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Screening Biological Activities of Grape Seed and Skin Extracts of Campbell Early (*Vitis labruscana* B.)

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Abstract

This study was conducted to determine biological activities, such as lipid peroxidation inhibition, cytotoxicity, sun blocker, inhibition of tyrosinase, and antioxidative effect, of ethanol extracts, and of solvent fractionated ethanol extracts obtained from grape seeds and skins. The strongest lipid oxidative inhibition of 66.9% and 67.6% was observed respectively, in the presence of 20 μg/mL of both ethanol extract and water fraction of grape seeds. Overall, the ethanol extracts and their fractions of grape seeds exhibited stronger lipid oxidative inhibition than that of skin extracts. On the other hand, the ethanol extracts of grape skins showed stronger cytotoxicity than that of seeds on MCF-7, Hep3B, and A549 cancer cell lines. However, the water fraction of seed ethanol extracts showed the strongest cytotoxic effect of 76.52% and 67.01% on MCF-7 and Hep3B, respectively among their fractions. Ethanol seed extracts obtained at 30°C had the strongest absorbance both at UVA region (350 nm) and UVB region (308 nm) and the chloroform fraction showed the strongest absorbance at UVB region and butanol fraction at UVA region among their fractions, respectively. In the meantime, the ethanol extracts obtained at 30°C and butanol fraction showed the strongest tyrosinase inhibitory effect of 39.4% and 37.6%, respectively. This study shows that ethanol extracts and their fractions of grape seeds and skins could be potential good materials for functional food and cosmetic products.

Key words: grape seed, grape skin, lipid peroxidation, cytotoxicity, UV protection, tyrosinase inhibition

INTRODUCTION

Biological activities have often been used as pointers to screen the bioactive materials from edible plants. Recently, more studies on the development of natural antioxidants having more safe and potent effects were reported (1-3).

Natural antioxidants can be obtained from various parts of a plant including leaves, flowers, seeds, trunks, roots and fruits. The developments of extraction, separation and purification technologies for the determination of major biological activities are recognized to be important (4). In addition, the natural components of the major biological activities such as antibacterial, antioxidant, anticancer and immune-strengthening effects were recognized to include polyphenols, vitamin C, α-tocopherol, β-carotine, and flavonoids, etc. Recently, edible plants containing lots of these natural components have been receving in renewal interests (5-7).

Grapes, originated in the Middle Asia, have been cultivated in different parts of the world because of their strong survival ability in unfavorable environment. The major species of grape in the world were known as European, American and a Cross species. Among them, American and a Cross species are widely cultivated in Korea.

Recently, both wines and juices of grape were reported to have biological activities, such as antimicrbial, antiinflammatory, anticancer activities and prevention of heart disease (8-11).

These biological activities are derived from polyphenols and flavonoids in grapes. The main components are (+)-catechin, (-)-epicatechin, procyanidin, resveratrol and viniferine (12,13).

The objectives of this study are to investigate biological activities of ethanol extracts and solvent fractionated ethanol extracts from grape seed and skin of Campbell Early (*Vitis labruscana* B.). The biological activities include lipid peroxidation inhibition, cytotoxicity, sun block activities, inhibition effect on tyrosinase, and antioxidative effect.

MATERIALS AND METHODS

Sample preparation

Grape used in this study was Campbell Early (*Vitis labruscana* B.) species which was cultivated in Chunchon area of the middle eastern part of Korea. Both grape seeds

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and skins were used.

Grape samples were dried, ground and extracted. The extraction was done by the reflux condenser technique. Ten times volume of ethanol was added to the ground sample in round flasks in water baths preheated at 78°C, 50°C, and 30°C, respectively and extracted twice for 12 hrs and each extract was filtered by a vacuum filtering device and concentrated by a rotary vacuum evaporator (EYELA N-N-Series, Japan) and dried by a freeze-drier.

The freeze-dried ethanol extract was mixed with other solvents in the ratio of 1:10:9 with ethanol extract, hexane, and water, respectively and fractionated by extraction using separatory funnel. The hexane fraction was collected and concentrated by a rotary vacuum evaporator. The remaining water-ethanol fraction was systematically fractionated with chloroform, ethylacetate, butanol and aqueous fractions. These fractions were concentrated and freeze-dried.

Measurement of lipid peroxidants

Lipid peroxidation of liver tissue obtained from rat was measured by the method of thiobarbituric acid reacting substance (TBARS) (3). Both ethanol extracts and their fractions of grape seed and skin were added to a mixture of 50 mM potassium phosphate buffer (pH 7.4), 100 μL microsome, 20 μL of 1.7 mM ADP and 20 μL of 0.1 mM $FeCl_3$. One hundred μL of 0.1 mM NADPH was added to the mixture. Lipid peroxidation was derived at 37°C for 60 min in a shaking water bath. The reaction was done by adding 100 μL of 20 mM EDTA. A 2 mL of mixture of TBA-TCA-HCl including 2% BHT was added. The mixture was boiled at 95°C for 10 min, and centrifuged at 3,000×g for 10 min. Finely, the upper fraction was collected. The absorbance of the fraction was measured at 535 nm with a spectrophotometer (UVIKON 922 Kontron Co.) and the amount of lipid peroxidant in the sample concentrate was compared with that of the control group which had no sample.

