한작지(Korean J. Crop Sci.), 60(2): 239~247(2015)

ISSN 0252-9777(Print)
ISSN 2287-8432(Online)
DOI : http://dx.doi.org/10.7740/kjcs.2015.60.2.239

Evaluation of Cytotoxicity, Antimicrobial and Antioxidant Enzyme Activity of Diploid and Tetraploid *Platycodon grandiflorum*

Hee-Ock Boo*†, Young-Sun Kim**, Hag-Hyun Kim***, Soo-Jeong Kwon***, and Sun-Hee Woo****

*WELLPHYTO Co. Ltd., BI Center, GIST, Gwangju 500-712, Korea
**Department of Korean Food, Jeonnam Provincial College, Damyang 517-802, Korea
***Faculty of Food Nutrition and Cookery, Woosong Information College, Daejeon 300-715, Korea
****Department of Crop Science, Chungbuk National University, Cheongju 361-763, Korea

ABSTRACT This experiment was conducted to obtain the have higher contents of pharmaceutical constituents as well as higher yield from colchicine induced diploid and tetraploid extracts of *Platycodon grandiflorum*. In order to determine the biological activity, this study was focused to evaluate the cytotoxicity, antimicrobial on the bronthus disease bacteria, antioxidant enzyme activity of diploid and tetraploid extracts in *P. grandiflorum*. The activities of antioxidant enzyme according to different solvent extracts were measured as superoxide dismutase (SOD), catalase (CAT), peroxidase (POD), and ascorbate peroxidase (APX). The cytotoxicity of methanol extracts of *P. grandiflorum* showed significant differences between tetraploid and diploid. That is, the cytotoxic effect against human cancer cell was higher in tetraploid than in diploid. At all extracts concentration, tetraploid samples showed high toxicity and the IC_{50} (concentration causing 50% cell death) value showed the highest on HCT-116 cell (105.91 μg/mL), and exhibited significant activity against the Hep 3B cell (140.67 μg/mL), SNU-1066 cell (154.01 μg/mL), Hela cell (158.37 μg/mL), SNU-601 cell (182.67 μg/mL), Calu-6 cell (190.42 μg/mL), MCF-7 cell (510.19 μg/mL). Antimicrobial activities of diploid *P. grandiflorum* were relatively low compared to tetraploid *P. grandiflorum* on most of the bacterial strains. In tetraploid *P. grandiflorum*, *K. pneumoniae* showed the clear zone formation (18~19 mm) of growth inhibition, followed by the clear zone formation of 13~15 mm on *C. diphtheria* and *S. pyogenes*. The antimicrobial activities in diploid *P. grandiflorum* were the highest on *K. pneumonia* (14~15 mm), and showed the clear zone formation of 11~12 mm on *C. diphtheria* and 12~13 mm on *S. pyogenes*. The antimicrobial activity is thought to look different depending on the bacterial strains and the polyploidy of *P. grandiflorum*. The root extract of *P. grandiflorum* had the highest (97.2%) SOD enzyme activity in ethyl acetate partition layer of tetraploid while water partition layer of diploid showed the lowest (48.6%) SOD enzyme activity. The activity of CAT showed higher values in the root of tetraploid than in the diploid of *P. grandiflorum* in all partition layers except butyl alcohol. The activities of APX and POD showed higher values in the root of tetraploid than in the diploid of *P. grandiflorum* in all fraction solvents except water layer. These results indicate that the tetraploid *P. grandiflorum* can be used as a source for developing cytotoxic agent and antimicrobials which can act against bronchus diseases bacterial strains.

Keywords : Cytotoxicity, Antimicrobial, Antioxidant enzyme, Diploid, Tetraploid, *Platycodon grandiflorum*

Many researches have shown that traditional medicinal plants have in vitro mutagenic or toxic and carcinogenic properties, thus it is important to explore the medicinal plants for their cytotoxicity. The cytotoxicity evaluation of plants is a major subject in pharmaceutical studies, particularly in the area of cancer research (Cuyacot *et al.,* 2014). However, there is a scarcity of data on the safety and tolerability of *Platycodon grandiflorum* when used as a health care materials. Medicinal plants including *P. grandiflorum* are good sources of antimicrobial agents. Many infectious diseases have been known to be treated with herbal extracts. The clinical efficacy of many existing antibiotics is being threatened by the emergence of multidrug-resistant pathogens (Goveas and Abraham, 2013). The evaluation of antimicrobial property of tetraploid *P. grandiflorum* is of great interest and importance. 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

†Corresponding author: (Phone) +82-10-6690-5636 (E-mail) swboo@hanmail.net, swboo@chosun.ac.kr
<Received 1 June, 2015; Accepted 4 June, 2015>

