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Antioxidant and Whitening Activities of Smilacis Glabrae Rhizoma Extracts

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Abstract: This study is finding out 4 kinds of extract's total amount of flavonoids and polyphenols these extracts are obtained by drying Smilacis Glabrae Rhizoma as 80% ethanol a solvent, extraction temperature 30, 70°C and distilled water as a solvent and extraction temperature 30, 100°C and confirming anti-aging active through antioxidant and whitening effect. As a result, the total polyphenol and total flavonoid content also extract ethanol 70°C each of 75.86, 2.38 mg/g showed the highest antioxidant effect. Tyrosinase inhibitory activity, 80% ethanol 30°C is the highest as 40.57%. From the above results, Smilacis Glabrae Rhizoma's extracts has tyrosinase inhibitory activity, effectiveness of antioxidant and since, it showed various effects in clinical practice, we confirmed that, it has the potential applications as natural cosmetics material.

Key words: Smilacis Chinae Radix, Smilacis Glabrae Rhizoma, antioxidant, anti-aging, whitening, cosmetics

INTRODUCTION

There is a growing interest in anti-aging due to improved living standards and longer life expectancy. It is known that, among the causes of aging, Reactive Oxygen Species (ROS) generated in the metabolic process of the human body not only cause aging by lowering biological functions such as the peroxidation and inactivation of lipids and proteins and DNA fragmentation but also involves in various diseases such as cancer, arteriosclerosis, diabetes and hypertension (Valko *et al*., 2007). Ultraviolet rays generate ROS in the skin which not only triggers an inflammatory response by causing damage to skin cells and tissues but also causes skin aging (Kwon, 2013) and produces spots, freckles and blotches (Chung *et al*., 2003) by reducing skin collagen and elastin which are the main constituents of the dermis layer of skin.

Synthetic antioxidants such as Butylated Hydroxy-Toluene (BHT) Butylated Hydroxy-Anisole (BHA) and Propyl Gallate (PG) have been developed to remove ROS and they are being widely used because of excellent antioxidative effects and economic efficiency. However, as they have been reported to have the possibility of causing cancer and problems with skin safety and stability when formulating cosmetics (Omaye *et al*., 1997), various efforts have recently been made to utilize different pharmacological effects of natural plants as functional cosmetic materials (Lee *et al*., 2014).

Smilacis Glabrae Rhizoma (SG) which is a deciduous climbing shrub belongs to the lily family is widely distributed in Asia including Korea, China and Japan and also referred to as Asian Supplejack or Korean Berchemia. This refers to the roots of Smilax China L. (Park *et al*., 2014). In the oriental medicine, SG is known to have the effects of hyperuricemia treatment and renal protection, gout treatment, detoxification, anti-inflammation, anticancer and antioxidant (Yuan *et al*., 2007; Lee *et al*., 2001). SG is also known to contain six types of steroid saponins as spirostane glycosides and furostane glycosides (Cha *et al*., 2005; Park *et al*., 2002) and to contain kaempfrol-7-O-β-Dglucoside which is a polyphenol component with antioxidant activity and kaempferol-7-O-α-L-rhamnopyranoside and kaempferol-3,7-O-α-L-dirhamnopyranoside with high antioxidant activity at α-tocopherol level (Cha and Lee, 2007). Based on this, this study intends to investigate antioxidant activity and tyrosinase activity inhibition using SG extract which is mainly used as a raw material of herbal medicine and to identify the possibility of using it as a raw material for cosmetics for antioxidant, anti-aging and whitening.

MATERIALS AND METHODS

Sample extraction: SG used in this study was purchased from a herbal medicine market in Seoul City, dried in the shade and grinded then used as an experimental material. 100 g of SG was each added to 600 mL of distilled water and 80% ethanol and the extracts were obtained for 2 h at room temperature. Also, 100 g of SG was each added to distilled water and 600 mL of 80% ethanol for 2 h in Outo Clave (KMC-1221, VISION, Deg.) made by Vision

Corp. and the extracts were obtained for 2 h at 100 and 70°C, respectively. The extracts were filtered, concentrated and lyophilized.

