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# Evaluation of Cancer Chemopreventive Potential of Various Grape Shoot Extracts and Refined Materials Using *in vitro* Bioassay Systems

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**Abstract** – Since reactive oxygen species, prostaglandins, and nitric oxide are closely involved in various pathological conditions and play important roles in the initiation, promotion, and progression of carcinogenesis, agents that modulate the production or activity of them might be considered as cancer chemopreventive agents. In the present study, we evaluated chemopreventive potential of some grape shoot extracts and their refined materials using various *in vitro* assay systems. As a result, both grape shoot extracts and refined materials possessed effective radical scavenging activities about 70~80% at the concentration of 500 μg/ml, and especially, the Sheridan shoot extract showed the most potent 1,1-diphenyl-2-picrylhydrazyl radical scavenging activity that was similar to resveratrol. In addition, refined materials from grape shoot extracts suppressed lipopolysaccharide-induced nitric oxide production in macrophage cells, and refined materials from Kyoho and Campbell shoot extracts exhibited similar inhibitory activities with $IC_{50}$ value of 22.4 μg/ml and 28.5 μg/ml, respectively. In addition, at the concentration of 50 μg/ml, all of refined materials inhibited cell proliferation against various human cancer cells about 30~40% compared to control. These findings suggest that grape shoot extract and their refined materials might be useful sources for the development of chemopreventive agents and/or functional foods.

**Keywords** ☐ grape shoot extracts, DPPH free radical scavenging activity, nitric oxide production, cancer cell growth inhibition

## INTRODUCTION

Cancer chemoprevention is defined as the use of non-toxic bioactive compounds derived from natural products or synthetic molecules to retard, reverse, or suppress the process of carcinogenesis (Morse and Stoner, 1993; Surh, 1999). It has been known that carcinogenesis is a multi-step process that progress gradually for over twenty years, and it may have limitations to the treatment of cancer, especially at a late stage. In this respect, chemoprevention have been regarded as a novel strategy to control, and ultimately, overcome cancer.

Chemopreventive agents can affect in each step of carcinogenesis with unique mechanism of action to modulate related biomarkers. Recent studies suggest that reactive oxygen species (ROS), prostaglandins (PGs), and nitric oxide (NO) play important roles in the process of carcinogenesis (Dreher and Junod, 1998; Dubois *et al*, 1998; Dannenberg *et al.*, 2001; Kröncke *et al.*, 1998). Although these molecules have been known to mediate tissue homeostasis and cellular signal transduction (Lander, 1997), they can also participate in various pathological conditions. For example, sustained damages of DNA, cell membrane, and protein, which are caused by ROS, can initiate and promote the transformation of normal cells to cancerous cells (Dreher and Junod, 1998). In addition, overexpression of inducible isoforms of cyclooxygenase (COX-2) and nitric oxide synthase (iNOS) can produce PGs and NO excessively, and over-produced NO and PGs can be important mediators to promote and progress carcinogenesis as well as to provoke chronic inflammatory responses (Dannenberg *et al.*, 2001; Kröncke *et al.*, 1998). Thus, agents that modulate the production or activity of ROS, NO, and PGs, including free radical scavengers and inhibitors of COX-2 or iNOS, might be considered as cancer chemopreventive agents.

It has been known that natural products have served as a source of lead compounds for developing new therapeutic agents. Many efforts are also in progress to develop cancer chemopreventive agents from natural products and dietary sub-

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stances, which isolate active principles or synthesize compounds by the modification of compounds from natural products, and evaluate their biological activities using various *in vitro* assay systems (Kinghorn *et al.*, 1998; Lee *et al.*, 1998; Gerhauser *et al.*, 2002; Rimando *et al.*, 2002). Recently, naturally occurring compounds, such as polyphenols, isoflavones, curcumin, sulforaphane, and resveratrol, have been shown to possess cancer chemopreventive activities (Surh, 1999; Kelloff *et al.*, 2000; Kwak *et al.*, 2001; Lambert and Yang, 2003). Among them, resveratrol is a phytoalexin that occurs in grapes and roots of *Polygonum* species (Fremont, 2000). Resveratrol shows various biological properties, including antioxidant effects such as free radical scavenging and inhibition of lipid peroxidation, anti-inflammatory activities through inhibition of COX and/or iNOS, anti-proliferative effects against various human cancer cells, and cancer chemopreventive activities (Jang *et al.*, 1997; Martinez and Moreno, 2000; Gusman *et al.*, 2001; Joe *et al.*, 2002). Therefore, resveratrol may be a lead principle for developing new therapeutics, preventive agents, and functional foods. However, it is difficult to obtain large amount of resveratrol from grapes themselves because of low yield and time-consuming extraction processes. In accordance with this respect, many studies are underway to develop more effective processes for which are able to produce and extract larger amount of resveratrol from grapes.

