Extraction conditions of white rose petals for the inhibition of enzymes related to skin aging

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In order to assess inhibitory potentials of white rose petal extracts (WRPE) on the activities of enzymes related to dermal aging according to the extraction conditions, three extraction methods were adopted. WRPE was prepared by extracting dried white rose (Rosa hybrida) petals with 50% ethanol (WRPE-EtOH), Pectinex® SMASH XXL enzyme (WRPE-enzyme) or high temperature-high pressure (WRPE-HTHP). In the inhibition of matrix metalloproteinase-1, although the enzyme activity was fully inhibited by all 3 extracts at 100 µg/mL in 60 min, partial inhibition (50-70%) was achieved only by WRPE-EtOH and WRPE-enzyme at 50 µg/mL. High concentrations (≥250 µg/mL) of all 3 extracts markedly inhibited the elastase activity. However, at low concentrations (15.6-125 µg/mL), only WRPE-EtOH inhibited the enzyme activity. Notably, WRPE-EtOH was superior to WRPE-enzyme and WRPE-HTHP in the inhibition of tyrosinase. WRPE-EtOH significantly inhibited the enzyme activity from 31.2 µM, reaching 80% inhibition at 125 µM. In addition to its strong antioxidative activity, the ethanol extract of white rose petals was confirmed to be effective in inhibiting skin aging-related enzymes. Therefore, it is suggested that WRPE-EtOH could be a good candidate for the improvement of skin aging such as wrinkle formation and pigmentation.

Keywords: Rosa hybrida, white rose petal extract, matrix metalloproteinase-1, elastase, tyrosinase

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With the improvement of economic level and the desire for beauty, the interest in health and skin care is increasing. For these demands, a variety of cosmetics and foods with novel bioactive ingredients for skin beauty are under research and development, attracting consumers’ attention [1].

Skin is influenced by various factors such as UV, stresses, hormones, and so on. During normal aging and photo-aging, the major features of the skin are wrinkle formation, decreased elasticity, and pigmentation. As a mechanism, increase in collagenase activity that causes skin wrinkles is one of the major factors that bring about the degeneration of collagen fiber and breakdown [1,2]. Reactive oxygen species (ROS) generated in the body also promote the activation of collagenase such as matrix metalloproteinases (MMPs) and restrain synthesis of collagen, which results in the decrease of skin elasticity and the acceleration of wrinkle formation [2,3]. Notably, skin elastase plays a pivotal role in wrinkling and/or sagging of the skin via the impairment of elastic fiber...
configuration and the subsequent loss of skin elasticity [4,5]. On the other hand, tyrosinase is a rate-limiting enzyme for the biosynthesis of melanin that causes the skin pigmentation such as melasma, freckle, and age spot [4,6-8]. Tyrosinase converts L-tyrosine into 3,4-dihydroxyphenylalanine (DOPA) as an initial step of melanin synthesis. In the pigmentation process, it is also known that ROS play an important role of mediator [2].

The rose (genus Rosa) might be one of the flowers most-widely developed to species and subspecies for decoration and as a source of aromatic oils for the perfume industry [9]. The pharmacological activities of rose flower extracts have been demonstrated in our recent series of reports. We have reported that extracts of rose flower have antioxidative and anti-inflammatory activities to assess their potential as a candidate for skin aging-related enzymes [10-12]. Especially, n-hexane and butanol fractions of white rose petal extract (WRPE) were found to have strong antioxidative activity [13,14] so that substantially prevented brain infarction in an ischemia-reperfusion model of stroke [15].

Particularly, it was suggested that rose petals and roots also contain organic molecules for natural resistance to microbial attack in the wild environment [16,17]. We demonstrated that n-hexane and butanol fractions of WRPE exerted antimicrobial activity [14] as well as anti-inflammatory potential [15,18]. Hexane fraction of WRPE was confirmed to contain high amounts of gallic acid and volatile compounds for the broad range of antioxidative, antimicrobial, antiallergic, and anti-inflammatory effects [13,14,18], however, it is still not clear whether such ingredients are responsible for the inhibition of enzymes related to skin aging.

