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Original Research Article

Immune Cells Activity, Nitrite Scanvenging and ABTS Radical Scavenging Activities of *Codonopsis lanceolata* Ethanol Extracts from Districts in Korea

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Abstract - This study was executed to evaluate the immune activity, nitrite scavenging activity and ABTS radical scavenging activity against extracts of various concentration of ethanol solvent from *Codonopsis lanceolata* cultured at 6 local regions. The immune responses from both human T and B cell line was significantly enhanced in the cell growth compared to control while the cell growth was influenced at a certain period of culture. The results revealed that the cell growth of both human T and B cell was altered in a time dependent manner. The nitrite scavenging activity of ethanol extracts from various solvent concentration of *C. lanceolata* were affected by pH. At a pH of 1.2, the nitrite scavenging effect of all of the extracts tested observed higher than that of the other two pH ranges. There was no distinct detection of nitrite scavenging effects of the pH range 6.0. The ABTS radical scavenging activity was progressively increased in a dose-dependent manner, and the activity was the highest in 100% ethanol extract. The result from this investigation suggests that the extract of *Codonopsis lanceolata* could be an addition to basic medicine for immune modulation and natural food additives.

Key words - Immune activity, Nitrite Scavenging Activity, ABTS radical scavenging activity, *Codonopsis lanceolata*

Introduction

Codonopsis lanceolata is a perennial flowering plant belonging to the family Campanulaceae and is grown commercially in East Asia. The roots of *C. lanceolata* have been used as a tonic crude drug and an edible plant in Korea, and mainly contain triterpenoid saponins including codonolaside, codonolaside Ⅰ-Ⅴ, lancemaside A-G. Their saponins have shown anti-inflammatory effects such as bronchitis and cough, insomnia, and hypomnesia. Lancemaside A, which is a main constituent of *C. lanceolata* was reported to potently inhibit LPS-stimulated, TLR-4-linked NF-jB activation of 293-hTLR4-hemagglutinin (HA) cells (Joh *et al*., 2010). *C. lanceolata* is well known to affect various pharmacological effects for human health and its consumption is increasing. Recently, plant and plant-derived products are treated a part of the healthcare system by applying the bioactive phytochemicals. Various chemical agents with strong apoptosis-inducing activity, but minimal toxicity have potential as anticancer drugs. As an herb, *C. lanceolata* is widely used in food preparation, but its medicinal application has not been explored yet in South Korea (Wang *et al*., 2011). Due to the adverse ecological conditions and stress factors, immune dysfunction occurs in humans. However, synthetic, biotechnological and natural and natural medicinal preparations are used in order to mitigate the immunological disorders (Isaykina *et al*., 2008). Furthermore, medicinal plant may aslo reduce the risk of oxidative stress and cell damage (Guizani *et al*., 2013). It has been revealed that the increasing immune response will improve the defense against various diseases such as microbial infections and leukemia (Paul *et*

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al., 2014). In the immune system, cell and molecules play an important role. In addition, the cellular dichotomy in adaptive immune responses is also reflected in functional division whereas T cells serve as effectors of cell. Mediated immune responses such as delayed type hypersensitivity and B cells serve as the helpers for the production of highly specific proteins (Janeway *et al*., 1999). Medicinal plants are belived to be a potential source for the research of new biologically active compounds. A part from the medicinal effects of traditional herbs, exploratoty researches have been executed and a vast variety of new biological activities from traditional medicinal plants have recently been reported, including anticancer activity (Pittella *et al*., 2009). Nitric oxide (NO) is free radicals originated from the interaction between NO with O_2 or reactive O_2 species. That is classified as free radical because of its unpaired electron and shows crucial reactivity with certain types of proteins and other free radicals such as superoxide (Boora *et al*., 2014; Kang *et al*., 2015; Park *et al*., 2016). However, NO plays many important roles as an effector molecules in diverse biological systems. NO is associated with various carcinomas and inflammatory conditions when it is exposed to chronic condition. Furthermore, the toxicity of NO aslo increases amazingly when it reacts with superoxide radical (Noh *et al*., 2014). Antioxidant compounds in food play an important role as a health protecting factor. Scientific evidence suggests that antioxidants reduce the risk for chronic diseases, including cancer and heart disease. Most of the antioxidant compounds in a typical diet are derived from plant sources and belong to various classes of compounds with a wide variety of physical and chemical properties. The main characteristic of an antioxidant is its ability to trap free radicals. Highly reactive free radicals and oxygen species are present in biological systems from a wide variety of sources. In response to the increased production of oxygen radicals the capacity of the antioxidant defense system is increased, but in most situations the response is moderate (Foyer *et al*., 1994). Consequently, we have focused on establishing a relationship between immune activity against T cell, B cell lines and nitrite scavenging activity with ABTS radical scavenging activity using root organ of *C. lanceolata*.