Previously we reported antioxidant and anti-inflammatory potential of some grape extracts (Min *et al.*, 2003). On the basis of this, in this study, we further developed a refining process of grape shoot extracts, and evaluated chemopreventive potential of various grape shoot extracts and their refined materials with antioxidant, anti-inflammation, and growth-inhibition against human cancer cells.

## MATERIALS and METHODS

### Chemicals

Dulbecco's modified Eagle medium (DMEM), minimun essential medium with Earle's salt (MEME), Roswell Park Memorial Institute medium 1640 (RPMI medium 1640), fetal bovine serum (FBS), non-essential amino acid solution, sodium pyruvate, L-glutamine, antibiotics-antimycotics solution, and trypsin-EDTA were purchased from Invitrogen Co. (Grand Island, NY, USA). 1,1-Diphenyl-2-picrylhydrazyl (DPPH), vitamin C, gallic acid, lipopolysaccharide (LPS, *E. coli* 0111: B4), N-(1-naphthyl)ethylnenediamine dihydrochloride, sulfanilamide, sodium nitrite, bovine serum albumin, sulforhodamine B, and 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) were from Sigma (St. Louis, MO, USA). $N^G$-monomethyl-L-arginine (L-NMMA), prostaglandin $E_2$ ($PGE_2$), and $PGE_2$-acetylcholinesterase tracer ($PGE_2$-AchE tracer) were from Cayman Chemical Co. (Ann Arbor, MI, USA). Anti-$PGE_2$ antibody was kindly provided from Pacific Corporation R&D Center (Kyounggi, Korea).

### Cell culture

Murine macrophage RAW 264.7 cells, human lung carcinoma (A549), colorectal carcinoma (HCT 116), colorectal adenocarcinoma (HT-29), fibrosarcoma (HT-1080), and stomach adenocarcinoma (SNU-638) cells were obtained from American Type Culture Collection (ATCC, Manassas, VA, USA) or Korean Cell Line Bank (KCLB, Seoul, Korea). RAW 264.7 and HT-1080 cells were cultured in DMEM. HCT 116, HT-29, and SNU-638 cells were maintained in RPMI medium 1640, and A549 cells were cultured in MEME. All media were supplemented with 10% heat-inactivated FBS and antibiotics. Cells were incubated at 37°C, 5% $CO_2$ in the humidified air.

### Preparation of test materials

Dried Campbell, Kyoho, and Sheridan grape shoots were pulverized, and then mixed solvent (ethanol : $H_2O$ = 8 : 2) was added to each pulverized shoot in the ratio of 8 g of shoots with 1 L of solvent. The suspension was sonicated for 10 min and filtered off non-extractable materials using Whatman No. 2 filter papers. The filtrate was centrifuged at 10,000 g for 15 min, and the supernatant was collected and then concentrated in a decompressed condition at 40°C. Extracts were dried in the oven for 4 hr, weighed, and then stored at a low temperature. For preparing refined materials, 1 g of extracts from grape shoots was added to 30 g of water and mixed well at room temperature. The mixture was centrifuged at 10,000 g for 15 min, and then non-extractable materials were collected. Thereafter, the refined materials were dried and stored at a low temperature.

### Evaluation of the antioxidant potential of test materials

The antioxidant properties of test materials were evaluated by 1,1-diphenyl-2-picrylhydrazyl (DPPH) free radical scavenging activity (Lee *et al.*, 1998). Test materials, dissolved in DMSO, were incubated with 300 μM of DPPH ethanol solution at 37°C for 30 min in 96 well plates. The absorbance was measured at 515 nm. % Inhibition, the degree of radical scavenging by test groups, was determined by comparison with vehicle-treated control group. $IC_{50}$ values, which denote the

concentration of test samples for bringing to scavenge 50% of DPPH radicals, were calculated using non-linear regression analysis (% inhibition versus concentration). Vitamin C and gallic acid were used as positive controls.

