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Full Length Research Paper

Anti-oxidant activity of *Rhus verniciflua* stokes by extract conditions

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In the present study, *Rhus verniciflua* extracts from various extract condition was evaluated by employing various *in vitro* anti-oxidant assay such as electron donation ability by 1,1-diphenyl-2-picryl-hydrazyl free radical (DPPH) scavenging, reducing power by Fe^{3+}-Fe^{2+} transformation method, and anti-lipid peroxidative effect by ferric thiocyanate. The electron donation ability of 100% ethanolic extract (94%) showed stronger activity than butylated hydroxyanisole (18%). One hundred percent ethanolic extract (0.54) showed the highest reducing power, and the other samples (0.29 to 0.44) showed stronger activity than α-tocopherol (0.13). Total phenol and flavonoid content showed the highest amount in 100% ethanolic extract (352.6 and 54.6 mg QE/g, respectively). The anti-lipid peroxidative effect of samples was greater than same concentration of α-tocopherol. Although higher extract yields were obtained using 80% methanol, higher anti-oxidant activities was obtained by 100% ethanol extracts.

Key words: *Rhus verniciflua*, anti-oxidant activity, electron donation ability, reducing power, anti-lipid peroxidative effect.

INTRODUCTION

Rhus verniciflua is a deciduous tree of the Anacardiaceae family that contains 250 over species, and is mainly cultivated in south-east Asian countries such as Korea, Japan and China. The sap of *R. verniciflua* has been used as a protective surface coating material for wood, porcelain, and metal wares in East Asia (Kobayashi et al., 2001; Onishi, 1995). It is interesting to see the old wares and woody relics coated with lacquer tree sap are well preserved, and this suggests that the sap might contain the strong anti-oxidants (Kim et al., 1997; Lee et al., 2002). In the orient, *R. verniciflua* has been used for medicinal and other uses by indigenous people.

Especially, some Korean still enjoyed eating chicken or duck soup boiled with *R. verniciflua* and sprout of *R. verniciflua* in the spring (Kim, 1996).

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Abbreviations: BHA, Butylated hydroxyanisole; **BHT,** butylated hydroxytoluene; **DPPH,** 1,1-Diphenyl-2-Picrylhydrazyl; **EDA,** electron donation ability; **FTC,** ferric thiocyanate.

The contact with poison ivy or *R. verniciflua* contains urushiol congeners cause irritation, inflammation, or blistering in sensitive individuals. For this reason, interest has been focused on the chemical structure of allergenic compound. Owing to a great deal of investigations on the major compound that induce dermatitis, Majima (1992) reported that main important components in urushi are urushiol, which has a mixture of olefinic catechols having an n-C_{15} or n-C_{17} alkyl side chain. Recently, due to allergy of urushiol, there has brought about many scientific studies and methods to remove urushiol. For example, there may be exemplified by a method involving heat treatment, solvent extraction, far-infrared radiation, and enzyme treatment (Choi et al., 2007; Kim, 2000; Kim et al., 2000; Park et al., 2009; Song, 2007).

Despite its allergic reaction, a number of researchers have been reported on various biological activities of *R. verniciflua* extracts. For example, anti-fibrogenic (Lee et al., 2003), anti-microbial (Kim et al., 1997), anti-mutagenic (Park et al., 2004; Son et al., 2005), anti-oxidant (Kim et al., 1997, 2002; Lee et al., 2002), anti-tumorigenic activity (Kitts and Lim, 2001; Lee et al., 2004), anti-cancer (Jeong et al., 2008; Lee et al., 2009), anti-platele (Jeon et al., 2006), anti-rheumatoid arthritis

(Choi et al., 2003), α-glucosidase inhibitory effect (Kim et al., 2010), and anti-obesity effect (Jeon et al., 2003) have reported.

Polar solvents (ethanol, methanol, and aqueous organic solvent mixture) are frequently used for the recovery of polyphenols from a plant material. The anti-oxidant activity depends on the extracting solvent polarity. The most suitable extract solvents are aqueous organic solvent mixtures than absolute organic solvent (Anwar et al., 2006; Sultana et al., 2007, 2009). In this present study, we reported the anti-oxidant properties activity from crude extract of *R. verniciflua* stem by different ratio of water/alcohol. To obtain a better understanding of the potent anti-oxidant extracts, we determined the amount of phenols and flavonoids. Most importantly, careful analysis using various assay like electron donation ability, reducing power, and anti-lipid peroxidative effect.