Materials and Methods

Plant material and extract preparation

C. lanceolata plant was grown in 6 regions (Hwasun, Uljin, Hoengseong, Jeju, Jecheon and Muju areas), and purchased from the cultivation farms of each region. The root samples were freeze dried and then ground into a fine powder. The powder was stored at -20℃ for further experiments. The freeze dried powder was immersed in 30%, 50%, 70% and 100% ethanol concentrations, and the filtrate was collected for three times with constant stirring of the mixture at every 24 hrs interval of a 72 hrs total collection period. The crude extracts were filtered through a Whatman filter paper No. 3. The collected filtrate was evaporated to dryness under vacuum at -45℃ using a rotary evaporator (IKA RV 10, Germany). The concentrated extract was stored at -20℃ until required.

Assay of immune activity

Immune enhancement effect was assayed in a similar method to the procedure described earlier (Lee *et al*., 2004) using T cell and B cell (RPMI 8226, KCLB No.10155). The cells were incubated for 24 hrs in RPMI-1640 medium containing 10% fetal bovine serum (FBS) at 37℃ under 5% CO_2 in a humidified incubator. After the incubation for 24 hrs, purified cells were cultured for durations of 1~10 days at densities ranging from 2.5×10^4 cells/well in 24 well microtiter plates with adding the extract of 0.5 ㎍ ㎖$^{-1}$ to each of the wells. After the incubation for 10 days, the immune enhancement effect of the treatment was determined as counting of the number of cells using hemacytometer, and then compared to untreated cell.

Assay of ABTS radical scavenging rate

The spectrophotomeric analysis of ABTS (2,2'-azinbis-(3-ethyl-benzothiazoline-6-sulfonic acid) radical cation ($ABTS^{\bullet+}$) scavenging activity of *C. lanceolata* was determined according to the method described previously (Re *et al*., 1999) Re *et al*., 1999). 7 mM ABTS solution with 2.45 mM potassium persulfate was mixed, and the mixture was incubated in the dark at room temperature for 15 hours, and then was diluted to the absorbance 0.7 at 734 ㎚. Fifty ㎕ of each sample prepared in

different concentrations with 950 μl diluted solution was added, and was shaken for 10 seconds by vortex mixer, and then was reacted for 5 min at room temperature, and the absorbance was read at 734 ㎚ using a spectrophotometer (Biochrom Co., England). The $ABTS^{\bullet+}$ scavenging activity showed as RAEAC (relative ascorbic acid equivalent antioxidant capacity), was calculated by the following equation:

$$RAEAC = \frac{Caa}{\Delta Aaa} \times \frac{\Delta As}{Cs}$$

ΔAaa: change of the absorbance after addition of ascorbic acid

Caa: concentration of ascorbic acid

ΔAs: change of the absorbance after addition of sample solution

Cs: concentration of sample

Assay of Nitrite scavenging rate

The nitrite scavenging activity (NSA) was determined according to a method using Griess reagent (Kato *et al*., 1987). First, 40 μl of each sample was mixed with 20 μl of 1 mM nitrite sodium. Then the mixture was added to 140 μl of 0.2 M citrate buffer (pH 1.2, 4.2, or 6.0). The final volume of each sample wad adjusted to 200 μl. After, the mixtures had been incubated for 1 h at 37℃, and added to 1000 μl of 2% acetic acid and 80 μl of Griess reagent (1% sulfanilic acid and 1% naphthylamine in a methanol solution containing 30% acetic acid). After vigorous mixing with a vortex, the mixture was placed at room temperature for 15 min, and absorbance was measured at 520 ㎚. The nitrite scavenging activity was determined based on the following formula:

$$NSA\ (\%) = ((1\text{-}A\text{-}C)/B) \times 100$$

Where A is the absorbance of the mixture sample during a reation with 1 mM $NaNO_2$ after a 1 h reaction, B is the absorbance of a mixture of distilled water and 1 mM $NaNO_2$ after a 1 h reaction and C is the absorbance of the sample.