It has been known that antiaging potentials are in part derived from antioxidative activity. Therefore, in addition to the antioxidative and anti-inflammatory capacities, the inhibitory activity of WRPE on the enzymes important for dermal aging was demonstrated in the present study, although active ingredients in the extracts are under analysis. We extracted white rose petals via three different extraction processes, the extracts were filtered through a Whatman filter paper (No. 4; Whatman, Clifton, NJ, USA) using a vacuum pump (BOC International, London, UK).

Male C57BL/6 mice to obtain blood neutrophils were purchased from the Daehan Biolink (Eumseong, Korea), and housed in a room with constant environmental conditions (23±2°C; 45-65% relative humidity; 12-hour light-dark cycle; 150-300 lux brightness). Pellet feed and purified water were available ad libitum. All the animal experiments were approved by the Institutional Animal Care and Use Committee (IACUC) of Chungbuk National University (CBNU), Korea, and conducted according to the Standard Operation Procedures (SOP) of the Laboratory Animal Research Center, CBNU.

MMP-1 activity was assayed using a SensoLyte® Generic MMP assay kit (AnaSpec, Fremont, CA, USA). In brief, Pro-MMP was incubated with 10 µg/mL trypsin for 20 min at room temperature in a 96-well microplate. Trypsin inhibitor (final concentration of 100 µg/mL) and serially-diluted WRPE (final concentrations of 20-100 µg/mL) were added to each well. After adding diluted enzyme and MMP substrate (50 µL each), mixed completely by shaking the plate. The absorbance change was recorded for 60 min at 412 nm.

Elastase activity was measured using a drug discovery kit following a protocol as in Enzo Life Science (Farmingdale, NY, USA). WRPE was serially diluted (final concentrations of 20-100 µg/mL) in 150 mM Tris-HCl buffer (pH 8.0), in a 96-well plate. Membrane homogenate of mouse blood neutrophils, as a source of elastase [19] and its substrate (100 µM N-succinyl-Ala-Ala-Ala-p-nitroanilide) were added into the wells. After incubation for 30 min at 25°C, the absorbance was measured at 410 nm using a microplate reader. The
Inhibition ratio of elastase was calculated as follows: Inhibition (%)=[(A−B)/A]×100, where A is the absorbance of control group, and B is the absorbance after reacting with each extract.

Tyrosinase activity was measured using a microplate spectrophotometer (Molecular Devices, Sunnyvale, CA, USA). Serially-diluted WRPE (final concentrations of 15.6-1,000 µg/mL) was pre-incubated with mushroom tyrosinase (2,500 U/mL; Sigma-Aldrich, St. Louis, MO, USA) in 100 mM sodium phosphate buffer (pH 6.5), and followed by adding substrate (1.5 mM tyrosine). After incubation for 30 min at 37°C, the absorbance was measured at 490 nm using a microplate reader. The inhibition ratio of tyrosinase was calculated as follows: Inhibition (%)=[(A−B)/A]×100, where A is the absorbance of control group, and B is the absorbance after reacting with each extract.

The statistical significance was determined by one-way analysis of variance, followed by post-hoc Tukey’s multiple-comparison test. P-values (<0.05, <0.01 or <0.001) were considered to be statistically significant.

In our previous studies, the pharmacological activities of WRPE were different according to the extraction solvents. For example, the antioxidative potential was strongest in hexane fraction, followed by butanol fraction and water extract. It was confirmed that the antioxidant activity of hexane fraction was comparable to well-known antioxidants butylated hydroxyanisole (BHA) and L-ascorbic acid (vitamin C) [13,14]. In the present study, we adopted ethanol, enzyme, and HTHP as solvents feasible for oral administration and dermal application. As results, all 3 kinds of extract exerted strong antioxidative activities in DPPH-scavenging assay as well as in lipid peroxidation test (data not shown), implying that the 3 types of WRPE might have antiaging potential [2].

