Journal of the Korean Applied Science and Technology
Vol. 39, No. 4. August, 2022. 580~587
ISSN 1225-9098 (Print)
ISSN 2288-1069 (Online)
http://dx.doi.org/10.12925/jkocs.2022.39.4.580

쥐참외뿌리 추출물의 항산화, 미백, 항염증 활성 연구

유선희[1] · 이은주[2†]

[1,2]연성대학교 뷰티스타일리스트과 스킨케어전공, 교수
(2022년 7월 31일 접수: 2022년 8월 25일 수정: 2022년 8월 28일 채택)

A Study on the Antioxidant, Whitening and Anti-Inflammatory Activities of T*richosanthis Cucumeroidis* Radix Extract

Seon-Hee You[1] · Eun-Ju Lee[2†]

[1,2]*Department of Beauty Stylist Major in Skin Care, Yeonsung University, Anyang, Gyeonggi-do, 14010, Republic of Korea*
(Received July 31, 2022; Revised August 25, 2022; Accepted August 28, 2022)

요 약 : 본 연구에서는 쥐참외뿌리 추출물의 항산화 활성 및 미백, 항염증 활성에 대한 생리활성 효과와 기능성 소재로써의 활용 가능성을 확인해 보고자 하였다. 쥐참외뿌리 추출물의 DPPH 라디칼 소거 활성을 통한 항산화 활성, 멜라닌 세포인 B16F10 melanoma 세포에 대한 멜라닌 생성 억제능을 통한 미백 활성 효과, 대식 세포인 RAW 264.7 세포에 대한 NO 생성 억제능을 통한 항염증 활성 효과를 확인해 보고자 하였다. 연구결과, 쥐참외뿌리 추출물은 농도 의존적으로 DPPH 라디칼 소거활성이 확인되었으며, 양성 대조군인 Ascorbic acid와 비슷한 DPPH 라디컬 소거활성이 확인되었다. 100 nM α-MSH로 유도된 멜라닌 생성 억제 활성과 LPS 1 ㎍/mL로 유도된 NO 생성 억제능을 유의하게 억제 하는 것을 확인되었다. 이에 따라 쥐참외뿌리 추출물은 항산화 및 미백, 항염증 효과를 가진 기능성 소재로써의 활용 가능성이 있음이 사료되어 진다.

주제어 : 미백 활성, 쥐참외뿌리 추출물, 항산화 활성, 항염증 활성, 기능성 소재

Abstract : In this study, the physiological activity effect of *trichosanthis cucumeroidis* radix extract on antioxidant activity, whitening, and anti-inflammatory activity was checked, and the possibility of its use as a functional material was checked. The purpose of this study was to confirm the antioxidant activity through DPPH radical scavenging activity of *trichosanthis cucumeroidis* radix extract, whitening activity effect through melanin production inhibition ability for melanin cell B16F10 melanoma cell, and anti-inflammatory activity effect through NO production inhibition ability for macrophage RAW 264.7 cell. As a result of the study, the concentration-dependent DPPH radical scavenging activity of the *trichosanthis cucumeroidis* radix extract was confirmed, and DPPH radical

†Corresponding author
(E-mail: ejlee@yeonsung.ac.kr)

scavenging activity similar to that of the positive control Ascorbic acid was confirmed. It was confirmed that the melanin production inhibitory activity induced by 100 nM α-MSH and the NO production inhibitory ability induced by LPS 1 ㎍/mL were significantly suppressed. Accordingly, it is considered that the *trichosanthis cucumeroidis* radix extract may be used as a functional material having antioxidant, whitening, and anti-inflammatory effects.

Keywords : Whitening activity, trichosanthis cucumeroidis radix extract, antioxidant activity, anti-inflammatory activity, functional materials

1. Introduction

In modern society, due to the rapid development of science and technology, interest in beauty along with health is also increasing due to the extension of average life expectancy and the improvement of living standards. In particular, due to environmental pollution, ozone layer destruction, various chemicals, and synthetic substances, interest in natural substances with fewer side effects and excellent effects on the skin is increasing[1,2].