### Nitrite assay

To evaluate the inhibitory activity of test materials on LPS-induced NO production, RAW 264.7 cells in 10% FBS-DMEM without phenol red were plated in 24 well plates ($5 \times 10^5$ cells/ml), and incubated for 24 hr. After incubation, cells were washed with PBS, replaced with fresh media, and then incubated with 1 μg/ml of LPS in a presence or absence of test samples. After additional 20 hr incubation, the media were collected and analyzed for nitrite accumulation as an indicator of NO production by the Griess reaction (Green *et al.*, 1982). Briefly, 180 μl of Griess reagents (0.1% *N*-(1-naphthyl)ethylenediamine dihydrochloride in $H_2O$ and 1% sulfanilamide in 5% $H_3PO_4$) were added to 100 μl of each supernatant from LPS or sample-treated cells in 96 well plates. The absorbance was measured at 540 nm using microplate reader (Bio-Rad Laboratories, Hercules, CA, USA), and nitrite concentration was determined by comparison with a sodium nitrite standard curve. % Inhibition was expressed as [1-(NO level of test samples/NO levels of vehicle-treated control)]×100. The $IC_{50}$ value, the sample concentration resulting in 50% inhibition of NO production, was determined using non-linear regression analysis (% inhibition versus concentration). $N^G$-monomethyl-L-arginine (L-NMMA) was used as a positive control.

### Measurement of cell viability (MTT assay)

To examine whether the inhibitory effects of test samples on LPS-induced NO production were related to their cytotoxicity, cell viability was determined directly by the mitochondrial-dependent reduction of MTT to formazan (Alley *et al.*, 1988) in this assay system. After Griess reaction, MTT solution was added to the media (final 500 μg/ml) and then incubated at 37 °C for 4 hr. The media were discarded, 1 ml of DMSO was added each well to dissolve the formazan, and then absorbance was measured at 570 nm.

### Measurement of the inhibitory activity of test samples on LPS-induced $PGE_2$ production

RAW 264.7 cells ($5 \times 10^5$ cells/ml) were seeded and incubated in 96 well plates for 24 hr. Cells were washed with PBS, and then replaced with fresh medium containing 1 μg/ml of LPS with or without test materials. After 20 hr, the supernatants were collected, and the amount of $PGE_2$ in this media was measured by enzyme-linked immunosorbent assay as follows. Briefly, the supernatants were incubated with $PGE_2$-AchE tracer in 96 well plates coated with $PGE_2$ antibody for more than 18 hr. Plates were washed with PBST (PBS with 0.05% Tween-20) five times, and then incubated with Ellmann's reagent for 5 hr. The absorbance was measured at 405 nm. $PGE_2$ levels in the supernatants of test groups were determined using a $PGE_2$ standard curve, and compared with those of vehicle-treated control group.

### Evaluation of growth-inhibitory activities of test samples against various human cancer cells

The growth-inhibitory potential of test materials against human cancer cells was examined by sulforhodamine B (SRB) assay. Various human cancer cells ($3\text{~}5 \times 10^4$ cells/ml) were incubated with test materials in 96 well plates for 72 hr. For zero-day control, cells were incubated for 30 min at 37°C in $CO_2$ incubator. After indicated incubation time, cells were fixed by the addition of cold 50% trichloroacetic acid (TCA) solution in each well (final 10% TCA solution), incubated for 30~60 min at 4°C, washed with tap water five times, and then dried in the air. The fixed cells were stained with 0.4% SRB solution (0.4% w/v SRB in 1% acetic acid solution) for 30~60 min. Unbound SRB solution was removed by washing with 1% acetic acid solution five times, and plates were dried again in the air. The bound dye was dissolved in 10 mM Tris solution (pH 10.0), and the absorbance was measured at 515 nm. Absorbance data from all groups were averaged, and that of zero-day control group was subtracted from those of each test group. % Survival was calculated compared with vehicle-treated control group.