Data analysis

All experiments were conducted for three to five independent replicates. The data are expressed in terms of mean and standard error. The statistical analysis was performed using the procedures of the Statistical Analysis System (SAS version 9.1). The ANOVA procedure followed by LSD (least significant difference) test was used to determine the significant difference at the $P < 0.05$ level.

Results and Discussion

Responses of immune activity towards T cell and B cell line

The immune response using medicinal plant products as a possible therapeutic measure has become a subject of active scientific investigations. The immune enhancement effect was assayed by hemacytometer using T cell and B cell. The cell growth of human T cell line of each extract from 6 regions cultured *C. lanceolata* is shown in Fig. 1. The cell growth of human T cell line enhanced gradually when the cell cultivation period increased. The extracts of all studied plants showed a pronounced cell growth till the 10^{th} day of cell culture while all extracts from *C. lanceolata* decreased its cell growth after the 10^{th} day of cell culture. However, 50% ethanol extract from *C. lanceolata* exhibited a promising immune activity, whereas 100% ethanol extract from *C. lanceolata* demonstrated the lowest immune activity. Compared to untreated cell (Control), the all extracts from *C. lanceolata* showed higher immune enhancement effect. The immune enhancement effect of human B cell line from 6 regions cultured *C. lanceolata* is shown in Fig. 2. The 30% ethanol extract from *C. coreana* exhibited the highest immune activity on the 10^{th} day of cell culture. On the cell culture day 10, the all extracts of the studied samples showed pronounced immune activity effects. The most of ethanol extracts from *C. lanceolata* demonstrated a promising cell growth compared to untreated cell line (Control). However, There was no significant difference between extracts cultured in 6 regions. This results in the present study is somewhat similar to the results obtained that reported earlier (Lee *et al*., 2004). However, the results indicate that the cell growth of human T and B cell is dependent to culture period and after a certain period, the cell number decreases when it increases the duration of cell culture. Between the two cell lines, the cell growth of T cell and B cell increased significantly till the 10^{th} day of cell culture and then it decreased gradually. So, the result revealed that both T and B cell responses were triggered by the period

