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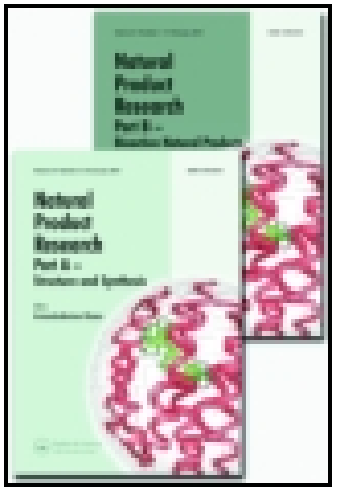

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Chemical investigation of metabolites produced by an endophytic Aspergillus sp. isolated from Limonia acidissima

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SHORT COMMUNICATION

Chemical investigation of metabolites produced by an endophytic *Aspergillus* sp. isolated from *Limonia acidissima*

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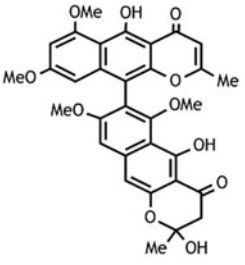

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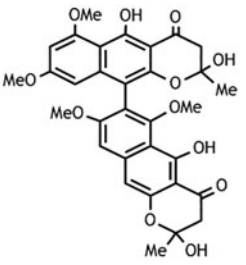

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Endophytic fungi are considered as a good source to produce important secondary metabolites with interesting bioactivities. In a continuation of our studies towards the search for environmentally friendly bioactive compounds from Sri Lankan flora, we investigated the secondary metabolites produced by the endophytic fungi *Aspergillus sp.* isolated from the seeds of the popular edible fruit *Limonia acidissima* L. of the family Rutaceae. The pure culture of the *Aspergillus* sp. was grown on potato dextrose broth media. After 4 weeks fermentation, fungal media were extracted with organic solvents. Chromatographic separation of the fungal extracts over silica gel, Sephadex LH-20 and RP-HPLC furnished flavasperone (**1**), rubrofusarin B (**2**), aurasperone A (**3**), fonsecinone D (**4**) and aurasperone B (**5**). Compounds **1**–**4** showed moderate activities in brine shrimp toxicity assay. This is the first report of the ^{13}C NMR data of compounds **4** and **5**.

Keywords: *Aspergillus*; aurasperone A; aurasperone B; fonsecinone D; flavasperone; rubrofusarin B

1. Introduction

Fungi can be categorised mainly into two groups, epiphytic fungi and endophytic fungi. Epiphytic fungi grow on the surface of the host, while endophytes are found in the inner tissues or even in the cell of their host (Zhang et al. 2009). Endophytes are considered as one of the few major sources which produce secondary metabolites with novel structures and interesting bioactivities (Strobel et al. 2004). Some endophytic fungal strains produce natural products that are either identical or closely related to those produced by the host plant. A well-known example is the production of Taxol, an anti-cancer drug obtained from the Pacific Yew tree *Taxus*

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brevifolia, which was also produced by the endophytic fungus *Taxomyces andreanae* isolated from its bark (Stierle et al. 1993).

Limonia acidissima L. of the family Rutaceae, native in India and Sri Lanka, is popularly cultivated for its edible fruits in tropical Asian countries. All parts of the plant have been used in the indigenous system of medicine for the treatment of various ailments. Fruits are refrigerant, stomachic, stimulant, astringent, aphrodisiac, diuretic, cardiotonic, tonic to liver and lungs, good for asthma and cure for cough and hiccup. Seeds are used in heart diseases. Furthermore, several pharmacological activities including antidiarrhoeal, antidiabetic, wound healing, anticancer, antioxidative, hepatoprotective, biosorbent, diuretic, antibacterial, antifungal, antispermatogenic, antihistaminic and larvicidal activities have been reported from the extracts of its various parts (Vijayvargia & Vijayvargia 2014). In a continuation of our studies towards the search for bioactive compounds from Sri Lankan flora we investigated the secondary metabolites of the endophytic fungi *Aspergillus sp.* isolated from the seeds of *L. acidissima* fruits. In this paper we report the identification of five secondary metabolites and their biological activities including brine shrimp toxicity assay against *Artemia salina*.