## Results and Discussion

It has been reported that ROS and pro-inflammatory mediators such as PGs and NO are closely implicated in various pathological processes, including atherosclerosis, several degenerative diseases, and cancer (Ames *et al.*, 1993; Patel *et al.*, 2000). Thus, the process that inhibits generation of free radicals, production of PGs and NO, and/or expression of COX-2 and iNOS might be a strategy for the treatment and prevention of many human diseases such as neurodegenerative diseases and cancer.

Natural products have been received much attention to be a source of lead candidates for developing new therapeutic or

preventive agents. Recent studies suggest that naturally occurring substances, which include polyphenols, stilbenoids, and organosulfur compounds, possess various pharmacological properties including prevention of cancer (Surh, 1999; Kelloff *et al.*, 2000). The biological mechanism of these substances is varying, which includes scavenging free radicals, suppressing pro-inflammatory processes, and inhibiting proliferation against human cancer cells. Especially, according to the protective effect of red wine against cardiovascular diseases, resveratrol, a naturally occurring stilbenoid from grapes, has extensively studied to evaluate its biological properties and elucidate the mechanism of action (Gusman *et al.*, 2001).

In the present study, we developed a refining process of grape shoot extracts to produce enrichment of polyphenols, and evaluated chemopreventive potential of various grape shoot extracts and their refined materials using *in vitro* bioassay systems. First, we examined antioxidant potential of grape shoot extracts and refined materials by the use of DPPH that stably generates free radicals in ethanol solution. As a result, all of test materials possessed radical scavenging activities about 70~80% at the concentration of 500 μg/ml, and the activity is corresponding to 500 μM of resveratrol (Table I). Accordingly, dose-responses on radical scavenging were also investigated and subsequently $IC_{50}$ values were determined. Among the extracts and refined materials, the Sheridan shoot extract (SSE) showed the most potent DPPH radical scavenging activity that was similar to that of resveratrol. In addition, the Kyoho shoot

**Table I.** Antioxidant potential of grape shoot extracts or their refined materials. Test materials were incubated with 300 μM of DPPH ethanol solution at 37°C. After 30 min, absorbance was measured at 515 nm. DPPH radical scavenging activity of each sample was determined in comparison with vehicle-treated control group. Values of % inhibition represent mean ± S.E.M.(n=3).

| Test samples | % Inhibition at 500 μg/ml | $IC_{50}$ (μg/ml) |
|---|---|---|
| Campbell shoot extract (CSE) | 85.3 ± 0.1 | 89.7 |
| Sheridan shoot extract (SSE) | 80.1 ± 1.2 | 39.2 |
| Kyoho shoot extract (KSE) | 85.3 ± 0.4 | 60.4 |
| Refined material from Campbell shoot extract (RCE) | 81.3 ± 1.8 | 144.6 |
| Refined material from Sheridan shoot extract (RSE) | 81.5 ± 2.1 | 109.2 |
| Refined material from Kyoho shoot extract (RKE) | 81.1 ± 0.6 | 65.8 |
| Resveratrol | 78.4 ± 0.7[a)] | 33.2 |
| Vitamin C | 81.1 ± 0.9 | 5.9 |
| Gallic acid | 88.3 ± 1.1 | 4.7 |

[a)]% Inhibition at 500 μM

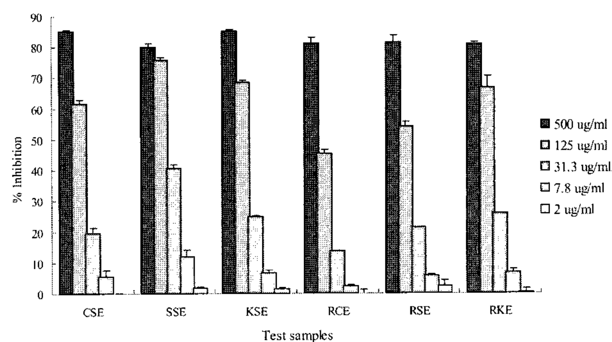


**Fig. 1.** DPPH radical scavenging activity of test materials. Test materials were incubated with 300 μM of DPPH ethanol solution at 37°C for 30 min. The absorbance was measured at 515 nm, and DPPH radical scavenging activity of each sample was determined in comparison with vehicle-treated control group. Abbreviations are as follows: CSE, Campbell shoot extract; SSE, Sheridan shoot extract; KSE, Kyoho shoot extract; RCE, Refined material from Campbell shoot extract; RSE, Refined material from Sheridan shoot extract; RKE, Refined material from Kyoho shoot extract.