Cancer cells and incubation

Cancer cell lines used in this study were A549 of human lung carcinoma, MCF-7 of human breast adenocarcinoma, and Hep3B of human hepatocellular carcinoma. The normal cell was 293 cell of the transformed primary human embryonal kidney. The media for A549 and MCF-7 cells were a mixed media containing RPMI1640. Media for Hep3B cell and 293 cell were Dulbecco's Modified Eagle Medium (DMEM) and Minimum Essential Medium (MEM), respectively. To these media, 10% fetal bovine serum was added and the cells were incubated in 5% CO_2 incubator at 37°C.

Measurement of cytotoxicity

Cell cytotoxicity was measured by SulfoRhodamine B (SRB) analysis. The analysis measures the cell growth by staining of cell protein (14). Five replicates of 100 μL of each type of cancer cells (A549, MCF-7, Hep3B) and the 293 normal cell at a concentration of 10^4 cells/mL in their corresponding medium (RPMI1640, DMEM, MEM including 10% fetal bovine serum) were transferred to wells. The samples were incubated for 24 hrs at 37°C in 5% CO_2 incubator. The 50 μL concentrations of 0.25, 0.5, 0.75, and 1.0 mg/mL samples were added and incubated again for 48 hrs. The 100 μL of 10% (v/v) trichloroacetic acid stored at 4°C was added and placed for 1 hr at 4°C. The mixture was washed 5 times with distilled water and dried. The dried sample was stained for 30 min with 100 μL of 0.4% SRB (w/v) dissolved in 1% acetic acid (v/v). The stained sample was washed 5 times with 1% acetic acid (v/v) and dried. The 100 μL of 10 mM tris buffer was added to the dried plate and the absorbance was measured at 540 nm of microplate reader (UVT 05975, Molecular Devices).

Measurement of blocking effects against ultraviolet light

The absorbance of 200 μg/mL concentrations of ethanol extracts and their fractions of grape seeds were measured at 308 nm and 350 nm using a UV-visible spectrophotometer (UVIKON 922 Kontron Co.). Screening power of 1% concentration of extracts against ultra-violet was measured at the absorbance of 1 cm diameter in a UV cell (15).

Measurements of inhibitory effects against tyrosinase

The 0.1 mg/mL concentration of ethanol extracts and their fractions of grape seeds were prepared. Each 0.5 mL extract was added to a 0.5-mL 50 mM phosphate buffer (pH 6.8) solution. A 0.5-mL tyrosinase (200 units/mL) and 0.5-mL 2.5 mM L-tyrosine were added and the mixture was reacted for 10 min at 37°C. The absorbance of the reacted mixture was measured at 475 nm by a spectrophotometer (UVIKON 922 Kontron Co.). The inhibition ratio against the control group was calculated from the value of the absorbance (16).

RESULTS AND DISCUSSION

Lipid peroxidation inhibition

The inhibitory effects of ethanol extracts and several fractions from grape seeds and skins on lipid peroxidation of liver tissues were analyzed by measuring the amounts of lipid peroxidants present. The inhibitory effects were compared with the control groups having neither extracts nor fractions.

The comparisons of inhibitory effects on lipid peroxidation by the ethanol extracts of grape seeds and of skins at different extraction temperatures were shown in Fig. 1.

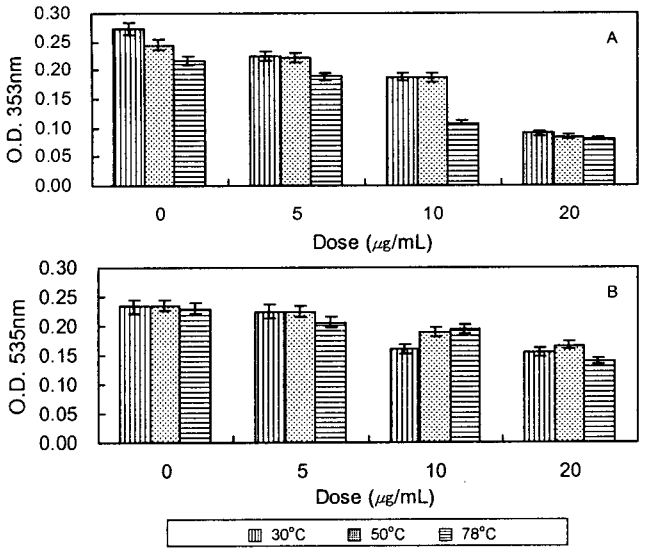

Fig. 1. Inhibitory effect of ethanol extracts of grape seeds (A) and skins (B) on lipid peroxidation at different extraction temperatures.

