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Original Research Article

Immune Cells Activity, Cytotoxicity and Nitrite Scavenging Activity of Extracts from Several Resource Plants

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Abstract - This study was conducted to explore the immune activity, anticancer activity and nitrile scavenging activities of methanol extracts from the various organs of four Korean resource plants. 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. Among tested several resource plants, the flower extract of *E. japonicum* demonstrated a pronounced cytotoxicity against HCT-116 cell with an IC50 value 132.08 ㎍ ㎖-1. The flower extract from *Corylopsis coreana* had a promising scavenging activity against pH 1.2 compared to other species. Taken together, the studied resource plants have influenced significantly in response to immunity and also have the potential cytotoxicity and nitric scavenging activities. However, the species *E. japonicum* exhibited the pronounced activities from several resource plants. The result from this investigation suggests that the extracts of studied resource plant could be an addition to basic medicine for some diseases.

Key words – *Corylopsis coreana*, Cytotoxicity, *Erythronium japonicum*, Immune activity, Nitrite scavenging activity, *Phragmites communis*, *Salicornia herbacea*

Introduction

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). Cardovascular and atherosclerosis disorders (Toh *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 al.*, 2014). To this end, research has taken to shed light on the identification of novel compounds from plants which may promote the immune response. In the immune system, cell and molecules play an important role. In addition, the cellular dichotomy in adaptive immune responses is also reflected in the 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 is believed 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). *Salicornia (S.) herbacea* L. (Chenopodiaceae) is known to be a salt marsh plant and one of the most salt tolerant species on Western coast of Korea and *Phragmites australis* (Cav.) Trin. ex Steud. is a perennial, emergent, salt-tolerant aquatic grass. From the ancient time, *S. herbacea* has been used as a folk medicine for the treatment of nephropathy, hepatitis and diarrhea or constipation in Korea (Rhee *et al.,* 2009). *Corylopsis coreana* Uyeki (Korean winter hazel; CL) belongs to the Hamamelidaceae and is cultivated widely as an ornamental plant in South Korea. Some species of the genus Corylopsis,

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such as *Hamamelis virginiana* (witch hazel) have been used as a folk medicine for the treatment of irritated skin and inflammatory disease (Wang *et al.,* 2003). *Erythronium japonicum* Decne. (Liliaceae) is a typical vernal plant that is distributed also in Korea, northeast China, Sakhalin, and the Kurile Islands (Ohwi and Kitagawa, 1983).

Plants have many phytochemicals with various bioactive componds including antioxidant, anti-inflammatory and anti-cancer activities. However, numerous studies have reported that extracts from natural products such as fruits, vegetables and medicinal herbs have positive effects against cancer (Pezzuto, 1997; Wu *et al.*, 2002). Therefore, many plants have been examined to identify new and effective antioxidant and anticancer compounds as well as to explore the mechanisms of cancer prevention and apoptosis (Kim, 1998; Pietta *et al.*, 1998; Swamy and Tan, 2000). 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). 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). Consequently, we have focused on establishing a relationship between immune activity and cytotoxicity with nitrite scavenging activities evaluating the activity against cancer cell lines using various organ obtained from four plant species. The present study aimed to investigate the effects of methanol extracts on the immune response, cytotoxicity and nitrite scavenging activities from several resource plants.

Materials and Methods

Plant material and extract preparation

In this experiment, four kinds of resource plant materials [*Salicornia herbacea* (aerial part), *Corylopsis coreana* (stem and flower), *Erythronium japonicum* (leaf, root and flower), *Phragmites communis* (root ad stem)] were used. These plants were chosen because of the possibility to obtain various physiological functionalities. The different organ samples were directly freeze-dried and then ground into a fine powder. Each sample powder was stored at -20℃ for experiments. Methanol extracts were prepared by soaking the sample powder into 100% methanol for 24 hours at room temperature. 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 methanol 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 x 10^4 cells/well in 24 well microtiter plates with adding the extract of 0.5 $\mu g\ ml^{-1}$ 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.

