New Anti-Aging Concept:

UJI TEA EXTRACT (B) BG inhibits calpain and blocks extremely harmful “late phase” oxidative shock in skin.
Inflammation and skin aging

UV

ROS

Dryness \rightarrow Fine wrinkle

proTEIN carbonylation in SC and dermal matrix

Elastase

Leucocytes

inflammation

Pro-MMP-1
TIMP-1

Deep wrinkle

Collagen, Elastin
UVB-irradiated cells were loaded with 20 μM H$_2$DCFDA (cell-permeant 2',7'-dichlorodihydrofluorescein diacetate) for 30 min and incubated further 30 min at each time. Intracellular ROS was measured by the fluorescence intensity (Ex: 485 nm/Em: 530 nm) of the cell lysates. Significance **<0.05.
Time course profile of IL-1α secretion after UVB irradiation

Cells were exposed to UVB (25 mJ/cm²). IL-1α secreted into the medium was quantified using commercial assay ELISA kits, Human IL-1α Immunoassay (Quantikine). Significance *<0.05, **<0.01.
Cells were loaded with 20 μM H₂DCFDA for 30 min and were then incubated with 10 ng/mL IL-1α. Intracellular ROS was measured by the fluorescence intensity (Ex: 485 nm/Em: 530 nm) of the cell lysates. Significance *<0.05, **<0.01.
Profiles of biphasic ROS generation after UVB irradiation

- Intensity
- ROS
- Cell damage
- Calpain
- pro-IL-1α
- IL-1α
- ROS
- Ca^{2+}

Time (h): 1, 3, 5, 7
Calpain is a calcium-dependent protein found in many parts of human organism. Several medical reports on the role of calpain in aging processes have been published.
Proposed scheme: $Ca^{2+}$-triggering cascade in ROS generation

NADPH oxidase (nicotinamide adenine dinucleotide phosphate-oxidase)

ROS

Calpain

Cell damage

Inflammation

Pigmentation

Wrinkling
After UVB irradiation (25 mJ/cm²), cells which had been loaded with 10 µM Suc-Leu-Leu-Val-Tyr-AMC for 1 h at each time (1, 2, 3, 4 and 6 h), were lysed and the fluorescence intensity (Ex: 380 nm/Em: 460 nm) was measured. Significance against sham-irradiation; *<0.05, **<0.01.
Effects of a Calpain inhibitor (ALLN) on UVB-induced reactions

After preloading with the calpain inhibitor, ALLN for 1 h, cells were exposed to UVB (25 mJ/cm²). Protease activation was measured at 2 h after UVB-R, Survival ratio was assessed at 24 h after UVB-R by neutral red incorporation, and ROS generation was measured at 7 h after UVB-R. Significance; *<0.05, **<0.01.

ALLN: N-Acetyl-L-leucyl-L-leucyl-L-norleucinal
**Short summary-1**

- The late phase ROS caused the UVB-induced cell damage.
- UVB-irradiated cells secreted IL-1α from 5 hr after UVB-irradiation.
- IL-1α significantly stimulated ROS generation.
- UVB irradiation induced rapid activation of calpain.
- A calpain inhibitor reduced ROS generation by UVB-irradiation.

*These findings strongly indicate the involvement of the calpain-IL-1α cascade on the late phase generation of ROS in UVB-irradiated HaCaT keratinocytes.*
Investigations

Applied research:
To identify a natural ingredient that could prevent photoaging according to the mechanism found in the basic research
Screening on calpain inhibition

Calpain
With Ca\(^{2+}\)

FITC (fluorescein isothiocyanate) - casein (substrate)

plant extract

37 °C
30 min

fluorescence (Ex=495 nm, Em=520 nm)

<table>
<thead>
<tr>
<th>Botanical name</th>
<th>IC(_{50}) value ((\mu g/mL))</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>UJI TEA EXTRACT</strong></td>
<td>13.80</td>
</tr>
<tr>
<td>Ocimum sanctum</td>
<td>&gt;100</td>
</tr>
<tr>
<td>Grifola frondosa</td>
<td>&gt;100</td>
</tr>
<tr>
<td>Acanthopanax senticosus</td>
<td>61.92</td>
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Summary 2