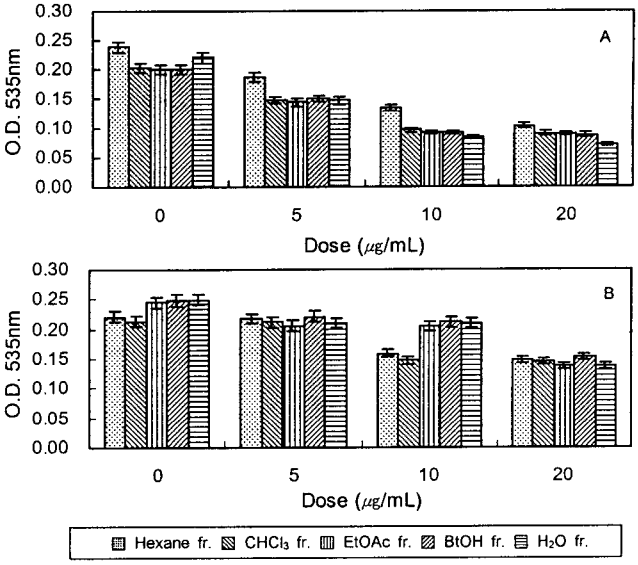

Fig. 2. Inhibitory effect of several organic solvent fractions from the ethanol extracts of grape seeds (A) and skin (B) on lipid peroxidation.

In both seed and skin ethanol extracts, the amounts of lipid peroxidants decreased with the increasing concentrations of the extracts. The ethanol extracts of grape seeds showed a reduction of 70% lipid peroxidants as compared with those of grape skins.

The comparisons of inhibitory effect on lipid peroxidation from several organic fractions of the original ethanol extracts of grape seeds and skins were shown in Fig. 2. In both organic solvent fractions from the seed and the skin ethanol extracts, the amounts of lipid peroxidants decreased with increasing concentrations of the extracts. The organic solvent fractions from ethanol extracts of grape seeds showed a higher inhibitory effects on lipid peroxidants than those of grape skins. Among the organic solvent fractions, the water fraction of seed ethanol extracts showed the highest inhibitory effects on lipid peroxidants at 67.7%.

In the results of Cho et al. (3), standard L-ascorbic acid solution at 250 μg/mL decreased the lipid peroxidant by 33.90% and tomato skins decreased the formation by 80.66%. By comparison, ethanol extracts from grape seeds and skins were shown to enhance the inhibitory effect on the formation of lipid peroxidants.

Since cell membranes consisted of lipid component, inhibition and control against the formation of lipid peroxidant in the lipid might be important for the prevention of several diseases related to aging. Current results proved that the extracts of grape seeds and skins could be valuable materials as natural antioxidants.

Cytotoxicity

In this study, the concentrations of 0, 0.25, 0.50, 0.75 and 1.00 mg/mL of the samples were tested for their inhibitions on the growth of each type of cancer cell lines as well as the toxicity against normal cells.

The results on breast cancer cells (MCF-7) were shown in Figs. 3 and 4, respectively. Both seed and skin ethanol extracts (1.00 mg/mL), at 30°C extraction temperature, had

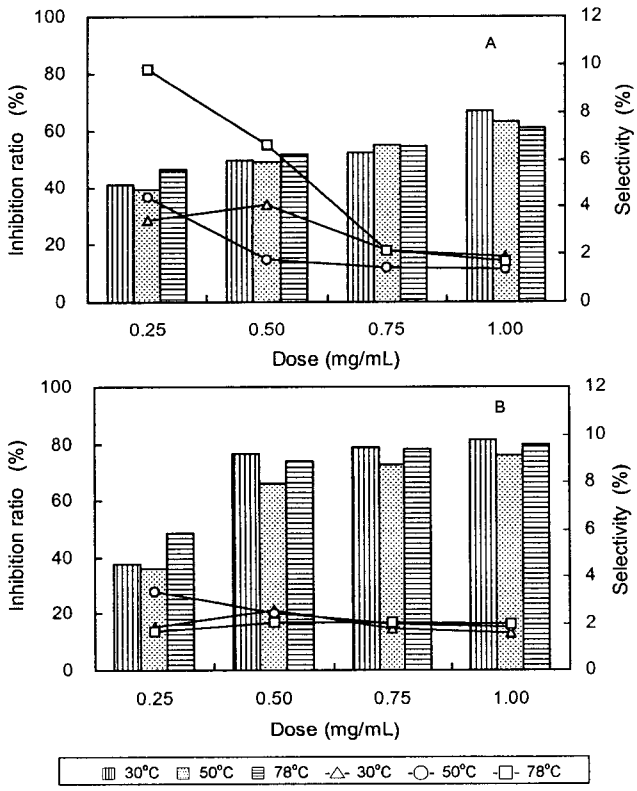

Fig. 3. Inhibition ratio of ethanol extracts of grape seeds (A) and skins (B) on the growth of MCF-7 (bar %) cell and selectivity (line %) at different concentrations.