characteristic of an antioxidant is its ability to eliminate free radicals. Highly reactive free radicals and oxygen species are present in biological systems from a wide variety of sources. The plants have antioxidant enzymes such as superoxide dismutase (SOD), catalase (CAT), peroxidase (POD), and ascorbate peroxidase (APX) against ROS (reactive oxygen species) (Zhou *et al.,* 2005). Both enzymatic and nonenzymatic antioxidant systems are present in plants. Superoxide radicals are detoxified by SOD and hydrogen peroxide is destroyed by CAT and different kinds of peroxidases (Kang and Saltveit, 2002). A major hydrogen peroxide-detoxifying system in plant is the ascorbate-glutathione cycle that includes APX and glutathione reductase (GR) (Asada, 1994). Ascorbate peroxidase, catalase and peroxidase, together with low-molecular mass scavengers such as ascorbate, glutathione and proline, act as the main defense against ROS produced in various parts of plant cells (Apel and Hirt, 2004). The induction of ROS-scavenging enzymes, such as SOD, POXs and CAT, is the most common mechanism for detoxifying ROS synthesized during stress responses (Wojtaszek, 1997; Mittler, 2002). *P. grandiflorum* is a perennial flowering plant belonging to the family Campanulaceae and is grown commercially in East Asia. Roots of *P. grandiflorum* have been used as a traditional oriental medicine and food for bronchial asthma, hepatic fibrosis, bone disorders (Lee *et al.,* 2004; Choi *et al.,* 2009; Jeong *et al.,* 2010; Lee, 1973), hypercholesterolemia and hyperlipidemia (Kim *et al.,* 1995). Roots of *P. grandiflorum* containing triterpenoid saponin, inulin, phytosterin, platycidinin, proteins, lipids, carbohydrates, iron, and fibers are similar to the ginseng roots and are being cultivated for food or medicine material. Recent studies indicate that platycodins are one of the most essential functional components in *P. grandiflorum* in terms of the inhibition of pancreatic lipase (Zhao and Kim, 2004), cholesterol lowering, and antiobesity effects (Zhao *et al.,* 2006). *P. grandiflorum* is well known to affect various pharmacological effects for human health and its consumption is increasing. In order to develop functional products using the physiological functionality, *P. grandiflorum* is needed a mass production of natural materials and the breeding of superior varieties. The creating of giant *P. grandiflorum* by the polyploidy breeding method can maximize its effects. The polyploidy breeding method in plants is a way to increase radically the emergence of new useful traits and the quantity by polyploidy obtained through quantitative doubling of the genome, which is a set of chromosomes. Polyploids, although frequently encounter low seed setting rates or complete sterility (Lewis, 1980), usually show larger organ size and superior cold tolerance (Kato and Birchler, 2006). For medicinal plants, polyploidy may increase the amounts of secondary metabolites (Thao *et al.,* 2003) which functional compounds accumulate in the vegetative parts such as purple coneflower (Gao *et al.,* 1996). So, polyploidy breeding is an effective approach of germplasm development for medicinal plants. Methods using colchinine for polyploidy induction are common for a wide range of plant species (Luckett, 1989; Ishizaka and Uematsu, 1994; Pinheiro *et al.,* 2000; Petersen *et al.,* 2003; liu *et al.,* 2007). Some reports were presented that the tetraploid induction of *P. grandiflorum* by colchicine treatment have also been available for breeding (Kim *et al.,* 2003; Wang *et al.,* 2006; Wu *et al.,* 2011). The present study was focused to evaluate the cytotoxicity, antimicrobial and antioxidant enzyme activity of diploid and tetraploid *P. grandiflorum*.

MATERIALS & METHODS

Plant material

The diploid plant of *Platycodon grandiflorum* was grown in Geumsan county, Chungcheong province and purchased from local market. Tetraploid plants were provided by the cooperative research laboratory of this study, Chungbuk National University. Root samples were freeze-dried, indoor-dried, hot-air dried, and microwave dried and then ground. Each sample powder was stored at -20℃ for experiments.

Induction of tetraploid

Tetraploid mutants were induced in a similar method to the procedure described by Kim *et al.* (2003). The colchicine treatment on seedlings were performed at the time of cotyledon emergence. After the treatment, seedlings were washed 3~4 times with distilled water and planted on 12cm pots containing of equal volume of perlite and coarse sand. Survival rate and chromosome numbers were counted 30 days after transplanting. After 4 months, measurement of stomates and morphological characters were made. To induce polyploids in adult plants, growing points were covered with cotton balls and sprayed with concentration of 0.05% colchicines solution 3 times a day

for 3 days. The ploidy level of *Platycodon grandiflorum* was estimated chromosome counting of root tips from obtained mutants by morphological characteristics.

Cytotoxicity measurement by the MTT assay

The cytotoxicity of *Platycodon grandiflorum* sample was assayed using human cancer cell lines, HeLa for human metrocarcinoma, Calu-6 for human pulmonary carcinoma, MCF-7 for human breast adenocarcinoma, HCT-116 for human colorectal carcinoma, SNU-1066 for human laryngeal squamous cell carcinoma, Hep 3B for human hepatocellular carcinoma and SNU-601 for human gastric 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 plated on 96 well plates at a concentration of 3 x 10^4 cells/mL. The cells were incubated for 24 hrs in RPMI-1640 medium at 37°C under 5% CO_2 in a humidified incubator, and treated with 2 μL of various concentrations (50, 100, 200, 400, and 800 μg mL^{-1}) of extracts. After the incubation for 48 hr, the cells were washed twice with phosphate buffer solution (PBS). MTT solution at 5 mg/mL was dissolved in 1mL of PBS, and 10 μL of it was added to each of the 96 wells. After the reaction for 4 hr, 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 minutes by plate shaker, and the absorbance was recorded using an ELISA reader (Bio-Rad model 550, USA) at a wavelength 540 nm. The viability of the treatment was determined as percentage of viability compared to untreated cell, and the values were then used to iteratively calculate the concentration of plant extracts required to cause a 50% reduction (IC_{50}) in growth for each cell line.