Cytotoxicity measurement by the MTT assay

The cytotoxicity of each plant sample was assayed by the method described earlier (Hansen *et al.*, 1989) using human cancer cell lines, Hep3B for human hepatocarcinoma, Calu-6 for human pulmonary carcinoma, SNU-601 for human gastric carcinoma and HCT-116 for human colorectal carcinoma. The cell lines were purchased from Korea Cell Line Bank (KCLB) for MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) assay. The cells were plated on 96-well plates at a concentration of 3×10^4 cells/㎖. The cells were incubated for 24 h in RPMI-1640 medium at 37℃ with 5% CO_2 in a humidified incubator, and then treated with 2 ㎕ of various concentrations (125, 250, 500, 1000, and 2000 $\mu g\ ml^{-1}$) of the extracts. After incubation for 48 h, the cells were washed twice with phosphate buffer solution (PBS). The MTT solution (5 $mg\ ml^{-1}$) was dissolved in 1 ㎖ of PBS, and 10 ㎕ of

this solution was added to each well. After a reaction period of 4 h, the solution in each well containing media, unbound MTT, and dead cells were removed by suction and 100 μl of DMSO was added to each well. The plates were shaken for 15 min using a plate shaker, and the absorbance was recorded using an enzyme-linked immunosorbent assay (ELISA) reader (Bio-Rad model 550, USA) at a wavelength of 540 ㎚. Cell viability was determined as the percent of the viability of treated cells compared with that of the untreated cell, and the values were then used to iteratively calculate the concentration of extract required to induce a 50% reduction (IC_{50}) in the growth of each cell line.

Nitrite scavenging assay

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˚C, 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:

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

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 statistical analysis was performed using the Statistical Analysis System software (SAS version 9.1). The analysis of variance (ANOVA), followed by Duncan test was used to determine significant difference ($p < 0.05$) between the treatment means.

Results

Responses of immune activity

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 enhanced gradually when the cell cultivation period increased. The extracts of all studied plants showed a pronounced cell growth till the 8th day of cell culture while two extracts from *P. communis* (stem) and *S. herbacea* decreased its cell growth after the 8th day of cell culture. However, the flower extract from *E. japonica* exhibited a promising immune activity, whereas the root extract of *P. communis* demonstrated the lowest immune activity. The cell growth of human T cell line of each extract from several resource plants is shown in Fig. 1. Compared to untreated cell (Control), the extracts from different organs (root, leaf and flower) of *E. japonica*, flower extract from *C. coreana* and stem extracts from *P. communis* showed higher immune enhancement effect. In contrast, the root extract from *P. communis*, stem extract from *C. coreana* and methanol extract of *S. herbaceae* observed the lower immune enhancement effect compared to untreated cell. The immune enhancement effect of human B cell line from several resource plants is shown in Fig. 2. The flower extract from *C. coreana* exhibited the highest immune activity on the 8th day of cell culture. On

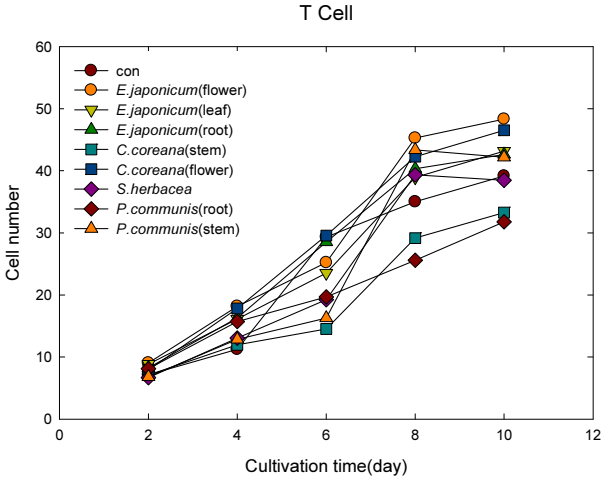

Fig. 1. The cell growth of human T cell line of each extract from several resource plants using hemocytometer.

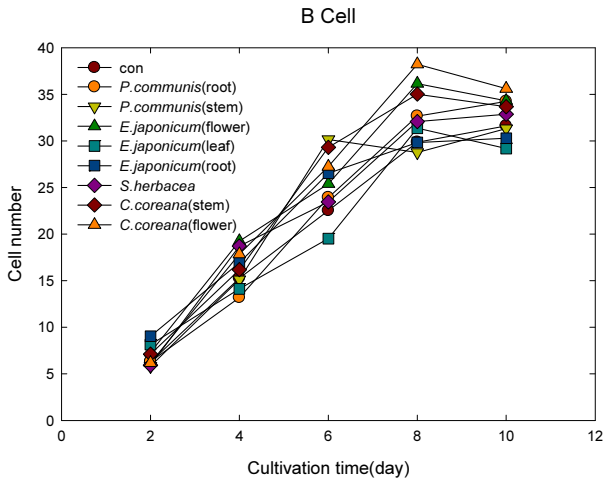

Fig. 2. The cell growth of human B cell line of each extract from several resource plants using hemocytometer.

the cell culture day 8, the all organs of the studied plants showed pronounced immune activity effects except the stem extract from *P. communis* that showed the lowest immune effect. However, the most of the organs from each plant demonstrated a promising cell growth compared to untreated cell line (Control).

