Enhancement of skin barrier function by the callus from Campanula Takesimana

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Introduction:

Campanula Takesimana (Korean bellflower, *Seomchorongkkot*) is a Korean endemic plant grown on Ulleng Island. We identified the potential effects of *Campanula Takesimana* callus extract on strengthening the skin barrier in order to develop an eco-friendly and sustainable cosmetic material. Atopic dermatitis (AD), a chronic inflammatory skin disease, is onset by structural and immunological dysfunction of human skin and by stimulation of environmental factors. However, specific pathologic mechanisms are not fully understood. Recently, many studies have identified that Th2-derived cytokines, IL-4 and IL-13, are elevated in AD lesions [1-6]. It has been reported that these cytokines impair barrier function by disrupting the expression of barrier-related proteins such as filaggrin, loricrin and tight junction proteins [2]. Therefore, we modeled atopic dermatitis-like skin inflammation by using IL-4 and IL-13 [3] and assessed the effect of *Campanula Takesimana* on AD in the model. *Campanula Takesimana* callus restored the expression of barrier proteins and tight jucntion proteins reduced by IL-4 and IL-13, suggesting that a native plant of Ulleng island can be a new eco-friencly cosmetic material for the skin barrier.

Materials & Methods:

- Induction of Callus and extraction of bioactive compounds
- Analysis of the bioactive compounds
- Evaluation of cell viability and proliferation
- Modeled AD-like skin inflammation using IL-4/IL-13
- Evaluation of the anti-inflammatory effect of C.T
- Evaluation of skin barrier function

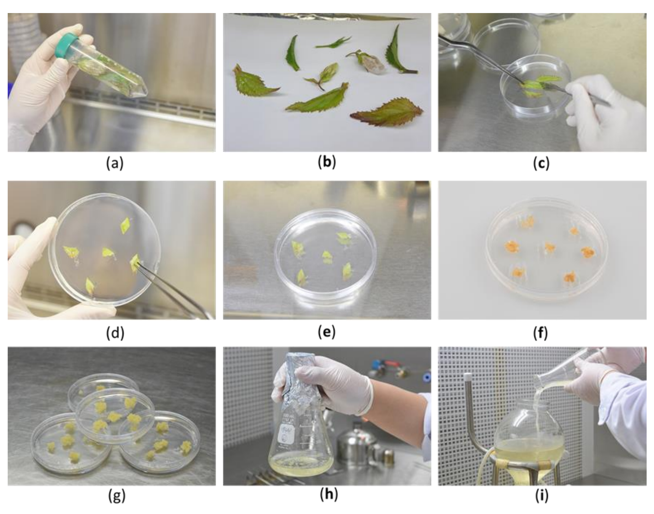


Fig 1. Callus induction of *Campanula Takesimana*. (a)~(b) Sterilized Leaf bloting. (d)~(e) Tissue plating. (f) Callus induction. (g) Callus line selection. (h~i) Bioreactor culture

Results & Discussion:

Cytotoxicity and cell proliferation by *Campanula Takesimana* callus

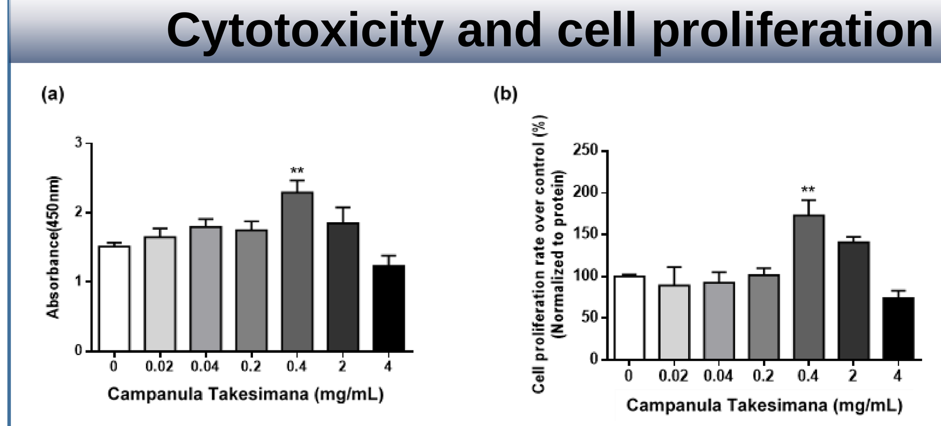


Fig 3. The effect of *Campanula Takesimana* callus extract on cell viability and proliferation. C.T callus extract was applied to culture medium for 24h. (a) Variation of cell viability by concentration of G.P. (b) Variation of cell proliferation rate by concentration of C.T. C.T : *Campanula Takesimana*; ***p*<0.01 vs C.P non-treated control; *****p*<0.001 vs C.P non-treated control. **0.4 mg/mL extract significantly raised the proliferation of keratinocytres.**

The callus extract doesn't improve IL-4/IL-13-induced inflammation

TSLP and IL-33, epithelial cytokines, are the key regulators of T_{h2}-mediated immune responses that cause skin itchy and damage to the skin barrier.

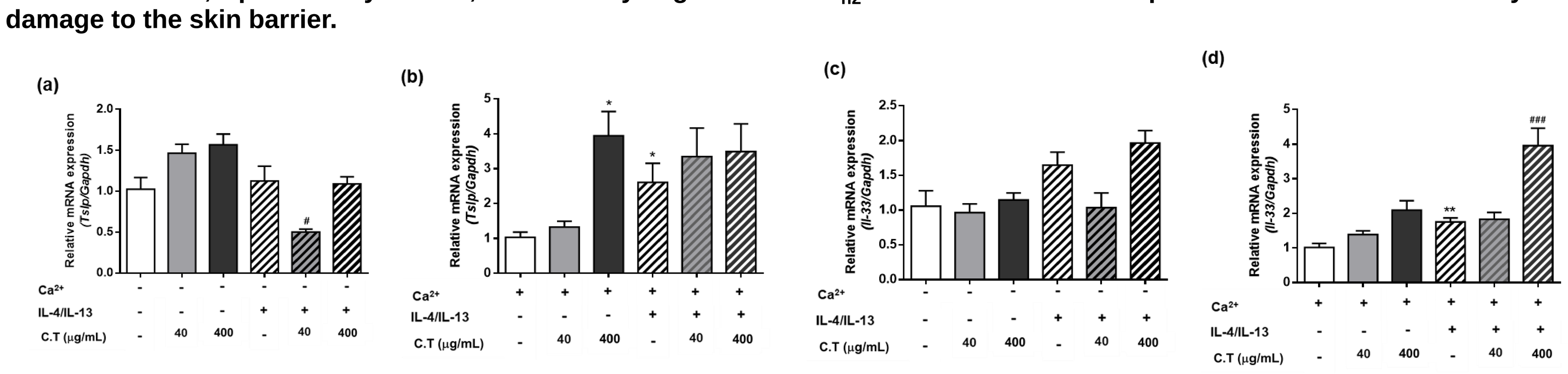
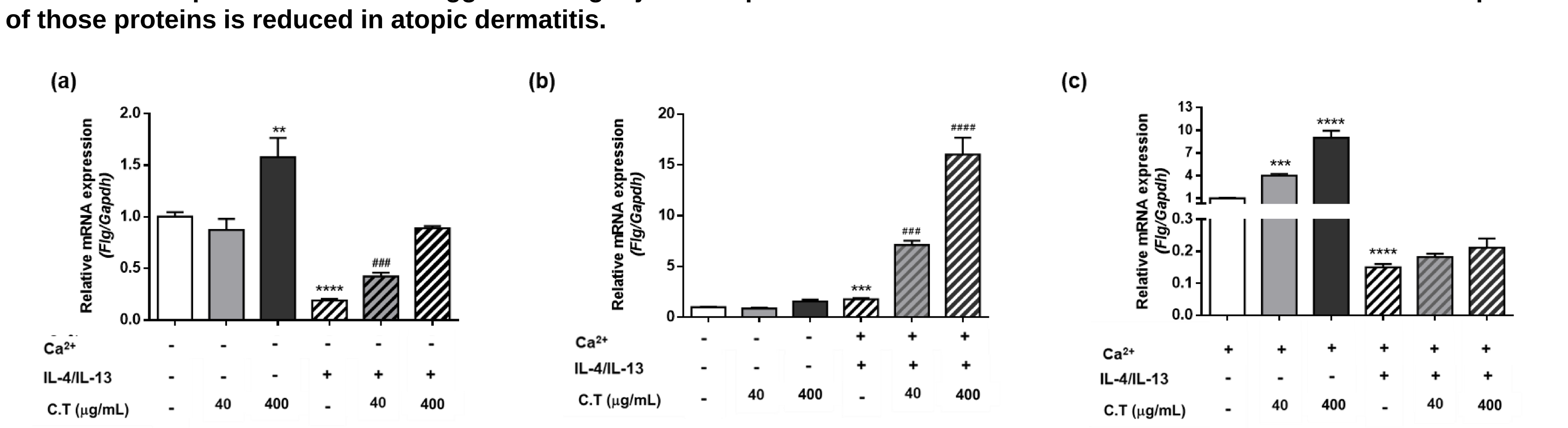


