

에이코사펜타엔산(EPA) 항산화활성 연구

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Eicosapentaenoic Acid (EPA) Antioxidant Activity Study

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요약 : 본 연구에서는 천연 생리활성물질인 에이코사펜타엔산을 다량 함유한 흑해삼의 항산화 효과를 조사하여 기능성 화장품 원료로서의 우수성을 제시하였다. 세포내 산화질소(NO) 생성 억제 효과에 대해 해삼 추출물의 활성산소 제거능을 평가하였다. 해삼의 생리활성물질은 활성산소를 제거하는 항산화 효과가 있으며, 평가 결과 우수한 NO 생성이 억제됨을 확인하였다. 연구에 따르면 생리활성 물질은 낮은 농도에서도 높은 항산화 효능을 보여 항산화 효과, 면역 및 염증 반응 조절에 관여하는 것으로 나타났다. 해삼의 항산화 제거능은 해삼 추출물의 EPA(eicosapentaenoic acid)를 함유하는 페놀 성분의 항산화 효능에 크게 기여하는 것으로 생각된다. 해삼의 항산화 성분이 작용한다는 보고와 유사한 것으로 밝혀졌다. 또한 해삼 추출물에 다량 함유된 에이코사펜타엔산(EPA) 성분이 염증 활성 억제 효과에 관여하는 것으로 입증돼 상처 치유에도 탁월하다. 본 연구의 결과, 해삼 추출물에 함유된 페놀 성분의 효과가 항산화 활성과 밀접한 인과관계가 있음을 확인하였다.

주제어 : 해삼추출물, 항산화효과, 에이코사펜타엔산, 생리활성물질

Abstract : In this study, the antioxidant effect of black sea ginseng containing a large amount of eicosapentaenoic acid, a natural physiologically active substance, was investigated, and its superiority as a functional cosmetic raw material was presented. The ability of the sea cucumber extract to remove active oxygen was evaluated for the inhibitory effect on intracellular nitric oxide (NO) production. The physiologically active substances of sea cucumber have an antioxidant effect that removes free radicals, and as a result of evaluation, it was confirmed that excellent NO production was suppressed. Studies have shown that bioactive substances are involved in antioxidant effects, immune and inflammatory response regulation by showing high antioxidant efficacy even at low concentrations. It is thought that the antioxidant removal ability of sea cucumber greatly contributes to the antioxidant efficacy of phenolic components containing EPA (eicosapentaenoic acid) of the sea

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cucumber extract. It was found to be similar to the report that the antioxidant component of sea cucumber works. In addition, it is excellent for wound healing with the eicosapentaenoic acid (EPA) component contained in a large amount of sea cucumber extract, which has been proven to be involved in the inhibitory effect of inflammatory activity. As a result of this study, it was confirmed that the effect of the phenol component contained in the sea cucumber extract had a close causal relationship with the antioxidant activity.

Keywords : *Sea cucumber extract, antioxidant effect, eicosapentaenoic acid, physiologically active substances*

1. Introduction

Various studies have been reported on antioxidants using natural substances that contain excessively produced oxidative active substances to inhibit human aging and are known to inhibit free radicals in the cellular metabolic process of the skin [1,2]. Recently, overeating, drinking, environmental pollution, stress, and health are important factors, and many functional products are being developed [3]. The skin is a major defense organ that surrounds the human body and is composed of proteins and functions to protect the inside of the human body from external physical and chemical factors [4]. In particular, polyphenol compounds are functional anti-inflammatory substances that show the highest physiological activity in the field of research and development of natural products [5]. It has been reported that phenolic hydroxyl groups widely distributed in animals and plants bind to polymers such as proteins and exhibit physiological activity [6]. Eicosapentaenoic acid contained in sea cucumber exists in cells and contains various physiologically active ingredients such as flavonoids and acid phenol compounds [7]. In this study, the function of sea cucumber extract, which is widely used as herbal medicine and health food, and has excellent antioxidant effect, was analyzed [8]. This was to provide eicosapentaenoic acid contained in sea cucumber extract as a raw material for functional cosmetics [9]. Although

the high content (EPA) component contained in sea cucumber extract is excellent for tissue recovery and wound healing, studies on natural antioxidants that prevent various diseases related to eicosapentaenoic acid are lacking [10]. Therefore, a comparative analysis on antioxidant activity was attempted based on previous studies [11].