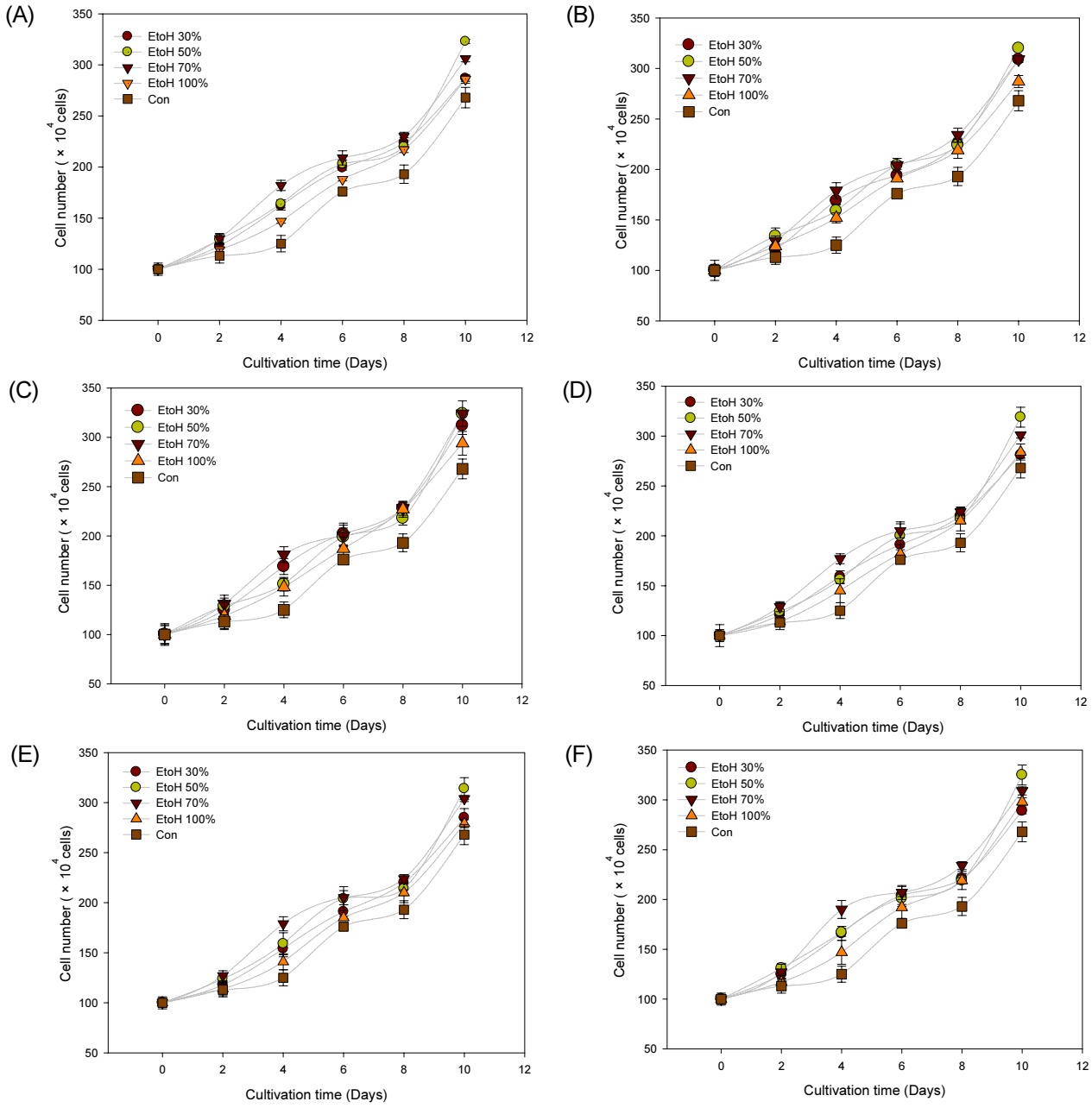

Fig. 1. The cell growth of human T cell line according to the concentration of ethanol solvent from 6 region cultured *Codonopsis lanceolata*. The bars represent the standard error. (A: Hwasun, B: Uljin, C: Hoengseong, D: Jeju, E: Jecheon, F: Muju).

of cell culture. Previous result revealed that severe combined immunodeficiency is the result of defects in more than 15 known genes that cause severe abnormal T cell and B cell immune function (Buckley *et al*., 1999).

Nitrite scavenging activity

Nitrite reacts with second and third grade amines to form nitrosamine in protein-rich foods, medicines, and residual pesticides. It is also present in large quantities in meat and both leafy and root vegetables. Nitrosamine is converted to diazoalkane (alkane nucleic acid), proteins, and intracellular components, which can increase the risk for cancer (Choi *et al*., 2008). Nitric oxide (NO) is basically generated from amino acid larginine by vascular endothelial cells, phagocytes