Oxidative stress includes the types of Reactive Oxygen Specifications (ROS) that occur during normal metabolic processes for generating energy in vivo, such as superoxide radical anon (· O2), hydroxyyl radicals (· OH), and hydrogen peroxide (H_2O_2). Active oxygen produced in vivo is not removed by the antioxidant system or is generated too much, resulting in oxidative damage due to the imbalance in free radical production and antioxidant defense systems[3,4]. Typical active oxygen problems are known to cause or promote damage to cell structures such as proteins and cell membranes, cancer, chronic diseases, skin diseases, and aging[5,6]. Antioxidants are known to reduce cell damage caused by free radicals and protect against various diseases, including cancer. Representatively, antioxidants such as polyphenols, flavonoids, and vitamins may help[7]. In particular, the various physiological activities of natural extracts are commonly derived from secondary metabolites such as polyphenols, flavonoids, carotenoids, and terphenoids, which contain a number of hydroxyl groups as a widely known secondary metabolite, thereby suppressing oxidative stress[8,9]. Recently, due to side effects on synthetic materials, many studies on antioxidants have been conducted to find the antioxidant activity of phenolic materials derived from natural and medicinal materials. Recent studies have included Evaluation of antioxidant, anti-inflammatory, and anti-wrinkle effects of the mushroom Leucopaxillus giganteus[10], Antioxidant and anti-inflammatory effects of seed ethanol extracts of Rubus coreanus miquel[11], Antioxidant and Anti-inflammatory Activities of Atractylodes japonica According to Extract Methods[12], A Study on the Antioxidant and Anti-inflammatory Effects of Prunella vulgaris Extract[13], Antioxidant Activities and Phenolic Compounds of Extract from Hallasan Wormwood[14].

Skin color is influenced by various factors such as melanin, carotene, and blood painting, and the double melanin pigment acts as the most important factor[15]. Skin pigmentation, such as blackening or smearing of the skin, is expressed by increased melanocyte-induced melanin synthesis. Melanin synthesis is mainly caused by the oxidative action of tyrosinase[16,17].

Accordingly, most of the studies for skin whitening have been related to inhibition of tyrosinase activity and inhibition of active oxygen. Studies such as Antioxidant Activities

and Whitening Effects of Extracts from Hippophae rhamnoides L.[18], Antioxidant Activity and Whitening Effect of Forsythiae Fructus Extracts[19], Whitening and Anti-oxidative Constituents from the Extracts of Hydrangea petiolaris Leaves[20] have been reported as previous studies related to inhibition of tyrosinase activity and inhibition of active oxygen.

Accordingly, this study used *trichosanthis cucumeroidis* radix extract to check antioxidant activity through DPPH radical scavenging activity and melanin production inhibition effect through melanin cell B16F10 melanoma cell, anti-inflammatory effect through NO production inhibition ability of RAW 264.7 cells, and to confirm its potential as a functional material using natural materials.

2. Research Method

2.1. DPPH Radical Scavenging Assay

In order to confirm the antioxidant activity of the *trichosanthis cucumeroidis* radix extract, the DPPH radical scavenging ability was measured using a 1,1-diphenyl-2-picryl-hydrazyl (DPPH; Sigma-Aldrich, USA) solution. The research method used in this study was measured using the colorimetric method introduced by Blois[21]. The *trichosanthis cucumeroidis* radix extract was diluted by concentrations of 6.25, 12.5, 25, 50, and 100 μg/mL. 20 μL of *trichosanthis cucumeroidis* radix extract and 180 μL of 10 mM DPPH solution were mixed with a 96-well plate, and then reacted at 37℃ for 30 min in a light shielding state.

After 30 min, absorbance was measured at 517 nm using a microplate reader. As a positive control group in this study, ascorbic acid (Sigma-Aldrich, USA) was used in the same way as the experimental group.

2.2. Cell Culture

B16F10 melanoma Cell and RAW 264.7 Cell were purchased and used by Korean Cell Line Bank (Korea). Maintain 10% fetal bovine serum (FBS; Sigma-Aldrich, USA) and 1% penicillin (100 IU/mL, GE Healthcare Life Sciences, USA), 1% streptomycin (50 μg/mL, GE Healthcare Life Sciences) in a high globule-Aldg, 5% CO_2 incubation medium (USDMA) at 37℃.