Fig 4. Effect of *Campanula Takesimana* callus extract on Th2 cytokine-induced *TSLP* and *IL-33* mRNA expression. Vehicle or C.T callus extract was applied to the culture medium for 5 days with or without IL-4/IL-13 in the (a, c) absence or (b, d) presence of 1.5 mM Ca^{2+}. (a, b) *TSLP* and (c, d) *IL-33* mRNA expression in keratinocytes. C.T : *Campanula takesimana* callus extract; **p*<0.05 vs G.P non-treated control; ***p*<0.01 vs non-treated control; ####*p*<0.001 vs IL-4/IL-13-treated control. ***Campanula Takesimana* callus extract had no effect on the *TSLP* and *IL-33* expression involved in the Th2 cytokine-induced immune response.**

Skin barrier function is improved by *Campanula Takesimana* callus extract

Barrier-related proteins such as filaggrin and tight junction proteins such as ZO-1 and Claudin-1 are known that the expression of those proteins is reduced in atopic dermatitis.



Results & Discussion:

Fig 5. *Campanula Takesimana* callus extract improved Th2 cytokine-induced *Filaggrin* mRNA downregulation in differentiated keratinocytes. Vehicle or C.T callus extract was added to the culture medium for 5 days with or without IL-4/IL-13 in the (a, b) absence or (b, c) presence of 1.5mM Ca^{2+}. C.T : *Campanula Takesimana* callus extract; ***p*<0.01 vs non-treated control; ****p*<0.005 vs non-treated control; *****p*<0.001 vs non-treated control; ###*p*<0.005 vs IL-4/IL-13-treated control. ***Filaggrin* mRNA expression was significantly decreased by IL-4 and Il-13 in differentiated and undifferentiated keratinocytes. However, *Campanula Takesimana* callus extract dramatically restored *filaggrin* mRNA expression in a concentration-dependent manner.**