MATERIALS AND METHODS

Plant materials

R. verniciflua stem was obtained from Hoengseong-gun, Gangwon-do, Korea. The samples were dried at room temperature and powdered, using a blender.

Chemicals

α-Tocopherol, butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT), and 1,1-diphenyl-2-picrylhydrazyl (DPPH) were obtained from Sigma Chemical Co. (St. Louis, MO). All other reagents were of analytical grade or better.

Extraction of *R. verniciflua*

The air-dried, powdered (30 g) of *R. verniciflua* stem were extracted three times with ethanol (60, 80 and 100%), methanol (60, 80 and 100%), and distil water, with a 10:1 solvent-sample ratio, for 24 h at room temperature. The solution was filtered, evaporated under reduced pressure and lyophilized to give dried powder extract. Sample was dissolved 80% EtOH (v/v). All the processes were triplicated.

Electron donation ability (EDA) assay

The EDA of sample was determined by the method of Kim et al. (2010). This assay is based on the capacity of a substance for scavenging stable DPPH free radicals. The EDA of *R. verniciflua* was measured as follows. The reaction mixture contained 1 ml of 1.5 mM DPPH-methanol solution, 3.98 ml of methanol and 20 µl of different concentration samples, or ascorbic acid, α-tocopherol, BHA, BHT, and 80% EtOH (control). The mixture was allowed to react at room temperature for 30 min and absorbance values were measured at 517 nm using a spectrophotometer (V-530, Jasco Co., Japan).

The experiment was performed in triplicate. The EDA was expressed as reduction rate of absorbance according to the following equation:

EDA (%) = [1-(absorbance value of sample/absorbance value of control)] × 100.

Determination of reducing power activity

The reducing power of sample was determined by the method of Oyaizu (1986) with some modifications. Reducing power activity is based on the reduction of (Fe^{3+}) ferricyanide in stoichiometric excess relative to the anti-oxidants (Benzie and Strain, 1996). Sample with different concentrations was mixed with 0.5 ml of 0.2 M sodium phosphate buffer (pH 6.6) and 0.5 ml of 1% potassium ferricyanide (w/v).

The mixture was incubated at 50°C for 20 min. After incubation, 2 ml of 10% trichloroacetic acid (w/v) was added to the mixture, followed by centrifugation at 650 rpm for 10 min. The upper layer (0.5 ml) was mixed with 0.5 ml of deionised water and 0.1 ml of 0.1% ferric chloride (w/v) and the absorbance of the resultant solution was measured at 700 nm. Ascorbic acid, α-tocopherol, BHA, and BHT were used as reference compound.

Total phenol and flavonoid analysis

The total phenolic content was determined using the Folin-Ciocalteu reagent according to the method described by Singleton and Rossi (1965). Briefly, 0.1 ml of sample and 50 µl of 2 N Folin-Ciocalteu reagents were added to a 5 ml volumetric flask. The solutions were mixed and allowed to stand for 3 to 5 min at room temperature. Next, 0.3 ml of a 20% sodium carbonate solution (w/v) was added. Solutions were mixed and kept aside for 15 min. Finally, 1 ml of distilled water was added. The blue color was measured against reagent blank at 725 nm using a UV-spectrophotometer.

The total phenolic content of sample was determined by comparing with the optical density values of different concentrations of a standard phenolic compound, gallic acid. This analysis for each sample was analyzed in triplicate, and a calibration curve of gallic acid was plotted by plotting absorbance vs concentration of gallic acid.

The total flavonoid content of extract was determined according to colorimetric method as described by Park et al. (1997). An aliquot of 0.2 ml was added to test tubes containing 0.1 ml of 10% aluminum nitrate (w/v), 0.1 ml of 1 M potassium acetate and 4.6 ml of 80% ethanol. After 40 min at room temperature, the absorbance was determined at wavelength 415 nm. The total flavonoid content was expressed in milligrams of quercetin equivalents (QE) per gram of samples.

Ferric thiocyanate test (FTC)

The test was performed according to FTC method in linoleic acid emulsion (Haraguchi et al., 1992) with some modifications. The reaction medium contained 0.02 ml of sample (10 mg/ml), 0.2 ml of 2.51% linoleic acid in ethanol, 0.4 ml of 0.04 M photassium phosphate buffer (pH 7.0) and 0.38 ml of distilled water. The solution (1 ml) was mixed and incubated at 70°C for 20 min in the dark.