2.3. Skin Melanin Inhibitory Activity Assay

Melanin production inhibition ability was measured to confirm the whitening effect of the *trichosanthis cucumeroidis* radix extract. B16F10 Melanoma cells were cultured on a 96-well plate at 37℃ for 24 h in a 5% CO_2 incubator. In order to induce melanin production, 5% FBS and 100 nM α-MSH (Sigma-Aldrich, USA) were exchanged as medium after 24 h. The *trichosanthis cucumeroidis* radix extract was diluted by concentrations of 0.5, 1, 2.5, and 5 μg/mL and cultured for 72 h. The amount of melanin secreted into the well plate was measured for absorbance at 490 nm using a microplate reader. As a positive control group, 100, 200, and 300 μg/mL Arbutin were used in the same manner as the experimental group.

2.4. NO (Nitric Oxide) Inhibitory Activity Assay

The ability to suppress NO production was measured to confirm the anti-inflammatory effect of the *trichosanthis cucumeroidis* radix extract. RAW 264.7 cells were divided into 96 well plates at a concentration of 5×10^4 cells/well, and then cultured at 37℃ for 24 h in a 5% CO_2 incubator. In order to remove the culture medium and induce NO production after culture, samples were added to the culture medium treated with lipopolysaccharide (LPS) 1 μg/mL concentration by concentration of 0.5, 1, 2.5, and 5 μg/mL for 48 hours.

After 48 h, 100 μL of grease region and 100 μL of cultured cell culture supernatant were added to a new 96-well plate, reacted 10 min in a shielded state, and the absorbance was measured at 540 nm using a microplate reader.

2.5. Statistical Method

The experiment of this study was independently measured three or more times under the same conditions, and the results of three or more measurements were obtained and used for analysis. Statistical processing was performed using SPSS Window Version 17.0 (SPSS Inc., Illinois, USA), and significance verification was performed using Student t-test.

3. Result and discussion

3.1. DPPH Radical Scavenging Activity Measurement Results

Natural antioxidants include polyphenols and flavonoid compounds, representatively quercetin and gatechin[22].

Most polyphenol and flavonoid compounds are often derived from plants, so research using various plant extracts is being actively conducted. In relation to this study, in a prior study of *trichosanthis cucumeroidis* radix extract antioxidants in You&Moon (2022), polyphenol and flavonoid contents, which are indicators of antioxidant activity, were reported as 308.2 mg/g and flavonoid 120.7 mg/g at 1% concentration[23].

As a result of the study, the *trichosanthis cucumeroidis* radix extract had a radical scavenging ability of 48.15%, 56.12%, 65.73%, 85.74%, and 98.44%, and the Ascorbic acid used as a positive control group had a radical scavenging ability of 42.6%, 49.3%, 64.7%, 82.9%, and 97.7% by concentration (Fig. 1). According to a previous study by Choi (2022), it has been reported that mixtures containing *trichosanthis cucumeroidis* radix extract have an excellent electron donor capacity of 50% or more regardless of the extraction method used in the DPPH radical analysis study[24]. In this study, as DPPH radical scavenging activity similar to Ascorbic acid used as a positive control group was shown, the *trichosanthis cucumeroidis* radix extract became a possibility as a natural antioxidant.

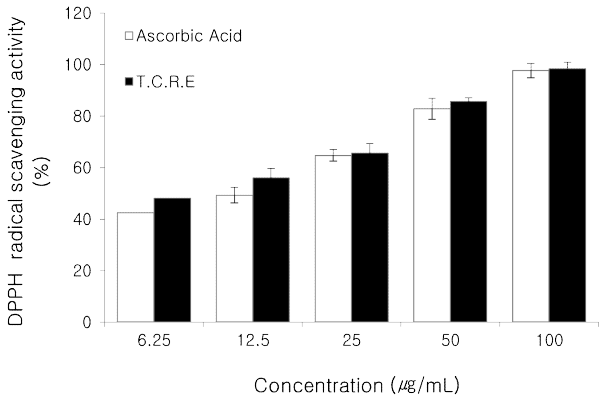

Fig. 1. DPPH radical scavenging activity of *trichosanthis cucumeroidis* radix extract, Experimental group: *trichosanthis cucumeroidis* radix extract(T.C.R.E), Positive control group: Ascorbic Acid.