1. **This study identified a novel cascade involving calpain-IL-1α on the UVB-induced generation of ROS in keratinocytes.**

2. **To block that cascade, we screened for a natural inhibitor of calpain activity.**

3. **As an effective candidate of inhibitor, we identified Uji Tea extract. We expect Uji Tea extract to suppress the cell damage induced by UVB irradiation through the calpain-IL-1α cascade.**
Ca^{2+}-triggering cascade in ROS production

UJI TEA EXTRACT (B) BG

Calpain

Ca^{2+} -> ROS

IL-1a

Pro-IL-1a

Cell damage

Inflammation

Pigmentation

Wrinkling
In vivo test 1: reduction of UV induced inflammation

Test sample: UJI TEA EXTRACT(B)-BG
Dosage: Placebo, 1%, 2% and 3%
Panel: 8 human subjects

Method: sites on the forearms of the panelists were treated with the test formulation (cream) and then irradiated with 1.5 MED (UVB+UVA) using Multiport Solar UV Simulator Model 601 (made by Solar Light). Test creams were applied immediately after application again, and the erythemas were evaluated 24 hours after irradiation.
# Test cream formulation

## Ingredients

<table>
<thead>
<tr>
<th>Formula</th>
<th>Placebo</th>
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<th>2%</th>
<th>3%</th>
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<tr>
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<td>wt%</td>
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<tr>
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<td>Phenoxyethanol</td>
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<td></td>
<td>Water</td>
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<tr>
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<td>Water</td>
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<td>D</td>
<td>UJI TEA EXTRACT(B)-BG</td>
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<tr>
<td>E</td>
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<tr>
<td><strong>Total</strong></td>
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</table>
In vivo test results: reduction of UV induced inflammation

Erythema condition 24 hours after UV irradiation

Placebo  1%  2%  3%

UJI TEA EXTRACT(B)-BG
In vivo test results: reduction of UV induced inflammation

Redness of treated sites 24 hours after UV irradiation

- Placebo
- 1%
- 2%
- 3%

UJI TEA EXTRACT(B)-BG
In vivo test: use of Uji Tea Extract (B) BG helps to increase MED in humans

Uji Tea Extract (B) BG shows significant effect of suppression of UV induced erythema in vivo. In a sunscreen formulation, Uji Tea Extract (B) BG at 1% will provide SPF boosting effect of approximately 21% and at 3% use level the SPF will increase by 87%.

Suppression of UV-induced erythema

<table>
<thead>
<tr>
<th>SPF</th>
<th>Placebo</th>
<th>UJI (1%)</th>
<th>UJI (2%)</th>
<th>UJI (3%)</th>
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<tr>
<td>10</td>
<td>100</td>
<td>121</td>
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Note: the SPF boosting effect is calculated on basis of UV-induced erythema suppression test results. The actual SPF increase may vary depending on the components of the actual formulation.
Conclusion: effects of Uji Tea Extract (B)-BG

1. Inhibition of calpain expression.

2. Suppression of the cell damage and reduction of erythema induced by UVB irradiation through the calpain-IL-1a cascade.
**Definition of Uji Tea**

- *Tea leaves are specifically cultivated in 4 prefectures, Kyoto, Nara, Shiga, and Mie prefectures*

- *Processed in Kyoto prefecture* by hand in a traditional way

*Uji city belongs to Kyoto pref.*

Ref: http://kyocha.or.jp/
**Botanical Information**

Name: *Camellia sinensis*
Family name: *Theaceae*
Part used: Leaf

Tea classification: Green tea (meets UJI TEA definition)

**Product Information**

Product name: UJI TEA EXTRACT (B)-BG

<table>
<thead>
<tr>
<th>INCI NAME</th>
<th>Composition</th>
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<tbody>
<tr>
<td>CAMELLIA SINENSIS LEAF EXTRACT BUTYLENE GLYCOL</td>
<td>0.2%</td>
</tr>
<tr>
<td>WATER</td>
<td>49.9%</td>
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</table>

- Japanese QD status: Listed as an additive
- Chinese INCI List 2007: 茶 (CAMELLIA SINENSIS) 叶提取物
- IECSC: Listed
- Origin of 1,3-butylene glycol: Plant derived
- ECOCERT: Certified