The same reaction medium, without any additive was taken as control sample. Synthetic anti-oxidants (BHA and α-tocopherol) were used for comparison, in the same concentration. At regular intervals during incubation, a 0.05 ml aliquot of the mixture was diluted with 2.85 ml of 75% ethanol, followed by the addition of 0.05 ml of 30% ammonium thiocyanate (w/v) and 0.05 ml of 20 mM of ferrous chloride in 3.5% HCl. The absorbance for the red colour was measured at 500 nm. These steps were repeated every 3 h until the control reached its maximum absorbance value.

Table 1. Yield, total phenolic content and total flavonoid content of the extracts from *R. verniciflua* by extraction conditions.

Sample	60E[d]	80E[d]	100E[d]	60M[d]	80M[d]	100M[d]	H_2O[d]
% Yield[a]	7.7	9.2	8.1	8.9	10.8	8.5	3.5
TPC[b]	272.7 ± 1.1	241.1 ± 3.0	352.6 ± 1.5	272.2 ± 0.3	274.2 ± 6.7	248.1 ± 4.3	220.8 ± 7.0
TFC[c]	35.4 ± 0.6	30.8 ± 1.0	54.6 ± 0.2	27.3 ± 0.1	25.4 ± 0.2	26.8 ± 0.1	17.4 ± 0.3

[a]Percentage yields from the dried plant material, Total phenol content analyzed as gallic acid equivalent (GAE) mg/g of extract, values are the average of triplicates, [c]Total flavonoid content analyzed as quercetin equivalent (QE) mg/g of extract, values are the average of triplicates,[d]60E, 60% ethanolic extract; 80E, 80% ethanolic extract; 100E, 100% ethanolic extract; 60M, 60% methanolic extract; 80 M, 80% methanolic extract; 100M, 100% methanolic extract; H_2O, distil water extract.

Statistical analysis

All data were expressed as mean value ± standard deviation (SD) of the number of experiments (n = 3). Microsoft excel program was used for data analysis.

RESULTS AND DISCUSSION

Extraction yield, total phenolic and total flavonoid content

The solvent extraction is frequently used for isolation of the anti-oxidants, both extraction yield and anti-oxidant activity of extracts strongly depend on a solvent polarity (Julkunen, 1985). To investigate the extraction yield percentage and total phenolic and flavonoid content of *R. verniflua*, we prepared the extract using distill water and different solvent solutions (methanol and ethanol) at room temperatures (Table 1). Extraction yield was significantly greater for using a mixture of alcohol and water (7.7 to 10.8%) than for using distill water (3.5%). The highest extraction yield was obtained using an 80% ethanol solution and the lowest using distill water. This result is consistent with previous reports of Kim and Kim (2006) and Sultana et al. (2009) that aqueous organic solvent is an effective solvent for extraction of plant materials.

The total phenolic content varied in different solvent extract condition and ranged from 220.8 to 352.6 mg GAE/g. In general, total phenolic content increased with the increment of the ethanol concentration up to 60% followed by a remarkable drop at 100% (Cacace and Mazza, 2003; Chan et al., 2009).

However, in this study, the total phenolic content of 100% EtOH extract was relatively higher compared to that of other samples. Distil water extract was the lowest total phenolic content. According to the recent reports, statistically significant relationship between total phenolics and anti-oxidant activity was found in traditional balsam vinegar (Verzelloni et al., 2007). The anti-oxidant activity power was correlated with the total polyphenolic content of the Indian Laburnum extracts (Siddhuraju et al., 2002).

The obtained results showed that the 100% EtOH extract had higher total flavonoid content, equal to 54.6 mg QE/g. Total flavonoid contents were found to contain in the following order: 100% EtOH extract > 60% EtOH extract > 80% EtOH extract > 60% MeOH extract > 80%, 100% MeOH extract > distil water extract (Table 1). Polyphenols are the major plant compounds with anti-oxidant activity. Green teas contain up to 30% of the dry weight as phenolic compounds, and tea polyphenols play an important part in the function of anti-oxidation and anti-proliferation (Lin et al., 1996). The anti-oxidant activity of phenolic compounds is mainly due to their redox properties, which allow them to act as reducing agents, hydrogen donors, and singlet-oxygen quenchers (Kang et al., 2006). This suggests that polyphenolic constituents from *R. verniflua* may contribute to the highest anti-oxidant activities in the DPPH assay.