Antimicrobial screening test on the bronchus disease bacteria

Strains and media

For the purpose of antimicrobial evaluation, 2 g of positive bacteria, and 4 g of negative bacteria were employed. These microorganisms were purchased from the Korean Collection for Type Culture (KCTC, Daejeon, Korea) and cultured in nutrient agar. Table 1 presents the test microorganisms and culture media.

Agar diffusion method

The effects of *P. grandiflorum* extracts on the bronchus disease bacteria (*Corynebacterium diphtheria*, *Klebsiella pneumoniae subsp. Pneumonia* and *Streptococcus pyogenes*) were evaluated using the agar diffusion method. Inocula of approximately 10^7 CFU were inoculated onto the surface of pre-dried agar. Sterile 8-mm filter paper discs were placed on the plates and impregnated with 40 μL of sample extract. After allowing 1 h at room temperature for the extracts to facilitate diffusion across the surface, the plates were incubated at 37℃ for 24 h for the bacteria. The antimicrobial activity was measured as the size of the clear zone of growth inhibition. The kanamycin was used as the control.

Assay of antioxidant enzyme on fraction extracts

SOD activity

The superoxide dismutase (SOD) activity was measured using SOD assay Kit-WST purchased from Sigma-Aldrich (Sigma-Aldrich Co., Japan). This assay is based on the colorimetric assay for the measurement of total antioxidant capacity of crude aqueous fractions. The 60 μL of sample solution (sample and blank2) or doubledistilled water (blank1 and blank3) was mixed with 600 μL of WST working solution. For Blank2 and Blank3, 60 μL of dilution buffer was added. Then, 60 μL of enzyme working solution was added to each

Table 1. List of strains and cultivation conditions used for screening of antimicrobial activity test.

Strains	Cultivation conditions
Corynebacterium diphtheriae (KCCM 40413)	37℃, Trypticase Soy Agar(BBL211043) with 5% defibrinated Sheep blood
Klebsiella pneumoniae subsp. pneumoniae (KCCM 41285)	37℃, Nutrient Agar
Streptococcus pygogenes (KCCM 11817)	37℃, Trypticase Soy Agar(BBL211043) with 5% defibrinated sheep blood

sample and blank1. The plate was incubated at 37℃ for 20 min, and the OD (Optical density) was determined at 450 nm using a spectrophotometer (Biochrom Co., England). SOD activity (inhibition rate percent) was calculated using the following equation:

$$\text{SOD activity}=\{[(\text{Ablank1}-\text{Ablank3})-(\text{Asample}-\text{Ablank2})]/(\text{Ablank 1}-\text{Ablank 3})\}\times 100.$$

CAT activity

Catalase (CAT) activity was assayed by the method of Mishra *et al*. (1993). The reaction mixture contained 50 mM potassium phosphate buffer (pH 7.0), 11 mM H_2O_2, and the crude enzyme extract. The reaction was initiated by addition of H_2O_2 to the mixture, and enzyme activity was determined by monitoring the decline in absorbance at 240 nm (ε=36 M$-$1 cm$-$1), because of H_2O_2 consumption.

APX activity

Ascorbate peroxidase (APX) activity was determined by monitoring the decline of absorbance at 290 nm as ascorbate (ε =2.8 mM^{-1} cm^{-1}) was oxidized, by the method of Chen and Asada (1989). The reaction mixture contained 100 mM potassium phosphate buffer (pH 7.5), 0.5 mM ascorbate, and 0.2 mM H_2O_2.

POX activity

Peroxidase (POX) activity was determined specifically with guaiacol at 470 nm (ε=26.6mM$-$1 cm$-$1), following the method of Egley *et al*. (1983). The reaction mixture contained 40 mM potassium phosphate buffer (pH 6.9), 1.5 mM guaiacol, and 6.5 mM H_2O_2 in 1 ml with crude enzyme extract. Control assays in which the enzyme extracts or substrates were replaced by buffer were performed.

Data analysis

The statistical analysis was performed using the procedures of the Statistical Analysis System. A ANOVA procedure followed by Duncan test was used to determine the significant difference ($p<0.05$) between treatment means.

RESULTS & DISCUSSION

Cytotoxicity

The cytotoxicity of *P. grandiflorum* on seven human cancer cell lines were evaluated by the MTT assay. When cells were treated for 48 hrs with various concentrations (50, 100, 200, 400 and 800 μg/mL) of methanol extracts, the rate of cell survival progressively decreased in a dose-dependent manner. Results of the cytotoxicity evaluation against human cancer cell lines from roots of *P. grandiflorum* are shown in Table 2. Overall, the cytotoxicity of methanol extracts of *P. grandiflorum* showed significant differences between tetraploid and diploid. However, the cytotoxic effect against human cancer cell was higher in tetraploid than in diploid. The extract of tetraploid at 200 μg/mL exhibited a pronounced cytotoxic effect (20.02%) on HCT-116 cell compare to that of diploid (60.45%). At all extracts concentration, tetraploid samples showed high toxicity and the IC_{50} (concentration causing 50% cell death) value showed the highest on HCT-116 cell (105.91 μg/mL), and exhibited significant activity against the Hep 3B cell (140.67 μg/mL), SNU-1066 cell (154.01 μg/mL), Hela cell (158.37 μg/mL), SNU-601 cell (182.67 μg/mL), Calu-6 cell (190.42 μg/mL), MCF-7 cell (510.19 μg/mL). The values of IC_{50} in diploid samples showed the highest on SNU-1066 cell (239.34 μg/mL), followed by 295.17 μg/mL on Hela cell, 309.12 μg/mL on HCT-116 cell, 393.17 μg/mL on Hep 3B cell, 432.25 μg/mL on SNU-601 cell. On the contrary, the extracts on MCF-7 cell and Calu-6 cell exhibited the weakest inhibition on cell viability, having an IC_{50} value of over 800 μg/mL against MCF-7 cell (831.91 μg/mL) and Calu-6 cell (947.64 μg/mL). The persistency search for new anticancer compounds in plant medicine and traditional foods is a realistic and promising strategy for its prevention. Numerous compounds found in plants with anticancer properties are such as alkaloids, phenylpropanoids, and terpenoids (Kintzios, 2006; Park *et al.,* 2008; Yan-Wei *et al.,* 2009; Vijayarathna and Sasidharan, 2012). Presently there is an increasing interest world wide in herbal medicines accompanied by an increased laboratory investigation into the pharmacological properties of the bioactive ingredients and their ability to treat various diseases (Lobo *et al.,* 2009). It is well known that chemicals and medicinal plant medicines, may produce toxic effects. Based on the results presented in this paper, tetraploid *P. grandiflorum* can be used

