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Study on the Application of Natural Substances to Cosmeics

Hye-Jin Kwon[1]
[1]Department of Chemical Engineering, University of Soongsil, Seoul, CO 06978, Korea

Abstract

Background/Objectives: This study was conducted on the application of natural cosmetics using Stachys sieboldii Miq. Roots and their Antioxidant, anti-aging and whitening. **Methods/Statistical analysis**: The DPPH radical scavenging activity of Stachys sieboldii Miq. root extract was increased in a concentration-dependent manner in an antioxidant activity test. Its ABTs radical scavenging activity was 94.32±0.12% at a concentration of 1000 μg/mL, and its collagenase inhibitory activity was 39.4±2.52% at the same level of concentration in an anti-aging activity test. **Findings**: These findings suggest that Stachys sieboldii Miq. roots have potential antioxidant, whitening and anti-aging effects. **Improvements/Applications**: It is deemed that Stachys sieboldii Miq. root extract can be effectively used in the development of new functional cosmetic ingredients.

Index Terms

Stachys sieboldii Miq. root extract, antioxidant, anti-aging, whitening, functional cosmetic

Corresponding author : H. J. Kwon
kwonhj0070@ssu.ac.kr

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I. INTRODUCTION

Unlike synthetic chemicals, physiologically active substances of natural products have relatively less toxicity issues on the human body, and above all, since natural products have the advantage of exhibiting various pharmacological activities at one time, those with proven pharmacological efficacy are used as ingredients for various foods, drugs, and cosmetics [1,2]. In general, exploring medicinal plants that have been traditionally used can be a very important step as a way to discover functional materials with excellent pharmacological activities and little side effects. The pharmacological effects of medicinal plants, which have been used empirically in the private sector, are often generally described as antioxidant effects. It has been reported that the antioxidant effects of medicinal plants are better than fruits and vegetables consumed in daily life [3,4]. Stachys sieboldii Miq. (Stachys sieboldii Miq.) is a perennial plant of the Lamiaceae family, and is known as a traditional health-functional vegetable in China [5]. It is a yearly herb and known to grow lush oval leaves in summer and 3 to 6-centimeter roots underground in winter, with an overall shape of a whelk, or Cordyceps militaris [6]. Stachys sieboldii Miq. extract has been reported to relieve dementia symptoms and maintain normal function of the brain in the animal brain tissue, while inhibiting damage to the brain tissue caused by free radicals [7-9]. In addition, Stachys sieboldii Miq. extract has been known for its antioxidant activities such as inhibiting the formation of lipid peroxides and scavenging nitrite. Recently in Korea, Stachys sieboldii Miq. has attracted attention for its excellent effect in preventing dementia, which leads to increasing cultivation areas for the production of this herb [10]. Therefore, this study aimed at providing basic data on the potential use of Stachys sieboldii Miq. as a functional ingredient by examining the effects of its root extract gained by extraction with 80% ethanol solvent on antioxidant activity, brightening, and anti-aging.

II. METHODS

A. Material preparation

After adding 500 ml of 80% ethanol to 50 g of Stachys sieboldii Miq. and extracting it under reflux for three hours, the filtrate was concentrated under reduced pressure with a rotary vacuum evaporator. The concentrated solution was freeze-dried with a freeze dryer, to obtain 31.6 g (yield: 63.2%) of powder. It was stored in a cryogenic freezer (-80°C) and diluted in distilled water to the concentration required for the test.

B. DPPH radical scavenging activity measurement

DPPH radical scavenging activity was measured by employing the Blois method. The sample concentration levels of 1 μg/mL, 10 μg/mL, 100 μg/mL, and 1000 μg/mL were equally applied to all tests. 150 μl of 0.2 mM DPPH solution was prepared, mixed, and reacted at 37°C for 30 minutes. The absorbance was measured at 515 nm.

C. ABTs cation radical scavenging activity assay

The measurement of antioxidant activity using ABTS radicals was performed by using the ABTS cation decolorization assay method. First, 7 mM 2,2–zino-bis-(3–ethyl–benthiazoline–6-sulfonic acid) and 2.45 mM potassium persulfate were mixed and left at room temperature for 24 hours to form ABTS+. It was then diluted with ethanol, and 100 μL of the sample was added to 100 μL of ABTS, and the absorbance was measured at 700 nm.

