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Cytotoxicity of *Paecilomyces tenuipes* Against Human Carcinoma Cells, HepG2 and MCF-7 *In Vitro*

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The methanolic extract of fruiting body of *Paecilomyces tenuipes* DGUM 32001 showed significant cytotoxicity against human cancer cells: HepG2 and MCF-7. The methanolic extract was further fractionated with organic solvents such as chloroform and ethyl acetate in that order. Among the fractions tested, the ethyl acetate fraction showed the highest cytotoxicity against the carcinoma tested. The IC_{50} values of ethyl acetate fraction against HepG and MCF-7 were 40 and 9.6 μg/ml, respectively.

KEYWORDS: Cytotoxicity, Fruiting body, *Paecilomyces tenuipes*

Dongchunghacho has been known to be distributed about 100 genuses, 750 species in the world, and 80 species has been reported in Korea. The representative genus includes the genus *Cordyceps*, *Paecilomyces*, *Isaria*, *Torrubiella*, *Podonetria*. Several *Cordyceps* species including *C. militaris*, *C. ophioglossoides*, and *C. sinensis* are known to be used as a traditional medicine in China, Japan, and Korea. Cordycepin, the active compound isolated from *C. militaris*, has been known to possess anti-bacterial, anti-fungal, anti-viral and anti-cancer activities (Marvin *et al.*, 1964; Helmut *et al.*, 1977; James and William, 1978). Ophiocordin, antibiotics, was isolated from submerged cultures of *Cordyceps ophioglossoides* (Helmut *et al.*, 1977).

Based upon these reports, we expected the possible existence of new anticancer compounds rather than cordycepin at other genera. *Paecilomyces tenuipes* is one of the most easily and artificially cultured species. Park *et al.* (2000) investigated the cytotoxic effect of *P. japonica* on several tumor cells of human. Polysaccharides isolated from *P. tenuipes* have been reported to possess anti-cancer activity *in vivo* (Ban *et al.*, 1998). This report suggested that the anti-cancer effect of the polysaccharides might be due to the stimulating of host immune system.

The cytotoxicity of methanolic extract of *P. tenuipes* DGUM 32001 has been reported, previously. The methanolic extracts of *P. tenuipes* showed significant cytotoxicity against cancer cell lines; HeLa, HeLaS3, and A-431 (Shim *et al.*, 2000). In this study, *in vitro* cytotoxicity of the methanolic extract and subsequent fraction of *P. tenuipes* DGUM 32001 will be reported against human carcinoma, HepG2 and MCF-7.

For cultivation of human cancer cells, DMEM, fetal bovine serum (FBS) and antibiotic-antimycotic were purchased from Gibco BRL (Grand Island, U.S.A.). For cultivation of the mycelia of *P. tenuipes*, media and other supplements were purchased from Difco Co. For determination of cytotoxicity, 3-(4,5-dimethylthiazole-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) and dimethyl sulfoxide (DMSO, photospectrometric grade) were purchased from Sigma Chem. Co. All other chemicals and reagents were 1st grade.

P. tenuipes DGUM 32001 used in this study was collected and isolated from Jeju Island (Shim *et al.*, 2000). The mycelia grown on the potato dextrose agar plate were collected by using a cork borer (dia, 8 mm) and inoculated into 500 m*l* of potato dextrose broth in 1-*l* flask. They were cultivated at 24°C for 7 days with shaking (120 rpm) and then were used as a inoculum. For the production of fruiting bodies, the cultured mycelia were inoculated into 850-m*l* polypropylene bottle containing of 100 g of silkworm pupae. After 10-day cultivation at 24°C, entire surface of pupae was covered with the mycelia. And then, the bottle was transferred to the culture condition of fruiting bodies formation (18°C, 500 lux) and incubated for 10 days.

The extraction and solvent fractionation was carried out by the method of Shim *et al.* (2000). The fruiting bodies of *P. tenuipes* DGUM 32001 (300 g) were collected and extracted at 80°C for three times with 5 *l* of methanol. After extraction, the solution was filtered through a filter paper (Toyo filter, No. 2) to discard the mycelial residues. The filtrate was evaporated and concentrated with a rotary evaporator (Eyela Co.) and then, dried by freeze dryer (Ilsin Co.). The methanol extract was further fractionated with organic solvents in a stepwise manner of increasing polarity: chloroform, ethyl acetate (Fig. 1).

Human carcinoma cells, HepG2 (hepato carcinoma) and MCF-7 (breast carcinoma), were kindly provided by KRIBB (Korean Research Institute of Bioscience and Biotechnology, Korea). The carcinoma cells were maintained at 37°C

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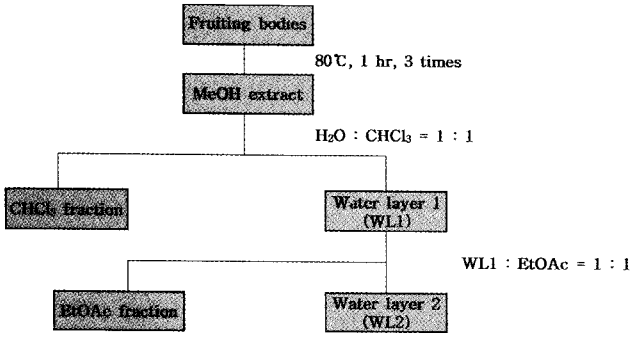

Fig. 1. Procedure for the extraction and fraction of *Paecilomyces tenuipes* DGUM 32001.

in DMEM medium supplemented with 10% of FBS and 1% antibiotic-antimycotic in a humidified atmosphere of CO_2 chamber (10% CO_2).