It was well known that degradation of a major portion (higher than 90%) of collagens affects directly on the decline of skin elasticity and wrinkle formation. There are many types of collagen-breakdown enzyme, wherein MMP-1 (collagenase) is one of the enzymes playing a central role [20-22]. The MMP-1 activity was inhibited by WRPE after 30 min-lag time (Figure 1). By comparison, it was found that WRPE-EtOH and WRPE-enzyme were more effective than WRPE-HTHP in the inhibition of MMP-1. Although the enzyme activity was fully inhibited by all 3 extracts at 100 µg/mL in 60 min, a partial inhibition (50-70%) was achieved only by WRPE-EtOH and WRPE-enzyme at 50 µg/mL.

It was known that up-regulated activity of skin fibroblast-derived elastase causes impairment of elastic fiber configuration and the subsequent loss of skin elasticity, in which UVA-exposed fibroblasts play important and additional roles in UVA-induced sagging and wrinkling by up-regulating neprilysin and MMP-1, respectively [5]. In the present study, high concentrations (≥250 µg/mL) of WRPE markedly inhibited the elastase activity (Figure 2). In addition, low concentrations (15.6-125 µg/mL) of WRPE-EtOH significantly inhibited the enzyme activity.

Melanocytes, activated by α-melanocyte-stimulating hormone (α-MSH) or UVB, are major cells responsible for synthesis of melanin pigment in the skin. Melanin is synthesized by tyrosinase which is supported by tyrosinase related protein-1 (TRP-1) and TRP-2 [23,24]. Being
substrates of tyrosinase for melanin biosynthesis, L-tyrosine and L-DOPA are components in melanosomes of melanocytes located in dermal stratum basale. Thus, screening bioactive ingredients for skin whitening is focused on the inhibition of tyrosinase activity [25]. In the present study, it was confirmed that tyrosinase was remarkably inhibited by WRPE (Figure 3). It is of interest to note that WRPE-EtOH was superior to WRPE-enzyme and WRPE-HTHP in the inhibition of tyrosinase. WRPE-EtOH significantly inhibited the enzyme activity from 31.2 µM, reaching 80% inhibition at 125 µM, a similar degree to that obtained with 500 µM vitamin C. WRPE-enzyme significantly inhibited tyrosinase activity from 62.5 µM.

In the present study, the inhibitory effects on MMP-1, elastase, and tyrosinase activities were assessed for 3 different extracts of white rose petals. By comparison, WRPE-EtOH and WRPE-enzyme were similar in MMP-1- and elastase-inhibitory capacities, exhibiting higher efficacy than WRPE-HTHP. Notably, there was a marked difference in tyrosinase inhibition among WRPE-EtOH, WRPE-enzyme, and WRPE-HTHP: i.e., WRPE-EtOH was the most effective. Although 36 peaks were obtained from GC/MS analysis of WRPE, and gartanin derivatives were identified as possible active ingredients during fractionation in our ongoing study (data not shown) [26], additional ingredients remain to be clarified. In addition to its strong antioxidative activity [27], the ethanol extract of white rose petals was confirmed to be effective in inhibiting skin aging-related enzymes. Therefore, it is suggested that WRPE-EtOH could be a good candidate for the improvement of skin aging such as wrinkle formation and pigmentation.

Figure 2. Inhibition of elastase by white rose petal extracts (WRPE). White rose petals were extracted with ethanol (WRPE-EtOH, A), enzyme (WRP-enzyme, B) or high temperature-high pressure (WRPE-HTHP, C). *, **, ***Significantly different from vehicle control (Ctrl) (*<0.05, **<0.01, ***<0.001).

Figure 3. Inhibition of tyrosinase by white rose petal extracts (WRPE). White rose petals were extracted with ethanol (WRPE-EtOH, A), enzyme (WRP-enzyme, B) or high temperature-high pressure (WRPE-HTHP, C). *, **, ***Significantly different from vehicle control (Ctrl) (*<0.05, **<0.01, ***<0.001).
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Conflict of interests  The authors declare that there is no financial conflict of interests to publish these results.

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