Cytotoxic Activities of Endophytic Fungi Isolated from the Endangered, Chinese Endemic Species *Dysosma pleiantha*

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Eleven strains of endophytic fungi which habitat in an endangered, Chinese endemic medicinal plant, *Dysosma pleiantha* (Hance) Woodson, were isolated and tested for their cytotoxic activity using the brine shrimp lethality bioassay. Six isolates were found to exhibit some cytotoxic activity. Extracts of F1273, F1276, and F1280, which were identified as *Trichoderma citrinoviride*, *Chaetomium globosum* and *Ascomycete* sp., in particular, showed most potent activity with LC_{50} values of 4.86, 7.71, and 14.88 μg/ml, respectively. These results indicate that endophytic fungi of *Dysosma pleiantha* could be a promising source for antitumour agents.

Key words: Dysosma pleiantha, Endophytic Fungi, Brine Shrimp, Cytotoxicity

Introduction

Dysosma pleiantha (Hance) Woodson (Berberidaceae), a rare and endangered species occurring solely in China, grows in the undergrowth of subtropical forests between 200 and 3,500 m elevation. It is a perennial rhizomatous herb that undergoes both sexual and asexual reproduction. Individual plants grow from rhizome and typically reach 40 to 150 cm in height, with an unforked stem bearing one or two alternately arranged leaves, which are rounded, centrally peltate, and 4- ~ 9-lobed with a finely dentate margin (Qiu and Qiu, 2002; Qiu *et al.*, 2005).

Podophyllotoxin, 4′-demethyldeoxypodophyllotoxin and deoxypodophyllotoxin have been extracted from *D. pleiantha* (Shang *et al.*, 1996; Zhang, 2001). It is used as an expectorant, carminative, antispasmodic, nervine sedative in Chinese folk medicine; it is also employed locally to remove warts, papillomas and condylomas. For external use, the poultice prepared from the fresh underground parts of it is beneficial to cure abscess maturation (Nanjing Medical University, 1994). The rhizome of *D. pleiantha* has also been found to be a source of podophyllotoxin, a active lignan used as a leading compound for the chemical semi-synthesis of etoposide (VP-16-213) and teniposide (VM-26), which are effective agents in the treatment of lung cancer, a variety of leukemias, and other solid tumour diseases (Jackson and Dewick, 1984, 1985). In recent years, natural populations have declined considerably due to anthropogenic activities such as habitat destruction and overcollection for medicinal applications. Nearly all remaining populations of *D. pleiantha* are now located within protected nature reserves, and the species is classified as endangered in the Chinese Plant Red Book (Fu, 1992). To our knowledge, there is no previous report on the compounds of fungal endophytes from *D. pleiantha*.

Material and Methods

Tested material

D. pleiantha was collected in November 2007 from Western Tianmushan, Zhejiang, China. A voucher specimen was deposited at the Herbarium of Zhejiang University, China (ZJUH 3024). The samples were kept at 4 ºC until processed.

Endophytic fungi which live inside other living plants are in a special biotope and account for some biological activity of their host (Schulz *et al.*, 2002). Some of these fungi are potential sources of diverse bioactive metabolites which may have potential for therapeutic purposes and could be used prolifically as research tools (Tan *et al.*, 2000; Tan and Zou, 2001). In our ongoing project aiming at the characterization of structurally novel and/or substantially bioactive metabolites from endophytic fungi of *D. pleiantha*, crude extracts of fermentation products obtained from 11 fungal isolates of this herb medicine were used for testing their cytotoxicity. Isolation and cultivation were carried out by general methods (Strobel *et al.*, 1996; Siva Sundara Kumar *et al.*, 2004). After fermenting for two weeks, the entire culture was blended and extracted exhaustively with ethyl acetate. The organic phase was dried over Na_2SO_4, filtered using silicon-coated Whatman filter paper and concentrated *in vacuo* at 35 ºC. Crude extracts obtained were stored at –20 ºC until assayed.

