A-6

In Vitro Antitumor Activity of Solvent Fractions from Ethanol Extract of Auricularia auricula-judae on Tumor Cell Lines

College of Veterinary Medicine, Kyungpook National University
Md. Ahsanur Reza*, Myung-Jin Choi, Seung-Hee Jang, Joong-Su Lee,
Dereje Damte, Seung-Chun Park

암 세포주에서 목이버섯의 에탄올 분획 추출물의 항암활성

경북대학교 : 아사눌 레자*, 최명진, 장승희, 이중수, 데레제 담테, 박승춘

Introduction

Auricularia auricula-judae, commonly called 'wood ear' ,'tree ear' or 'black fungus' has long been used as food and traditional remedies in Asian countries such as Korea and China. The fruiting body of this mushroom is rich in carbohydrates and protein and its active constituents include beta (1-3) and (1-6) D glucans. Despite the well-documented pharmacological and therapeutic properties of other Basidiomycota mushrooms, there is lack of available literatures on the biological activities of Auricularia auricula-judae. In this study, we evaluated the in vitro anti-tumor activity of various fractions from the ethanol extracts of Auricularia auricula-judae using various tumor cell lines.

Materials and Methods

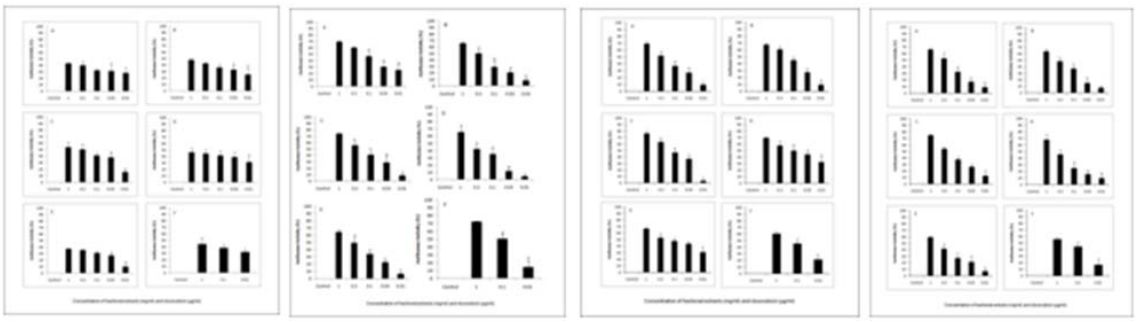
The mesh of Auricularia auricula-judae was mixed with 80% ethanol and heated at 1000C for 6 hrs and ethanol extract (ETOH) was collected. Thereafter, ethanol extract was successively fractionated with the same volume of dichloromethane (DCM), ethyl acetate, n-butanol and a water extract at room temperature as well as concentrated to dry materials in a vacuum concentrator at a controlled temperature (< 500C). The P388D1 macrophage cells and Sarcoma 180 cells, human NSCLC NCI H358 (bronchioalveolar) cells and SNU1 cells (Gastric carcinoma) were cultured in RPMI-1640 medium supplemented with FBS, L-glutamine, penicillin and streptomycin. All cell cultures were incubated at 370 C with 5% CO2. The cytotoxicity of different fractions to tumor cells was measured by MTT and SRB assays. Doxorubicin was used as positive control. All values were expressed as the mean ± S.D. Statistical analysis was done by one way analysis of variance (ANOVA) using SAS program. GraphPad Prism program was used to calculate IC50 values. P-values less than 0.05 were considered significant.