Cytotoxicity was measured using the MTT assay. The MTT assay was performed using a modified method of Plumb (Plumb *et al.*, 1989). Exponentially growing cells were trypsinized and carefully pipetted to yield single cells. Cell numbers were counted using Coulter Counter (Coulter, UK). These single cells were then inoculated at 3×10^3 cells/well on 96-well plates supplemented with 100 μl of DMEM. After 24 hr, various concentrations of extract were added. After 3 days, 50 μl of MTT (2 mg/m*l*) were added and the plates were incubated for an additional 4 h. DMSO (150 μl) was added to dissolve the formazan, and after 10 min of gentle shaking, each plate was read at 570 nm using a microplate reader (Molecular Devices, California). The IC_{50} values were determined by plotting the concentration of extract or fraction versus the survival ratio of the treated cells.

The 2×10^3 cells were seeded in 6-well plates and after 2 days were treated with ethyl acetate fraction at various concentration. Cells were harvested with a cell scraper and washed twice with PBS, then centrifuged at 2000 rpm for 5 min at 4°C. Cells were lysed in a 500 μl lysis buffer at 37°C for 2 hr and centrifuged at 1000 g for 10 min to remove cell debris. Supernatants were incubated for at least 2 hr or overnight in 50 μg/m*l* RNase A and 120 μg/m*l* proteinase K. Then, DNA was extracted with phenol in 0.5 M Tris (pH 8.0), phenol/chloroform/isoamylalcohol (25 : 24 : 1). The supernatant containing DNA was then precipitated with 100% ethanol and 0.3 M sodium acetate at -70°C, then washed with 70% ethanol. DNA samples were electrophoresed through a 1.5% agarose gel containing ethidium bromide, and DNA bands were visualized under UV light.

The cytotoxic effect of *P. tenuipes* was tested against two carcinoma cells. Among the tested cells, MCF-7 cells were most sensitive to the methanolic extract of *P. tenuipes* as shown in Table 1. The IC_{50} value of the methanolic extract against MCF-7 and HepG2 cells were 110 and 120 μg/m*l*, respectively. When the solvent fraction from methanolic extract were examined, ethyl acetate fraction showed much better cytotoxicity than chloroform and butanol fractions. The IC_{50} values of ethyl acetate fraction against HepG2 and MCF-7 were 40 and 9.6 μg/m*l* respectively (Table 1). These results coincided with the result of Shim *et al.* (2000) previously reported on other human carcinomas, HeLa, HeLaS3 and A-431.

Table 1. Cytotoxicity of extract and solvent fractions of the fruiting bodies of *P. tenuipes* DGUM 32001 against cancer cells

Cell line	Carcinoma	IC_{50} value (μg/m*l*)		
		Extraction	Fraction	
		Methanol	Chloroform	EtOAc
HepG2	Hepato	120	80	40
MCF-7	Breast	110	30	9.6

Among the solvent fractions tested, ethyl acetate fraction revealed the highest cytotoxicity against HepG2 and MCF-7 carcinoma cells. The cytotoxicity of ethyl acetate fraction exhibited the characteristic dose-response survival curves against two carcinoma cells (Fig. 2). MCF-7 carcinoma cells were more sensitive to the ethyl acetate fraction than HepG2 cacinoma cells (Fig. 2, Table 1). In order to further investigation of cell death mode induced by ethyl acetate fraction, we examined the biochemical changes of the genomic DNA MCF-7 carcinoma cells treated by ethyl acetate fraction. DNA fragmentation is one of many traits of apoptotic cells undergo. The DNA fragmentation was not induced treatment with ethyl acetate fraction. Apototic cells undergo various morphological changes such as cell shrinkage, membrane blebbing but MCF-7 was not

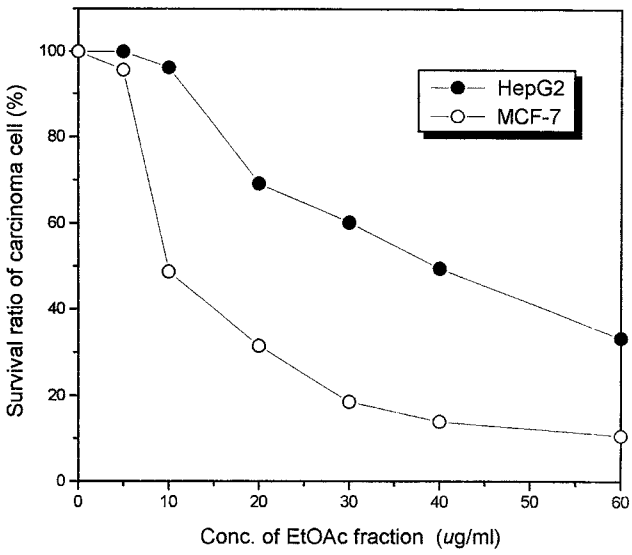

Fig. 2. Relative survival ratio of human cancer cell lines treated with ethyl acetate soluble fraction from the fruiting bodies of *Paecilomyces tenuipes* DGUM 32001.

shrinked treament with ethyl acetate fraction. The cytotoxicity of HepG2 and MCF-7 carcinoma cells might not be due to apoptosis (data not shown).

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