

# *YUZU SEED EXTRACT*

## Ingredients for Enhancement of Beauty and Relaxation

- **YUZU SEED EXTRACT – P**  
(Powder, Food Grade)
- **YUZU SEED EXTRACT – WSP**  
(Water Soluble Powder, Food Grade)
- **YUZU SEED EXTRACT – PC**  
(Powder, Cosmetic Grade)
- **YUZU SEED EXTRACT – LC**  
(Liquid, Cosmetic Grade)

# YUZU SEED EXTRACT

For Enhancement of  
Beauty and Relaxation

## 1. Introduction

Yuzu (*Citrus junos*), or Japanese grapefruit, is originated in Sichuan and Yunnan, located in the upstream region of the Yangtze River in China. It was brought to Japan during the Heian period (about a thousand years ago).

Yuzu fruits become bright yellow, as they get ripe in late November. It possesses acidic taste and citrus aroma. Its juice, peel and seeds have been utilized in various life scenes since ancient times. For example, a small slice of Yuzu is commonly used in sophisticated Japanese dishes as fine flavoring agents, characterized by its rich ingredients of limonene and pinene. Yuzu has also been used to make flavorful cakes and jam called *Yuzuneri*. Yuzu-in-the-bath is helpful in relaxation, revitalization, and relief of cold symptoms with its outstanding ingredients.

The intake of pulverized and charred seeds of Yuzu is beneficial for alleviating symptoms of body ache is a statement described in the herbal list of a pharmaceutical bibliography, edited during the Edo period (about three hundred years ago). Following this statement, Yuzu is used in the folk remedies for treatment of rheumatism, stiffness in the shoulders. The juice is beneficial for relaxation, pain relief, and cosmetics with its rich content of organic acids, such as citric acid, tartaric acid and ascorbic acid (vitamin C). Furthermore, the limonoid content of Yuzu are known to enhance blood circulation. Pectin, existing in the surface of seeds, is expected to moderate blood sugar and cholesterol levels.

Oryza Oil & Fat Chemical Co., Ltd. conducted various studies on the diverse pharmacological functions of Yuzu. It was confirmed that Yuzu Seed Extract is an excellent skin whitening and smoothing agent. Its revitalizing functionality in psychosomatic health is widely applicable to food and nutraceutical industries. The solution is here for beauty enhancement and as relaxation booster.