extract (KSE) and the refined material from Kyoho shoot extract (RKE) effectively scavenged DPPH free radical generation. Other materials also exhibited moderate scavenging properties, however, these activities were less potent than those of positive controls, vitamin C, gallic acid, and resveratrol. The mechanism of antioxidant effects of these materials is probably related to their direct radical scavenging activities because resveratrol, a main active principle contained in these materials, possessed direct radical scavenging activity *in vitro* (Rimando *et al.*, 2002). This result suggests that grape shoots extracts and their refined materials may possess antioxidant potential by scavenging free radical generation.

Next, we investigated anti-inflammatory effects of test materials by the inhibition of NO and $PGE_2$ production as parameters. iNOS and COX-2, which produce NO and $PGE_2$, respectively, are induced by the response of several pro-inflammatory stimuli such as bacterial lipopolysaccharide (LPS), interleukin-1 (IL-1), and interferon-γ (IFN-γ) (Dubois *et al.*, 1998; Kröncke *et al.*, 1998). These enzymes and their products, NO and $PGE_2$, may play important roles in chronic inflammatory responses and promotion and/or progression of cancer (Kröncke *et al.*, 1998; Dannenberg *et al.*, 2001). According to this, we evaluated the inhibitory effects of test materials by the determination of NO and $PGE_2$ accumulation in the cultured media using colorimetric chemical reaction and enzyme-linked immunosorbent assay. As shown in Table II and Fig. 2, all of refined materials showed inhibitory activities on LPS-induced

**Table II.** Inhibitory effects of test materials on LPS-induced nitrite formation in cultured mouse macrophage cells. Cells were stimulated by treatment of LPS (1 μg/ml) in a presence or absence of test samples. After 20 hr, supernatants were collected and analyzed to determine the level of NO accumulation using Griess reaction. Values of % inhibition represent mean ± S.E.M.(n=4)

| Test samples | % Inhibition at 50 μg/ml | $IC_{50}$ (μg/ml) |
|---|---|---|
| Campbell shoot extract (CSE) | 1.0 ± 6.6 | > 50 |
| Sheridan shoot extract (SSE) | 1.0 ± 5.3 | > 50 |
| Kyoho shoot extract (KSE) | 26.3 ± 6.0 | > 50 |
| Refined material from Campbell shoot extract (RCE) | 64.7 ± 0.7 | 28.5 |
| Refined material from Sheridan shoot extract (RSE) | 56.7 ± 4.7 | 46.1 |
| Refined material from Kyoho shoot extract (RKE) | 65.9 ± 1.2 | 22.4 |
| Resveratrol | 78.4 ± 2.3 | 3.6 |

[a)]% Inhibition at 50 μM

NO accumulation. At the concentration of 50 μg/ml, they inhibited iNOS-induced NO production about 60~70%. However, grapes shoot extracts did not suppress the NO production in this assay system. Among the refined materials, Kyoho and Campbell refined materials showed similar inhibitory potency, and Sheridan refined materials were about twice less potent than Kyoho and Campbell. In addition, the inhibitory activities of NO production by these refined materials were not related to their cytotoxicity. However, all of test materials did not show any significant inhibitory effects on LPS-induced $PGE_2$ production (Table III). The difference of results between these assay systems might be due to the difference of assay procedure and/or the relevant signaling to regulate the expression of iNOS and COX-2 and the production of NO and $PGE_2$. This result indicates that refined materials from grape shoot extracts may possess anti-inflammatory potential on the basis of the inhibition of LPS-induced NO production.