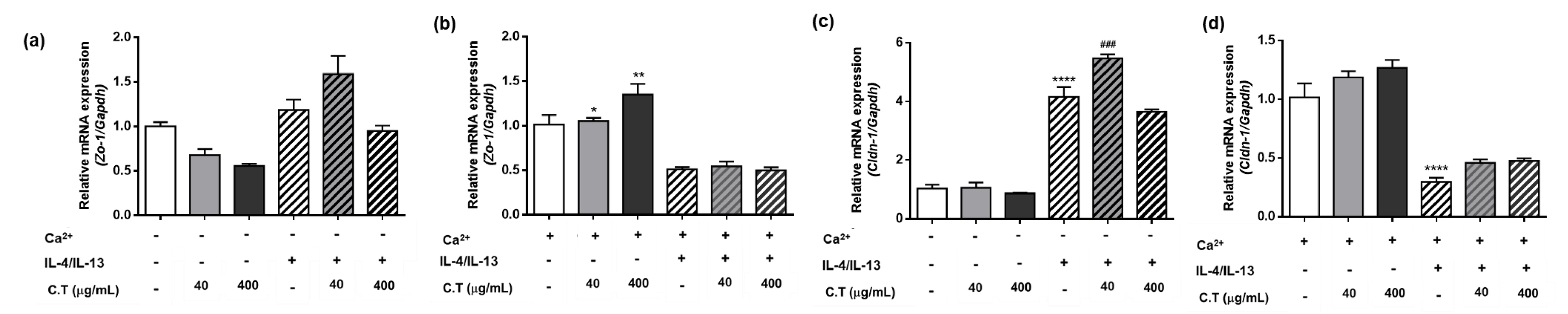


Fig 6. *Campanula Takesimana* callus extract increased *ZO-1* and *CLDN-1* mRNA downregulation in differentiated keratinocytes. Vehicle or C.T callus extract was added to the culture medium for 5 days with or without IL-4/IL-13 in the (a, c) absence or (b, d) presence of 1.5mM Ca^{2+}. (a, b) *ZO-1* mRNA and (c, d) *CLDN-1* mRNA expression. C.T : *Campanula Takesimana* callus extract; ***p*<0.01 vs non-treated control; ****p*<0.005 vs non-treated control; *****p*<0.001 vs non-treated control; ###*p*<0.005 vs IL-4/IL-13-treated control. **Tight junction protein Zo-1 expression was increased in differentiated keratinocytes in the absence of IL-4 and IL-13, whereas, Claudin-1 expression was elevated by stimulation of IL-4 and IL-13 and more increased by treatment of 0.04 mg/mL extract in undifferentiated keratinocytes.**