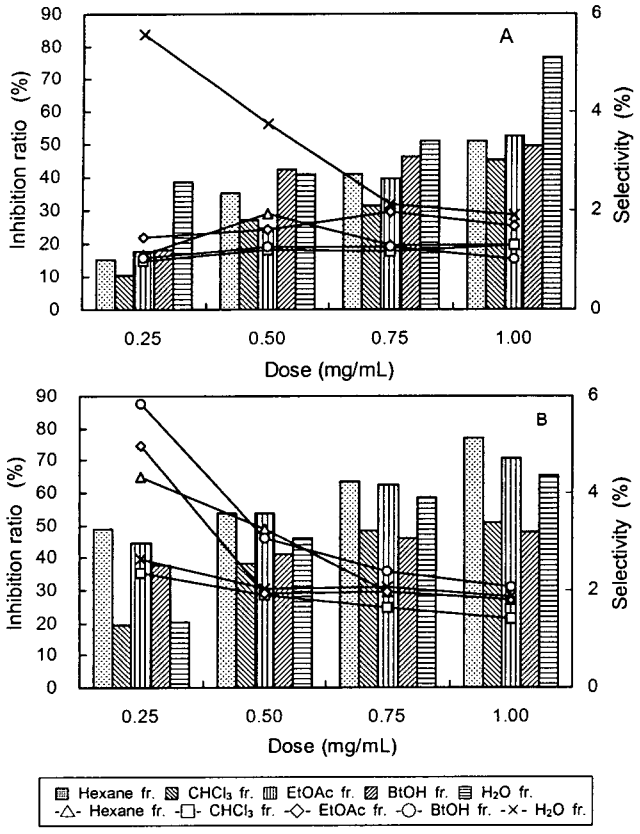

Fig. 4. Inhibition ratio of several organic solvent fractions from the ethanol extracts of grape seeds (A) and skin (B) on the growth of MCF-7 (bar %) cell and selectivity (line %) at different concentrations.

the maximum growth inhibitory activities of 67.06% and 81.18%, respectively. Generally, skin ethanol extracts showed higher growth inhibitory activities than those of seed extracts. The selectivity of seed ethanol extracts on normal cell was a little higher than that of skin ethanol extracts.

In the growth inhibitory activities of several organic solvent fractions from seed and skin ethanol extracts, the water fraction of the seed extracts (1.00 mg/mL) had a 76.52% activity and the hexane fraction of the skin extracts (1.00 mg/mL) had a 76.82% activity. The water fraction of the seed ethanol extracts showed the selectivity on normal cells of 1.92~5.59. Similary, the hexane fraction of the skin ethanol extracts had a selectivity on normal cells of 1.80~4.43.

The results related to the liver cancer cells (Hep3B) were shown in Fig. 5 and 6, respectively. Seed ethanol extracts (1.00 mg/mL) at 30°C had the highest growth inhibitory activity of 57.14%, whereas the skin extracts at 78°C showed the highest inhibitory activity of 64.63%. Seed ethanol extracts had a slightly higher selectivity on normal cells than skin ethanol extracts, and its selectivity decreased as the concentration increased.

The highest inhibition of water fraction in the both seed and skin of grape on the growth of Hep3B cell was 67.01% and 64.07%, respectively. In the selectivity on normal cells, the water fraction of ethanol extract of grape seeds showed high values of 1.68 to 3.84, but the selectivity decreased with concentration. The ethanol extract of grape skins showed a little lower values of 1.54 to 1.87.

The results on A459 lung cancer cell lines were shown in Figs. 7 and 8. The ethanol extract (1.00 mg/mL) of grape seeds at 30°C showed the highest inhibition of 59.03%, and the ethanol extract (1.00 mg/mL) of grape skins, the extract at 78°C showed the highest value of 67.82%. Therefore, the ethanol extract of grape skins had higher inhibition activity than that of grape seeds.

For the growth inhibitory activity of the organic solvent fractions obtained from ethanol extracts of grape seeds and skins, the hexane fraction (1.00 mg/mL) from the ethanol extracts of the seeds showed the highest growth inhibitory activity value of 61.70%, and the water fraction (1.00 mg/mL) from the ethanol fraction of the skins showed the highest value of 67.98%.