Table 2. Cytotoxicity of extracts on seven human cancer cell lines of diploid and tetraploid in *Platycodon gradinflorum*.

Plant	Cell line	Cell viability, % of control					
		Concentration (μg/mL)					
		50	100	200	400	800	IC_{50}
Diploid	SNU-601	80.45±2.13^{c}	78.23±3.31^{b}	70.77±5.83^{b}	64.64±2.59^{c}	11.39±1.55^{cd}	432.25
	SNU-1066	104.82±7.14^{a}	102.32±5.79^{a}	77.23±8.08^{ab}	26.99±3.44^{d}	5.91±0.48^{d}	239.34
	MCF-7	90.39±3.11^{abc}	82.66±4.83^{b}	77.01±3.05^{ab}	75.05±1.06^{b}	50.34±2.65^{b}	831.91
	HCT-116	75.75±8.81^{c}	68.80±4.97^{b}	60.45±5.09^{b}	34.18±3.96^{d}	10.50±2.18^{d}	309.12
	Calu-6	106.24±5.29^{a}	104.65±8.22^{a}	94.32±6.78^{a}	89.98±3.14^{a}	57.60±4.34^{a}	947.64
	Hep 3B	99.60±5.39^{ab}	74.12±8.86^{b}	70.77±8.59^{b}	30.50±4.26^{d}	17.50±1.08^{c}	393.17
	Hela	84.43±6.86^{bc}	70.94±5.90^{b}	63.88±7.67^{b}	16.65±2.05^{e}	6.22±0.35^{d}	295.17
Tetraploid	SNU-601	100.23±9.51^{a}	94.40±9.06^{a}	49.13±5.32^{b}	16.03±2.34^{c}	5.71±0.29^{d}	182.67
	SNU-1066	102.01±5.29^{a}	82.53±7.10^{ab}	31.58±2.74^{d}	8.21±0.73^{bc}	7.00±0.39^{c}	154.01
	MCF-7	96.74±5.91^{ab}	94.86±5.01^{a}	90.66±7.69^{a}	55.51±6.11^{a}	20.21±3.99^{a}	510.19
	HCT-116	68.65±3.23^{c}	61.05±9.01^{b}	20.02±2.83^{d}	4.76±0.67^{c}	3.55±0.22^{c}	105.91
	Calu-6	79.94±3.42^{bc}	75.02±8.80^{ab}	69.56±4.37^{b}	13.51±2.64^{b}	3.66±0.21^{c}	190.42
	Hep 3B	86.48±6.78^{abc}	77.29±4.21^{ab}	22.56±1.39^{d}	15.11±2.34^{b}	12.82±2.10^{b}	140.67
	Hela	96.05±4.81^{ab}	91.24±9.83^{a}	33.95±5.82^{d}	8.18±1.00^{bc}	5.88±0.36^{c}	158.37

[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. SNU-601: human gastric carcinoma, SNU-1066: human laryngeal squamous cell carcinoma, MCF-7: human breast adenocarcinoma, HCT-116: human colorectal carcinoma, Calu-6: human pulmonary carcinoma, Hep 3B: human hepatocellular carcinoma, Hela: human metrocarcinoma.

as a source of cytotoxic agent.

Antimicrobial activity

The antimicrobial activity of methanolic extracts of roots of *P. grandiflorum* was assayed *in vitro* by agar disc diffusion method against three different bronchus diseases bacterial strains (*Corynebacterium diphtheria*, *Klebsiella pneumoniae subsp. Pneumonia* and *Streptococcus pyogenes*). The comparative analysis results are shown in Fig. 1 and Table 3. Antimicrobial activities of diploid *P. grandiflorum* were relatively low compared to tetraploid *P. grandiflorum* on most of bacterial strains. In tetraploid *P. grandiflorum*, *K. pneumoniae* showed the clear zone formation (18~19 mm) of growth inhibition, followed the clear zone formation of 13~15 mm on *C. diphtheria* and *S. pyogenes*. The antimicrobial activities in diploid *P. grandiflorum* was the highest on *K. pneumonia* (14~15 mm), and showed the clear zone formation of 11~12 mm on *C. diphtheria* and 12~13 mm on *S. pyogenes*. The antimicrobial activity is thought to look different depending on the bacterial strains and the polyploidy of *P. grandiflorum*. It was reported that the antimicrobial activities against bronchus diseases bacterial strains of extracts from ethyl ether and petroleum ether of diploid *P. grandiflorum* were high (Lee *et al.,* 2000). Currently a wide range of natural substances known to have antimicrobial activity, and also active research underway thereto (Chan *et al.,* 2008; Goveas and Abraham, 2013; Modaressi *et al.,* 2013), but few studies related to the antimicrobial efficacy against bronchus diseases and the application of the tetraploid *P. grandiflorum* has been done. These results indicate that the tetraploid *P. grandiflorum* can be used for developing antimicrobials which can act against bronchus diseases bacterial strains.