D. Tyrosinase inhibitory activity measurement

Tyrosinase inhibitory activity was measured in accordance with the method of Yagi. In the reaction zone, 40 μL of 200 U/mL mushroom tyrosinase was added to a mixture of 40 μL of a substrate with 10 mM L-DOPA dissolved in 80 μL of 67 mM sodium phosphate buffer (pH 6.8) and 40 μL of the sample solution. The mixture was reacted at 37°C for 10 minutes and the DOPA chrome generated in it was measured at 492 nm. The tyrosinase inhibitory activity was expressed as the rate of decrease in the absorbance of the sample solution-added and no-added groups.

E. Elastase inhibitory activity measurement

Elastase inhibitory activity was measured in accordance with the method of Cannell. Using N-succinyl- (L-Ala)3-p-nitroanilide (Sigma, U.S.A.) as a substrate, the amount of p-nitroanilide produced from the substrate was measured at 445 nm for 30 minutes at 37°C. More specifically, each test solution was prepared to a certain concentration and then taken into a 96-well plate by 40 μL, and 40 μL of porcine pancreas elastase (2.5 U/mL) (Sigma, U.S.A.) dissolved in 50 mM tris-HCl buffer (pH 8.6) was applied to it. Thereafter, 80 μL of N-succinyl-(L-Ala)3-p-nitroanilide (0.5 mg/mL) dissolved in 50 mM tris-HCl buffer (pH 8.6) was added as a substrate to react for 30 minutes. The amount of p-nitroanilide produced from the substrate was then measured at 445 nm. The elastase inhibitory activity was expressed as the rate of decrease in the absorbance of the sample solution-added and no-added groups.

F. Collagenase inhibitory activity measurement

Collagenase inhibitory activity was measured in accordance with the method of Wűnsch E and Heindrich HG. In the reaction zone, 75 μL of 0.2 mg/mL collagenase (Sigma, U.S.A.) was added to a mixture of 75 μL of a substrate obtained by dissolving 0.3 mg/mL of 4-phenylazobenzyloxycarbonyl-Pro-Leu-Gly-Pro-D-Arg (Sigma, U.S.A.) in 0.1 M tris-HCl buffer (pH 7.5) with 4 mM CaCl2 added and 50 μL of the sample solution. It was then left for 20 minutes at room temperature, and by the addition of 250 μL of 6% citric acid, the reaction was halted. Thereafter, by adding 1.5 mL of ethyl acetate, absorbance was measured at 320 nm. The collagenase inhibitory activity was expressed as the rate of decrease in the absorbance of the sample solution-added and no-added groups.

III. Results and Discussion

A. DPPH radical scavenging activity

This factor is widely used to determine the free radical scavenging ability of natural extracts containing phenolic compounds. The electron donating ability of the ethanol extract of Stachys sieboldii Miq. root was 39.47% at 1000 μg/mL, which indicates its excellent antioxidant activity (Fig. 1)

B. ABTs cation radical scavenging activity

The ABTs radical scavenging activity at each concentration level was measured and charted in Fig. 2. As seen in Fig. 1, ABTs radical scavenging activity tended to increase in a concentration-dependent manner, and a high activity of about 94.32±0.12% was shown at a concentration of 1,000 μg/mL, which is similar to that observed in the control solution, 0.1% BHT (108.19±6.51%). The ABTs radical scavenging activity in the extract were 0.00±0.31%, 1.97±0.49%, 25.27±1.61%, and 94.32±0.12%, respectively. Overall, when the concentration of the extract is 1000 μg/mL or higher, 90% or higher levels of ABTs scavenging activity were observed, which demonstrates a high scavenging activity. In general, differences in the levels of DPPH and ABTs radical scavenging activity can be attributed to differences in radical removal mechanism, degrees of substrate binding, and compositions of polar and non-polar substances in the extract according to the types of antioxidants [11].

C. Tyrosinase inhibitory activity

Tyrosinase, as a type of polyphenol oxidase, is an

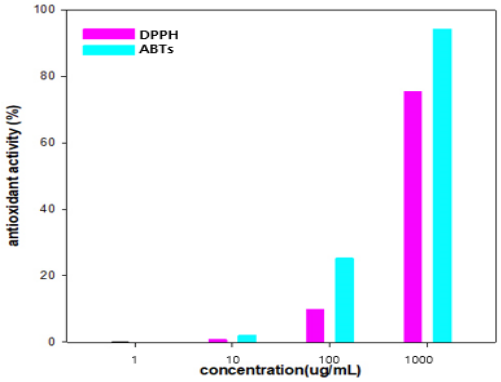

Fig. 1. Antioxidant activity of Stachys sieboldii Miq. Root extracts

enzyme containing Cu2+ and involved in the biosynthesis of the melanin pigment. Melanin increases the skin's resistance to UV rays, dryness, and extreme temperatures, but excessive melanin production causes pigmentation such as spots, freckles, and age spots on the skin and accelerates skin aging. Therefore, tyrosinase inhibitory activity is one of the very important factors involved in the browning of organisms [12]. As shown in Table 1, the tyrosinase inhibitory activity of the extract increased according to concentration levels, measured as 20.70±0.47% at a concentration of 1000 μg/mL.

