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Skin whitening and anti-corrugation activities of glycoprotein fractions from liquid extracts of boiled sea cucumber

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ABSTRACT

Objective: To determine skin whitening and wrinkle improvement efficacy, glycoprotein fractions were extracted from liquid extracts of boiled sea cucumber and their effects on tyrosine and elastase inhibitory activities were assayed.

Methods: Fractions above and below 50 kDa (>50 kDa and <50 kDa) were extracted via a series of steps involving: boiling, filtering, desalting and freeze drying. Cytotoxicity, skin whitening and wrinkle-removing effects of boiled liquid were determined.

Results: Our MTT data showed that neither glycoprotein fraction of boiled liquid induces cellular cytotoxicity up to a concentration of 10 mg/mL treatment of the mouse melanoma cell line, B16F10, with 10 mg/mL >50 kDa enhanced tyrosinase and elastase inhibitory activities by 50.84% and 28.78%, respectively. Correlations of the >50 kDa concentration with tyrosinase inhibitory (R2 = 0.968) and elastase inhibitory (R2 = 0.983) efficacy were significant.

Conclusions: >50 kDa glycoprotein fraction isolated from liquid extracts of boiled sea cucumber, which can serve as a functional cosmetic ingredient for whitening and wrinkle improvement of skin.

1. Introduction

Sea cucumbers are marine animals from the class Holothuroidea. About 1 200 known species of sea cucumber exist in worldwide, with using in food or folk remedies [1]. Sea cucumbers have traditionally been employed as medicines owing to their therapeutic efficacy in various diseases such as hypertension, asthma, rheumatism, incisions, burns, erectile dysfunction, and constipation. Over 300 compounds, including saponins, cerebrosides, polysaccharides, and peptides, have been identified in sea cucumber extract [2–5], with anti-angiogenic, anti-coagulation, anti-bacterial, anti-oxidation, and anti-tumor activities [6–10]. In terms of food science, sea cucumbers contain 90.5% water, 3.2% protein, 0.2% fat, and 3.5% minerals [11,12]. In addition, these animals are known as "ginseng of the sea" due to their high saponin content known for its efficacious ginseng ingredient [6,13]. The key components of dried sea cucumber are glycoprotein and chondroitin. Earlier research has focused on mucous-like polysaccharides and sulfate chondroitin structure [14]. In particular, glycoprotein, derived from marine organisms, has been shown to enhance anticancer activities and immune functions [15,16]. However, the mechanisms underlying the effects of these compounds on diseases at the molecular level remain to be elucidated.

Liquid extracts of boiled sea cucumber are byproducts of the dried cucumber production process. In general, the majority of this liquid is discarded, and results in large economic losses and raising concerns regarding environmental pollution. In addition, despite the high content of functional ingredients (for instance, polyphenol, protein, and glycogen), most studies on sea cucumbers to date have been related to food product development [17] and limited research has focused on extracting functional ingredients from the aspect of sea cucumber cooked liquid or developing functional products that based on the constituents.

In this study, liquid extracts of boiled sea cucumber were extracted from Korean sea cucumbers and glycoproteins that

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separated by purifying the fractionated liquid based on molecular size. We evaluated the skin whitening and anti-corrugation activities of each glycoprotein fraction, with a view to determining the feasibility of using functional ingredients from sea cucumber cooked liquid for preparation of cosmetics.

2. Materials and methods

2.1. Production of liquid extracts

We used approximately 200 g of Korean sea cucumbers (*Stichopus japonicus*, Selenka) harvested on the day of testing. Animals were eviscerated via a 3 cm incision from the anus to the center of the back and cleaned using fresh water for removal of salt and impurities. About 50 kg of eviscerated sea cucumbers were placed in boiling water and the mixture continuously stirred, and causes leakage of liquid extracts. Heating temperature was maintained at 80–90 °C. After removal of solid materials, the collected liquid extracts were stored at −20 °C until separation of glycoprotein fractions.