1.1. Eicosapentaenoic Acid (EPA)

EPA, which is abundant in sea cucumber, is an unsaturated fatty acid and has pharmacological effects on adult diseases such as cardiovascular hypertension, asthma, myocardial infarction, arteriosclerosis, and pulmonary fibrosis. The best known source of sea fish oil is n-3 polyunsaturated fatty acids (PUFAs) or eicosapentaenoic acids (EPAs). It is known to have several health benefits associated with cardiovascular disease, including low triglyceride and anti-inflammatory effects [12]. In addition, there are prior research reports that it is effective in anticancer, antioxidant, antidepressant, anti-aging, and arthritis. Recent studies have shown that these fatty acids also have superior insulin and anti-inflammatory roles in metabolic disorders [13]. EPA increases the body's energy metabolism by eliminating triglycerides and cholesterol, the causes of diabetes and obesity [14]. EPA is known as an important bioactive substance in several diseases such as obesity, diabetes, asthma, anemia and cancer [15].

2. Experiment

2.1. Experimental materials and methods

Fresh domestic sea cucumber is a polar skin and its scientific name is *Holothria*. Buy it at the fish market, wash it clean with distilled water, cut it into small pieces, and use it as a raw material for experimental materials.

2.1.1. Reagents and devices

The reagents used in the experiment were butylated hydroxytoluene (BHT), ascorbic acid, 1,1-diphenyl-2-picrylhydrazyl (DPPH), lipopolysaccharide (LPS), quercetin, arbutin, pyrogallolmercuric, glycolytic acid, gulphin, threptomycin, formaldehyde, petal bobbin serum phosphate buffered saline solution (PBS) was purchased from Sigma Chemical Co. (USA). Other first-class reagents were used.

2.1.2. Production of sea cucumber extract

In this experiment, 50% ethanol was made 10 times the sea cucumber sample to extract polyphenol and flavonoids from antioxidants contained in sea cucumber. Next, it was incubated for 72 hours in an incubator at 2

5°C and 150 rpm. It was centrifuged at 8,000 rpm for 20 minutes in a centrifuge. After that, it was filtered with Whatman No. 2 filter paper, the supernatant was filtered, and then filtered with a sterile filter paper. In addition, ethanol was removed by a reduced pressure concentration method (EYELA, Japan) and freeze-dried to be used as a powdered sample for sea cucumber extract.

2.2. Experiments and Methods

2.2.1. Measurement of antioxidant capacity

DPPH Radical Clearing was performed with a variation of Blois' method. In general, active oxygen is a stable material, and a method of bleaching by reacting with an antioxidant was used. When evaluating the antioxidant activity of sea cucumber extract, 0.05%, 0.1%, 0.25%, 0.5%, and 1% concentration experiments were used, respectively. Accordingly, the sea cucumber extract was dissolved in ethanol in a 96-well plate, transferred to a 96-well plate containing 20 μ l of a sea cucumber extract sample in 180 μ l of DPPH solution, and incubated at 37°C for 30 minutes. The reaction

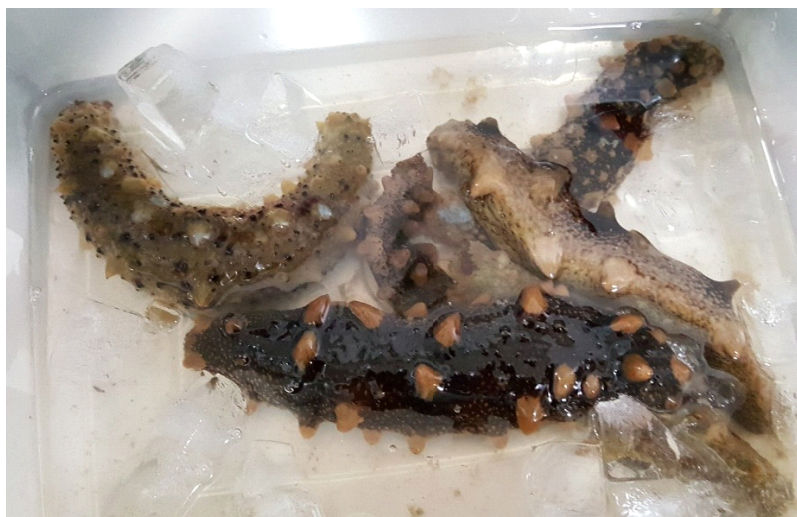


Fig. 1. An image of a sample of domestic fresh sea cucumber experimental material purchased at a fish market.

used ascorbic acid as a comparative material, and absorbance was measured with a 517 nm microplate reader[16].