Determination of electron donation ability

The determination of the EDA, *R. verniciflua* extracts were carried out according to the DPPH method. The EDA of extract from *R. verniciflua* showed stronger activity than BHT, but it did not exhibit higher anti-oxidant activity than ascorbic acid and α-tocopherol. Each extract showed different activities particularly, 100% EtOH extract showed the highest activity, which was about 5.2-fold more active than that of a positive control, BHT (Figure 1).

Reducing power activity

In this assay, the reducing power was determined using reaction solution colour changing at 700 nm, which the yellow colour of the test solution change to green and blue depending on the reducing power of sample concentration. Higher absorbance of the reaction mixture indicates a higher reducing power. Figure 2 showed a dose-dependent reducing power of *R. verniciflua* extracts. The reducing power of extracts and positive control increased steadily with increasing concentration of sample. One hundred percent EtOH extracts (0.54) showed the highest activity, and the other extracts (0.29

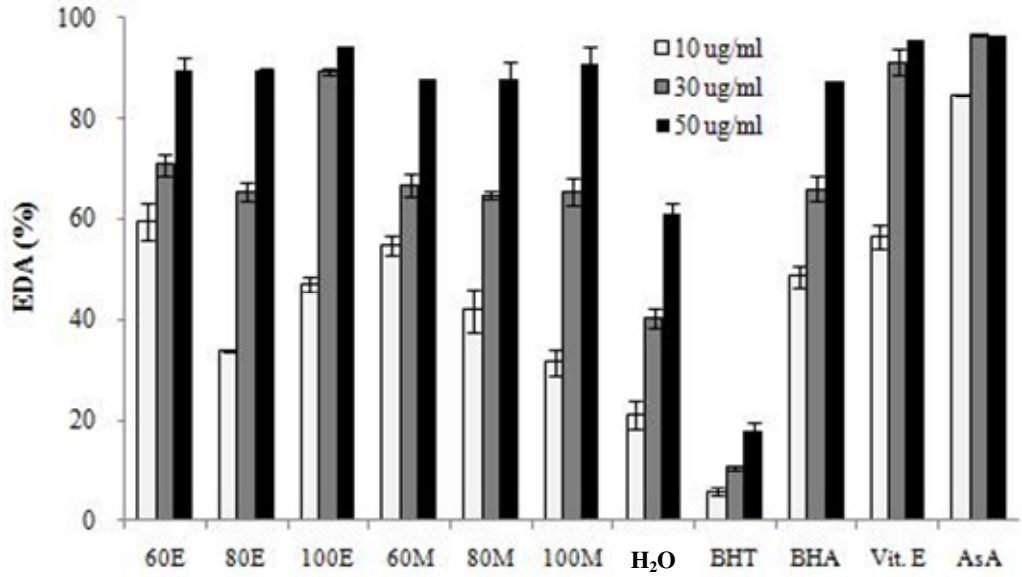

Figure 1. Electron donation ability (EDA) of extracts from *R. verniciflua* by extraction conditions. 60E, 60% ethanolic extract; 80E, 80% ethanolic extract; 100E, 100% ethanolic extract; 60 M, 60% methanolic extract; 80 M, 80% methanolic extract; 100M, 100% methanolic extract; H_2O, distil water extract; BHT, butylated hydroxytoluene; BHA, butylated hydroxyanisole; Vitamin E, α-tocopherol; AsA, ascorbic acid.

