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SHORT COMMUNICATION

Chemical investigation of metabolites produced by an endophytic fungi *Phialemonium curvatum* from the leaves of *Passiflora edulis*

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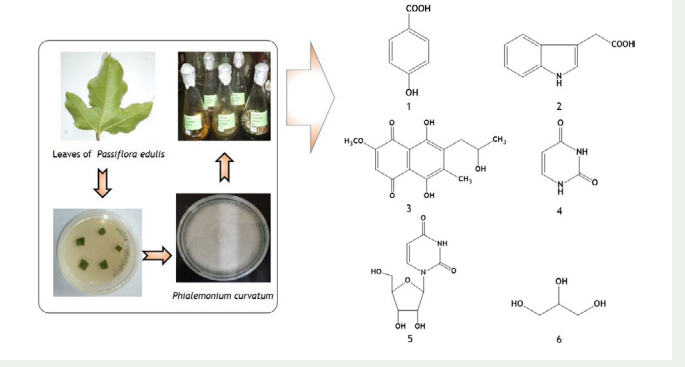
ABSTRACT

Phialemonium curvatum, an endophytic fungus isolated from the leaves of *Passiflora edulis* was cultured in potato dextrose broth (PDB) media and chromatographic separation of the EtOAc extract of the broth and mycelium led to the isolation of 4-hydroxybenzoic acid (**1**), 3-indole acetic acid (**2**), solaniol (**3**), uracil (**4**), uridine (**5**) and glycerol (**6**). Compound **2** showed a weak antifungal activity against *Cladosporium cladosporioides*. This is the first report of the isolation of the endophytic fungus *P. curvatum* from *P. edulis* and complete ^{13}CNMR assignments of **3**.



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Passiflora edulis; endophytic fungi; *Phialemonium curvatum*

1. Introduction

Endophytic micro-organisms that reside in the tissues of living plants have been attracting considerable attention in recent years since they are potential sources of novel natural products for exploitation in medicine, agriculture and industry (Strobel et al. 2005). In a

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continuation of our studies towards the search for environmentally friendly bioactive compounds from Sri Lankan flora, we investigated the secondary metabolites produced by an endophytic fungus isolated from the leaves of the popular medicinal plant *Passiflora edulis*. Medicinal uses, phytochemistry and pharmacology of the *P. edulis* plant have been described in several reviews (Patel 2009). Here, we report the isolation of secondary metabolites 4-hydroxybenzoic acid (**1**), 3-indole acetic acid (IAA) (**2**), solaniol (**3**), uracil (**4**), uridine (**5**) and glycerol (**6**) (Figure 1) from an endophytic fungus *Phialemonium curvatum* isolated from the leaves of *P. edulis*.

2. Results and discussion

An endophytic fungus isolated from the leaves of *P. edulis* was identified as *P. curvatum* by sequence analysis of the ITS region of the rDNA gene. Amplification of the ITS region was carried out using the universal eukaryotic primers of ITS1 and ITS4. BLAST search indicated that the sequence of the ITS region had 100% similarity to that of *P. curvatum* UTHSC 06-4324 (GenBank Accession No. EU035984.1). A pure culture of the fungal strain *P. curvatum* (IFS/N/PE/1/2014) and the photographic evidence of the leaves of the *P. edulis* and the fungal strain are deposited at the National Institute of Fundamental Studies. This is the first report of the isolation of endophytic fungus *P. curvatum* from *P. edulis*. To our knowledge, there is only one paper describing the isolation and identification of endophytic fungi from *Passiflora* genus (*Passiflora incarnata*) (Seetharaman et al. 2017). The EtOAc extract obtained from the culture broth and mycelium of *P. curvatum* in PDB media displayed antifungal activity against