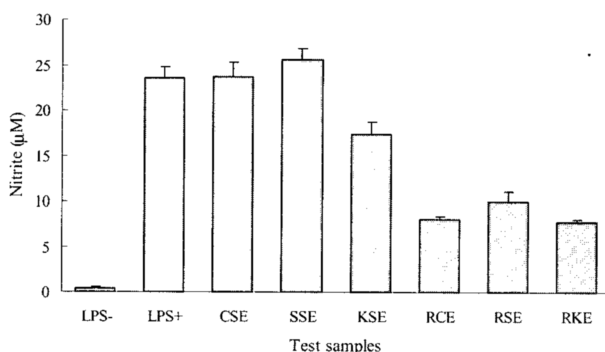


**Fig. 2.** Inhibitory effects of test materials on iNOS-mediated NO accumulation in LPS-stimulated mouse macrophage cells. Cells($5\times10^5$ cells/ml) were incubated in 24 well plates for 24 hr, then stimulated by the treatment of LPS (1 μg/ml) with or without test samples. After 20 hr, the media were collected and examined the amount of NO production using colorimetric Griess reaction. Abbreviations are as follows: CSE, Campbell shoot extract; SSE, Sheridan shoot extract; KSE, Kyoho shoot extract; RCE, Refined material from Campbell shoot extract; RSE, Refined material from Sheridan shoot extract; RKE, Refined material from Kyoho shoot extract.

Further, we examined the anti-proliferative activities of grape shoot extracts and refined materials against several human cancer cells. Previous studies demonstrate that resveratrol inhibited cell proliferation against human cancer cells and induced apoptosis, and these anti-proliferative mechanisms of resveratrol were diverse depending on cell lines and experimental conditions (Ahmad *et al.*, 2001; Joe *et al.*, 2002; Liang *et al.*, 2003). Accordingly, we evaluated the inhibitory effects of test materials on the proliferation of human lung, colon, stomach cancer cells, and fibrosarcoma cells compared with resveratrol. As a result, at the concentration of 50 μg/ml, all of refined materials showed the growth-inhibitory activities about 30~40% compared to control, but any of shoot extracts did not significantly inhibit cell proliferation against human cancer cells (Table IV), and inhibitory activities of refined materials were less potent than resveratrol. Although the difference of inhibitory potency either species of grapes or cancer cell lines

**Table III.** Effects of test materials on LPS-induced $PGE_2$ production in RAW 264.7 cells. Cells were stimulated by the treatment of LPS (1 μg/ml) with or without test samples. After 20 hr, supernatants were collected and analyzed to determine the amount of $PGE_2$ production using enzyme immunoassay

| Test samples | % Inhibition at 50 μg/ml |
|---|---|
| Campbell shoot extract (CSE) | 16.7 |
| Sheridan shoot extract (SSE) | 26.1 |
| Kyoho shoot extract (KSE) | 27.2 |
| Refined material from Campbell shoot extract (RCE) | 22.8 |
| Refined material from Sheridan shoot extract (RSE) | 45.7 |
| Refined material from Kyoho shoot extract (RKE) | 1.0 |

**Table IV.** Effects of test materials on the proliferation of various human cancer cells. Cells were incubated with test materials for 72 hr. After incubation, the inhibitory effects of test materials on the cell proliferation were examined by SRB assay. Values represent mean ± S.E.M. (n=3). Abbreviations are as follows: CSE, Campbell shoot extract; SSE, Sheridan shoots extract; KSE, Kyoho shoot extract; RCE, Refined material from Campbell shoot extract; RSE, Refined material from Sheridan shoot extract; RKE, Refined material from Kyoho shoot extract

| Test samples | % Survival at 50 μg/ml | | | | |
|---|---|---|---|---|---|
| | A549 | HCT 116 | HT-29 | HT-1080 | SNU-638 |
| CSE | 83.4 ± 5.0 | 83.3 ± 6.4 | 95.9 ± 2.6 | 84.7 1.5 | 93.0 ± 3.1 |
| SSE | 100.0 ± 2.2 | 100.0 ± 5.5 | 97.4 ± 3.3 | 100.0 1.1 | 99.9 ± 5.8 |
| KSE | 94.7 ± 8.7 | 97.7 ± 9.3 | 87.1 ± 3.7 | 97.8 3.1 | 94.4 ± 11.8 |
| RCE | 59.8 ± 2.7 | 55.2 ± 4.2 | 59.4 ± 2.6 | 71.1 3.5 | 61.5 ± 2.4 |
| RSE | 61.2 ± 6.5 | 59.9 ± 2.2 | 68.3 ± 3.8 | 69.4 1.8 | 60.5 ± 3.0 |
| RKE | 62.5 ± 1.9 | 61.0 ± 4.9 | 70.5 ± 2.8 | 74.3 3.2 | 69.3 ± 2.9 |
| Resveratrol[a)] | 21.2 | 19.8 | 20.0 | 18.6 | 7.0 |