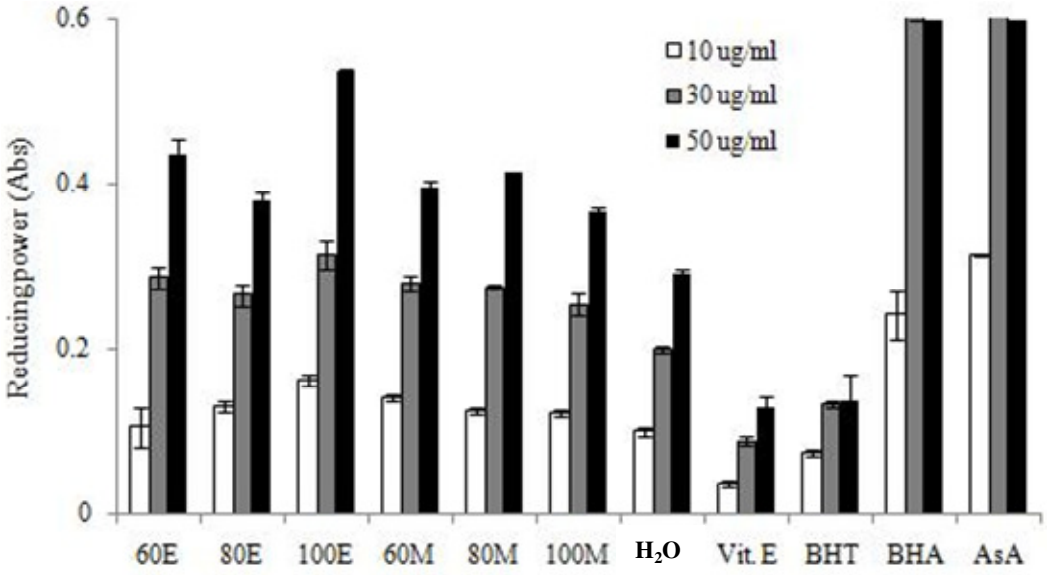

Figure 2. Reducing power of extracts from *R. verniciflua* by extraction conditions. 60E, 60% ethanolic extract; 80E, 80% ethanolic extract; 100E, 100% ethanolic extract; 60 M, 60% methanolic extract; 80 M, 80% methanolic extract; 100 M, 100% methanolic extract; H_2O, distil water extract; Vit. E, α-tocopherol; BHT, butylated hydroxytoluene; BHA, butylated hydroxyanisole; AsA, ascorbic acid.

to 0.44) showed stronger activity than α-tocopherol (0.13) and BHT (0.14). Distill water extract showed some degree of reducing power. Although EDA of tested α-positive control were in increasing order, ascorbic acid > tocopherol > BHA > BHT, but reducing power order were ascorbic acid > BHA > BHT > α-tocopherol. The reducing capacity may used as an indicator of the potential activity. Total phenolic content increased proportionally to the

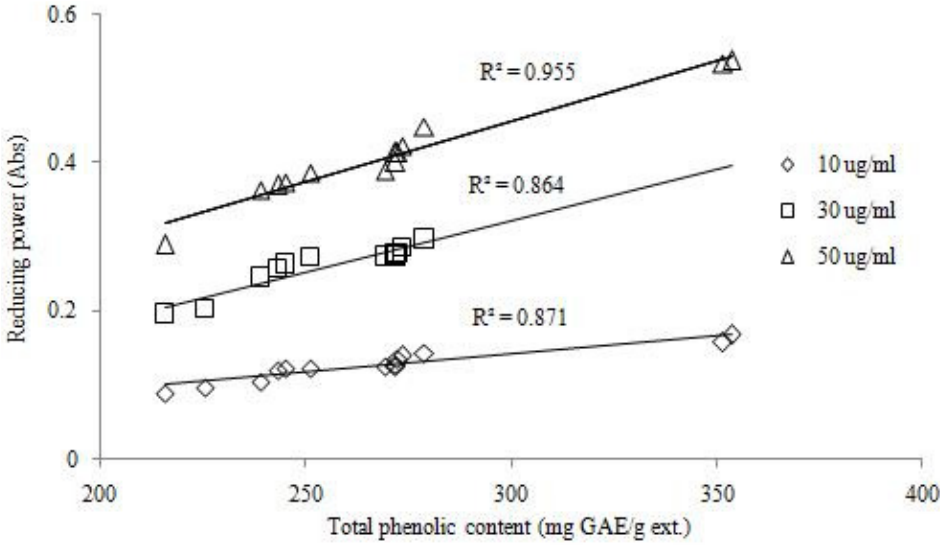

Figure 3. Correlation established between reducing power and total phenolic content values in *R. verniciflua*