3.2. Melanin Inhibition Activity Measurement Results

In order to confirm the whitening effect of the *trichosanthis cucumeroidis* radix extract, it was intended to confirm the whitening activity by performing melanin production inhibition ability that represents the color of the skin. In relation to this study, according to the results of a prior study using *trichosanthis cucumeroidis* radix extract of You&Moon (2022), cell survival rate of 80% or less in 10 μg/mL concentration was confirmed in B16F10 melanoma cells, which are melanocytes[23]. In this study, a melanin production inhibition experiment was conducted at a concentration of 5 μg/mL in which a survival rate of more than 80% was confirmed.

As a result of this study, α-MSH was treated on B16F10 melanoma cells, which significantly increased melanin production by α-MSH. After α-MSH treatment, the melanin production inhibition rate was 38.55% at the concentration of 100 μg/mL of albutin, a representative whitening component, and 45.14% at the concentration of 200 μg/mL and 53.3% at the concentration of 300 μg/mL. In the *trichosanthis cucumeroidis* radix extract, 64.8% melanin production inhibitory effect was found at a concentration of 0.5 μg/mL, and 74.32%, 75.09% and 75.22% melanin production inhibitory effect were found at each concentration (Fig. 2). According to a previous study by Choi (2022), it has been reported that a mixture containing *trichosanthis cucumeroidis* radix extracts inhibits tyrosinase activity and melanin production, thereby having excellent skin whitening activity[24]. Through the above research results, it was confirmed that *trichosanthis cucumeroidis* radix extract inhibits melanin production in B16F10 melanoma cells, which are melanocytes, and the possibility of a whitening agent using natural materials is confirmed.

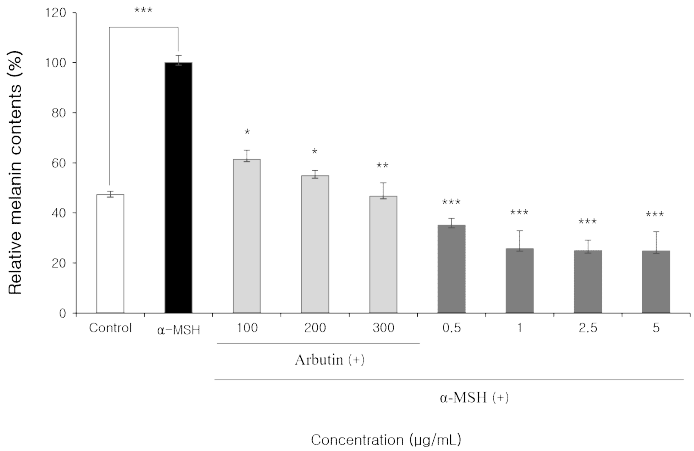

Fig. 2. Melanin production inhibitory activity of *trichosanthis cucumeroidis* radix extract, Experimental group: *trichosanthis cucumeroidis* radix extract, Positive control group: Arbutin.

3.3. NO Inhibition Activity Measurement Results

The effect of inhibiting NO production in RAW 264.7 cells was measured. According to the results of a prior study of the cytotoxicity experiment using *trichosanthis cucumeroidis* radix extract of You&Moon (2022), *trichosanthis cucumeroidis* radix extract increased cell survival at a concentration of 1.25% at RAW 264.7 Cell. However, as it was confirmed that the cell survival rate was lowered at the 2.5 μg/mL concentration, an NO production inhibition effect experiment was conducted at the 5ug/mL concentration, where the survival rate of more than 76% was confirmed[23].

As a result of the experiment, the NO generation inhibition effects of 49.81%, 55.67%, 61.31%, and 64.45% were confirmed for each used concentration of *trichosanthis cucumeroidis* radix extract, respectively. Through the above research results, it was confirmed that *trichosanthis cucumeroidis* radix extract inhibits NO production in RAW 264.7 cells, which are macrophages, and the possibility as an anti-inflammatory agent using natural substances was confirmed(Fig. 3).