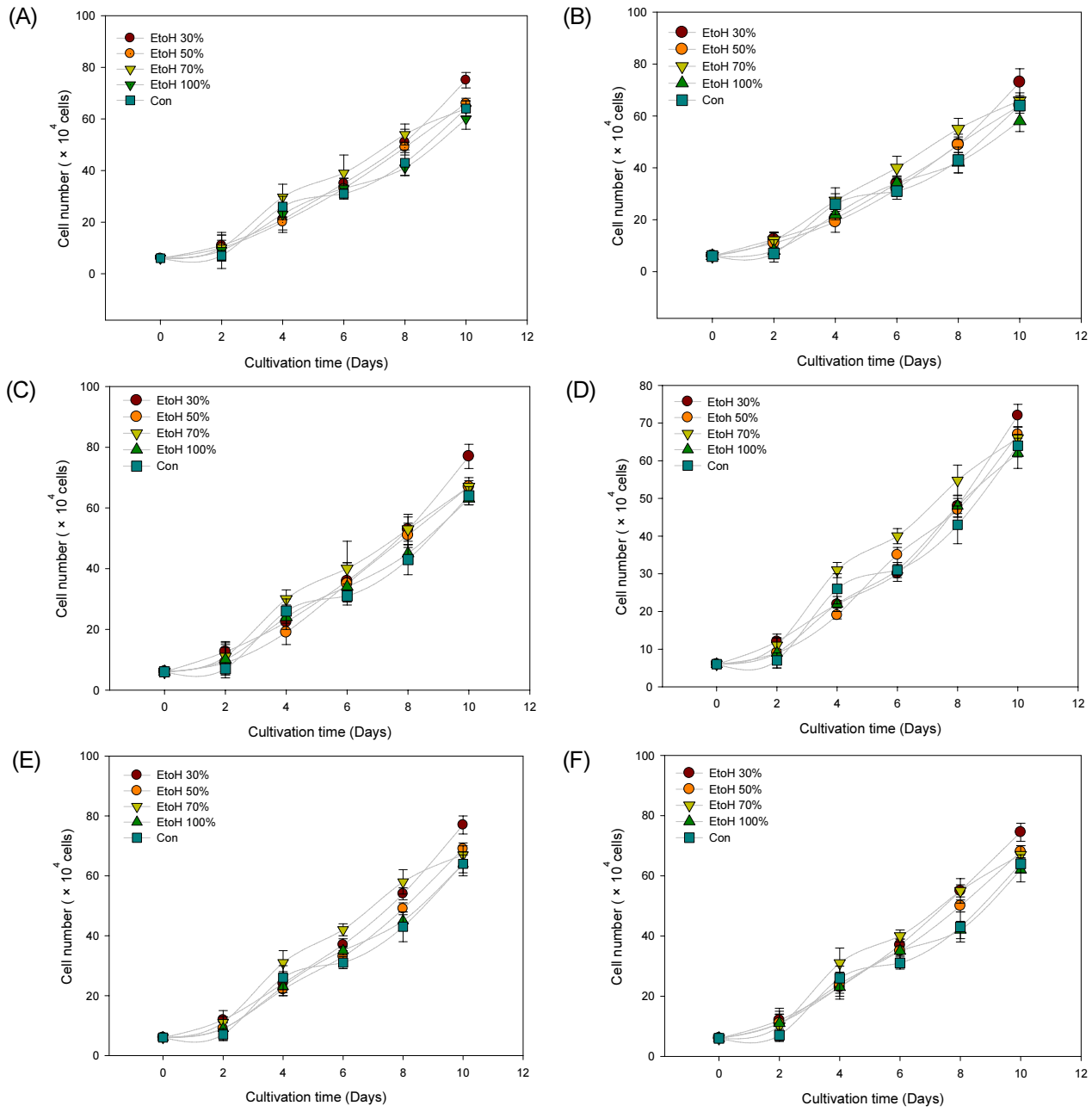

Fig. 2. The cell growth of human B cell line according to the concentration of ethanol solvent from 6 regions cultured *Codonopsis lanceolata*. The bars represent the standard error. (A: Hwasun, B: Uljin, C: Hoengseong, D: Jeju, E: Jecheon, F: Muju).

and certain cells of the brain. The toxicity of nitric oxide becomes adverse when it reacts with superoxide radical, forming a highly reactive peroxyritrite amino ($ONOO^-$) (Boora *et al*., 2014). The results of the determination of nitrite scavenging activity of *C. lanceolata* are summarized in the Table 1. As can be seen, the ethanol extracts from various solvent concentration of the studied plant were affected by pH. Three pH ranges (pH 1.2, pH 4.2 and pH 6.0) were considered in the present study. At a pH of 1.2, the scavenging effect of all of the extracts tested observed higher than that of the other two pH ranges. In addition, there was no distinct detection of nitrite scavenging effects of the pH range 6.0. The highest scavenging ability was observed from 30% ethanol solvent extract of *C. lanceolata* at a pH of 1.2

Table 1. Nitrite scavenging activities according to the concentration of ethanol solvent from 6 regions cultured *Codonopsis lanceolata*