Cytotoxic activity test

The brine shrimp lethality assay, which has been proven to be an effective and rapid assay method to screen compounds for potential cytotoxic activity (Meyer *et al.*, 1982), was applied to determine the general toxicity of these eleven endophytic fungal strains from *D. pleiantha*. Brine shrimp (*Artemia salina*) nauplii (eggs obtained commercially from Bo Hai, China), hatched for 48 h, were applied for the cytotoxicity study (Meyer *et al.*, 1982). Podophyllotoxin was used as a positive control, DMSO (1%) as a solvent and negative control. Tests were done in triplicate.

Results and Discussion

The identification of a total of 11 representative fungi and the result of the cytotoxic activity assay are listed in Table I.

The endophytic fungal strains were identified through scrutiny of their morphology, *i.e.* characters of fruiting structures and spores. Moreover, the result was reinforced by comparing the entire ITS (ITS1-5.8s-ITS2) rDNA sequences with the available data of GenBank databases through the NCBI BLAST search algorithm (Altschul *et al.*, 1997; Liang and Gao, 2000; Girlanda *et al.*, 2002; Landwehr *et al.*, 2002; Bougoure and John, 2005). Due to the large number of isolates and complications of identification of the endophytic fungi, 5 strains were identified at the genus or species level, whereas 6 unidentified strains belonging to ascomycetes are still unnamed.

The cytotoxicity assay result showed that their LC_{50} values were quite diverse, ranging from 4.86 μg/ml to more than 1000 μg/ml, whereas that of the positive control podophyllotoxin, a well known cytotoxic lignan, was 2.72 μg/ml. All the material showed some cytotoxic activity except for F1270 and F1272. Additionally, it was observed that F1273 (4.86 μg/ml), F1276 (7.71 μg/ml), and F1280 (14.88 μg/ml) displayed meaning-

Table I. Identification and cytotoxicity of eleven endophytic fungi isolated from *Dysosma pleiantha*[a].

Test material code	Endophytic fungi taxa	Concentration [μg/ml]	LC_{50}-24 h [μg/ml]
F1266	*Acremonium furcatum*	50, 100, 500, 1000	325.72 (198.00 – 792.90)
F1268	*Ascomycete* sp. (Fungal endophyte MS6 IS133)[b]	50, 100, 500, 1000	490.79 (258.30 – 682.34)
F1269	*Ascomycete* sp.	10, 50, 100, 200, 500	61.57 (36.53 – 103.61)
F1270	*Paecilomyces marquandii*	50, 100, 500, 1000	>1000
F1272	*Cylindrocarpon* sp.	50, 100, 500, 1000	>1000
F1273	*Trichoderma citrinoviride*	2, 4, 8, 10, 15, 25	4.86 (2.95 – 7.55)
F1276	*Chaetomium globosum*	2, 4, 8, 10, 15, 25	7.71 (7.30 – 8.12)
F1277	*Ascomycete* sp.	10, 50, 100, 200, 500	124.54 (67.51 – 183.57)
F1278	*Ascomycete* sp.	50, 100, 500, 1000	228.32 (170.54 – 311.08)
F1279	*Ascomycete* sp.	10, 50, 100, 200, 500	179.09 (131.23 – 243.95)
F1280	*Ascomycete* sp. (Fungal endophyte R51)[b]	2, 4, 8, 10, 15, 25	14.88 (14.05 – 15.81)
Podophyllotoxin[c]	–	1, 2, 4	2.72 (2.38 – 3.50)

[a] All determinations were done in triplicate, 95% confidence limits in parentheses. No mortality with the negative control group (1% DMSO).
[b] BLAST search indicated relatives.
[c] Positive control group.

ful toxicity which was ca. 2-times, 3-times and 6-times less than that of podophyllotoxin. Significant correlations with brine shrimp toxicity have previously been shown for cytotoxicity and antitumour activity (Anderson *et al.*, 1991). Therefore, from the LC_{50} values of these materials, it can be speculated that extracts of some endophytic fungi isolated from their host possess some anticancer potential.

In conclusion, the results indicate that endophytic fungi accreting with pharmaceutical plants have significant functions in research and development of bioactive substances with antitumour activity.

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