2.2.2. Measurement of nitrogen oxide generation suppression ability

As a functional raw material of the sea cucumber extract, the amount of NO in the cell culture was evaluated according to the Griess measurement method to measure the NO production inhibitory effect. RAW 264.7 cells were distributed at a concentration of 5×10^4 cells/well and cultured in 96-well plates for 24 hours. After incubation, the medium was removed and each sea cucumber extract was treated in a medium treated with LPS $1 \mu\text{g}/\text{mL}$ for 48 hours [17]. After adding $100 \mu\text{L}$ of cell supernatant and $100 \mu\text{L}$ of Griess reaction solution to a new plate, light was removed and reacted for 10 minutes, and absorbance at 540 nm was indicated using a microplate reader. It was expressed as a percentage compared to the LPS-only treatment group.

3. Results and review

3.1. Antioxidant activity evaluation and analysis

It is known that DPPH radical scavenging ability is related to the prevention of aging in the human body and the prevention of fat oxidation in food. The biggest factor in human aging is UV rays that repeatedly irritate the skin. UV-induced free radicals cause external photooxidative damage to the skin and irritate the skin [18]. As a result of measuring the antioxidant activity of sea cucumber extract, the sample concentration showed a tendency to increase from 0.05% to 32.73%, 0.1%, 34.74%, 0.25% to 34.74%, 0.5% to 37.36%, and 1%. At a concentration of $50 \mu\text{g}/\text{mL}$ of sea cucumber extract at 42.24%, a scavenging activity of 63.28% was reported [19]. The effects of seafood spirulina

0.1%, cheongcheong 13.1%, seaweed 10.57%, kelp 10.67%, and seaweed 14% have been reported [20, 21]. Sea cucumber extract showed higher scavenging activity than various seaweeds at all concentrations, and in particular, at 0.1% concentration, the scavenging activity was evaluated to be 33.34% higher. The increase in the concentration of the sea cucumber extract did not show a significant change in the scavenging ability, and it was confirmed that the physiological activity effect was high even at a low concentration. As a result of the analysis, it was reported that the phenol content of the sea cucumber extract was related to the antioxidant activity effect according to the appropriate concentration [22]. In this case study, it can be evaluated that the antioxidant activity effect appears depending on the phenolic compound. In general, certain ingredients are interpreted to be derived from phenolic compounds present in plants and animals [23]. Therefore, the antioxidant of Eicosapentaenoic Acid, which is abundant in sea cucumber samples, was identified as an antioxidant with excellent immune activity [24].

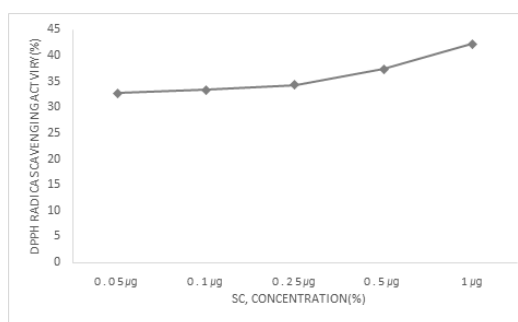


Fig. 2. Sea cucumber extract was highly evaluated with an antioxidant activity of 37.36% even at a concentration of 0.5%. It was confirmed that the physiological activity effect was high at a low concentration.

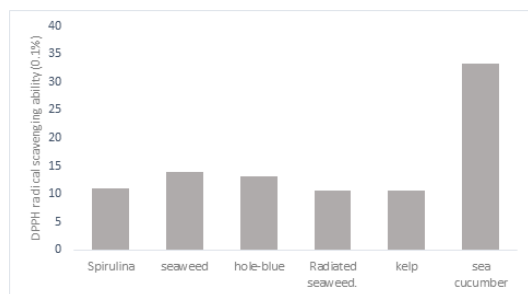


Fig. 3. As a result of comparing sea cucumber extracts with research cases (sample concentration 0.1%), spirulina erasing ability was 11%, radiation pattern erasing ability was 10.57%, holblue 13.1%, oak kelp erasing ability was 10.67%, and seaweed erasing ability was 14%. Sea cucumber accounts for 33.34%.