Table. 1. Tyrosinase inhibitory activity of Stachys sieboldii Miq. Root extracts

Concentration	Tyrosinase inhibitory activity(%)
1	0.62±2.04
10	3.36±0.14
100	11.16±1.53
1000	20.70±0.47

D. Elastase inhibitory activity

The extract inhibited elastase in a concentration-dependent manner at concentrations of 1 μg/mL, 10 μg/mL, 100 μg/mL, and 1000 μg/mL, respectively, but the degree of activity was slightly insignificant (Fig. 2). The elastase inhibitory activity was 0.89±3.30%, 2.09±3.51%, 11.98±3.29%, and 28.11±2.53%, respectively.

E. Collagenase inhibitory activity

Collagen is ECM fibrillar molecules that make up the dermis and plays a role in maintaining the firmness of the skin, divided into five types (I, II, III,

IV, V). The decrease in collagen is due to a decrease in the synthesis ability of skin fibroblasts or an increase in resolution by collagenase. Collagen decreases not only by aging but also by photoaging caused by UV irradiation, which is known to be closely related to the formation of wrinkles on the skin[13-15]. In the test, collagenase inhibitory activity was measured as 1.61±3.06%, 4.29±1.03%, 18.08±4.73%, and 39.47±2.52% (Fig. 3). The anti-aging effect of Stachys sieboldii Miq. roots is thought to play a role in inhibiting the generation of ROS caused by photoaging due to ultraviolet rays and suppressing the formation of wrinkles by scavenging the generated ROS.

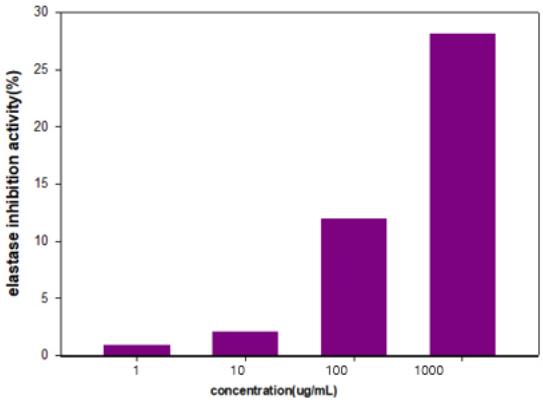

Fig. 2. Elastase inhibitory activity of Stachys sieboldii Miq. Root extract.

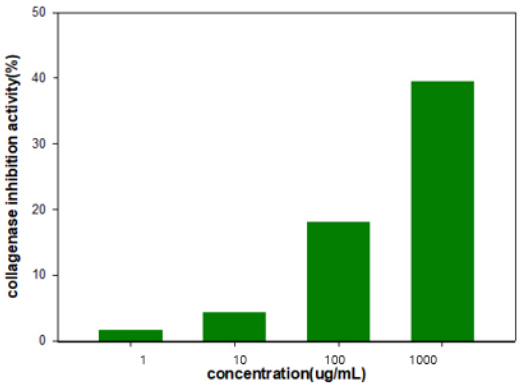

Fig. 3. Collagenase inhibitory activity of Stachys sieboldii Miq. Root extract.

IV. Conclusion

This study was conducted to find ways to use Stachys sieboldii Miq. roots rich in choline and phenylethanoids as functional cosmetic ingredients in the future by examining their antioxidant, brightening, and anti-aging effects. The study results can be summarized as follows. The Stachys sieboldii Miq. root extract showed a DPPH radical scavenging activity of 39.47% at a concentration of 1000 μg/mL, and its ABTs radical scavenging activity was 94.32±0.12% at a concentration of 1000 μg/mL. This indicates that it possesses an excellent antioxidant activity. In the anti-aging effect test, it showed a somewhat insignificant level of elastase inhibitory activity, but its collagenase inhibitory activity was measured as 39.47% at a concentration of 1000 μg/mL. Overall, it is deemed that Stachys sieboldii Miq. root extract can be effectively used in the development of new functional cosmetic ingredients.

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