Cytotoxicity on human cancer cell

The cytotoxic activities of the different organs from the four studied plants were investigated using a MTT assay on four human cell lines Hep-3B, Calu-6, SNU-601 and HCT- 116. The rate of cell survival progressively decreased in a dose-dependent manner (Table 1). The cytotoxic effect against the cancer cell lines was higher in the flower of *C. coreana* and *E. japonicum* than in the other plant organs. The IC_{50} value on the Hep3B cell line was the highest on the flower of *C. coreana* (512.00 $\mu g\ ml^{-1}$), followed by the stem of *C. coreana* (693.22 $\mu g\ ml^{-1}$), the flower of *E. japonicum* (709.28 $\mu g\ ml^{-1}$) and the leaf of *E. japonicum* (752.82 $\mu g\ ml^{-1}$). The IC_{50} value on the Calu-6 and HCT-116 cell lines was the highest on the flower of *E. japonicum* (660.37 and 132.08 $\mu g\ ml^{-1}$). The IC_{50} value on the SNU-601 cell line was the highest on the flower of *C. coreana* (154.58 $\mu g\ ml^{-1}$). At investigated all plant organs, the SNU-601 cell line showed a relatively high cytotoxic effect. Overall, the cytotoxicity on SNU-601 cell and HCT-116 cell showed higher on Hep3B cell and Calu-6 cell. also showed similar tendency to the Hep3B cell line. It was found that the concentrations of 1,000 $\mu g\ ml^{-1}$ or more of plant extracts was remarkably low the cell viability percentage. That is, at the highest concentration (2,000 $\mu g\ ml^{-1}$) of all organs showed the highest toxicity against all cancer cell lines.

Nitrite scavenging activity

The results of the determination of nitrite scavenging activity of the studied plants are summarized in the Table 2. As can be seen, the methanol extracts from various organs of the studied plants 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 (96.4%) was observed from the flower organ of *Corylopsis coreana* at a pH of 1.2 while the lowest scavenging activity (58.2%) was observed from the root extract of *Phragmites communis.* The result revealed that among all of the tested plants, *Corylopsis coreana* showed the highest scavenging activity followed by *Erythronium japonica*, *Salocornia herbacea* and *Phagmites communis* respectively. Also it was prevailed that the organ, root showed more weak scavenging activity than that of other organs used in the present study.

Discussion

Medicinal plants have been useful source for the research of new biologically active compounds. However, there are several medicinal plants are employed in different systems of medicine throughout the world to improve the immunological disorders. Several medicinal plants are considered as toxic and can cause serious damage to the health of patients. Therefore, the assessment of the toxicity of medicinal plants as well as their herbal preparations, is essential to determine the applicability of the sample as a pharmacological drug (Junior *et al.,* 2005). In the present study, the cell growth of human T and B cell line of each extract was carried out from several economic plants using hemocytometer. Compared to other studied plants, the species *E. japonicum* demonstrated

Table 1. Cytotoxicity of extracts from the different organs of several resource plants on four human cancer cell lines