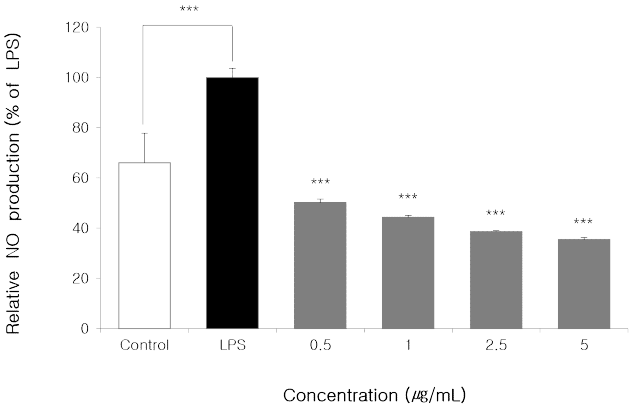

Fig. 3. NO inhibitory activity of *trichosanthis cucumeroidis* radix extract.

4. Conclusion

In this study, as a follow-up study of, the physiological effect of *trichosanthis cucumeroidis* radix extract on antioxidant, whitening, and anti-inflammatory activity was checked, and the possibility of use as a functional material was checked.

First, as a result of measuring the DPPH radical scavenging activity to confirm the antioxidant activity of the *trichosanthis cucumeroidis* radix extract, the *trichosanthis cucumeroidis* radix extract confirmed an excellent DPPH radical scavenging activity effect. As a result, it is believed that the *trichosanthis cucumeroidis* radix extract will play an important role in suppressing oxidative stress by erasing free radicals, while reducing and protecting cell damage.

Second, the *trichosanthis cucumeroidis* radix extract significantly increased melanin production with α-MSH in B16F10 melanoma cells, which are melanin cells, and then melanin production inhibition activity was measured. As a result of the measurement, excellent melanin production inhibition activity of 75.22% was confirmed at a concentration of 5 μg/mL.

Finally, NO production inhibition activity was measured by significantly increasing inflammation with LPS for RAW 264.7 cells, which are macrophages, and then treating *trichosanthis cucumeroidis* radix extract As a result of the measurement, excellent NO production inhibition activity of 64.45% was confirmed at a concentration of 5 μg/mL. Based on these research results, *trichosanthis cucumeroidis* radix extract can be used as a functional material using natural materials as it is confirmed that it has excellent antioxidant activity through DPPH radical scavenging activity, whitening effect through melanin production inhibition, and anti-inflammatory effect through NO production inhibition.

References

1. K. Maeda, M. Fukud, "In vitro effectiveness of several whitening cosmetic components in human melanocytes", *The Journal of Society of Cosmetic Scientists of Korea,* Vol.42, pp. 361-368, (1991).
2. T. H Lee, H. J. Kim, Y. B. Kim, "Depigmentation Activity of Barley, Unpolished Rice, Job's-tear", *The Journal of Oriental Medical Ophthalmology & Otolaryngololgy & Dermatology*, Vol.16, No.2 pp. 57-78, (2003).
3. H. Hwangbo, J. S. Jeung, M. Y. Kim, S. Y. Ji, S. H. Yoon, T. H. Kim, S. O. Kim, Y. H. Choi, "A Study on Antioxidant and Anti-inflammatory Effects Based on Analysis of Functional Components of Cornus officinalis Siebold & Zucc", *Journal of Life Science,* Vol.31, No.3 pp. 287-297, (2021).
4. J. Lee, N. Koo, D. B. Min, "Reactive oxygen species, aging, and antioxidative nutraceuticals", *Comprehensive reviews in food science and food safey*, Vol.3, No.1 pp. 21-33, (2004).
5. H. Kang, S. G. Lee, "Antioxidant Capacity of Ethanol Extracts and Fractions from Rubus coreanus Miq", *Journal of Plant Biotechnology*, Vol.48, pp. 264-270, (2021).
6. M. J. Jang, S. J. Seo, Y. S. Lee, "Antioxidant, Anti-inflammatory and Anti-aging Activities of Elaeagnus multiflora Fruit Extracts as a Cosmetic Material", *Journal of Investigative Cosmetology,* Vol.17, No.2 pp. 159-167, (2021).
7. I. Gulcin I, "Antioxidants and antioxidant methods: An updated overview", *Archives of toxicology,* Vol.94, No.3 pp. 651-751, (2020).
8. J. B. Park, K. M. Lee, J. S. Park, J. Kim, J. D. Lee, "Selection of high functional red paprika (Capsicum annuum L.) germplasms by antioxidant activity, polyphenol content, and red pigment amount", *Korean J. Breed. Sci*, Vol.53, pp.96-104, (2021).
9. H. J. Kwon, S. H. Beom, J. A. Hyun, E. B. Kang, H. E. Park, D. G. Han, E. Y. Choi, B. J. An, "Analysis of antioxidant activity, total phenol content, and flavonoid content of Abelmoschus manihot flower extracts", *Korean J. Food Preserv*, Vol.29, pp. 157-165, (2022).
10. N. Guo, J. A. Lee, "Evaluation of antioxidant, anti-inflammatory, and anti-wrinkle effects of the mushroom Leucopaxillus giganteus", *Korean Society of Cosmetics and Cosmetology*. Vol.12, No.1, pp. 113-120, (2022).
11. J. W. Hwang, H. Kang, S G. Lee, "Antioxidant and anti-inflammatory effects of seed ethanol extracts of Rubus coreanus miquel", *J Plant Biotechnol,* Vol.49, pp. 155-161, (2022).
12. H. K. Oh, "Antioxidant and Anti-inflammatory Activities of Atractylodes japonica According to Extract Methods", *Journal of the Korean Applied Science and Technology*, Vol.38, No.6 pp. 1543-1552, (2021).
13. J. C. Yang, "A Study on the Antioxidant

