

Anti-inflammatory and barrier enhancing effect of cultured coconut extract against *ex-vivo* UVB radiation

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Abstract

Natural plant oils have been used as a translational alternative to modern medicine. Particularly, virgin coconut oil (VCO) has gained popularity because of its potential benefits in pharmaceutical, nutritional, and cosmetic applications. Cultured coconut extract (CCE) is an alternative end product of VCO, which undergoes a further bacterial fermentation process. This study aimed to investigate the effects of CCE on human skin. We analyzed the expression of skin barrier molecules and collagen after its application on explant human skin. To evaluate the anti-inflammatory properties, the expression of inflammatory markers was analyzed after ultraviolet B (UVB) irradiation. The CCE-treated group showed increased expression of cornified cell envelope components, which contribute to protective barrier functions of the stratum corneum. Furthermore, the expression of inflammatory markers was muchlower in the CCE-treated group after exposure to UVB radiation. From such results, we expect an anti-inflammatory effect of CCE against UVB irradiation-induced inflammation. Additionally, the CCE-treated group showed increased collagen and hyaluronan synthase-3 expression.

In our study, CCE showed a barrier-enhancing effect and anti-inflammatory properties against ex vivo UVB irradiation induced inflammation. Due to its beneficial molecular profile, we can expect various clinical implications of CCE in both diseased and healthy skin.

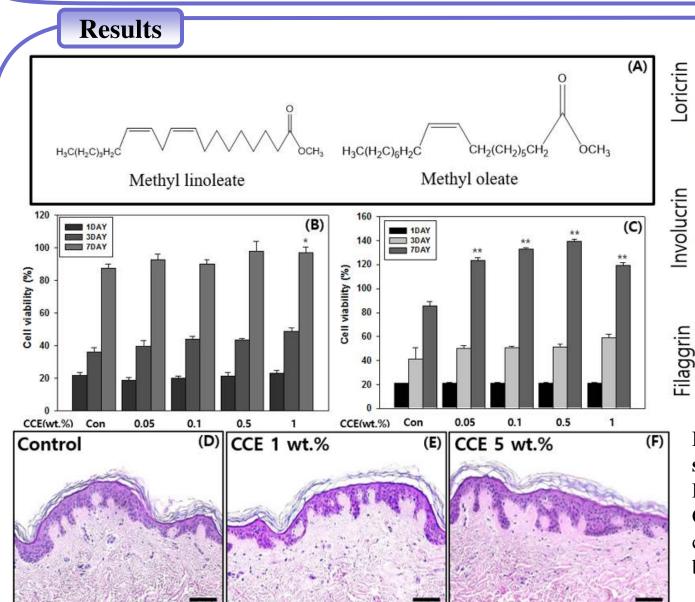


Figure 1. Biocompatibility of CCE. (A) Chemical structure of fatty acids in CCE. Cell viability of HDFs(B) and HaCaT(C) was evaluated by CCK-8 assay kit. Cell viability rate was increased as CCE concentration was increased in both cells. Explant human skin tissue was treated with CCE(1-5 wt.%). To conform the biocompatibility of CCE on human skin, H&E staining(D-F) was performed. *p<0.05, **p<0.01 indicate a significant difference from the control. (Scale bar=100 μ m)

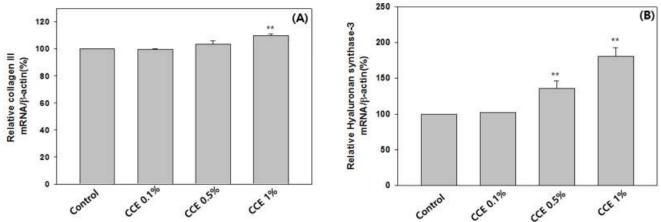
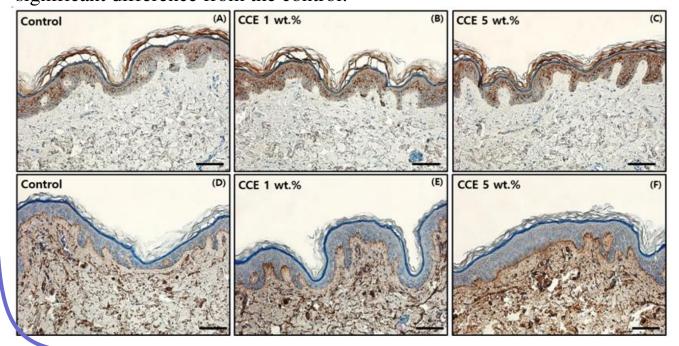