The selectivity of hexane fraction from ethanol extract of seeds on normal cells was 1.56 to 2.92, and the selectivity of water fraction from ethanol extract of grape skins was 1.25 to 2.11. Shirataki et al. (10) reported that the

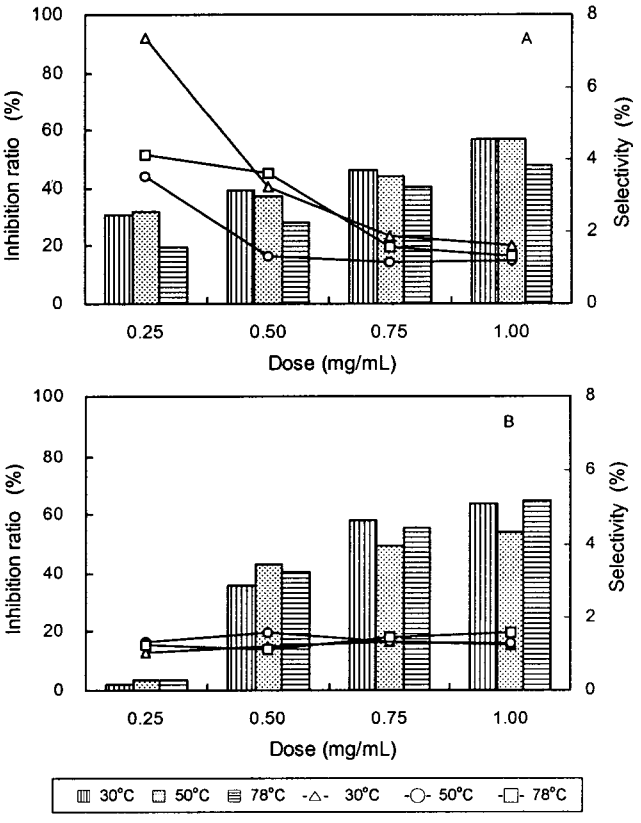

Fig. 5. Inhibition ratio of ethanol extracts of grape seeds (A) and skins (B) on the growth of Hep3B (bar %) cell and selectivity (line %) at different concentrations.

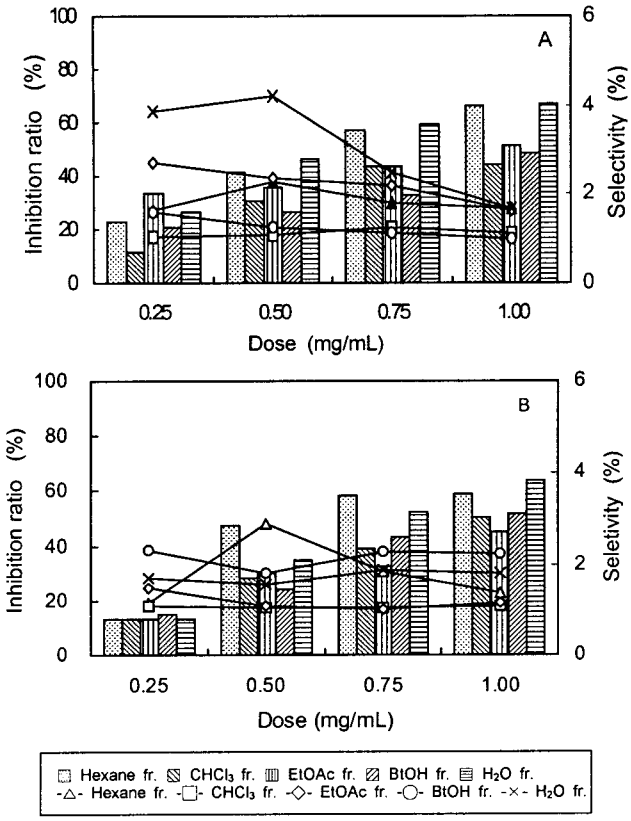

Fig. 6. Inhibition ratio of several organic solvent fractions from the ethanol extracts of grape seeds (A) and skin (B) on the growth of Hep3B (bar %) cell and selectivity (line %) at different concentrations.