Plant	Culture region	Solvent	Nitrite scavenging activity (%)[z]		
			pH 1.2	pH 4.2	pH 6.0
Codonopsis lanceolata	Hwasun	EtOH30%	76.94[ay]	27.60[ay]	1.44[ay]
		EtOH50%	65.27[b]	10.51[b]	N.D[b]
		EtOH70%	63.40[b]	3.53[c]	N.D[b]
		EtOH100%	33.80[c]	N.D[d]	N.D[b]
	Uljin	EtOH30%	74.14[a]	21.20[a]	2.47[a]
		EtOH50%	60.78[b]	14.52[b]	N.D[b]
		EtOH70%	57.80[b]	8.12[c]	N.D[b]
		EtOH100%	34.30[c]	N.D[d]	N.D[b]
	Hoengseong	EtOH30%	76.66[a]	26.25[a]	N.D
		EtOH50%	65.27[b]	12.42[b]	N.D
		EtOH70%	61.16[b]	8.98[b]	N.D
		EtOH100%	32.03[c]	N.D[c]	N.D
	Jeju	EtOH30%	77.40[a]	21.49[a]	1.34[a]
		EtOH50%	65.27[b]	12.89[b]	N.D[b]
		EtOH70%	59.29[b]	7.83[c]	N.D[b]
		EtOH100%	32.87[c]	N.D[d]	N.D[b]
	Jecheon	EtOH30%	73.86[a]	22.54[a]	1.54[a]
		EtOH50%	66.11[b]	11.37[b]	N.D[b]
		EtOH70%	61.06[b]	7.35[bc]	N.D[b]
		EtOH100%	35.95[c]	N.D[c]	N.D[b]
	Muju	EtOH30%	77.87[a]	25.98[a]	N.D
		EtOH50%	66.67[b]	13.28[b]	N.D
		EtOH70%	63.49[b]	6.02[c]	N.D
		EtOH100%	33.89[c]	N.D[d]	N.D

[z]Data represent the mean values±SE of three independent experiments. [y]Means with the same letter in column are not significantly different at $p<0.05$ level by Duncan's multiple range test.

while the lowest scavenging activity was observed from 100% ethanol solvent extract of *C. lanceolata.* The results prevailed that the nitrite scavenging activity was influenced by the ethanol concentration and was recognized the significance between ethanol solvent extracts. However, The nitrite scavenging activity was no significant difference between extracts cultured in 6 regions. The fact that the nitrite scavenging activity was high at pH 1.2 suggests that nitrosamine production can be inhibited *in vivo* (Choi *et al*., 2008). These results were consistent with other findings that had the highest the nitrite scavenging at pH of 1.2 in fermented pine extract (Hong *et al*., 2004) and extracts from different parts of citron (Shin *et al*., 2005).

ABTS radical scavenging activity

In order to evaluate the radical scavenging activities of extracts according to the concentration of ethanol solvent from *C. lanceolata*, ABTS assays were performed. The results of the ABTS radical scavenging activity were shown in Table 2. When the experimental samples from *C. lanceolata* were treated with various concentrations (500, 1000, 2500, 5000, 10000 and 20000 ㎎ L^{-1}) of extracts, the ABTS radical scavenging activity was progressively increased in a dose-dependent manner. The ABTS radical scavenging activity was the highest in 100% ethanol extract. Particularly, the ABTS radical scavenging activity showed that the increase was proportional to the ethanol concentration except 30%

Table 2. ABTS radical scavenging activities according to the concentration of ethanol solvent from 6 regions cultured *Codonopsis lanceolata*

