Skin moisturizing efficacy of *Salicornia herbacea* L. extract by enhanced skin barrier

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**Summary**
To examine enhancing of the skin barrier in a *Salicornia herbacea* L., several tests were carried out. Expression of Transglutaminase-1 increased dose dependently in T-Gase-1 assay. Also, cornified envelope expression was increased when the treated *Salicornia herbacea* L. extract. A result of RT-PCR performing to check the expression of involucrin, filaggrin and tissue inhibitor of metalloproteinase-1 (TIMP-1), expression of involucrin increased similar to the positive control when the treated *Salicornia herbacea* L. extract. Filaggrin and TIMP-1 were also increased dose dependently. On the other hand, expression of matrix metalloproteinase-1 (MMP-1) decreased dose dependently. Finally, hyaluronic acid and glycosaminoglycan (GAGs) were increased when the treated *Salicornia herbacea* L. extract. Therefore, these results were indicated that a *Salicornia herbacea* L. extract was effective in skin barrier strengthen and moisturizing.

**INTRODUCTION**
The skin, an organ that exists in the outermost, protects the water of body and acts as a barrier to prevent the invasion of foreign substances. The protective role of the skin can be maintained the function when stratum corneum consists of normally formed keratinocytes only. When the stratum corneum is damaged, moisture evaporates easily and the skin becomes dry. Corneous also occurs at the same time. To enhance skin barrier and moisturizing, many cosmetic products have been developed. But chemical components contained in general cosmetic products caused skin side effects. So our experiments were carried out using a natural extract to enhancing skin barrier and increasing moisturizing. *Salicornia* was used as a natural extract. *Salicornia* (*salicornia herbacea* L.) has been widely used in folk medicine plant since ancient. *Salicornia* is known to be effective to cancer, sinusitis, arthritis, high blood pressure, back pain, obesity, hemorrhoids, diabetes, thyroiditis, asthma, bronchitis, and liver disease. *Salicornia* grows in the flock in the west coast and south
Therefore, in this study, we have conducted experiments to confirm that moisturizing and enhancing the skin barrier of salicornia.

**MATERIAL AND METHODS**

Preparation of extract
50g dried Salicornia were pulverized using a pulverizer. Pulverized Salicornia extracted at room temperature for three hours using 1L 70% ethanol. Salicornia extract was filtered with a filter paper. Filtered Salicornia extracts were concentrated completely using rotary evaporator.

Cell culture
HaCaT human keratinocyte cells and Human dermal fibroblasts (HDF) were grown in Dulbecco’s modified Eagle’s medium (DMEM) containing 10% fetal bovine serum (FBS) and 1% penicillin/streptomycin in a 5% CO₂ air atmosphere at 37°C.

MTT cell viability assay
Cytotoxicity of HaCaT and HDF were evaluated by the MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide) assay. MTT was dissolved in PBS at 5mg/ml. MTT solution was added to all wells of an assay plate, and plates were incubated at 37°C for 4hr. Dark blue crystals were dissolved in acid-isopropanol and absorbance values were measured [1].

Transglutaminase-1 assay
HaCaT cells were incubated for 48hr with salicornia in CaCl₂ media. After three additional freeze-thaw cycles, acetone : ethanol (1:1) solution treated to 4°C for 30min. Primary and secondary anti-body were incubated. The buffer containing the O-phenylenediamine dihydrochloride and the urea hydrogen peroxide tablet was added and after the reactions had proceeded in darkness for 20min. Stop solution is treated and absorbance values were measured. [2]

Cornified envelope assay
HaCaT cells were incubated for 48hr with salicornia in CaCl₂ media. Cells were collected
and washed with PBS. The remainder of the cells were centrifuged, resuspended in 1 mL of cell envelope dissociation buffer [2% SDS, 20 mM DTT, 5 mM EDTA, 0.1 M Tris-HCl (pH 8.5)], and boiled for 5 min. Detergent-insoluble cornified cell envelope were cooled, centrifuged, and resuspended in PBS. Absorbance values were measured [3].

Reverse transcription polymerase chain reaction
Involucrin, filaggrin and TIMP-1 expression was demonstrated to use RT-PCR. HaCaT cells and HDF were incubated for 48 hr with salicornia in serum free DMEM media. Total RNA was isolated from the cells with QIAzol (QIAGEN science, USA) according to the instructions of the manufacturer. After RT-PCR performing, the PCR products were identified by electrophoresis on 1.5% agarose gel and EtBR staining. PCR fragments were analysis with Gel Logic 212 Pro [4].

Matrix metalloproteinase-1 assay
HDF were incubated for 48 hr with salicornia in serum free DMEM media. Cell culture medium was collected after 48 hr, and MMP-1 production was quantified using a human MMP-1 ELISA kit respectively [5].

Hyaluronic acid assay
HDF were incubated for 48 hr with salicornia in serum free DMEM media. Cell culture medium was collected after 48 hr, and Hyaluronic acid production was quantified using a human hyaluronic acid ELISA kit respectively [6].

Glycosaminoglycan assay
HDF were incubated for 48 hr with salicornia in serum free DMEM media. Cell culture medium was collected after 48 hr, and glycosaminoglycan production was quantified using a human sulfated glycosaminoglycan ELISA kit respectively [7].

RESULTS
MTT cell viability assay
MTT assay was performed to confirm cytotoxicity. In cytotoxicity test of HaCaT and HDF, we confirmed more than 80% viability at all concentration (Figure 1, 2). Therefore, all
experiments in the next step were used highest concentration of 100 and 200μg/ml.

Figure 1. Effects of salicornia herbacea L. extract on the cell viability in HaCaT cells.

Figure 2. Effects of salicornia herbacea L. extract on the cell viability in HDF.

