

# Identification of a molecular-level moisturizing effect on the entire skin layer of a mixture composed of seaweed extract from Jeju Island

Poster ID 300

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## Introduction:

Water is absolutely essential for the normal functioning of the skin, and the loss of water from the skin must be carefully regulated. Otherwise, various skin problems such as dry skin, itchiness, and damage to skin tissue may occur and reduced skin elasticity and wrinkles; thus, moisturizing is the most basic and essential function in cosmetics. Traditionally, skin care strategies for moisturizing have mainly utilized methods that prevent moisture evaporation from the skin by providing water or moisturizing ingredients directly to the skin or by forming a film on the skin. However, in recent years, substances have been revealed that act as a moisturizing agent at the molecular level. For example, glycerol is present as a natural endogenous wetting agent in the stratum corneum (SC). Hyaluronan, which has been considered a predominantly dermal component, is found in the epidermis and is important for maintaining a normal SC structure and epidermal barrier function. The presence of aquaporin-3, a water transport protein in the epidermis, and the presence of tight junction structures at the junction between the granular layer and the SC are also important findings [1]. Rather than simply supplying water to the skin from the outside, it will be possible to achieve stronger skin hydration through a molecular-level moisturizing approach. This study was conducted to develop a new ingredient with moisturizing and water-loss prevention functions at the molecular-level to fundamentally prevent and solve the problem of the lack of water in the skin.

## Results & Discussion:

### 2. The seaweed extract can contribute to moisturizing the entire skin layer by activating different AQP3s for each skin layer

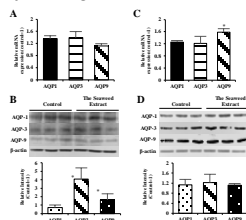


Figure 2. Effects of the seaweed extract on the expression of AQP3s in HaCaT and HDFn

Among several skin molecular-level moisturizing strategies to retain water and prevent water loss, we focused on aquaporins. Aquaporins are membrane proteins that form water channels across the cell membrane. They facilitate the transport of water and solutes like glycerol or urea. AQP3 is expressed from the basal layer up to one layer of cells below the SC [2]. When the seaweed extract was applied to the skin cells and artificial skin, it was confirmed that the expression level of AQP3 was increased in the epidermal cells and epidermal layer (Fig. 2A, 2B, 3A). In the case of AQP1, no significant increase or decrease in expression was observed by the seaweed extract (Fig. 2). The expression of AQP9 in the dermal cells was increased when our new moisturizing ingredient was applied (Fig. 2C, 2D, 3B).

## Materials & Methods:

### Preparation of the seaweed extract

We prepared a new moisturizing ingredient with seaweed extract containing *Gelidium Cartilagineum*, *Hizikia Fusiforme*, *Codium Fragile*, *Ecklonia Cava*, and *Sargassum Fulvellum* (Table 1). The seaweeds were collected from the Jeju sea area. By applying a special extraction method, we obtained hydrolyzed *Gelidium Cartilagineum* extract containing agarobiose, a functional substance not found in the hot-water *Gelidium Cartilagineum* extract. Finally, a mixture of the hydrolyzed agar extract and the extracts 2, 3, 4, and 5 (Table 1) were mixed in a 1:1 ratio to prepare the new seaweed extract.

Table 1. Composition of the seaweeds extract.

NO.	INCI name	%
1	<i>Gelidium Cartilagineum</i> Extract	50
2	<i>Hizikia Fusiforme</i> Extract	12.5
3	<i>Codium Fragile</i> Extract	12.5
4	<i>Ecklonia Cava</i> Extract	12.5
5	<i>Sargassum Fulvellum</i> Extract	12.5

### Cell cultures and reagents

Keratinocyte (HaCaT) cells and human primary fibroblast cells (HDFn, PromoCell, Germany) were used.

### RT-PCR and Quantitative Real-Time polymerase chain reaction (qRT PCR)

RNA extraction and RT-PCR were performed. Using a SYBR Green Realtime PCR Master Mix and QuantStudio™ 3 (Thermo Fisher Scientific, Inc.), the gene expression levels were standardized to the housekeeping gene glyceraldehyde 3-phosphate dehydrogenase (Gapdh).