3.2. Measurement result of inhibition of nitric oxide production

Nitric oxide is produced by an enzyme called NOS (nitric oxide synthase), is overproduced in various metabolic processes in the body, and is reported to play an important role in vascular infections and various acute and chronic inflammations[25]. L-Arginine (NOS (nitric oxide synthase)) Produced NO has the ability to kill bacteria, fungi and tumors, but overproduction leads to intracellular inflammation, tissue damage and genetic modification[26]. As a result of this study, a sea cucumber extract was obtained with a 50% ethanol solvent and used as a sample. As a result of measuring the ability to inhibit NO production by LPS in RAW 264.7 cells, sea cucumber extract 5g/mL 17.07%, 10g/mL 8.7%, 20g/mL 41.46%, 50g/mL 43.55%, 100 μ g/mL, 51.22% LPS group and Conversely, as the concentration increased, the inhibition rate of NO production increased, and it was confirmed that the inhibition rate of NO production was 41.46% at a concentration of 20 μ g/mL. The antioxidant effect of sea cucumber extract was confirmed as antioxidant, immune, and inflammatory action [27]. As a result, the ability of sea

cucumber EPA (eicopentaenoic acid) to inhibit NO production reported to be involved in wound regeneration and to inhibit the promotion of inflammatory activity was confirmed.

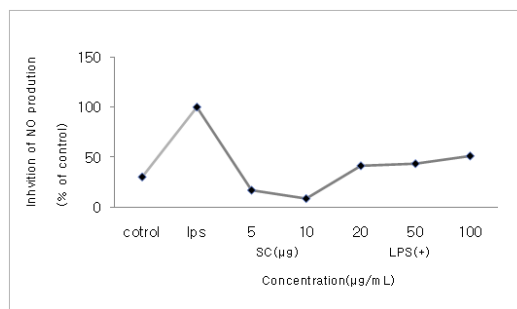


Fig. 4. Nitrogen oxide generation low performance measurement results.

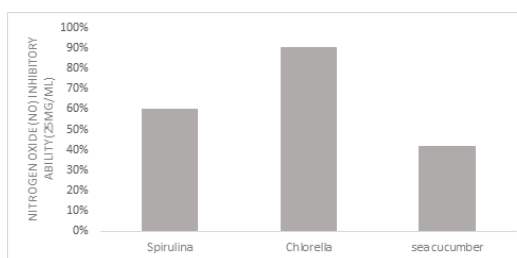


Fig. 5. Antioxidant activity comparison: 41.46% sea cucumber, 60% spirunira, 90% crorella, and ability to remove free radicals (20 μ g/mL concentration)

4. Conclusion

In this study, the antioxidant activity of sea cucumber containing EPA (eicosapentaenoic acid), a physiologically active solvent extract, was confirmed. Based on the analysis of phenolic compounds of sea cucumber extract, active oxygen removal and antioxidant activity of free radicals were compared and analyzed with previous studies on the inhibitory effect of nitrogen oxides (NOS) in cells. The active ingredient of sea cucumber has an antioxidant effect that removes free radicals and is

evaluated to be excellent in suppressing NO production. The result of high antioxidant activity of sea cucumber extract at low concentration proves that bioactive substances are involved in the regulation of antioxidant response. It was found that the antioxidant scavenging ability of sea cucumber greatly contributes to the antioxidant effect of the phenol component containing eicosapentaenoic acid (EPA) contained in the sea cucumber extract. In this study, it was confirmed that the phenol effect contained in the sea cucumber extract was related to the antioxidant ability. In particular, the antioxidant activity was highly evaluated at a low concentration of the sea cucumber extract. Reports on the antioxidant effect and immunity of sea cucumber extract were similar to the results of this study. It also contains a large amount of eicosapentaenoic acid (EPA) extracted from sea cucumber extract, which is excellent for wound healing. Inhibition of NO production has also been reported in previous reports of its inhibitory effect on inflammatory activity. Through this study, it is expected that the study of eicosapentaenoic acid contained in sea cucumber, which can be developed as a material for a functional cosmetic composition that can effectively identify the antioxidant reaction of phenolic compounds in the human body, will continue.

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