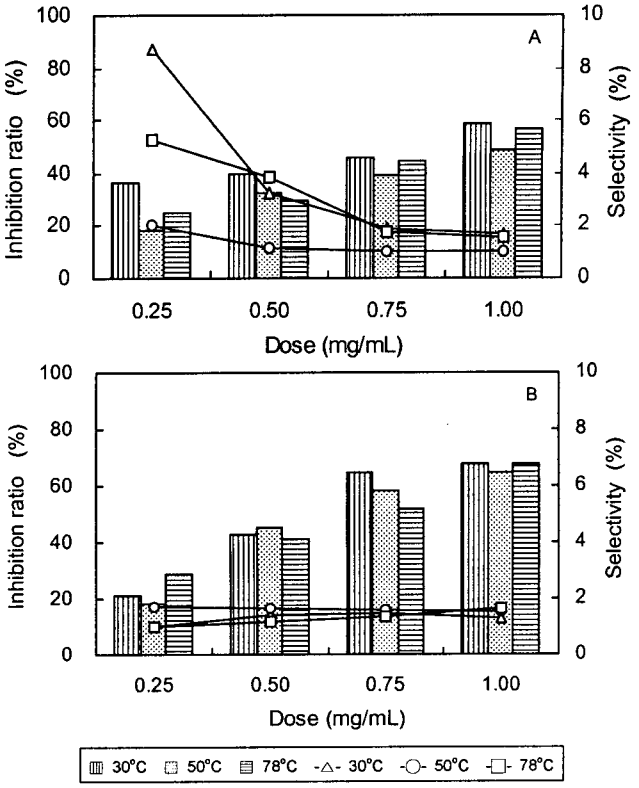

Fig. 7. Inhibition ratio of ethanol extracts of grape seeds (A) and skins (B) on the growth of A549 (bar %) cell and selectivity (line %) at different concentrations.

growth inhibitory effect of methanol extract of grape seeds was higher than that of grape skins.

In this study, the ethanol extract of grape skins showed higher growth inhibitory effect than that of grape seeds. The best extraction temperature for higher growth inhibition was 30°C, compared to other temperatures.

Bioactive materials for inhibiting the growth of cancer cells were thought to be sensitive to heat, therefore, the inhibition activity decreased as the extraction temperature increased. Also, the selectivity of the ethanol extract of grape seeds on the normal cells decreased as the concentration of the extract increased. The inhibitory effect on the normal cells increased depending on the concentration, and the reason behind such observations should be further investigated in the future study. Ethanol extracts of grape seeds and skins showed a slightly higher growth inhibition than each of those fractions from the organic solvents. The reason was thought to be the combined effects from several growth inhibition materials of each fraction to inhibit the growth of cancer cells.

Also, Carnesecchi et al. (17) reported that 50 μg/mL of cocoa extract containing high quantity of polyphenols, like those in grape seeds, showed an inhibition of 70% against Caco-2 cell of human lung carcinoma. Besides, Singletary and Meline (18) reported that proanthocyanidin of grape seeds inhibited the growth of human lung carcinoma and human breast adenocarcinoma.

Resveratrol and other bioactive materials are thought to be found in grape seeds and skins, and to have a high inhibition effect against several type of cancer cells, Nevertheless, further studies on these materials are required for their suggested functions.

Blocking effects against ultraviolet light

For the blocking effects against ultraviolet light, ethanol extracts and fractions from grape seeds were evaluated by measuring the absorbance of ultraviolet at long wavelengths (UVA 320 to 400 nm) and short wavelength (UVB 280 to 320 nm) ranges. Then, their absorbance coefficients (E% cm) were compared.

Ultraviolet absorbance coefficients of seed ethanol extracts and several organic solvent fractions on extraction temperatures were shown in Table 1.

The seed extracts at 30°C had the highest absorbance coefficients of 68.2 and 21.5 for UVA and UVB, respectively. In the fractions of several organic solvents, chloroform fraction had the highest value of 69.4 at UVA and butanol fraction showed the highest value of 20.8 at UVB.

Compared with aloe vera, a natural ultraviolet interceptor, a high absorbance coefficient in UVA was found which

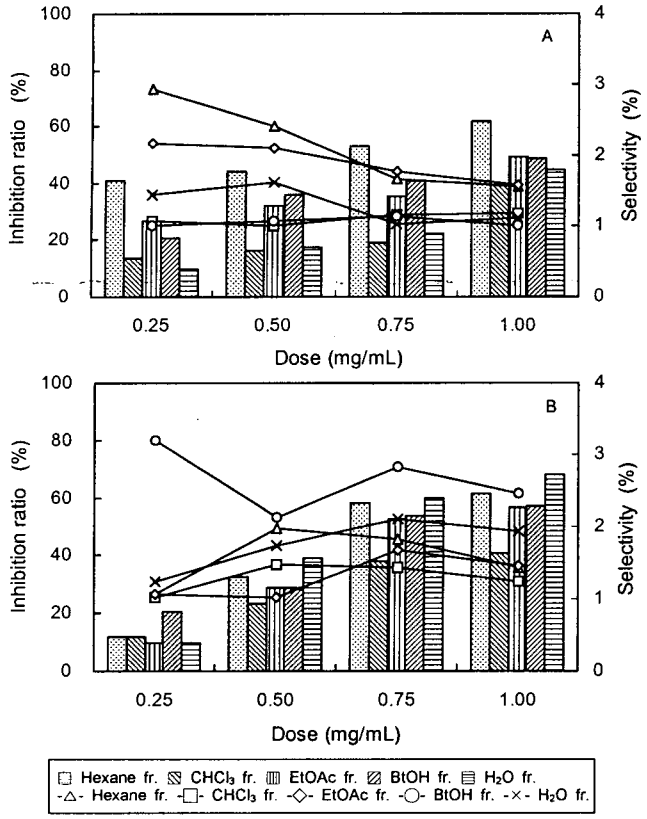

Fig. 8. Inhibition ratio of several organic solvent fractions from the ethanol extracts of grape seeds (A) and skin (B) on the growth of A549 (bar %) cell and selectivity (line %) at different concentrations.