Figure 2. Collagen III and hyaluronan synthases-3 mRNA expression in human dermal fibroblasts. HDFs were treated with different concentrations (0.1-1 wt.%) of CCE for 7 days. Collagen III(A) and HAS-3(B) mRNA expression levels were evaluated by RT-PCR. Compared with control group, cells incubated with 1 wt.% CCE. **p<0.01 indicates a significant difference from the control.



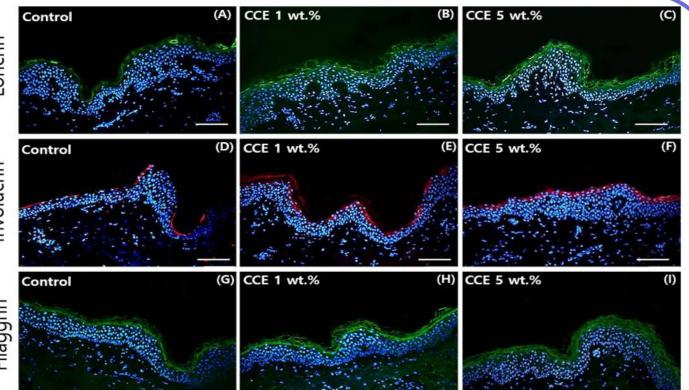


Figure 4. **Effect of CCE on barrier molecules expression in explant human skin tissue.** Immunofluorescence staining of Loricrin (a-c), Involucrin (d-f) and Filaggrin (g-i) in explant human skin tissue. The skin tissue were cultured with CCE (1-5 wt.%) and harvested after 5 days of tissue culture. Compared with control, tissues treated with CCE showed stronger expression profile of skin barrier molecules. (Scale bar=100µm)

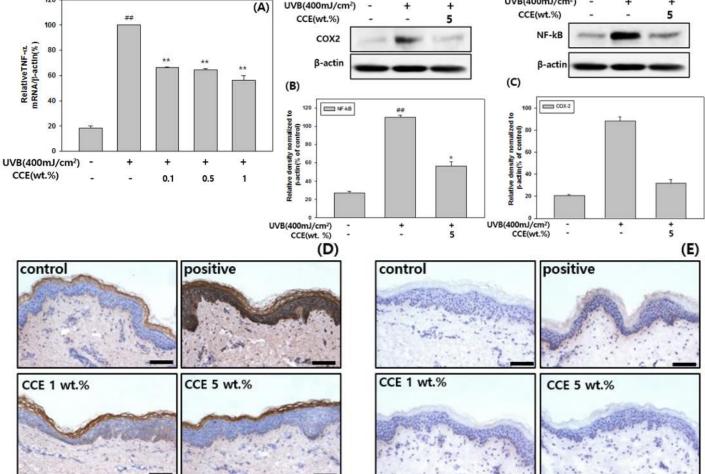


Figure 5. Anti-inflammatory effect of CCE on human skin. HDFs were cultured with and without different concentrations of CCE for 48 hrs. The cells were expressed under UVB (15 mJ/cm²) and mRNA was extracted after 24 hrs. TNF- α mRNA expression(A) was evaluated by RT-PCR. Compared with positive control, CCE treated groups showed lower expression level of TNF- α . To evaluated the anti-inflammatory effect of CCE on human skin, western blot analysis (B-C) and immunohistochemistry staining(D-E) was performed on defected human skin tissue by UVB (400 mJ/cm²) exposure. Group treated with CCE, The expression of NF-κB and COX-2 were lower in CCE treated group than non-treated group (positive). ##p<0.01 indicates a significant difference from the control; *p<0.05, **p<0.01 indicate a significant difference from the positive control. (Scale bar=100μm)

Figure 3. Effect of CCE on collagen I and III expression in explant human skin tissue. The explant human skin tissue were treated with different concentrations (1-5 wt.%) of CCE for 5 days. immunohistochemistry staining for collagens was performed. The staining intensity of collagens showed higher intensity as CCE concentration increased. (Scale bar=100µm)