Yuzu Fruits

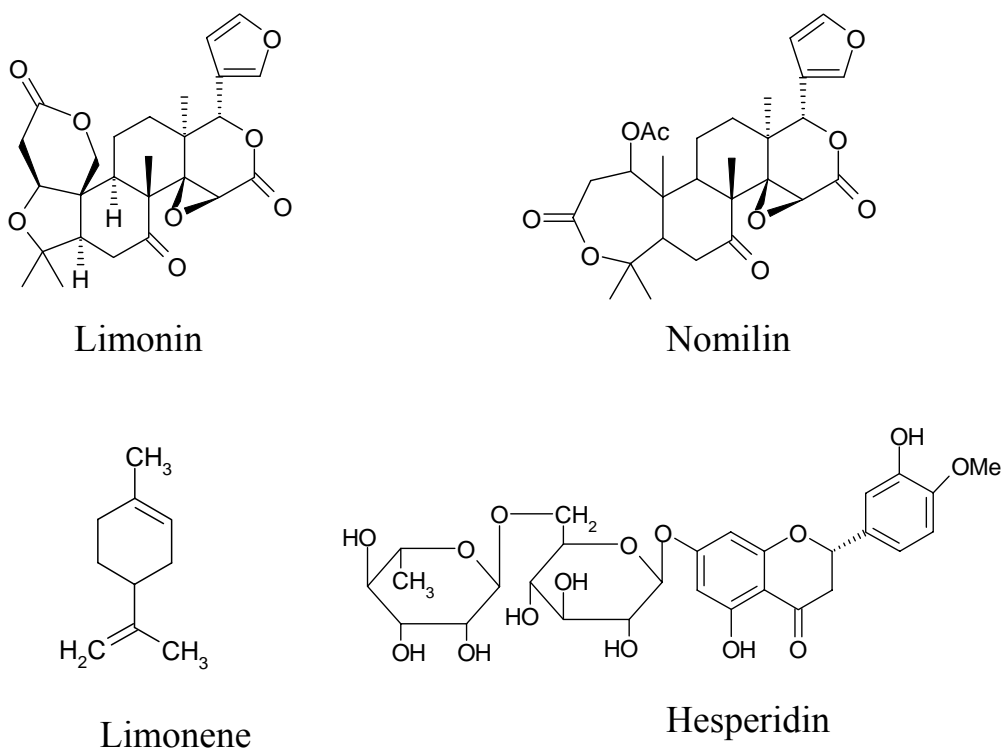


Yuzu Seeds

## 2. Functional Ingredients of YUZU SEED EXTRACT

### (1) Recent Studies

The Designer Food Project in the United States listed citrus to be beneficial for cancer prevention. Epidemiological studies from the National Cancer Institute of United States showed an inverse relationship between the intake of citrus and the prevalence of cancer. Identified as effective cancer preventive agents are *d-limonene*, *limonoids* and *hesperidin*<sup>1)</sup>, all of which are in Yuzu.<sup>2)</sup> Limonoid is a general term for triterpene derivatives found only in the *Rutaceae* and *Meliaceae* species. Thirty-six types of aglycones and glucose glycosides have been identified and isolated so far.<sup>3)</sup> A limonoid *limonin* is a source of bitterness, and is found 1,800-fold richer in seeds than in juice (our original data). Limonoid glycosides are not bitter, and are found mainly in juice. *In vivo* studies using mice and hamsters showed that limonoids *nomilin* and *obacunon* activate glutathione-S-transferase, an enzyme that suppresses tumor formation. In addition, limonin and obacunone were reported to suppress colon cancer in rats.<sup>4)</sup>



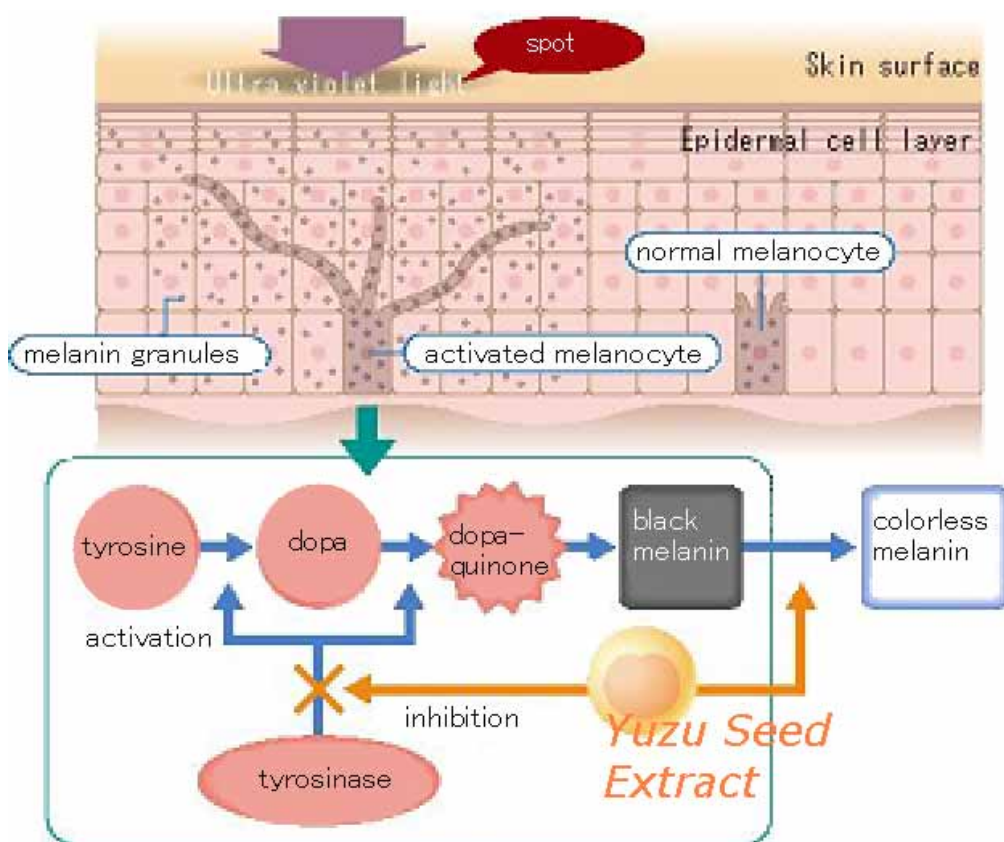
**Figure 1. Chemical Structures of the Ingredients in Yuzu Seeds**