Table 1. Ultraviolet absorbance coefficient of seed ethanol extracts and several organic solvent fractions on the different extraction temperatures

Sample		E% cm[1)]	
		308 nm	350 nm
Extracts	30°C ex.	68.2	21.5
	50°C ex.	58.2	17.5
	78°C ex.	55.2	20.2
Fractions	Hexane fr.	53.6	18.9
	$CHCl_3$ fr.	69.4	14.9
	EtOAc fr.	60.3	18.8
	BuOH fr.	69.1	20.8
	H_2O fr.	11.3	2.8
Dioxybenzone		412.6	208.5
Oxybenzone		423.8	216.1
Aloe vera		73.8	20.0

[1)]The theorical absorbance of a 1% solution over an optic path of 1 cm.

was known to oxidize the reduced melanin from human skins. But a low absorbance coefficient in UVB was observed, which was caused by skin diseases and cancers.

Compared with the results of Jeong et al. (15) on 300 kinds of wild plants for the blocking effects against ultraviolet light, grape seed ethanol extracts except for a few species generally showed high absorbance coefficients.

Additional studies are needed to effectively recover the natural ultraviolet interceptor from the grape seed ethanol extract.

Tyrosinase inhibition

Melanin is a natural and macromolecular pigment which is widely distributed in animals and plants. It is synthesized via dopaquinone obtained from tyrosine by a tyrosinase. By controlling the enzymatic oxidation of a browning reaction, the shelf-life of commercial products containing the pigment can be extended.

Inhibitor effects on tyrosinase by seed ethanol extracts and each fraction were evaluated by comparing the inhibition ratios calculated from the absorbances between control groups (without adding samples) and the treatment groups of the extracts extracted at different temperatures.

Inhibitory effects on tyrosinase by ethanol extracts at different extraction temperatures were shown in Fig. 9. All extracts appeared to have the inhibition rate of more than 30% at the concentration of 0.1 mg/mL. The inhibition rate of the extracts extracted at 30°C had the highest inhibitory rate of 39.4%% against tyrosinase activity.

Inhibitory effects on tyrosinase by different fractions of several organic solvents from the seed extracts were shown in Fig. 10. Most fractions except for the ethyl acetate fraction appeared to have a inhibition rate of more than 30%.

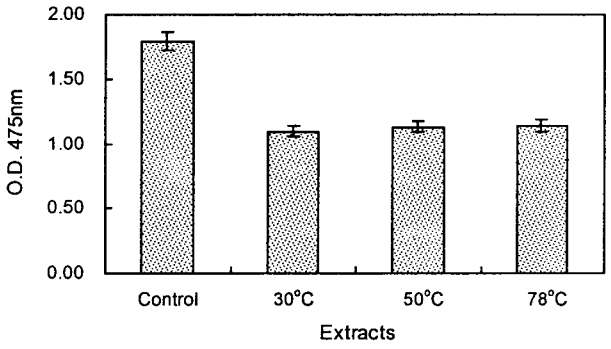

Fig. 9. Effect of ethanol extracts of grape seeds on tyrosinase activity at different extraction temperatures.

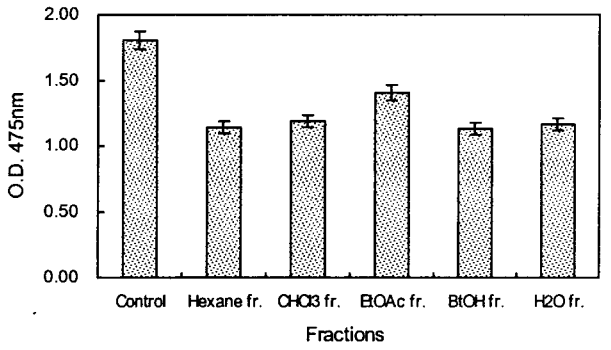

Fig. 10. Effect of several organic solvent fractions from the ethanol extracts of grape seeds on tyrosinase activity.

Butanol fraction showed the highest inhibitory rate of 37.6% on tyrosinase activity.

Vegetables such as watermelon, cucumber, old pumpkin, melon, blue pepper and tomato, and fruits such as orange, pear, grape, banana and apple showed high browning rates. The reason was that the enzymatic activity of tyrosinase in these fruits and vegetables was high or that they had activators for tyrosinase (17). In this study, the inhibitory effects on tyrosinase by grape seed ethanol extract and fractions were proved to exist.