Antioxidant enzyme activity

Freeze-dried samples of *P. grandiflorum* were extracted in methanol at room temperature and fractionated into five different solvents (hexane, methylene chloride, ethyl acetate, butyl alcohol, and water). This study shows that the plants induced polyploidy mutants can be achieved the activities of SOD, CAT, APX, and POD. The comparative results of the

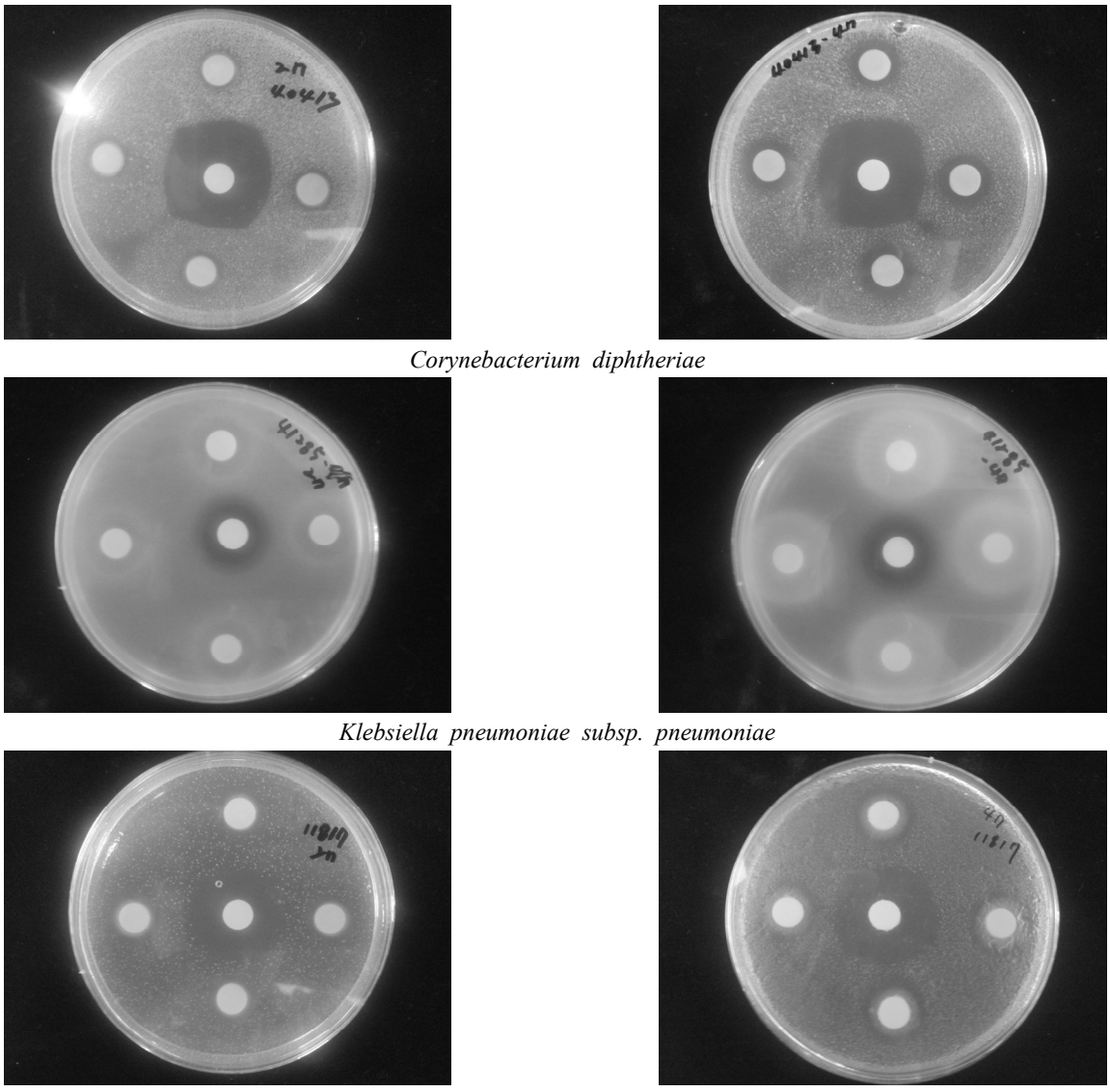

Fig. 1. Inhibition activity of *Platycodon gradinflorum* against three microorganisms in paper disc diffusion assay. A: Diploid of *Platycodon gradinflorum*, B: Tetraploid of *Platycodon gradinflorum*.