[a)]represented as $EC_{50}$ values(μM)

tested was not significant, the refined material from Campbell shoot extract (RCE) was the most potent than other refined materials. This result suggests that refined materials from grape shoot extracts may have anti-proliferative potential against various human cancer cells.

In summary, we evaluated chemopreventive potential of some grape shoot extracts and their refined materials using *in vitro* assay system. Both grape shoot extracts and their refined materials effectively showed scavenging activities on generated DPPH free radicals, and also refined materials from grape shoot extracts suppressed LPS-induced NO production in macrophage cells, and also moderately inhibited cell proliferation against human cancer cells. Although biological activities of these materials were less effective than those of a positive control, resveratrol, it is more reasonable to evaluate their biological potential considering the possibility of co-existence of some substances that may inhibit biological activities in grape shoot extracts and refined materials. Further studies are underway to investigate additional biological effects of shoot extracts and their refined materials. In conclusion, these grape shoot extracts and their refined materials might be useful sources for the development of chemopreventive agents and/or functional foods.

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## REFERENCE

Ahmad, N., Adhami, V. M., Afaq, F., Feyes, D. K., and Mukhtar, H. (2001). Resverartrol causes WAF1/p21-mediated $G_1$ phase arrest of cell cycle and induction of apoptosis in human epidermoid carcinoma A431 cells. *Clin. Cancer Res.* **7**, 1466-1473.

Alley, M. C., Dominic, A., and Shoemaker, R. H. (1988). Feasibility of drug screening with panels of human tumor cell lines using a microculture tetrazolium assay. *Cancer Res.* **48**, 589-601.

Ames, B. N., Shigenaga, M. K., and Hagen, T. M. (1993). Oxidants, antioxidants, and the degenerative disease of aging. *Proc. Natl. Acad. Sci. USA* **90**, 7915-7922.

Dannenberg, A. J., Altorki, N. K., Boyle, J. O., Dang, C., Howe, L. R., Weksler, B. B., and Subbaramaiah, K. (2001). Cyclooxygenase-2: a pharmacological target for the prevention of cancer. *Lancet Oncol.* **2**, 544-551.

Dreher D. and Junod, A. F. (1998). Role of oxygen free radicals in cancer development. *Eur. J. Cancer* **32A**, 30-38.

Dubois, R. N., Abramson, S. B., Crofford, L., Gupta, R. A., Simon, L. S., Van De Putte, L. B., and Lipsky, P. E. (1998). Cyclooxygenase in biology and disease. *FASEB J.* **12**, 1063-1073.

Fremont, L. (2000). Biological effects of resveratrol. *Life Sci.* **66**, 663-673.

Gerhauser, C., Alt, A., Heiss, E., Gamal-Eldeen, A., Klimo, K., Knauft, J., Neumann, I., Scherf, H.-R., Frank, N., Bartsch, H., and Becker, H. (2002). Cancer chemopreventive activity of xanthohumol, a natural product derived from hop. *Mol. Cancer Ther.* **1**, 959-969.

Green, L. C., Wanger, D. A., Glogowski, J., Skipper, P. L., Wishnok, J. S., and Tannenbaum, S. R. (1982). Analysis of nitrate, nitrite, and [$^{15}$N]nitrate in biological fluids. *Anal. Biochem.* **126**, 131-138.

Gusman, J., Malonne, H., Atassi, G. (2001). A reappraisal of the potential chemopreventive and chemotherapeutic properties of resveratrol. *Carcinogenesis* **22**, 1111-1117.

Jang, M., Cai, L., Udeani, G. O., Slowing, K. V., Thomas, C. F., Beecher, C. W., Fong, H. H. S., Farnsworth, N. R., kinghorn, A. D., Mehta, R. G., Moon, R. C., and Pezzuto, J. M. (1997). Cancer chemopreventive activity of resveratrol,

a natural product derived from grapes. *Science* **275**, 218-220.