Measurement of DPPH radical scavenging ability: The radical scavenging activity for 1, 1-diphenyl-2-picryl hydrazyl (DPPH) radical was measured using Bloi's method (Blois, 1958). Four extracts under each condition were prepared at the concentration of 0.01 g/mL and used for measurement. About 0.1 mL of the extract was added in increments of 100 and 500 mg/L to 3.9 mL of DPPH methanol solution made by dissolving 0.0013 g of DPPH solution in 50 mL of methanol solution. After the mixture was reacted at room temperature for 30 min, the absorbance was measured at 515 nm using a UV/VIS spectrophotometer. The difference in absorbance between the group of treated sample and untreated sample was expressed by percentage.

Measurement of the total polyphenol content: Total polyphenol content was measured by Folin-Deni's method. About 1 mL of SG extract sample was mixed with 60 μL of $FeCl_3$ (0.1 M), 20 μL of HCl (0.1 M) and 60 μL of $Fe(CN)_6$ (0.008 M) solution and the mixture was reacted at room temperature for 10 min. After that, the absorbance was measured at 720 nm using a UV/VIS Spectrophotometer. The standard calibration curve was taken to give final concentrations of 2, 4, 6 and 8 μg/mL using tannic acid (10 mg/L) and the absorbance was measured using the method described above to calculate the polyphenolic compound content contained each extract.

Measurement of the total flavonoid content: In the case of total flavonoid content, SG exract sample was dissolved in increments of 0.02 g in 1 mL of water using a modified Moreno *et al*. (2000) method. The mixture was centrifuged which was mixed in increments of 500 μL with water at a ratio of 1:1 to prepare a 2% solution. After 1 mL of DMACA solution (4-Dimethylamimo Cinnamaldehyde 0.1% in 1 mol/L of HCl in MeOH) was mixed with the 2% solution and that was reacted for 10 min, the absorbance was measured at 640 nm using a UV/VIS spectrophotometer. Total flavonoid content was determined using catechin so that the final concentrations of catechin were 2, 4, 6, 8 and 10 μg/mL and the absorbance was measured and calculated using the method described above.

Measurement of tyrosinase inhibition activity: Yagi method was adjusted to measure tyrosinase inhibition activity. The concentration of extracts under each condition to 0.001 g/mL was adjusted to make a sample which was used to measure tyrosinase inhibition activity. After 100 μL of 0.03% tyrosine in phosphate buffer (pH 6.8) and 100 μL of the extract were mixed and left in a 37°C water bath for 10 min. About 100 μL (200 units/mL) of mushroom tyrosinase was added to the mixture and reacted at 37°C. Then, the absorbance was measured at 472 nm using a UV/VIS spectrophotometer and the rate of decrease in absorbance of the group of treated extract sample and untreated extract sample was calculated as percentage and expressed as tyrosinase inhibition rate.

Statistical processing: The results of the experiment were processed statistically using unpaired student's t-test in SPSS 20.0 and ANOVA. Statistical significance was tested at $p<0.05$, <0.01 and <0.001.

RESULTS AND DISCUSSION

DPPH radical scavenging ability: DPPH (1, 1-diphenyl-2-picrylhydrazyl) which is a stable free radical is reduced by aromatic compounds and amines due to the electron donating effect of antioxidants and it is a method for measuring antioxidant activity using the degree of discoloration of purple color. The ability to significantly reduce or offset free radicals implies the high antioxidant effects and scavenging activity against ROS (Blois, 1958). Table 1 shows the result of measuring antioxidant activity using DPPH to investigate the antioxidant effect of SG extract. It was compared with the antioxidant effect of SG extract using vitamin C which is known to have antioxidant activity as a positive control group. The free-radical scavenging activity assay was performed for 1% SG extract using DPPH method. As a result, at 100 mg/L, the ability was 93.57% for SGW3, 97.19% for SGW 10, 90.80% for SGE3, 98.71% for SGE7 and at 500 mg/L, the ability was 94.74% for SGW3, 97.75% for SGW10, 91.82% for SGE3, 99.42% for SGE7. It was highest in SGE7. The higher the extraction temperature was the higher the free radical scavenging ability was in a concentration dependent manner. The control group, vitamin C showed 47.46% scavenging ability per 1 mg/mL and SG extract showed over 2 times higher free radical scavenging ability than vitamin C. This result shows that the electron donating activity of broadleaf liriope root extract is over 3 times higher than 12.78 and 28.91% (Seo and Kim, 2010). This suggests that SG extract can exert the anti-aging effect of an antioxidant superior to conventional herbal medicine.