Table 3. Antimicrobial activity of *Platycodon gradinflorum* extracts on the bronchus diseases bacteria

P. gradinflorum extract	Clear zone on plate (mm)		
	C. diphtheria	*K. pneumonia*	*S. pygogenes*
Diploid	11~12	14~15	12~13
Tetraploid	13~15	18~19	13~15

antioxidant enzyme activity in fraction extracts of diploid and tetraploid in *P. grandiflorum* plants are shown in Fig. 2. The root extract of *P. grandiflorum* had the highest (97.2%) SOD enzyme activity in ethyl acetate partition layer of tetraploid and while water partition layer of diploid showed the lowest (48.6%) SOD enzyme activity. The activity of CAT showed higher values in the root of tetraploid than in the diploid of *P. grandiflorum* in all partition layers except butyl alcohol. The

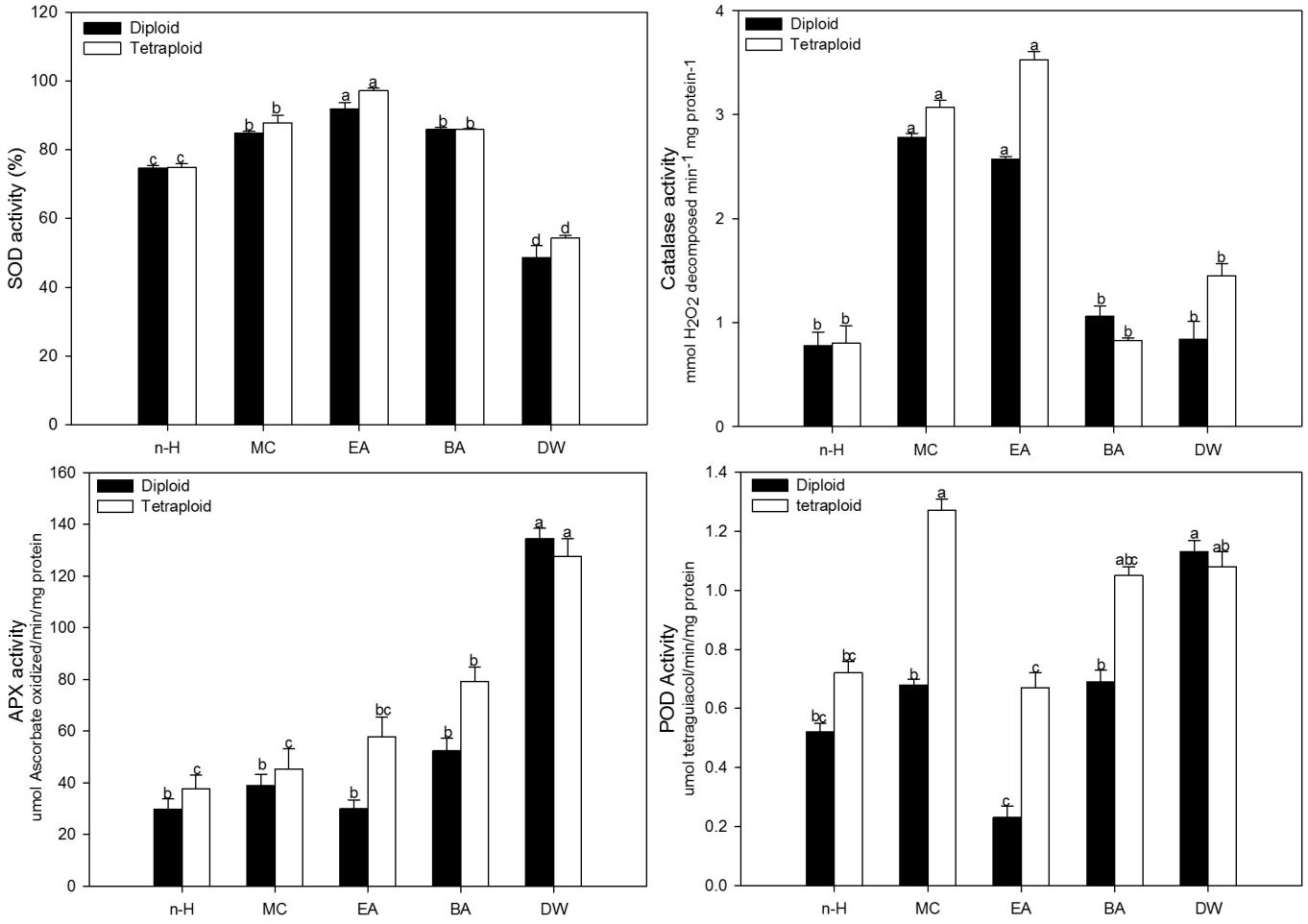

Fig. 2. Antioxidant enzyme(SOD, CAT, APX and POD) activities according to extract solvent fractions of diploid and tetraploid in *Platycodon grandiflorum*. The bars represent the standard error. n-H: n-hexane, MC: methylene chloride, EA: ethyl acetate, BA: butyl alcohol, DW: distilled water.