Joe, A. K., Liu, H., Suzui, M., Vural, M. E., Xiao, D., and Weinstein, I. B. (2002). Resveratrol induced growth inhibition, S-phase arrest, apoptosis, and changes in biomarker expression in several human cancer cell lines. *Clin. Cancer Res.* **8**, 893-903.

Kelloff, G. J., Crowell, J. A., Steele, V. E., Lubet, R. A., Malone, W. A., Boone, C. W., Kopelovich, L., Hawk, E. T., Lieberman, R., Lawrence, J. A., Ali, I., Viner, J. L., and Sigman, C. C. Progress in cancer chemoprevention: development of diet-derived chemopreventive agents. (2000). *J. Nutr.* **130**, 467S-471S.

Kinghorn, A. D., Fong, H. H. S., Farnsworth, N. R., Mehta, R. G., Moon, R. C., Moriarty, R. M., and Pezzuto, J. M. (1998). Cancer chemopreventive agents discovered by activity-guided fractionation: a review. *Curr. Org. Chem.* **2**, 597-612.

Kröncke, K. D., Fehsel, K., and Kolb-Bachofen, V. (1998). Inducible nitric oxide synthase in human diseases. *Clin. Exp. Immunol.* **113**, 147-156.

Kwak, M. K., Egner, P. A., Dolan, P. M., Ramos-Gomez, M., Groopman, J. D., Itoh, K., Yamamoto, M., and Kensler, T. W. (2001). Role of phase 2 enzyme induction in chemoprotection by dithiolethiones. *Mutat. Res.* **480-481**, 305-315.

Lambert, J. D., and Yang, C. S. (2003). Cancer chemopreventive activity and bioavailability of tea and tea polyphenols. *Mutat. Res.* **523-524**, 201-208.

Lander, H. M. (1997). An essential role for free radicals and derived species in signal transduction. *FASEB J.* **11**, 118-124.

Lee, S. K., Mbwambo, Z. H., Chung, H., Luyengi, L., Gamez, E. J. C., Mehta, R. G., Kinghorn, A. D., and Pezzuto, J. M. (1998). Evaluation of the antioxidant potential of natural products. *Comb. Chem. & High Through. Screen.* **1**, 35-46.

Liang, Y. C., Tsai, S. H., Chen, L., Lin-Shiau, S. Y., and Lin, J. K. (2003). Resveratrol-induced $G_2$ arrest through the inhibition of CDK7 and $p34^{CDC2}$ kinases in colon carcinoma HT29 cells. *Biochem. Pharmacol.* **65**, 1053-1060.

Martinez, J. and Moreno, J. J. (2000). Effect of resveratrol, a natural polyphenolic compound, on reactive oxygen species and prostaglandin production. *Biochem. Pharmacol.* **59**, 865-870.

Min, H. Y., Park, E. J., Lee, S. K., and Cho, Y. J. (2003). Effects of grape extracts on free radical scavenging activity and inhibition of pro-inflammatory mediator production in mouse macrophage cells. *Korean J. Food Sci. Technol.* **35**, 132-137.

Morse, M. A., and Stoner, G. D. (1993). Cancer chemoprevention: principles and prospects. *Carcinogenesis* **14**, 1737-1746.

Patel, R. P., Moelliring, D., Murphy-Ullrich, J., Jo, H., Beckman, J. S., and Darley-Usmar, V. M. (2000). Cell signaling by reactive nitrogen and oxygen species in atherosclerosis. *Free. Radic. Biol. Med.* **28**, 1780-1794.

Rimando, A. M., Cuendet, M., Desmarchelider, C., Mehta, R.G., Pezzuto, J. M., and Duke, S. O. (2002). Cancer chemopreventive and antioxidant activities of pterostilbene, a naturally occurring analogue of resveratrol. *J. Agric. Food Chem.* **50**, 3453-3457.

Surh, Y. J. (1999). Molecular mechanisms of chemopreventive effects of selected dietary and medicinal phenolic substances. *Mutat. Res.* **428**, 305-327.