Compared with the results of Choi et al. (19) that *Saururus chinensis* Baill and *Rhus japonica* L. showed inhibition rates of 93% and 91% at the concentration of 2.0 mg/mL, the ethanol extracts from grape seeds in this study had the inhibitory rate of more than 30% at the concentration of 0.1 mg/mL. These results suggest that low concentration of ethanol extracts from grape seeds showed the high inhibition on tyrosinase activity.

Overall, these results are likely due to the presence of polyphenol compounds such as tannins and flavonoids. And the ethanol extracts of grape seeds appeared to contain valuable inhibitors of natural tyrosinases.

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REFERENCES

1. Manzocco L, Anese M, Nicoli MC. 1998. Antioxidant properties of tea extracts as affected by processing. *Lebensm-Wiss U Technol* 31: 694-698.
2. Wang JN, Chen YJ, Hano Y, Nomura T, Tan RX. 2000. Antioxidant activity of polyphenols from seeds of *Vitis amurensis in vitro*. *Acta Pharmacol Sin* 21: 633-636.
3. Cho SY, Han YB, Shin KH. 2001. Screening for antioxidant activity of edible plants. *J Korean Soc Food Sci Nutr* 30: 133-137.
4. Chung HS. 2001. Isolation of new bioactive phytochemicals from natural products. *Food Ind Nutr* 6: 53-59.
5. Peterson DM. 2001. Oat antioxidants. *J Cereal Sci* 33: 115-129.
6. Halliwell B. 1996. Antioxidants in human health and disease. *Annual Review of Nutrition* 16: 33-50.
7. Wang JF, Schramm DD, Holt RR, Ensunsa JL, Fraga CG, Schmitz HH, Keen CL. 2000. A dose-response effect from chocolate consumption on plasma epicatechin and oxidative damage. *J Nutr* 130: 2115S-2119S.
8. Lee MC, Kim GP, Kim SH, Choung NH, Yim MH. 1997. Antimicrobial activity of extract from gall-nut and red-grape husk. *Korean J Food and Nutr* 10: 174-179.
9. Hur SK, Kim SS, Heo YH, Ahn SM, Lee BG, Lee SK. 2001. Effects of the grapevine shoot extract on free radical scavenging activity and inhibition of pro-inflammatory mediator production. *J Applied Pharmacology* 9: 188-193.
10. Shirataki Y, Kawase M, Saito S, Kurihara T, Tanaka W, Satoh K, Sakagami H, Motohashi N. 2000. Selective cytotoxic activity of grape peel and seed extracts against oral tumor cell lines. *Anticancer Res* 20: 423-426.
11. Sato M, Maulik G, Ray PS, Bagchi D, Das DK. 1999. Cardioprotective effects of grape seed proanthocyanidin against ischemic reperfusion injury. *J Mol Cell Cardiol* 31: 1289-1297
12. Zhao J, Wang J, Chen Y, Agarwal R. 1999. Anti-tumor-promoting activity of a polyphenolic fraction isolated from grape seeds in the mouse skin two-stage initiation-promotion protocol and identification of procyanidin B5-3'-gallate as the most effective antioxidant constituent. *Carcinogenesis* 20: 1737-1745.
13. Saucier C, Mirabel M, Daviaud F, Longieras A, Glories Y. 2001. Rapid fractionation of grape seed proanthocyanidins. *J Agric Food Chem* 49: 5732-5735.
14. Kim SK, Kim YG, Lee MK, Han JS, Lee JH, Lee HY. 2000. Comparison of biological activity according to extracting solvents of four *Acanthopanax* root Bark. *J Medicinal Crop Sci* 8: 21-28.
15. Jeong KJ, Sa JH, Rhu MJ, Kim JB, Lee W, Han KS, Oh HS, Choi KY, Park SK and Cheung EH. 2000. Screening on sunsreen agents from domestic plants grown in the area of Kangwon province. *Rep Inst Health and Environ* 11: 69-83.
16. Jung SW, Lee NK, Kim SJ, Han DS. 1995. Screening of tyrosinase inhibitor from plants *Korean J Food Sci Technol* 27: 891-896.
17. Carnesecchi S, Schneider Y, Lazarus AS, Coehlo D, Gosse F, Raul F. 2002. Flavanols and procyanidins of cocoa and chocolate inhibit growth and polyamine biosynthesis of human colonic cancer cells. *Cancer Letters* 175: 147-155.
18. Singletary KW, Meline B. 2001. Effect of grape seed proanthocyanidins on colon aberrant crypts and breast tumors in a rat dual-organ tumor model. *Nutr Cancer* 39: 252-258.
19. Choi S, Kim H, Chang E, Sapers GM. 1997. Inhibition of tyrosinase activity by plant extracts. *Foods and Biotechnology* 6: 44-49.

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