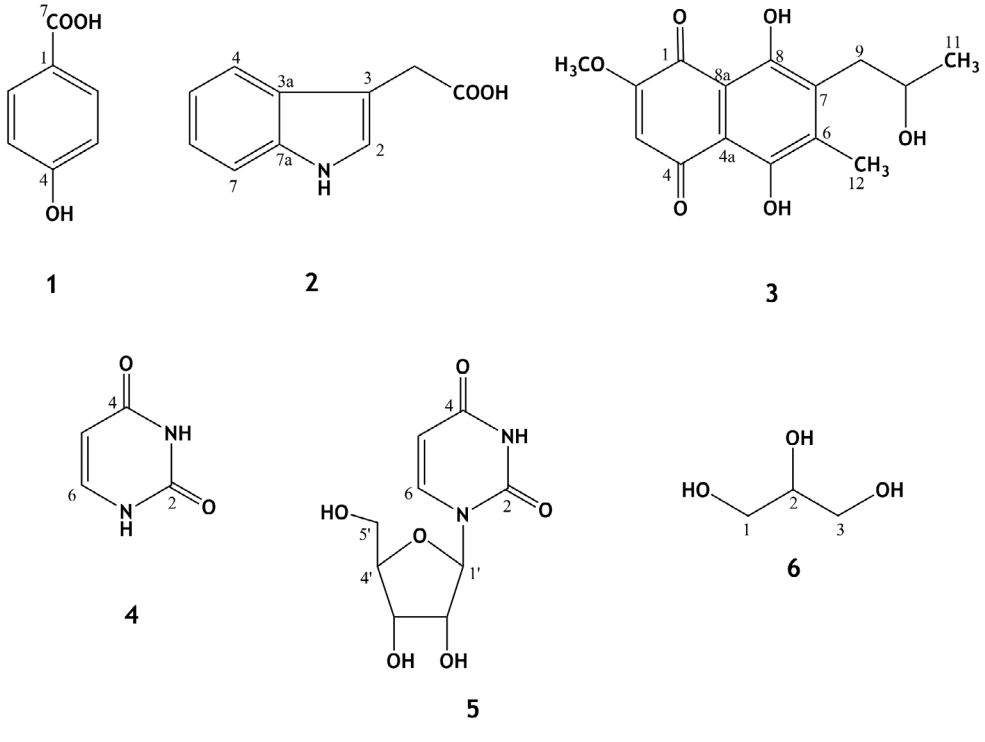

Figure 1. Structures of compounds **1**–**6.**

Cladosporium cladosporioides on TLC bioassay, antioxidant activity against DPPH (IC_{50} 109 ppm; positive control ascorbic acid, IC_{50} 6.5 ppm), brine shrimp lethality against *Artemia salina* (LD_{50} 583 ppm; positive control atropine, LD_{50} 0.8 ppm), phytotoxic activity against *Lactuca sativa* (root growth inhibition at IC_{50} 494 ppm and shoot growth inhibition at IC_{50} 559 ppm). The EtOAc extract did not show any anticandidal activity at 5000 ppm. Chromatographic separation of the EtOAc extract over silica gel, Sephadex LH-20 and preparative thin-layer chromatography furnished compounds **1**–**6** (Figure 1), which were identified as 4-hydroxybenzoic acid (**1**), 3-indole acetic acid (**2**), solaniol (**3**), uracil (**4**), uridine (**5**) and glycerol (**6**) by detailed analysis of NMR and MS data. TLC analysis indicated that none of these compounds were present in the extracts of the leaves of *P. edulis*, which confirm that these compounds are not carryover from the leaves of the plant.

4-Hydroxybenzoic acid (**1**) has been previously reported from several endophytic fungi including *Fusarium oxysporum* isolated from healthy tomato roots (Bogner et al. 2017). 4-Hydroxybenzoic acid is a compound with a broad range of biological activities and is used as a preservative of drugs, cosmetic products, food and beverages, etc. (Khadem and Marles 2010; Manuja et al. 2013). 3-Indole acetic acid (IAA) (**2**) is a well-known plant hormone, which regulates some physiological activities of plants. IAA is a common product of L-tryptophan metabolism in some micro-organisms (Shahab et al. 2009). There are only a few reports of IAA production by endophytic fungi, e.g. an unidentified *Colletotrichum* sp. isolated from *Artemisia annua* (Lu et al. 2000) and *Purpureocillium lilacinum* (Cavello et al. 2015). This is the first report of the isolation of IAA from *Phialemonium* sp. IAA (**2**) was further subjected to the bioassays due to the poor yields of the other isolates. In this investigation IAA showed a weak antifungal activity against *C. cladosporioides* on TLC bioautography method with a MIC value of 62.5 ppm (benlate MIC 4 ppm), although it was almost inactive in DPPH radical scavenging assay (IC_{50} 152 ppm) and brine shrimp toxicity assay (LD_{50} 105 ppm). Solaniol (**3**) is a naphthoquinone derivative, which has been previously reported from *Fusarium* sp. (Arsenault 1968; Kimura et al. 1988). ^{13}CNMR data of solaniol is reported here for the first time and the assignments (δ 177.5 (C-1), 160.4 (C-2), 109.4 (C-3), 184.0 (C-4), 107.8 (C-4a), 160.7 (C-5), 142.4 (C-6), 138.5 (C-7), 162.4 (C-8), 109.4 (C-8a), 36.2 (C-9), 67.6 (C-10), 23.9 (C-11), 13.0 (C-12), 56.7 (OCH_3)) were based on the hmbc spectrum. Uracil (**4**) is one of the four nucleobases in the nucleic acid of RNA and has been often isolated from fungal species including endophytic fungi such as *Neofusicoccum* sp (Qin et al. 2014), *Colletotrichum gloeosporioides* (Chapla et al. 2014) and *Penicillium commune* (Yan et al. 2010). Uridine is a glycosylated pyrimidine analogue containing uracil attached to a ribose ring. It is a component of RNA that has positive health effects on humans. It has been isolated from fungal species including *Phomopsis* sp. PSU-D15 (Rukachaisirikul et al. 2008) and *Alternaria alternate* (Ma et al. 2010). Physiological functions of the isolated compounds to *P. curvatum* were not determined in this study.

3. Conclusion

Chemical investigation of secondary metabolites produced by an endophytic fungus *P. curvatum* isolated from the leaves of *P. edulis* furnished 4-hydroxybenzoic acid (**1**), 3-indole acetic acid (**2**), solaniol (**3**), uracil (**4**), uridine (**5**) and glycerol (**6**). Compound **2** was found to show a weak antifungal activity against *C. cladosporioides*. Further study on endophytic fungi associated with *P. edulis* and their secondary metabolites will be of value in view of the medicinal uses of this plant.

Disclosure statement

No potential conflict of interest was reported by the authors.

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