주저자 연락처 (Corresponding author) : 아사눌 레자 E-mail : happya514@hanmail.net Tel : 053-950-5964

Results

The cytotoxicity of the fractional extracts decreased significantly ($P < 0.05$) in a dose-dependent manner. The anti-tumor activity of dichloromethane extract (1 mg/ml) was the highest ($P < 0.05$) in all experimental cell lines. There was also a significantly different sensitivity ($P < 0.05$) among the P388D1, Sarcoma 180, NCI H358 and SNU1 cells for the fractional extracts. According to IC50 values, the most potent cytotoxic activity of dichloromethane fraction was found in Sarcoma 180 and NCI H358 cell lines. Butanol fraction appeared more cytotoxic to SNU1 cell line and water fraction had the highest cytotoxicity in P388D1 cell line. We did not find any significant difference between MTT and SRB assays in their ability to estimate cytotoxicity in all cell lines.

시험성적



Anti-tumor activity of solvent fractions; A. ethyl acetate (EtOAc), B. butanol (BuOH), C. dichloromethane (DCM), D. ethanol (ETOH) and E. Water of ethanol extract from Auricularia auricula-judae at the various concentrations and doxorubicin as a positive control on P388D1 (Fig.1), Sarcoma 180 (Fig. 2), NCI H358 (Fig. 3) and SNU1 (Fig. 4) cell line by MTT assay. All values are presented as percentages of the results from control, and are expressed as mean ± SD, * for the dose-dependent significant decreasing manner of anti-tumor activity at $P<0.05$

Table 1 IC_{50} values of different solvent fractions of *Auricularia auricula-judae* extract on tumor cell lines, while doxorubicin was used as positive control.

Cell lines	Fractions of *Auricularia auricula-judae* (μg/ml)					Doxorubicin (positive control) (ng/ml)
	EtOAc	BuOH	DCM	ETOH	Water	
P388D1	143.8	94.6	38.3	44.0	28.2	100.0
Sarcoma 180	108.9	134.1	94.2	133.0	102.3	95.0
NCI H358	106.7	66.0	57.2	103.4	108.1	95.0
SNU1	126.5	107.9	134.3	201.1	129.6	92.0

Data are presented as IC_{50} values by MTT assay from three independent experiments, performed in triplicate on tumor cell lines, obtained by nonlinear regression using the GRAPHPAD Prism program. EtOAc, BuOH, DCM and ETOH are stand for ethyl acetate, butanol, dichloromethane and ethanol respectively

Table 2 Comparative estimation of inhibitory activities solvent fractions of *A. auricula-judae* extract between MTT and SRB assays (1 mg/ml), while doxorubicin (1 μg/ml) was used as positive control).

Solvent Fractions (1 mg/ml)	P388D1 cells		Sarcoma 180 cells		NCI H358 cells		SNU1 cells	
	MTT assay	SRB assay	MTT assay	SRB assay	MTT assay	SRB assay	MTT assay	SRB assay
EtOAc	42.78±0.78	46.01±2.12*	69.61±2.19	69.08±4.03	69.44±2.99	64.95±1.70	65.88±1.54	64.41±3.70
BuOH	48.13±3.16	51.17±2.07	65.74±2.44	67.53±1.30	67.43±2.44	65.64±4.78	63.64±4.37	63.24±3.39
DCM	53.95±7.67	51.71±3.71	73.97±1.11	72.67±2.47	76.29±4.18	74.33±1.57	75.03±1.52	74.84±3.49
ETOH	56.57±6.32	47.42±6.33	65.71±9.14	59.67±1.97	69.76±1.33	65.26±2.13*	68.01±7.62	64.56±1.36
Water	37.23±0.87	37.93±1.70	64.48±3.51	58.39±4.59	66.94±2.76	62.23±2.20	58.96±1.99	59.68±4.31
Dox 1 μg/ml)	44.33±9.11	46.04±2.76	72.54±0.26	72.81±2.09	60.44±3.88	61.93±4.08	56.19±3.13	55.83±0.98

All values are presented as percentages of the results from control, and are expressed as mean ± SD of three independent (triplicate wells) experiments, * is expressed as significant different that compared to MTT values of the respective fraction in same cell line (1 mg/ml) at $P<0.05$. EtOAc, BuOH, DCM, ETOH and dox are stand for ethyl acetate, butanol, dichloromethane, ethanol and doxorubicin respectively.