- 1) Yano M. Cancer prevention by citrus. *Nippon Shokuhin Kagaku Kogaku Kaishi*, **49**, 139-144 (2002).
- 2) Hashinaga F., Herman Z, Hasegawa S. Limonoids in Seeds of Yuzu , *Nippon Shokuhin Kogyo Gakkaishi*, **37**, 380-382 (1990).
- 3) Hasegawa S, Ifuku Y. Biochemistry of citrus limonoids, *Nippon Shokuhin Kogyo Gakkaishi*, **41**, 372-380 (1990).
- 4) Tanaka T., Maeda M., Kohno H., Murakami M., Kagami S., Miyake M., Wada K. Inhibition of azoxymethane-induced colon carcinogenesis in male F344 rats by the citrus limonoids obacunone and limonin. *Carcinogenesis*, **22**, 193-198 (2000).

**(2) Mechanism of Melanin Production and Pigmentation**

What we call spots is melanin pigmentation on skin. The melanin pigmentation is caused by aging, changes in hormone balance, inflammation and exposure to ultraviolet (UV) light. Upon exposure to UV light, a signal transduction takes place from epidermis to melanocytes mediated by endothelin and phospholipase. Melanocytes are melanin-producing cells in skin, and work for defence against harmful UV light. In melanocytes, an enzyme called tyrosinase produces melanin as they receive the signal. Tyrosinase converts tyrosine, an amino acid, to dopa, then dopa quinone. Dopa quinone is further oxidized to melanin. In addition, a protein called “stem cell factor” (SCF) has been found to play an important role in skin hyperpigmentation. UV light activates SCF and endothelin to stimulate melanocytes to enhance melanin production.

These reactions are natural defense mechanisms to protect skin from harmful UV radiation. If skin is in a good health, old cells go away with melanin, and are replaced by new melanin-less skin cells in a 28-day cycle skin turn over. However, as metabolic rates decline with ageing, the turn over does not occur regularly, resulting in accumulation of melanin pigments, that is, hyperpigmentation.

