruns T, Lee SB, Taylor JW (1990) Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. In: Innis M, Gelfand DH, Sninsky JJ, White TJ (eds) PCR Protocols: A Guide to Methods and Applications. Academic Press, New York, pp 315–322