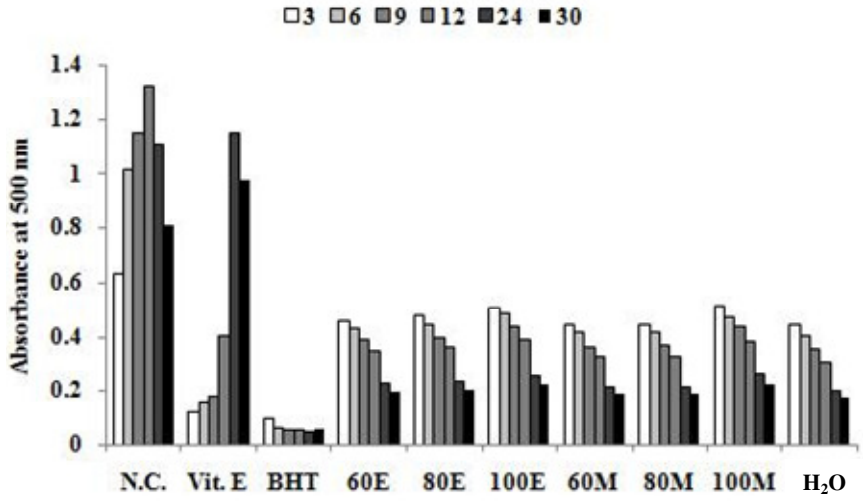

Figure 4. Antioxidative activity of *R. verniciflua* extracts based on a ferric thiocyanate method. N.C., negative control; Vitamin E, α-tocopherol; BHT, butylated hydroxytoluene; 60E, 60% ethanolic extract; 80E, 80% ethanolic extract; 100E, 100% ethanolic extract; 60 M, 60% methanolic extract; 80 M, 80% methanolic extract; 100 M, 100% methanolic extract; H_2O, distil water extract

reducing power. As showed in (Figure 3), a high correlation ($r^2 = 0.955$) was found between reducing power and total phenolic content in *R. verniciflua* extracts. A similar finding was reported by Sousa et al. (2008), who suggested that high correlation established between the total phenolic content in the samples and its anti-oxidant activity (reducing power and radical scavenging effect on DPPH radicals). Also, linear relationships between these parameters have been found for extracts of several plant materials (Hajimahmoodi et al., 2008; Vásquez et al., 2008). Anti-oxidant activity of pulp extracts is due to the presence of phenolic compounds; about 85% of the anti-oxidant activity of phenolics is attributable to their redox properties (Hajimahmoodi et al., 2008).

Ferric thiocyanate test (FTC)

The anti-oxidant activities of the extracts are compared with two commercial anti-oxidants, α–tocopherol and BHT, as determined by the ferric thiocyanate method, which measures the amount of peroxide produced by linoleic acid emulsion during incubation. Low absorbance values in the FTC method indicate high level of anti-oxidant activity. Figure 4 shows the changes in the

absorbance for each sample during 30 h of incubation at 70°C. It is seen that absorbance increases with time, the autoxidation of linoleic acid emulsion without sample or BHT was accompanied by a rapid increase of peroxide product.

The significantly lower absorbances of the extracts and BHT indicate the greater anti-oxidant activity of the extracts. α–Tocopherol was slowly increased until 9 h, but suddenly increased after 9 h. The increasing orders of anti-oxidant activity may be given as distill water extract > 60, 80% MeOH extract > 60, 80% EtOH extract > 100% MeOH or EtOH extract.

In conclusion, the aim of this study was to obtain a better understanding of the potent antioxidant extracts of *R. verniciflua* using different water/alcohol ratios. So, we determined the amount of phenols and flavonoids, and conducted using various assays, including electron donation ability, reducing power, and anti-lipid peroxidative effects. As a result, the 100% EtOH extracts showed stronger antioxidative activity than other extracts.

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REFERENCES

Anwar F, Jamil A, Iqbal S, Sheikh MA (2006). Antioxidant activity of various plant extracts under ambient and accelerated storage of sunflower oil. Grasas Aceites Sevilla, 57: 189-197.

Benzie IFF, Strain JJ (1996). The ferric reducing ability of plasma as a measure of 'antioxidant power': the FRAP assay. Anal. Biochem., 239: 70-76.

Cacace JE, Mazza G (2003). Optimization of extraction of anthocyanins from black currants with aqueous ethanol. J. Food Sci., 68: 240-248.

Chan SW, Lee CY, Yap CF, Wan AWM, Ho CW (2009). Optimisation of extraction conditions for phenolic compounds from limau purut (*Citrus hystrix*) peels. Int. Food Res. J., 16: 203-213.

Choi HS, Kim MK, Park HS, Yun SE, Mun SP, Kim JS, Sapkota K, Kim S, Kim TY, Kim SJ (2007). Biological detoxification of lacquer tree (*Rhus verniciflua* Stokes) stem bark by mushroom species. Food Sci. Biotechnol., 16: 935-942.