Transglutaminase-1 assay

Transglutaminase-1 (TGase-1) is most important factor to make cornified envelope, the skin barrier layer. TGase-1 assay was performed to confirm TGase-1 expression in HaCaT cells. Calcium was used as a control because calcium is materials that regulate a development and differentiation of keratinocyte and gene expression. 0.03mM calcium medium was used as a negative control group, and 1.2mM calcium medium was used as a positive control group. As a result, transglutaminase-1 assay show that increasing the expression of TGase-1 dose-dependently when compared to control group (Figure 3).
Figure 3. Effects of Salicornia herbacea L. extract on the Transglutaminase-1 (Tgase-1) activity in HaCaT cells. (n=3) ***p < 0.001, *p < 0.1 compared to control; one way ANOVA, followed by Tukey’s Multiple Comparison test.

Cornified envelope assay
Cornified envelope plays a role of a physical barrier on the skin. Cornified envelope were formed the number of proteins cross-linked by transglutaminase-1. Measure of cornified envelope is good standard to judge of skin barrier protection.
Cornified envelope assay used calcium control. Cornified envelope expression was increased when the treated salicornia extracts (Figure 4).

Figure 4. Effects of Salicornia herbacea L. extract on the proliferation and differentiation of HaCaT cells. (n=3) ***p < 0.001, *p < 0.1 compared to control; one way ANOVA, followed by Tukey’s Multiple Comparison test

Reverse transcription polymerase chain reaction
The expression of involucrin, filaggrin and TIMP-1 was confirmed by RT-PCR. Involucrin is a structure protein that makes cornified envelope. Involucrin uses calcium control. A result of RT-PCR performing to check the expression of involucrin, expression of involucrin increased similar to the positive control when the treated salicornia extracts (Figure 5).

Filaggrin is a precursor of natural moisturizing factors (NMFs) that show skin moisturizing ability. A result of RT-PCR performing to check the expression of filaggrin, expression of filaggrin was increased. (Figure 6).

TIMP-1 is an inhibitor of MMP-1. A result of RT-PCR performing to check the expression of TIMP-1, expression of TIMP-1 was increased dose-dependently when the treated salicornia extracts (Figure 7).

![Figure 5. Effects of Salicornia herbacea L. extract on the expression of involucrin mRNA in HaCaT cells.](image)

![Figure 6. Effects of Salicornia herbacea L. extract on the expression of filaggrin mRNA in HaCaT cells.](image)
Figure 7. Effects of Salicornia herbacea L. extract on the expression of tissue inhibitor of metalloproteinase-1 (TIMP-1) mRNA in HDF.

Matrix metalloproteinase-1 assay
Matrix metalloproteinase-1 (MMP-1) is degradation enzyme of collagen. The degradation of collagen is weakening the skin barrier because collagen is an important factor of skin barrier. Results of matrix metalloproteinase-1 (MMP-1) assay, TGF-β was reduced to expression of MMP-1 by 50%. Salicornia extract also reducing the expression of MMP-1 dose-dependently (Figure 8).

Figure 8. Effects of Salicornia herbacea L. extract on the Matrix metalloproteinase-1 (MMP-1) inhibition in of HDF. (n=2) **p < 0.01, compared to control; one way ANOVA, followed by Tukey’s Multiple Comparison test

Hyaluronic acid assay
Hyaluronic acid is the most important factor in skin moisture. Hyaluronic acid is known as natural moisturizing factors. Results of hyaluronic acid assay, EGF was increased to expression of hyaluronic acid. Salicornia extract also increasing the expression of hyaluronic acid (Figure 9).

![Figure 9. Effects of Salicornia herbacea L. extract on the hyaluronic expression in of HDF.](image)

Glycosaminoglycan assay
Glycosaminoglycan is the most important factor in skin moisture. Glycosaminoglycan has a water-holding ability of the dermal layer. Results of glycosaminoglycan assay, EGF was increased to expression level of glycosaminoglycan. Salicornia extract also increasing the expression of glycosaminoglycan dose-dependently (Figure 10).

![Figure 10. Effects of Salicornia herbacea L. extract on the glycosaminoglycan expression in of HDF.](image)

**CONCLUSION**
The skin is damaged in various ways. Damaged skin have weak skin barrier and increase water loss. Recently, for damaged skin, use of natural extracts is increasing. Among the various natural products, *Salicornia herbacea* L. has been studied in various fields, but insufficient study on skin effects. So, we investigated enhancement of the skin barrier and moisturizing capacity to using *Salicornia herbacea* L. *Salicornia herbacea* L. extract was increased to expression of TGase-1, cornified envelope, involucrin, filaggrin, TIMP-1, hyaluronic acid, glycosaminoglycan and decreased to expression of MMP-1. TGase-1 is the most important factor to make a cornified envelope. Cornified envelope plays a role of a physical barrier in the skin. Also, cornified envelope has been formed crosslinked proteins such as involucrin. Filaggrin is a precursor of a natural moisturizing factors(NMFs) that shows a skin moisturizing ability. Also, filaggrin plays the role of join together the keratin in keratinocyte. Therefore increasing expression of above factor may be enhanced skin barrier. MMP-1 is decomposed collagen, and TIMP-1 inhibit to MMP-1. The combination of MMP-1 and TIMP-1 will inhibit the degradation of collagen. Collagen affects all of the skin barrier strength and moisturizing. Hyaluronic acid and glycosaminoglycan is the most important factor in skin moisture. Hyaluronic acid is known as natural moisturizing factors. And glycosaminoglycan has a water-holding ability of the dermal layer.

As a result, *Salicornia herbacea* L. extract is considered to the development as moisturizing cosmetics by skin barrier strengthening function.

**REFERENCE**

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