2.2. Separation and purification of glycoprotein fractions

After defrosting of the liquid extracts at 4–6 °C, the first decompression and filtration steps were conducted using a SUPRAcap depth filter (K500 P grade), followed by second depression and filtration to remove impurities using a SUPRAcap depth filter 10 inch capsule. Glycoprotein fractions with molecular weights of above and below 50 kDa (>50 kDa and <50 kDa) were separated using by an ultrafiltration system (BTR), lyophilized, and used for bioactivity assay. The separation and purification steps of glycoprotein fractions are shown in Figure 1.

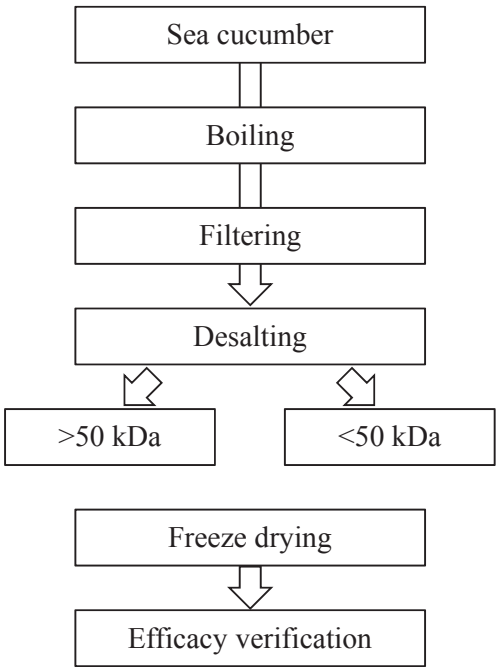

Figure 1. Schematic diagram of isolation of glycoprotein fractions from sea cucumber.

2.3. Separation and purification of glycoprotein fractions

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2.4. Cytotoxicity assay of glycoprotein fractions

Mouse melanoma cell line suspensions (100 μL; 5 000 cells/well) (B16-F10, ATCC LOT. 60508145) were dispensed into a 96-well plate and preincubated at 37 °C in 5% CO_2 for 24 h for cell adherence to the well plate. The control contained 100 μL Dulbecco's Modified Eagle Medium (ATCC) without cells. A 10 μL aliquot of fractions of >50 kDa and <50 kDa of liquid extracts was added to each well for each concentration (0.1, 1, 2, and 5 mg/mL). For the negative control, 10 μL medium was added and the mixtures reacted at 37 °C in 5% CO_2 for 24 h. After incubation of each well with 10 μL CCK-8 solution for 1 h, absorbance was measured at 450 nm.

$$\text{Survival rate}\ (\%) = \left(A_{\text{sample}} - A_b\right)/\left(A_c - A_b\right) \times 100$$

A_{sample}: sample absorbance, A_b: blank absorbance (value obtained with sample containing no cells), A_c: negative control absorbance (value obtained with distilled water containing cells). Experiments were performed in triplicate, and the results recorded as means ± SD.

2.5. Measurement of tyrosinase inhibitory activity

Tyrosinase inhibitory activity was assessed according to the method of Kim *et al.* [18]. A 500 μL cell suspension (50 000 cells/well) was dispensed into a 24-well plate (B16-F10, ATCC LOT. 60508145), and cells adhered by pre-incubating at 37 °C in 5% CO_2 for 24 h. Fractions >50 kDa and <50 kDa of liquid extracts and vitamin C were added to each well for all the concentrations tested and incubated at 37 °C in 5% CO_2 for 24 h. The pellet obtained from trypsin treatment was dissolved in 1% Triton X-100 PBS (0.5 mL), mixed with 0.2% L-DOPA (0.1 M) and 0.5 mL sodium phosphate buffer, and incubated at 37 °C for 2 h. Absorbance was measured at 490 nm.

$$\text{Inhibition}(\%) = (1 - A_1/A_0) \times 100$$

(A_0 = control group absorbance, A_1 = experimental group absorbance). Experiments were performed in triplicate, and the results recorded as means ± SD.

2.6. Elastase inhibitory activity assay

The elastase inhibitory effect was measured using the method of Kim *et al.* [19]. Fractions >50 kDa and <50 kDa of liquid extracts and vitamin C were prepared, and an aliquot of 10 μL pipetted into a test tube and mixed with 50 μL porcine

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