and Anti-inflammatory Effects of Prunella vulgaris Extract", *Culture and Convergence*, Vol.43, No.11 pp. 1037-1048, (2021).
14. K. C. Kim, J. S. kim, "Antioxidant Activities and Phenolic Compounds of Extract from Hallasan Wormwood", *korean journal medicinal crop science*, Vol.29, No.5 pp. 345-352, (2021).
15. Department of Dermatology. Seoul National University College of Medicine. Dermatology for medical students. 4th edition. Seoul:Korea Medical Book Publishing Co. pp. 237-243, (2017).
16. S. N. Youn, Y. J. Kim, Y. J. Lee, M. R. Kim, W. K. Yoo, "Effect of ethanol extract from mixture including Angelicae Dahuricae Radix on Dermal Anti-aging and Whitening", *The Korea Jounrnal of Herbology*, Vol.34, No.6 pp. 109-115, (2019).
17. J. C. Lee, S. Y. Park, J. H. Choi, J. H. Kim, "Effects of Paeoniae radix alba(PRA) on Skin whitening and Elasticity using Melanoma cells", *The Journal of Korean Oriental Medical Ophthalmology & Otolaryngology & Dermatology*, Vol.25, No.1 pp.1-11, (2012).
18. M. S. Ko, H. J. Lee, M. J. Kang, "Antioxidant Activities and Whitening Effects of Extracts from Hippophae rhamnoides L," *J East Asian Soc Dietary Life*, Vol.22, No.6 pp. 812-817, (2012).
19. S. J. Yang, T. B Choe, "Antioxidant Activity and Whitening Effect of Forsythiae Fructus Extracts", *Korean J. Medicinal Crop Sci*, Vol.19, No.6 pp. 472-477, (2011).
20. M. J. Seong, J. E. Kim, N. H. Lee, "Whitening and Anti-oxidative Constituents from the Extracts of Hydrangea petiolaris Leaves", *journal of the society of cosmetic scientists of korea*, Vol.48, No.2 pp. 123-134, (2022).
21. M. S. Blois, "Antioxidant determinations by the use of a stable free radical", Nature. Vol.181, pp. 1199-1200, (1958).
22. H. Kang, S. G. Lee, "Antioxidant capacity of ethanol extracts and fractions from Rubus coreanus Miq", *J. Plant Biotechnol*, Vol.48, pp. 264-270, (2021).
23. S. H. You, J. S. Moon, "Antioxidant and Cytotoxicity in Skin Cell of the Trichosanthis Cucumeroidis Radix Extract", *Journal of the Korean Applied Science and Technology*, Vol.39, No.3 pp. 417-422, (2022).
24. M. J. Choi, "Skin Whitening Efficacy of Mixed Extracts of Crassostrea gigas, Trichosanthes cucumeroides, Angelica dahurica and Asarum sieboldii", *Asian J Beauty Cosmetol*, Vol.20, No.2 pp. 157-167, (2022).