activities of APX and POD showed higher values in the root of tetraploid than in the diploid of *P. grandiflorum* in all fraction solvents except water layer (Fig. 2). Significant roles of POD have been suggested in plant development processes (Gaspar *et al.,* 1985), which was involved in scavenging of H_2O_2 produced in chloroplasts (Asish and Anath, 2005). The antioxidant enzyme activities differ significantly in different plants. The SOD is one of the enzymes, in vivo, to catalyze the reaction that converts the harmful reduced oxygen formed in cell due to rancidity into hydrogen peroxide; is generated in most aerobic or anaerobic biological organisms; is switched to water and oxygen by the CAT and APX, and loses then its toxicity. APX activity, which is an important component of the antioxidant system, plays a key role in eliminating H_2O_2 molecules and in the modulation of its steady-state levels in various plant subcellular compartments (Najami *et al.,* 2008). The CAT is also an antioxidant enzyme that protects cells by dispatching of in vivo harmful oxygen and is a typical enzyme that acts to decompose and scavenge the H_2O_2 together with APX. We consider that relatively high antioxidant enzyme activity in the tetraploid of *P. grandiflorum* is influenced by providing some stress on the plant under forced polyploidy induction (Boo *et al.,* 2013). The antioxidant enzymes, indicating a high activity to remove harmful free radicals, have the effect of prevention and inhibition of various diseases and aging. In the tetraploid of *P. grandiflorum*, we can also expect to see these benefits for the next variety of natural foods and cosmetics where the need to apply functional substances may also be required. In conclusion, to use of tetraploid *P. grandiflorum* in healthy functional foods and medicinal industry with regard to its high

antioxidant potentials, it is suggested that complement experiments is required to accomplish on fraction extracts of *P. grandiflorum.*

ACKNOWLEDGEMENT

This research was supported by High Value-added Food Technology Development Program of IPET, Ministry of Agriculture, Food and Rural Affairs, Republic of Korea.

REFERENCES

Apel, K. and H. Hirt. 2004. Reactive oxygen species: Metabolism, oxidative stress, and signal transduction. Annu. Rev. Plant Biol. 5 : 373-99.

Asada, K. 1994. Production and action of active oxygen species in photosynthetic tissues. *In* Foyer C.H., P.M. Mullineaux (eds.), Causes of Photooxidative Stress and Amelioration of Defense Systems in Plants. CRC Press, London, England. pp.77-105.

Asish, K. P. and B. D. Anath. 2005. Salt tolerance and salinity effects on plants. A review Ecotoxicol. Environ. Saf. 60 : 324-349.

Boo, H. O., J. H. Shin, Y. S. Kim, H. J. Park, H. H. Kim, S. J. Kwon, and S. H. Woo. 2013. Comparative Antioxidant Enzyme Activity of Diploid and Tetraploid *Platycodon grandiflorum* by Different Drying Methods. Korean J. Plant Res. 26(3) : 389-396.

Chan, L. W., E. L. C. Cheah, C. L. L. Saw, W. Weng, and P. W. Heng. 2008. Antimicrobial and antioxidant activities of *Cortex Magnoliae Officinalis* and some other medicinal plants commonly used in South-East Asia. Chinese Medicine. 3 : 15.

Choi, J. H., Y. P. Hwang, H. S. Lee, and H. G. Jeong. 2009. Inhibitory effect of Platycodi Radix on ovalbumin-induced airway inflammation in a murine model of asthma. Food Chem. Toxicol. 47 : 1272-1279.

Cuyacot, A. R., J. J. M. Mahilum, and M. R. S. B. Madamba. 2014. Cytotoxicity potentials of some medicinal plants in Mindanao, Philippines. Pelagia Research Library. 4(1) : 81-89.

Gao, S. L., D. N. Zhu, Z. H. Cai, and D. R. Xu. 1996. Autotetraploid plants from colchicine-treated bud culture of Salvia miltior-rhiza Bge. Plant Cell, Tissue Org. Cult. 47(1) : 73-77.

Goveas, S. W. and A. Abraham. 2013. Evaluation of antimicrobial and antioxidant activity of stem and leaf extracts of *Coscinium fenestratum*. Asian Journal of Pharmaceutical and Clinical Research. 6(3) : 218-221.

Gaspar, T., C. Penel, F. J. Castillo, and H. Greppin. 1985. A two step control of basic and acid peroxidases and its significance for growth and development. Plant Physiol. 64 : 418-423.

Ishizaka, H. and J. Uematau. 1994. Amphidiploids between *Cyclamen persicum* Mill . and *C. hederifolium* Aiton induced through colchicine treatment of ovules *in vitro* and plants. Breed. Sci. 44 : 161-166.

Jeong, H. M., E. H. Han, Y. H. Jin, Y. P. Hwang, H. G. Kim, B. H. Park, J. Y. Kim, Y. C. Chung, K. Y. Lee, and H. G. Jeong. 2010. Saponins from the roots of *Platycodon grandiflorum* stimulate osteoblast differentiation via p38 MAPK- and ERK-dependent RUNX2 activation. Food Chem. Toxicol. 48 : 3362-3368.

Kang, H. M. and M. E. Saltveit. 2002. Chilling tolerance of maize, cucumber and rice seedling leaves and roots are differentially affected by salicylic acid. Physiol. Plant. 115 : 571-576.

Kata, A. and J. A. Birchler. 2006. Induction of tetraploid derivatives of maize inbred lines by nitrous oxide gas treatment. J. Hered. 97(1) : 39-44.

Kim, I. K., H. H. Kim, E. Y. Hong, J. S. Yun, T. Yun, J. K. Hwang, and C. H. Lee. 2003. Breeding of tetraploid in *Platycodon grandiflorum* (Jacq.) A. DC. by colchicine treatment. Korean J. Plant Res. 6(3) : 188-194.

Kim, K. S., O. Ezaki, S. Ikemoto, and H. Itakura. 1995. Effects of *Platycodon grandiflorum* feeding on serum and liver lipid concentrations in rats with diet-induced hyperlipidemia. J. Nutr. Sci. Vitaminol. 41 : 485-491.