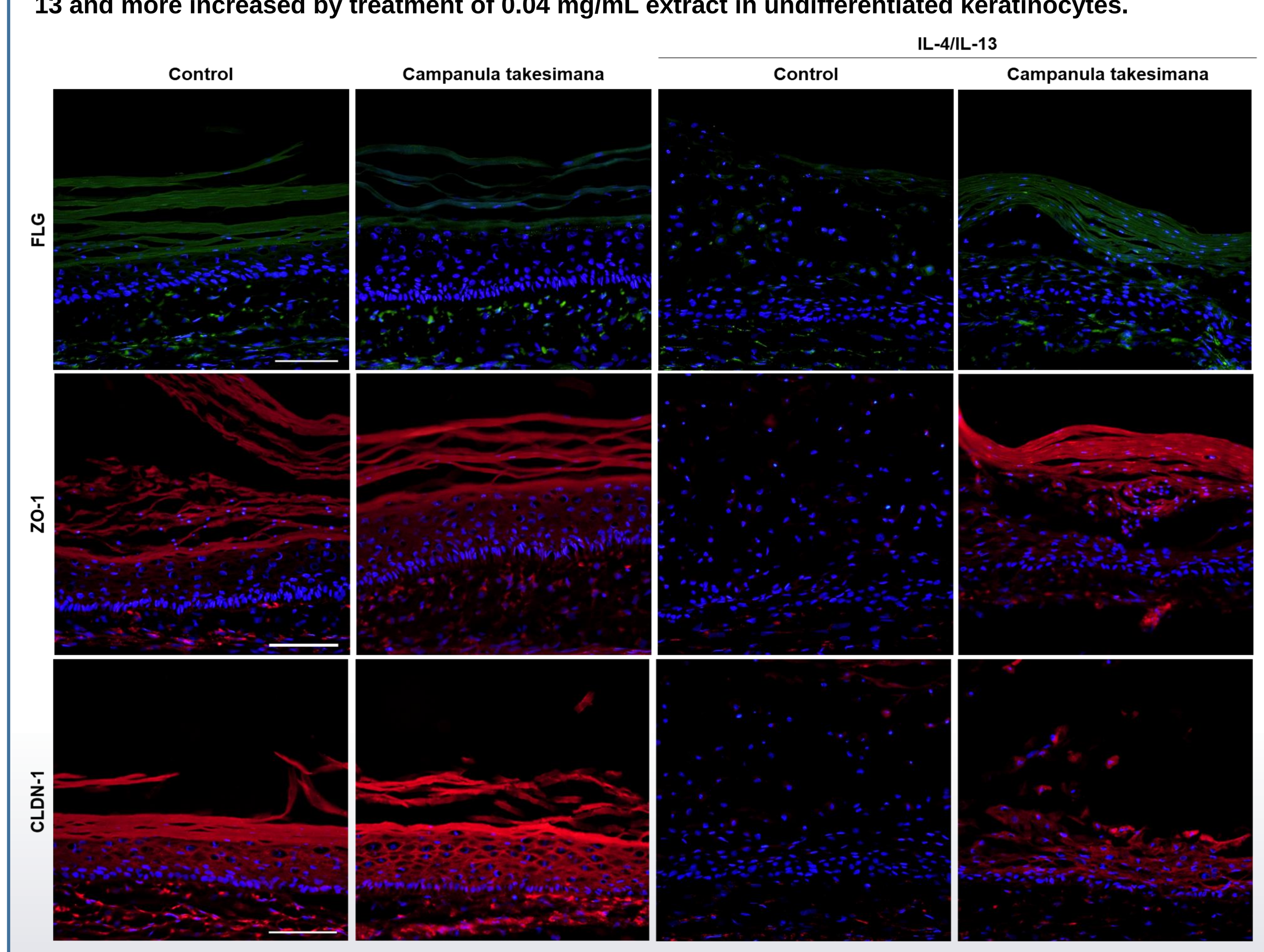


Fig 7. *Campanula Takersimana* callus extract improved skin barrier function we assessed barrier-related protein expression in reconstructed human skin model. C.T callus extract was added to the culture medium with or without IL-4/IL-13 during epidermal differentiation. C.T : *Campanula Takesimana.* **Skin barrier-related proteins such as Filaggrin, ZO-1 and CLDN-1 were barely expressed in AD model whereas they were highly expressed in the Campanula Takesimana treated model.**

***Campanula Takesimana* callus extract has an effect on the strengthening of skin barrier function**

Discussion

Aberrant expression of skin barrier-related proteins, such as filaggrin, is a classic hallmark of atopic dermatitis [4]. As filaggrin is an important protein for skin barrier homeostasis, several studies have shown that a lack of filaggrin interferes with epidermal maturation function, as well as alters skin lipid composition and organization. In addition, filaggrin mutation causes reduced natural moisturizing factors, which increases skin pH and accelerates barrier dysfunction [4-6].

Campanula Takesimana, a Korean endemic plant grown on Ulleng island, is known that it is beneficial for improving allergic diseases such as bronchitis and asthma. We hypothesized that the extract of *Campanula Takesimana* callus can improve atopic inflammation in the skin which is one of the most common allergic disease. Therefore, we investigated the role of the extract of *Campanula Takesimana* callus on Th2 cytokine-induced inflammation and barrier-related proteins in the keratinocytes. Although the extract *Campanula takesimana* callus did not ameliorated Th2-induced TSLP and IL-33, it increased filaggrin and tight junction proteins. Moreover, Th2 cytokine-induced downregulation of filaggrin expression was restored by the callus extract in undifferentiated keratinocytes. Taken together, *Campanula takesimana* callus extract could help atopic dermatitis by maintaining epidermal homeostasis and strengthening the skin barrier function.

Conclusions:

The extracts from *Campanula Takesimana* callus can be effective in strengthening the skin barrier by inducing filaggrin expression. Considering the advantage of the callus that it is a useful source of sustainable, eco-friendly active material, *Campanula Takesimana* callus extracts could be widely used as a cosmetic material for improving skin barrier function.

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