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2. Results and discussion

Black coloured densely sporulated endophytic fungus isolated on potato dextrose agar media from surface-sterilised seeds of *L. acidissima* was identified as *Aspergillus* sp. based on its morphological characteristics (Mitard & Riba 1988). Fermentation of the fungus in PDB for 4 weeks and extraction into EtOAc gave a dark brown sticky solid E1 while the extraction of mycelium with EtOAc gave E2. Preliminary studies indicated a similar TLC pattern for both E1 and E2. E1 and E2 were combined and chromatographed to give five compounds **1**–**5**, identified by a detailed analysis of NMR and MS data. These compounds were identified as flavasperone (**1**) {FABMS(+): *m/z* 287$[M + H]^+$} (Gorst-Allman et al. 1980), rubrofusarin B (**2**) {FABMS (+): *m/z* 287$[M + H]^+$} (Shaaban et al. 2012), aurasperone A (**3**) {FABMS(+): *m/z* 571 $[M + H]^+$} (Campos et al. 2005), fonsecinone D (**4**) {FABMS(+): *m/z* 589$[M + H]^+$} (Priestap 1984) and aurasperone B (**5**) {FABMS(+): *m/z* 607$[M + H]^+$} (Shaaban et al. 2012), respectively, by comparison of their NMR data with literature values and FABMS data (Figure 1). The ^{13}C NMR data ($CDCl_3$, 125 MHz) of **4** and **5** are reported here for the first time; compound **4**: δ_c 196.6, 184.6, 167.6, 164.1, 162.6, 161.4, 161.0, 161.0, 159.4, 153.3, 150.8, 142.7, 140.7, 116.8, 110.7, 108.6, 107.2, 105.1, 104.2, 103.5, 102.7, 101.9, 100.1, 96.9, 96.4, 61.9, 56.2, 55.9, 55.2, 47.2, 28.8, 20.7), and compound **5**: δ_c 197.5, 196.5, 164.9, 164.1, 162.2, 161.8, 161.0, 158.2, 153.0, 151.2, 142.6, 142.3, 117.8, 110.7, 107.8, 106.6, 103.8, 103.5, 102.6, 102.2, 100.2, 100.1, 97.2, 96.3, 61.7, 56.2, 55.9, 55.2, 47.2, 46.7, 29.0, 28.7).

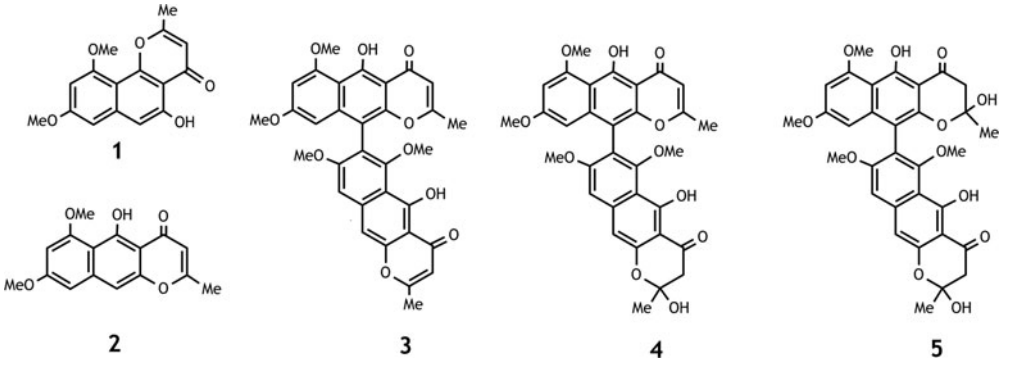

Figure 1. Structures of compounds **1**–**5**.

Compounds and extracts were subjected to bioassays for antifungal activity against *Cladosporium cladosporioides* by TLC bioautographic method (Homans & Fuchs 1970), antioxidant activity against DPPH radical scavenging using spectrophotometry method (Tepe et al. 2007), and brine shrimp toxicity assay (Meyer et al. 1982). In the brine shrimp assay, compounds **1**–**4** showed LD_{50} values of 32, 50, 9 and 40 ppm, respectively. It has been reported that the percentage mortalities of brine shrimps at 10 ppm of compounds **2**, **3** and **5** were 11, 9.7 and 6.4, respectively (Shaaban et al. 2012). None of compounds **1**–**5** showed significant antifungal, antioxidant and phytotoxic activities.

3. Conclusion

This is the first paper for the isolation of endophytic fungi from *L. acidissima*. Many endophytic *Aspergillus* species have been isolated from a wide variety of plant sources: *A. fumigates* from soybean roots (Khan et al. 2011), 22 *Aspergillus* sp. from leaves and seeds of *Gloriosa superba* (Budhiraja et al. 2013), *A. versicolor* from the marine green alga *Codium fragile* (Liu et al. 2012) and *Aspergillus* sp. from mangrove (Xiao et al. 2013). Monomeric and dimeric naphthopyrones produced by these fungi have been attracting the interest of many investigators due to their broad-range biological activities (Bouras et al. 2005).

The five naphthopyrones isolated in this study belong to a polyketide class of secondary metabolites, which include many pharmacologically and economically useful compounds of commercial interest. The five naphthopyrones showed moderate toxicity in brine shrimp assay. The assay is considered as a useful tool for preliminary assessment of toxicity. Toxicity indicated by this assay is useful as an indication of metabolites with potential cytotoxic activity. These compounds produced by an endophytic fungus from seeds of an edible fruit, an environmentally friendly source without any toxicological issues, may have the ability to destroy unfavourable cells related to various health issues. It has been reported that flavasperone (**1**) showed weak antiproliferative activities to Hep 3B and U87 MG cells (Huang et al. 2011). Rubrofusarin B (**2**) exhibited a significant cytotoxicity against colon cancer SW1116 cell line (Song et al. 2004) and fonsecinone D (**4**) showed a significant inhibitory activity against the proliferation of SMMC-7721 and A549 tumour cell lines (Zhang et al. 2007).

Supplementary material

Experimental details relating to this paper are available online.

Disclosure statement

No potential conflict of interest was reported by the authors.

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