Cell line	Plant organ	Cell viability (% of control)					
		Concentration (㎍/㎖)					
		125	250	500	1000	2000	IC_{50}
Hep3B	*S. herbacea*	80.13±1.83abc[z]	69.54±2.70ab	54.25±2.94ab	34.84±0.70bc	12.37±0.07de	795.88
	C. *coreana* (stem)	69.64±4.73c	65.34±2.60bc	55.24±1.18ab	33.32±1.43cd	11.05±1.85cd	693.22
	C. *coreana* (flower)	75.53±1.18abc	55.36±3.44d	44.85±3.39b	20.82±1.18e	12.23±2.28de	512.00
	E. *japonicum* (leaf)	87.26±4.77a	58.15±2.71cd	54.53±3.30ab	27.25±1.36dde	16.21±1.54e	752.82
	E. *japonicum* (root)	79.96±3.20abc	71.95±3.06ab	51.56±3.67ab	42.26±2.31ab	27.48±1.23a	957.30
	E. *japonicum* (flower)	70.27±4.80bc	63.17±3.92bcd	52.93±4.14ab	36.28±1.04bc	14.76±0.11de	709.28
	P. *communis* (stem)	77.82±3.79abc	75.12±0.56a	52.43±3.73ab	34.73±3.97bc	23.43±2.24ab	873.79
	P. *communis* (root)	82.84±4.35ab	77.11±1.48a	58.04±1.77a	45.56±3.95a	20.15±1.01bc	995.31
Calu-6	*S. herbacea*	74.82±3.09bc	67.93±3.52a	56.75±3.30ab	34.95±2.98b	15.36±1.21c	786.12
	C. *coreana* (stem)	87.81±0.35a	55.72±1.81b	51.23±1.09ab	34.42±2.10b	12.52±1.70c	730.60
	C. *coreana* (flower)	74.05±1.19bc	61.32±0.54ab	41.15±1.66c	37.41±1.51ab	20.92±1.19b	673.33
	E. *japonicum* (leaf)	81.33±4.03ab	59.63±0.52ab	47.06±3.32bc	40.07±4.10ab	12.52±2.58c	721.61
	E. *japonicum* (root)	79.34±2.67ab	65.25±3.02a	57.24±1.86ab	41.75±0.34ab	13.75±0.13c	830.31
	E. *japonicum* (flower)	66.64±1.72c	64.94±3.71a	50.94±3.75ab	34.92±2.14b	16.83±195bc	660.37
	P. *communis* (stem)	77.52±0.02b	61.77±2.22ab	59.53±2.02a	38.03±0.57ab	11.45±0.23c	787.18
	P. *communis* (root)	71.65±5.66bc	64.72±2.61a	51.51±5.44ab	43.43±3.51a	26.42±2.57a	866.81
SNU-601	*S. herbacea*	71.33±2.43a	56.92±2.71ab	49.63±1.37ab	26.05±0.64cd	5.54±1.14d	464.21
	C. *coreana* (stem)	62.24±2.10b	50.35±1.39b	47.56±1.34dbc	37.73±1.09a	9.96±0.35cd	452.80
	C. *coreana* (flower)	70.15±3.02ab	31.23±3.79c	39.54±2.07c	24.05±0.55d	8.46±0.84d	154.58
	E. *japonicum* (leaf)	68.53±3.34ab	49.45±3.79b	49.02±3.81ab	28.33±1.19bcd	8.92±0.67d	466.78
	E. *japonicum* (root)	74.93±3.02a	58.17±1.86ab	44.66±0.55bc	32.78±0.42b	23.15±0.97a	638.75
	E. *japonicum* (flower)	63.16±1.71b	57.92±1.34ab	42.15±4.66bc	28.24±2.20bcd	14.97±2.82b	429.60
	P. *communis* (stem)	74.62±1.49a	58.75±3.98ab	44.35±2.03bc	32.26±2.71b	14.14±2.31bc	612.14
	P. *communis* (root)	69.13±1.80ab	60.14±3.06a	57.45±3.55a	29.83±1.40bc	8.15±1.14d	629.37
HCT-116	*S. herbacea*	70.53±2.26c	58.11±1.72cd	37.36±1.30bc	28.11±1.72abc	13.83±1.99ab	470.37
	C. *coreana* (stem)	83.53±2.08b	55.52±5.20cd	40.15±2.43bc	25.52±1.20bcd	10.93±1.87b	570.00
	C. *coreana* (flower)	67.76±2.23cd	45.74±1.50ef	42.84±1.84b	25.74±1.50bcd	11.45±2.05b	348.40
	E. *japonicum* (leaf)	69.16±1.66c	49.73±1.06de	39.04±1.10bc	29.73±1.06ab	8.96±1.59b	396.29
	E. *japonicum* (root)	83.04±1.75b	72.36±4.20b	60.13±3.98a	22.36±4.20cd	9.43±2.19b	771.79
	E. *japonicum* (flower)	65.95±1.73d	40.33±2.25f	34.72±1.29c	20.33±2.25d	10.04±1.91b	132.08
	P. *communis* (stem)	72.23±2.42c	62.82±1.65c	40.63±1.86bc	32.82±1.65a	12.14±1.32ab	588.62
	P. *communis* (root)	91.61±0.65a	87.23±1.93a	65.32±2.18a	30.23±1.93ab	17.36±1.19a	977.80

[z]Data represent the mean values±SE of three independent experiments. Means with the same letter in column are not significantly different at $p<0.05$ level by Duncan's multiple range test. Hep3B: human hepatocarcinoma cell, Calu-6: human pulmonary carcinomacell, SNU-601: human gastric carcinoma cell, HCT-116: human colorectal carcinoma cell.

the highest immune responses. The cell number also have been increased significantly compared to control plants. This results 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

Table 2. Nitrite scavenging activities of the different organs extract in several resource plants