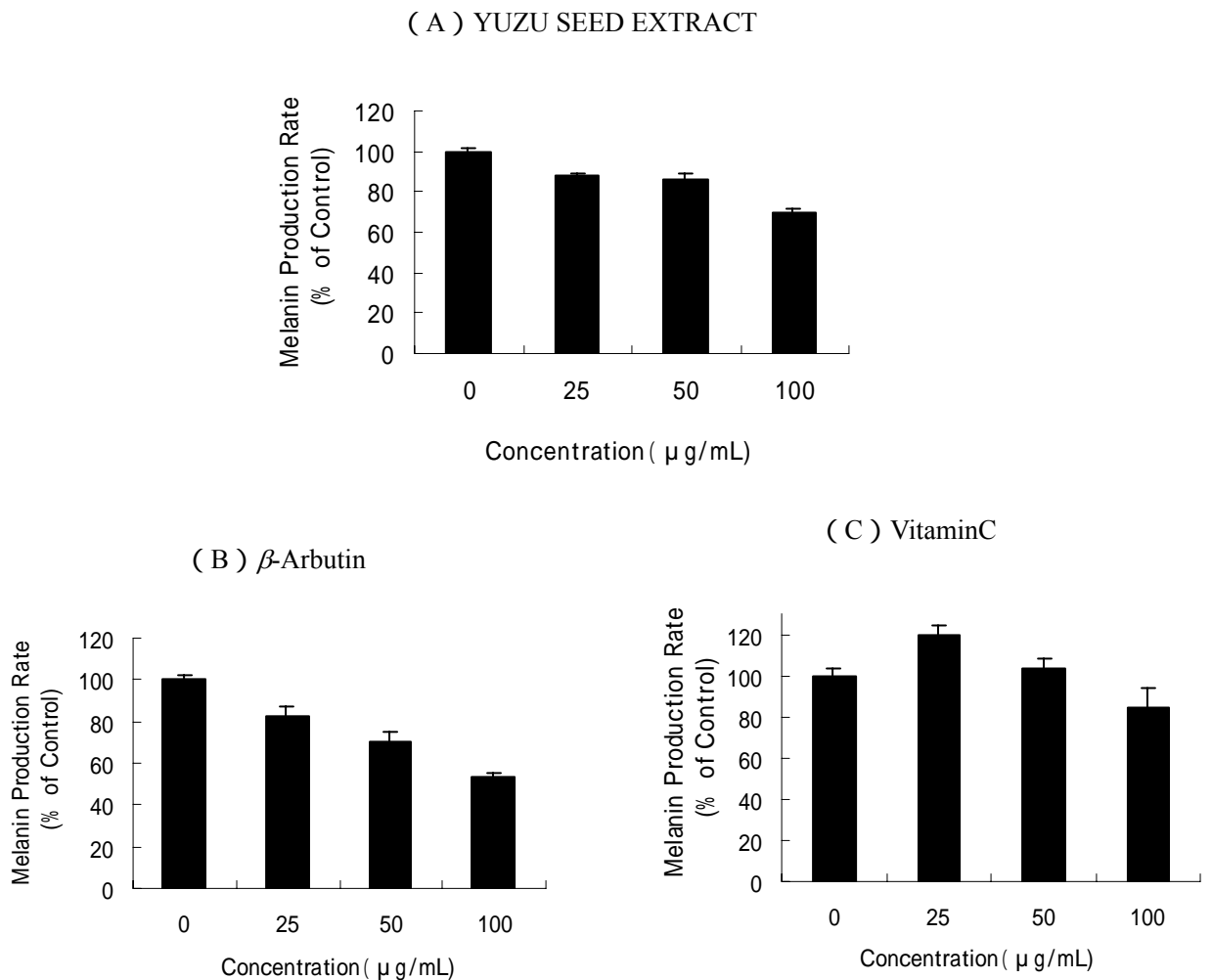


**Figure 2. Mechanism of Melanin Production**

### 3. Physiological Functions of YUZU SEED EXTRACT

#### (1) Skin Whitening (*In Vitro*)

B16 melanoma cells were used to examine the skin whitening effects of YUZU SEED EXTRACT,  $\beta$ -arbutin and vitamin C (ascorbic acid). YUZU SEED EXTRACT was found to suppress the melanin formation in a dose-dependent manner, as illustrated in Figure 3A. YUZU SEED EXTRACT showed comparative or superior inhibitory effects on melanin formation, compared to  $\beta$ -arbutin (Figure 3B) and vitamin C (Figure 3C), respectively.



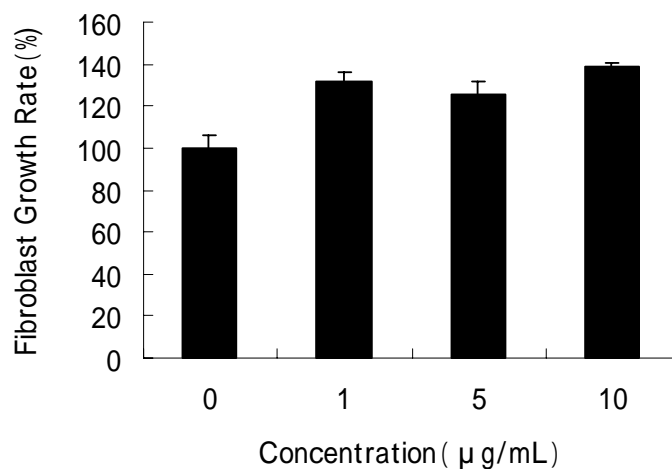
**Figure 3. Effects of Plant-Origin Ingredients on Melanin Formation in B16 Melanoma Cells ( mean  $\pm$  S.E., n=4 ).**

(Experimental Method)

B16 melanoma cells ( $2.5 \times 10^4$  cells/well) were plated in a 24-well plate in MEM with 10% fetal bovine serum, 100 units/ml of penicillin, 100  $\mu$ g/ml of streptomycin and 2 mM theophylline. Solution (55 $\mu$ l) of YUZU SEED EXTRACT,  $\beta$ -arbutin and vitamin C was added to each well, followed by 3-day incubation. The culture was crushed by ultrasonication in 300  $\mu$ l of PBS. The cell crush was transferred to in a 96-well assay plate and absorbance was measured at the wavelength of 415 nm using a microplate reader.

## (2) Fibroblast Growth (*In Vitro*)

YUZU SEED EXTRACT is shown to stimulate fibroblast growth as illustrated in Figure 4, from the examination using human neonatal skin fibroblast, NB1RGB. YUZU SEED EXTRACT enhances growth of new skin cells, thus promotes turn over of skin.