Choi J, Yoon BJ, Han YN, Lee KT, Ha J, Jung HJ, Park HJ (2003). Antirheumatoid arthritis effect of *Rhus verniciflua* and of the active component, sulfuretin. Planta. Med., 69: 899-904.

Hajimahmoodi M, Sadeghi N, Jannat B, Oveisi MR, Madani S, Kiayi M, Akrami MR, Ranjbar AM (2008). Antioxidant activity, reducing power and total phenolic content of Iranian olive cultivar. J. Biol. Sci., 8: 779-783.

Haraguchi H, Hashimoto K, Yagi A (1992). Antioxidative substances in leaves of *Polygonum hydropiper*. J. Agric. Food Chem., 40: 1349-1351.

Jeon WK, Kim JH, Lee HW, Ko BS, Kim HK (2003). Effects of *Rhus verniciflua* Stokes (RVS) extract on diet-induced obesity in C57BL/6 mouse. Korean J. Pharmacol., 34: 339-343.

Jeon WK, Lee JH, Kim HK, Lee AY, Lee SO, Kim YS, Ryu SY, Kim SY, Lee YJ, Ko BS (2006). Anti-platelet effects of bioactive compounds isolated from the bark of *Rhus verniciflua* Stokes. J. Ethnopharmacol., 106: 62-69.

Jeong JS, Park JW, Yoon SW (2008). Carcinostatic effect of allergen removed *Rhus Verniciflua* Stokes based traditional Korean medicine on a patient with lung adenocarcinoma; single case report. Orient. Pharm. Exp. Med., 7: 573-578.

Julkunen TR (1985). Phenolic constituents in the leaves of northern willows, methods for the analysis of certain phenolics. J. Agric. Food Chem., 33: 213-217.

Kang KA, Zhang R, Piao MJ, Park S, Park J, Kim JS, Kang SS, Hyun JW (2006). Screening of antioxidant and anticancer effects from flavonoids. Cancer Prevention Res., 11: 235-239.

Kim JB (2000). Method for extracting the extract of detoxicated *Rhus verniciflua*. KR Patent No. KR/10/2000/0055559.

Kim JH, Kim JK (2006). Antioxidant activity and functional component analysis of Korean mountain Ginseng's different sections. J. Korean Soc. Food Sci. Nutr., 35: 1315-1321.

Kim JS, Kwon YS, Chun WJ, Kim TY, Sun J, Yu CY, Kim MJ (2010). *Rhus verniciflua* Stokes flavonoid extracts have anti-oxidant, anti-microbial and α-glucosidase inhibitory effect. Food Chem., 120: 539-543.

Kim MJ, Choi WC, Barshinikov AM (2002). Anticancer and antioxidant activity of allergen-removed extract in *Rhus verniciflua* Stokes. Korean. J. Med. Crop Sci., 10: 288-293.

Kim MJ, Choi YH, Kim WG, Kwak SS (1997). Antioxidative activity of urushiol derivatives from the sap of lacquer tree (*Rhus vernicifera* Stokes). Korean. J. Plant Res., 10: 227-230.

Kim MJ, Kim CJ, Kwak SS (1997). Antifungal activity of urushiol components in the sap of Korean lacquer tree (*Rhus vernicifera* Stokes). Korean J. Plant Res., 10: 231-234.

Kim MJ, Lee YS, Lee YJ, Kim JS, Choi WC, Kwon YS, Rim YS, Kim EH, Kim CM, Yu CY (2000). Allergen-removed-extract of *Rush verniciflua*. KR Patent No. KR10/2000/0031328.

Kim TJ (1996). Korea resource plants. Seoul National University Press, Seoul, Korea, 2: 294.

Kitts DD, Lim KT (2001). Antitumorigenic and cytotoxic properties of an ethanol extract derived from *Rhus verniciflua* Stokes (RVS). J. Toxicol. Environ. Health, Part A, 64: 357-371.

Kobayashi S, Uyama H, Ikeda R (2001). Arttificial urushi. Chem. A Eur. J., 7: 4755-4760.

Lee JC, Lee KY, Kim J, Na CS, Jung NC, Chung GH, Jang YS (2004). Extract from *Rhus verniciflua* Stokes is capable of inhibiting the growth of human lymphoma cells. Food Chem. Toxicol., 42: 1383-1388.

Lee JC, Lim KT, Jang YS (2002). Identification of *Rhus verniciflua* Stokes compounds that exhibit free radical scavenging and anti-apoptotic properties. Biochem. Biophys. Acta., 1570: 181-191.