Total polyphenol and flavonoid content: Phenolic compounds including flavonoids contained in edible and

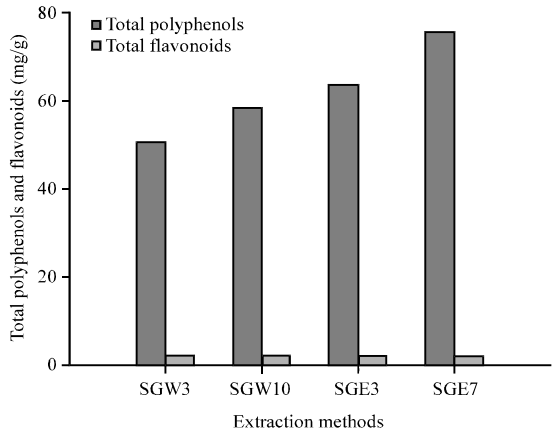

Fig. 1: Total polyphenol and flavonoids in each extracts from Smilacis glabrae Rhizoma. Values are mean of three independent measurements. SGW3: Smilacis Glabrae Rhizoma water extract at room temperature, SGW10: Smilacis Glabrae Rhizoma water extract at 100°C, SGE3: Smilacis Glabrae Rhizoma ethanol extract at room temperature, SGE7: Smilacis glabrae Rhizoma ethanol extract at 70°C

Table 1: Comparison of DPPH radical scavenging activity of extracts with different extraction methods from smilacis glabrae rhizoma

Extract	100 mg/L (%)	500 mg/L (%)
SGW3	93.57	94.74
SGW10	97.19	97.75
SGE3	90.80	91.82
SGE7	98.71	99.42

medicinal plants are hydroxyl group-bearing aromatic compounds and also physiologically active ingredients that exhibit various physiological and pharmacological activities such as antioxidation, anti-aging, anti-inflammation, UV exposure and anti-allergy and enhance the safety and functionality of foods and cosmetics (Ku *et al*., 2015).

Total polyphenol contents in SG extract were measured using tanic acid as a reference material and total flavonoid contents were measured using catechin as a reference material. The results are shown in Fig. 1. The total polyphenol contents in SG were 50.97 mg/g in SGW3, 58.32 mg/g in SGW10, 63.98 mg/g in SGE3 and 75.86 mg/g in SGE7. Total flavonoid contents were 2.33 mg/g in SGW3, 2.31 mg/g in SGW10, 2.33 mg/g in SGE3 and 2.38 mg/g in SGE7. There was no significant difference in flavonoid content among solvents but total polyphenol content was relatively high in ethanolic extract. Total polyphenol and flavonoid contents at each temperature were higher in the extract using 95% ethanol than distilled water as a solvent. This, therefore, shows that polyphenol and flavonoid contents in extracts are highest in 80% ethanol at 70°C. According to the results of a study conducted to measure the content of polyphenol