Kintzios, E. 2006. Terrestrial plant-derived anticancer agents and plant species used in anticancer research. Crit. Rev. Plant Sci. 25 : 79-113.

Lee, E. B. 1973. Pharmacological studies on *Platycodon grandiflorum* A. DC. IV. A comparison of experimental pharmacological effects of crude platycodin with clinical indications of Platycodi Radix. Yakugaku Zasshi. 93 : 1188-1194.

Lee, K. J., J. Y. Kim, K. S. Jung, C. Y. Choi, Y. C. Chung, D. H. Kim, and H. G. Jeong. 2004. Suppressive effects of *Platycodon grandiflorum* on the progress of carbon tetrachloride-induced hepatic fibrosis. Arch. Pharm. Res. 27 : 1238-1244.

Lewis, W. H. 1980. Ploidy: Biological Relevance. Plenum, NewYork, USA.

Liu, G., Z. Li, and M. Bao. 2007. Colchicine-induced chromosome doubling in *Platanus acerifolia* and its effect on plant morphology. Euphytica 157(1-2) : 145-154.

Lobo, R., K. S. Prabhu, and A. Shirwaikar. 2009. *Curcuma zedoaria* Rosc. (white turmeric): a review of its chemical, pharmacological and ethnomedicinal properties. J. Pharm. Pharmacol. 61 : 13-21.

Luckett, D. J. 1989. Colchicine mutagenesis is associated with substantial heritable variation in cotton. Euphytica 42(1-2) : 177-182.

Mittler, R. 2002. Oxidative stress, antioxidants and stress tolerance. Trends Plant Sci. 7 : 405-410.

Moftah, A. H. and B. E. Michel. 1987. The effect of sodium chloride on solute potential and proline accumulation in soybean leaves. Plant Physiol. 83 : 238-240.

Modaressi, M., R. Shahsavari, F. Ahmadi, M. Rahimi-Nasrabadi,

R. Abiri, A. Mikaeli, and H. Batoli. 2013. The evaluation of antibacterial, antifungal and antioxidant activity of methanolic extract of *Mindium Laevigatum* (Vent.) Rech. F., from central part of Iran. Jundishapur J. Nat. Pharm. Prod. 8(1) : 34-40.

Najami, N., J. Tibor, W. Barriah, G. Kayam, T. Moshe, M. Guy, and M. Volokita. 2008. Ascorbate peroxidase gene family in tomato: its identification and characterization. Mol. Genet. Genom. 279 : 171-182.

Park, H. J., M. J. Kim, E. Ha, J. H. Chung. 2008. Apoptotic effect of hesperidin through caspase 3 activation in human colon cancer cells, SNU-C4. Phytomedicine. 15 : 147-151.

Petersen, K. K., P. Hagberg, and K. Kristiansenk. 2003. Colchicine and oryzalin mediated chromosome doubling in different genotypes of Miscanthus sinensis. Plant Cell, Tissue and Organ Culture. 73(2) : 137-146.

Pinheiro, A. A., M. T. Pozzobon, C. B. Do Valle, M. I. O. Penteado, and V. T. C. Carneiro. 2000. Duplication of the chromosome number of diploid Brachiaria brizantha plants using colchicine. Plant Cell Reports 19(3) : 274-278.

Thao, N. T. P., K. Ureshino, I. Miyajima, Y. Ozaki, and H. Okubo. 2003. Induction of tetraploids in ornamental Alocasia through colchicine and oryzalin treatments. Plant Cell, Tiss. Org. Cult. 72 : 19-25.

Vijayarathna, S. and S. Sasidharan. 2012. Cytotoxicity of methanol extracts of *Elaeis guineensis* on MCF-7 and Vero cell lines. Asian Pac. J. Trop Biomed. 2(10) : 826-829.

Wang, X. H., L. Xiong, Y. H. Qu, J. Yang, and Z. J. Gu. 2006. The polyploidy induction and identification of *Platycodon grandiflorus* (Campanulaceae) in China. Acta Botanica Yunnanica 28(6) : 593-598.

Wojtaszek, P. 1997. Oxidative burst: an early plant response to pathogen infection. Biochem. J. 322 : 681-692.

Wu, Y., F. Yang, X. Zhao, and W. Yang. 2011. Identification of tetraploid mutants of *Platycodon grandiflorus* by colchicines induction. Caryologia. 64(3) : 343-349.

Yan-Wei, H., L. Chun-Yu, D. Chong-Min, W. Wen-Qian, and G. Zhen-Lun. 2009. Induction of apoptosis in human hepatocarcinoma SMMC-7721 cells *in vitro* by flavonoids from Astragalus complanatus. J. Ethnopharmacol. 123 : 293-301.

Zhao, H. L., K. H. Cho, Y. W. Ha, T. S. Jeong, W. S. Lee, and Y. S. Kim. 2006. Cholesterol lowering effect of platycodin D in hyper cholesterolemic ICR mice. European journal of pharmacology. 537 : 166-173.

Zhao, H. L. and Y. S. Kim. 2004. Determination of the kinetic properties of platycodin D for the inhibition of pancreatic lipase using a 1, 2 − diglyceride based colorimetric assay. Archives of pharmacal research. 27 : 1048-1052.

Zhou, B. Y., Z. F. Guo, and Z. L. Liu. 2005. Effects of abscisic acid on antioxidant systems of Stylosanthes guianensis (Aublet) Sw. under chilling stress. Crop Science. 45 : 599-605.