Plant	Culture region	Solvent	ABTS radical scavenging activity, % of control[z]					
			Concentration (㎎/㎖)					
			0.5	1	2.5	5	10	20
Codonopsis lanceolata	Hwasun	EtOH30%	4.95±0.58^{by}	13.94±0.21^{ay}	19.87±1.28^{ay}	42.27±0.07^{ay}	62.05±0.78^{ay}	82.91±0.19^{ay}
		EtOH50%	5.35±0.59^{ab}	6.13±0.25^{b}	10.47±0.13^{b}	29.05±0.34^{c}	38.08±1.08^{d}	73.04±0.54^{c}
		EtOH70%	7.21±0.18^{a}	13.21±0.71^{a}	20.23±0.34^{a}	35.45±1.27^{b}	55.96±1.04^{b}	77.33±1.15^{b}
		EtOH100%	4.32±0.97^{b}	13.17±1.45^{a}	18.89±0.96^{a}	33.50±0.52^{b}	46.87±0.41^{c}	79.35±0.30^{b}
	Uljin	EtOH30%	7.21±0.46^{a}	10.02±0.26^{b}	16.11±1.57^{bc}	37.56±1.24^{a}	55.26±0.87^{b}	77.20±1.30^{b}
		EtOH50%	3.40±0.64^{b}	7.78±0.27^{c}	13.58±0.38^{c}	27.53±1.08^{b}	40.12±1.12^{d}	76.83±0.18^{b}
		EtOH70%	5.98±0.40^{a}	15.15±0.27^{a}	23.83±0.58^{a}	42.42±0.89^{a}	58.94±0.65^{a}	83.61±0.50^{a}
		EtOH100%	5.52±0.69^{a}	10.59±0.64^{b}	17.83±0.69^{b}	30.84±2.92^{b}	46.79±0.28^{c}	79.31±0.58^{b}
	Hoengseong	EtOH30%	5.61±0.70^{ab}	12.06±0.23^{a}	19.70±1.20^{a}	38.73±0.18^{a}	58.21±1.23^{a}	79.52±0.84^{ab}
		EtOH50%	4.78±0.74^{b}	5.86±0.22^{b}	14.70±0.71^{b}	29.02±1.20^{b}	38.51±0.94^{c}	73.63±0.20^{c}
		EtOH70%	7.86±0.70^{a}	12.72±0.85^{a}	21.40±0.92^{a}	37.30±0.93^{a}	52.02±0.78^{b}	78.23±1.48^{b}
		EtOH100%	6.20±0.66^{ab}	13.68±0.80^{a}	22.69±1.17^{a}	38.11±0.44^{a}	54.72±1.11^{ab}	81.77±0.33^{a}
	Jeju	EtOH30%	5.91±0.43^{b}	11.96±0.38^{b}	19.75±0.30^{b}	38.00±2.41^{b}	57.51±1.08^{b}	81.62±1.17^{b}
		EtOH50%	6.86±0.68^{b}	8.28±0.42^{c}	17.21±0.61^{b}	30.01±1.80^{c}	44.46±0.78^{c}	80.50±0.37^{b}
		EtOH70%	8.83±0.46^{a}	13.53±0.41^{ab}	25.74±1.47^{a}	35.38±0.23^{b}	59.46±1.05^{b}	84.11±0.13^{a}
		EtOH100%	7.00±0.29^{b}	14.05±0.88^{a}	25.91±0.55^{a}	44.46±0.05^{a}	63.15±0.26^{a}	85.62±0.02^{a}
	Jecheon	EtOH30%	5.04±0.80^{b}	11.93±0.40^{b}	16.44±0.27^{c}	34.59±0.82^{bc}	59.03±1.60^{b}	77.88±1.70^{b}
		EtOH50%	4.83±0.79^{b}	5.63±0.53^{c}	12.47±0.89^{d}	31.23±2.13^{c}	48.86±0.75^{c}	77.15±0.51^{b}
		EtOH70%	7.83±0.38^{a}	11.77±0.71^{b}	22.42±0.82^{b}	38.28±1.95^{b}	55.64±1.37^{b}	80.37±1.07^{b}
		EtOH100%	4.89±0.78^{b}	15.52±0.50^{a}	25.12±0.56^{a}	44.77±0.51^{a}	65.31±1.82^{a}	85.08±0.20^{a}
	Muju	EtOH30%	7.26±0.90^{a}	11.98±0.63^{a}	15.08±1.04^{b}	32.33±0.58^{b}	52.62±0.44^{b}	77.62±0.66^{b}
		EtOH50%	5.25±0.33^{b}	7.22±0.31^{c}	11.45±0.97^{c}	31.67±1.78^{b}	38.73±0.81^{c}	73.60±0.20^{c}
		EtOH70%	5.03±0.37^{b}	10.14±0.39^{b}	23.16±0.98^{a}	33.53±1.36^{ab}	53.58±1.12^{b}	77.36±0.80^{b}
		EtOH100%	7.57±0.41^{a}	12.01±0.30^{a}	21.22±0.90^{a}	37.80±1.33^{a}	59.89±0.95^{a}	83.04±0.51^{a}

[z]Data represent the mean values±SE of three independent experiments. [y]Means with the same letter in column are not significantly different at $p<0.05$ level by Duncan's multiple range test.

ethanol extract. There was little difference in the ABTS activity between extracts cultured in 6 regions. However, the extracts cultured in Jeju and Jecheon region showed slightly high the ABTS activity. Overall, ABTS activity of *C. lanceolata* exhibited slightly lower at 50% ethanol solvent extract than any other concentration of ethanol. In the present evaluation, The results of the nitrite scavenging activity and ABTS radical scavenging activity of *C. lanceolata* was not consistent with each other. These results were supposed that there is a difference in the nitrite scavenging activity and ABTS radical scavenging activity according to plant species.

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