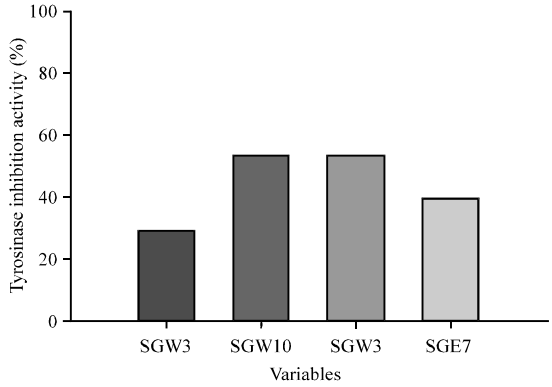

Fig. 2: Tyrosinase inhibition activity of extracts with different extraction methods from Smilacis glabrae Rhizoma. All value presents the mean of triplicate determinations. SGW3: Smilacis Glabrae Rhizoma water extract at room temperature, SGW10: Smilacis Glabrae Rhizoma water extract at 100°, SGE3: Smilacis Glabrae Rhizoma ethanol extract at room temperature, SGE7: Smilacis Glabrae Rhizoma ethanol extract at 70°C (SGE3>SGW10>SGE7> SGW3)

compounds, the content of polyphenols in ginseng and Solomon's-seal was 3.97 and 2.62 mg/g, respectively (Kim *et al*., 2004) and that of ultrasonification ethanolic extract of *Portulaca oleracea* was 43.73 mg/g (Ku *et al*., 2015). By contrast, the total polyphenol content in SGE7 of this study was 75.86 mg/g. This indicates that the ethanolic extract of SG can be used as an antioxidant activity material based on the result of a previous study (Rice-Evans *et al*., 1997) that the higher the total polyphenol content is the greater the effect of physiological activity such as antioxidation is. In addition, the extraction contents depending on solvents should be considered when making SG extract.

Tyrosinase inhibition activity: Tyrosinase is an enzyme that converts tyrosine to Dihydroxyphenyl-alanine (DOPA) and DOPA to DOPA-quinone. It involves in the production of black brown melanin pigment. Thus, the inhibition of tyrosinase activity is recognized as a useful evaluation method for the development of whitening materials and development of anti-aging skin products (Prota, 1980).

Figure 2 shows the tyrosinase inhibition activity of SG extract. The activity in SGW3 was 21.70%, SGW10 40.28%, SGE3 40.57% and SGE7 29.69%. Among them, SGE3 showed the highest activity with 40.57% which was lower than that of vitamin C (1000 mg/L, 60.78%), the control group.

Tyrosinase inhibition activity showed a high activity with 40.57% in SGE3, compared to the inhibitory rate of 29.10% in ultrasonification ethanolic extract of

Portulaca oleracea (Ku *et al*., 2015), 22% in peppermint, 20% in rosemary and 5% in *Acorus gramineus* Soland (Jung *et al*., 1995). Tyrosinase inhibition is known to be more effective as the content in polyphenol compounds is higher (Seo *et al*., 2013). This suggests that tyrosinase inhibition activity is high due to a high polyphenol content in SG extract. Thus, it seems that SG extract in 80% ethanol at 30°C can be used as a whitening cosmetic material.

CONCLUSION

This study conducted an experiment on the antioxidant and tyrosinase inhibitory effects of SG extracts in order to investigate the possibility of using the SG extract as a functional cosmetic material. The yield of SG extract was higher in distilled water than ethanol and the extract in distilled water at 100°C showed the highest yield with 8.27%. According to the result of verifying the antioxidant effect using electron donating ability, the antioxidant effect was highest with 99.42% at SGE7 500 mg/L which has twice as high antioxidant effect as vitamin C, the control group. Total polyphenol content was highest with 75.86 mg/g in SGE7 and relatively high in ethanol extract. Total flavonoid content was highest with 2.38 mg/g in SGE7 but the difference depending on solvents and temperature gap was not significant. Tyrosinase inhibition activity which affects the whitening effect was highest with 40.57% in SGE3 but lower than that of vitamin C (60.78%), the control group.

In conclusion, SG extract has the highest level of total polyphenol and flavonoid contents and electron donating ability in 80% ethanol and 70°C extractionn which means high antioxidant and anti-aging effects. SG for the whitening effect was most effective in 80% ethanol and 30°C extraction. It seems that SG extract in ethanol at high temperature can be used as a functional material for antioxidant and anti-aging.

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