### Western blot analysis

The primary antibodies were as follows: anti-AQP1, anti-AQP3, anti-AQP9, anti-HAS1 (LSBIO, USA), anti-HAS2 (LSBIO, USA), and anti-HAS3 (LSBIO, USA)

### Immunocytochemistry

The primary antibodies: anti-HA (Invitrogen, USA), anti-Zo-1 (Invitrogen, USA) and anti-Claudin (Bethyl Laboratories, Montgomery, USA). Staining was examined under an SP8 X Confocal Laser Scanning Microscope (Leica, Germany).

### 3D reconstructed human skin model

3D reconstructed human full skin model (Keraskin-FT™) and Keraskin-FT™ culture media were purchased from Biosolution Co., Ltd. (Seoul, Korea).

## Results & Discussion:

### 1. The seaweed extract did not significantly reduce the viability of HaCaT and HDFn at concentrations below 0.1%.

The seaweed extract did not significantly reduce the viability of human epidermal cells and fibroblasts at concentrations of 0.1% or less (Fig. 1). Mild cytotoxicity was observed above 0.5%. Therefore, it is safe to use seaweed extract at a concentration of 0.1% or less.

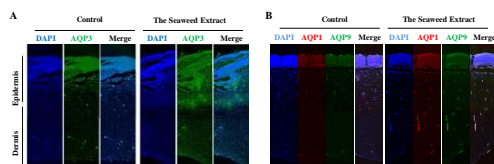


Figure 3. Effects of the seaweed extract on the expression of AQP3s in the 3D skin model.

### 3. The Seaweed extract can increase HA synthesis by activating different types of hyaluronic acid synthase for each skin layer.

HA is known to be mainly expressed in the dermis, and its existence was recently confirmed in the SC [1, 5]. Therefore, it can be said that HA is generated throughout the skin layer and has a role in supplying moisture to the skin. In this study, we confirmed that the seaweed extract increases the expression level of the enzyme that promotes hyaluronic acid synthesis and synthesis of HA in HDFn and the effect of activating hyaluronic acid synthase which could be involved in hyaluronic acid synthesis in HaCaT (Fig. 4). Through this, it can be seen that the seaweed extract has the effect of activating different hyaluronic acid synthases in all layers of the dermis and epidermis while accelerating the synthesis of hyaluronic acid according to the characteristics of each skin layer.

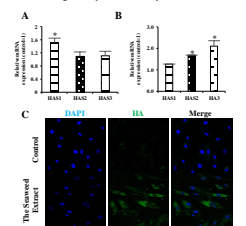


Figure 4. Effects of the seaweed extract on expression of HAS and HA.

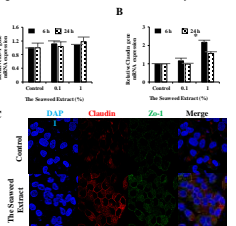


Figure 5. Effects of the seaweed extract on expression of tight junction molecules.

### 4. The effect of the seaweed extract on tight junctions which prevent the evaporation of skin moisture

Tight junctions consist of proteins such as claudin, occludin, Zo-1, Zo-2, and Zo-3. In this study, we confirmed that the seaweed extract increased the gene expression and protein production of Zo-1 and Claudin among proteins constituting the Tight junctions (Fig. 5).

## Conclusions:

We wanted to confirm that our new moisturizing ingredient has a strong molecular-level moisturizing effect in all layers including the dermis, epidermis and stratum corneum of the skin. Our new moisturizing functional ingredient, the seaweed extract, activates different AQP3s and hyaluronic acid synthases in the dermal and epidermal layers to increase the moisturizing ability of the skin. In addition, it is a powerful and effective molecular-level moisturizing material that prevents skin water loss by strengthening the Tight Junctions. Therefore, the new seaweed extract was shown to have potential as a powerful moisturizing ingredient.

## References:

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