Plants	Nitrite scavenging activity (%)		
	pH 1.2	pH 4.2	pH 6.0
Salicornia herbacea	80.2±1.65[cz]	29.5±1.54[a]	ND[y]
Corylopsis coreana (Stem)	91.5±1.89[a]	28.3±2.28[a]	ND
Corylopsis coreana (Flower)	96.4±1.18[a]	25.4±1.85[b]	ND
Erythronium japonicum (Leaf)	85.2±1.36[b]	22.1±1.38[c]	ND
Erythronium japonicum (Root)	58.5±1.35[d]	14.1±1.53[d]	ND
Erythronium japonicum (Flower)	85.3±1.78[b]	17.4±0.92[d]	ND
Phragmites communis (Root)	58.2±1.26[d]	15.3±0.95[d]	ND
Phragmites communis (Stem)	60.1±1.08[d]	13.9±1.07[d]	ND

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

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 8th 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 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). Cancer is believed to be a multi-step disease incorporating, physical environmental, metabolic, chemical and genetic factors that play a crucial role in the induction and deterioration of cancers (Rahman *et al.,* 2011). By this way, it is becoming evident that certain phytochemicals, particularly those included our daily diet, many have important cancer chemopreveutive properties (Samaha *et al.,* 1997). In the present study, the methanol extracts of the organ of the studied plants were investigated for their cytotoxic activity on four human cancer cell lines (Hep 3B, Calu-6, SNU- 601, HCT-116) by the MTT assay (Heo *et al.,* 2007). Our present result demonstrated that extract from various organs from four resource plants exhibited a promising cytotoxic activity against all human cell lines at relatively higher concentration. In all extracts investigated, the lowest IC_{50} value was observed from the flower extract of *E. japonicum* (132.08 $\mu g\ ml^{-1}$) against HCT-116 cell line and the highest IC_{50} value was observed from the root extract of *P. communis* (995.31 $\mu g\ ml^{-1}$) against Hep3B cell line. However, the obtained results from our investigation is similar to the results obtained from previous investigation (Rahman *et al.,* 2011). Compared to other cell line, the HCT-116 cell line showed the highest toxicity. The leaf and flower extract from *E. japonicum* showed the highest cytotoxicity compared to other extract of the studied plants. But, the root extract from this species (*E. japonicum*) showed the lowest cytotoxicity against all cancer cells. In previous investigation, it was revealved that the higher the concentration were the lower the cell viability percentage (Heo et al., 2007). On an average, the species *E. japonicum* showed the highest cytotoxicity compared to other species. Therefore, the result revealed that the species *E. japonicum* is susceptible to cytotoxicity against all cancer cell lines. Nitric oxide (NO) is basically generated from amino acid larginine by vascular endothelial cells, phagocytes 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; Choung and Sohn, 2011). Nitric radical scavenging assay was carried out on the ethanol extracts of *E. japonicum*, *C. coreana*, *P. communis* and *S. herbacea* organs with an pH range of 1.2-6.0. Each fraction of ethanol extraction of the studied plants exhibited a pH dependent nitric scavenging activity. However, the results prevailed that the nitrite scavenging effect of the organ extracts was adversely affected by pH. The highest nitrite scavenging activity (96.4%) was observed from the flower extract of *C. coreana* and the lowest activity (58.2%) was observed from the root extract *of P. communis*. The scavenging effect all of the

extracts tested was higher at pH 1.2 than that the other pH concentrations. Earlier studies report that the methanol extracts of organ have a very high scavenging effect at a pH of 1.2 (92.87~96.51 and 93.00~98.86% respectively) (Joung and Kim, 2006; Noh *et al.,* 2014; Boo *et al.,* 2016). Few previous reports indicate that the nitrite scavenging effect of Korean ginseng, Rubus Coreamus and *Angelica dahurica* leaves decreases with increasing pH (Lee, 2007; Park and Chang, 2003; Ye *et al.,* 2010). Compared to other studied plants, the *C. coreana* exhibited the highest nitrite scavenging activity 96.4% and 91.5% respectively, and there was no distinct difference in the nitrite scavenging effects of flower and stem organs in the present study. In conclusion, the results obtained in the present study demonstrated the immune responses of the aerial parts of four resource plant related to its cytotoxic activities against cancer cell lines combined with nitrite scavenging activity. The results prevailed that the immune responses from both T and B cells were influenced by the time dependant manner and most of the studied resource plants have potential cytotoxic activity. The nitrite scavenging effects revealed that the organ extracts was affected by pH. It was highest at a pH of 1.2 and decreased as pH increased, suggesting that the nitrite scavenging effect was higher in more acidic conditions. Taken Together, this study indicates that bioactive molecule present in those plants could be helpful for the development of new drugs and / or as a source of basic medicine in the treatment of some diseases.

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