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CinderellaCare Test Method

Western blot analysis

Melanocytes were cultured with Wild Thyme Extract. After 72 hrs, the cells were lysed with RIPA buffer. Approximately 5ug of cell lysate was boiled at 95° C for 5 minutes in the sample buffer. The samples were then separated by SDS- PAGE, followed by protein blotting onto a PVDF membrane. The protein-blotted membranes were blocked with 5% (w/v) fat-free dry milk in PBS with 0.05% Tween 20 (PBS-T) for over-night at 4° C. They were then incubated with anti-kinesinantibody (Abcam, MA), and treated with goat anti-mouse IgG antibody coupled to horseradish peroxidase. The expressions of the proteins were detected by ECL detection system.

Localization of fluorescence beads

We used the fluorescence beads in this experiment as a mimic of themelanosome. The melanocyte were treated with fluorescence beads for 72hrs. The melanocyte including fluorescence beads has seeded into a chamber-slide, and Wild Thyme Extract was added. 24hrs later, the localization of the beads were observed by Fluorescent Microscopy.

Melanin degradation assay by 3D model skin

3D model skin was cultured for 10 days according to the manufacturer's protocol. The exchange of culture media and the apply sample were performed every other day and all steps were performed five times. After having washed cells in PBS, the cells irradiated UVB of 5mJ from the second culture media exchange. Therefore, the UV irradiated four times in total during an incubation period (total 20mJ/10days). After 10-days culture, we washed insert in PBS and photographed the whole tissue with a digital camera. Additionally, we observed a state of the interstitial melanocyte by a microscope and photographed it. The tissues were soaked into PSB and performed crushing and centrifugal separation (2,000rpm,10min). After remove supernatant, add 1 ml of ethanol and ether mixed solution (3:1) and performed stirring and centrifugal separation (2,000rpm, 10min). After removed supernatant that sediment were dried. We heat 1N NaOHwith 10% DMSO in a bath of 90 degrees for 20 minutes, and measured the absorbance (405nm) of the liquid which dissolved.

Human skin test

Twenty healthy volunteers were recruited to examine the effects of Wild Thyme Extract on skin-photoaging such as pigment spots and skin whitening. Before the start of human volunteer test, an informed written consent was obtained and examined skin conditions. The experimental protocol was approved by the institutional Ethical Committees. The twenty onevolunteers (age from 20's to 60's, 9 males and 12 female)were treated with 1% Wild Thyme Extract and control on their each half face twice a day for two months. The melanin index was measured by Mexameter® MX18(Courage and Khazaka, Germany). The spots of the face were measured by VISIA® (integral, Tokyo, Japan).