Lee JH, Lee HJ, Lee HJ, Choi WC, Yoon SW, Ko SG, Ahn KS, Choi SH, Ahn KS, Lieske JC, Kim SH (2009). *Rhus verniciflua* Stokes prevents cisplatin-induced cytotoxicity and reactive oxygen species production in MDCK-I renal cells and intact mice. Phytomedicine 16: 188-197.

Lee SH, Nan JX, Zhao YZ, Woo SW, Park EJ, Kang TH, Seo GS, Kim YC, Sohn DH (2003). The chalcone butein from *Rhus verniciflua* shows antifibrogenic activity. Planta Med., 69: 990-994.

Lin Y, Juan I, Chen Y, Liang Y, Lin J (1996). Composition of polyphenols in fresh tea leaves and associations of their oxygen-radical-absorbing capacity with antiproliferative actions in fibroblast cells. J. Agric. Food Chem., 44: 1387-1394.

Majima R (1992). Über den hauptbestandteil des Japan-lacks, VIII. Mitteilung: Stellung der doppelbindungen in der seitenkette des urushiols und beweisführung, daß das urushiol eine mischung ist. Berichte der Deutschen Chemischen Gesellschaft B, 55: 172-191.

Onishi N (1995). Urushi. Elegant Asia, NEC Kurieitibu,Japan, p. 18.

Oyaizu M (1986). Studies on products of browning reactions: Antioxidative activities of products of browning reaction prepared from glucosamine. Jap. J. Nutr., 44: 307-315.

Park KY, Jung GO, Lee KT, Choi J, Choi MY, Kim GT, Jung HJ, Park HJ (2004). Antimutagenic activity of flavonoids from the heartwood of *Rhus verniciflua*. J. Ethnopharmacol., 90: 73-79.

Park SJ, Kim KH, Choi WC, Kwon SP, Wang YK, Kang BS, Lee SS, Shin, MJ (2009). Allergen free *Rhus verniciflua* extract and composition containing the same. KR Patent No. KR10/2009/0035684.

Park YK, Koo MH, Ikegaki M, Contado JL (1997). Comparison of the

flavonoid aglycone contents of *Apis mellifera* propolis from various regions of Brazil. Arquivos de Biologiae Technologia,, 40: 97-106.
Siddhuraju P, Mohan PS, Becker K (2002). Studies on the antioxidant activity of Indian Laburnum (*Cassia fistula* L.): A preliminary assessment of crude extracts from stem bark, leaves, flower and fruit pulp. Food Chem., 79: 61-67.
Singleton VL, Rossi JA, (1965). Colorimetry of total phenolics with phosphomolybdic-phosphotungstic acid reagents. Am. J. Enol. Viticul., 16: 144-158.
Son YO, Lee KY, Lee JC, Jang HS, Kim JG, Jeon YM, Jang YS (2005). Selective antiproliferative and apoptotic effects of flavonoids purified from *Rhus verniciflua* Stokes on normal versus transformed hepatic cell lines. Toxicol. Lett., 155: 115-125.
Song SH (2007). Method of detoxifying lacquer. KR Patent No. KR10/2007/0049009.
Sousa A, Ferreira ICFR, Barros L, Bento A, Pereira JA (2008). Effect of solvent and extraction temperatures on the antioxidant potential of traditional stoned table olives “alcaparras”. Food Sci. Technol., 41: 739-745.
Sultana B, Anwar F, Ashraf M (2009). Effect of extraction solvent/technique on the antioxidant activity of selected medicinal plant extracts. Molecule, 14: 2167-2180.
Sultana B, Anwar F, Przybylski R (2007). Antioxidant activity of phenolic components present in barks of *Azadirachta indica*, *Terminalia arjuna*, *Acacia nilotica*, and *Eugenia jambolana* Lam. trees. Food Chem., 104: 1106-1114.
Vázquez G, Fontenla E, Santos J, Freire MS, González ÁJ, Antorrena G (2008). Antioxidant activity and phenolic content of chestnut (*Castanea sativa*) shell and eucalyptus (*Eucalyptus globulus*) bark extracts. Ind. Crop Prod., 28: 279-285.
Verzelloni E, Tagliazucchi D, Conte A (2007). Relationship between the antioxidant properties and the phenolic and flavonoid content in traditional balsam vinegar. Food Chem., 105: 564-571.