**Figure 4. Effects of YUZU SEED EXTRACT on Growth of NBIRGB Fibroblast (mean  $\pm$  S.E.: n=6 )**

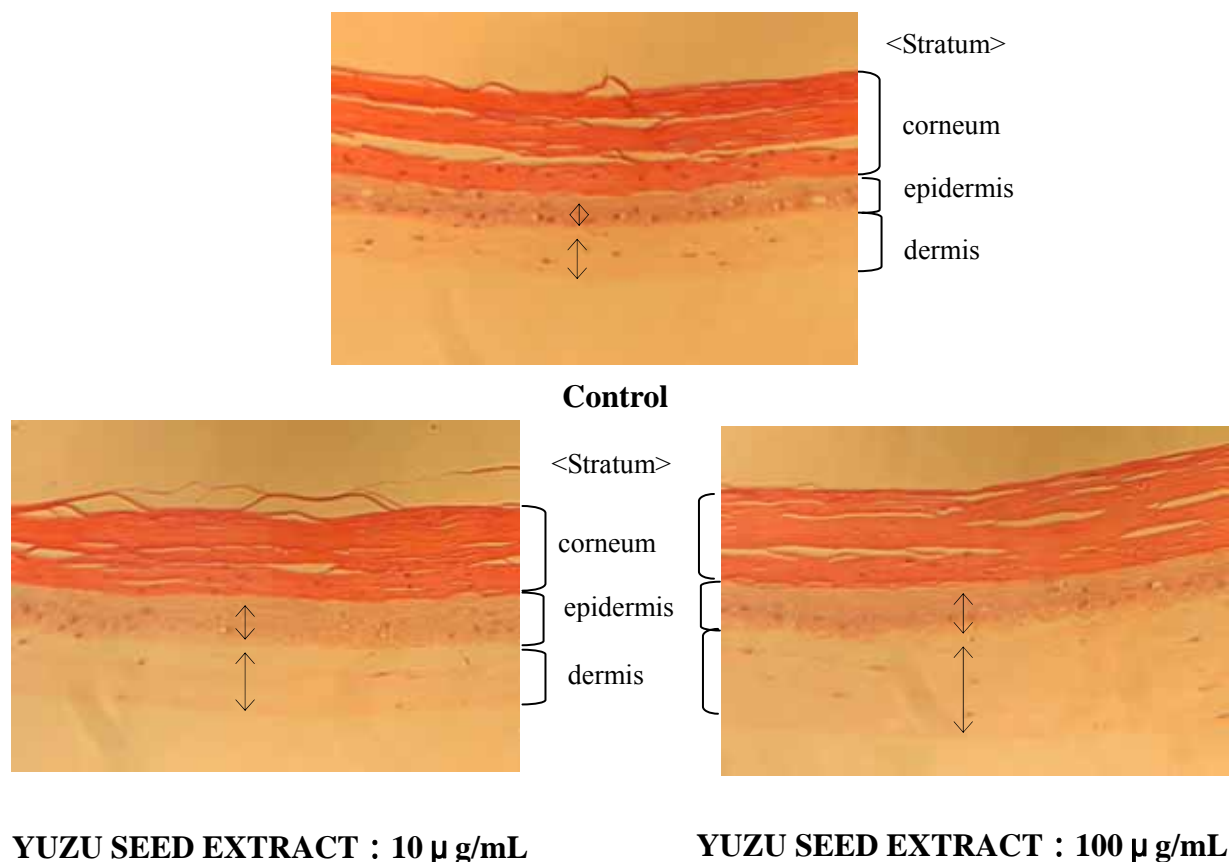
(Experimental Method)

NB1RGB cells were plated ( $2 \times 10^4$  cells/well) in a 96-well plate in  $\alpha$ -MEM with 10% fetal bovine serum, 100 units/ml of penicillin and 100 $\mu$ g/ml of streptomycin. YUZU SEED EXTRACT solution (10 $\mu$ l) was added to each well, followed by 2-day incubation. Cell growth was determined by an MTT assay.

### (3) Skin Turn Over

A reconstructed artificial human skin cell model was used to examine the effect of YUZU SEED EXTRACT on skin turn over. The microscopic illustrations of skin dermal layer are shown in Figure 5.

A pronounced thickening effect on skin dermal layers (epidermis and dermis) was observed in the models treated with YUZU SEED EXTRACT in a dose-dependent manner. It was hypothesized that YUZU SEED EXTRACT promoted collagen production and fibroblast growth, resulting in thickening of the skin dermal layer.



**Figure 5. Microscopic Illustration of Reconstructed Skin Cell**

(Experimental Method)

Reconstructed Human Skin model (TESTSKIN™) was purchased from Toyobo Co., Ltd. DMSO solution of YUZU SEED EXTRACT was injected into the dermis layer of the skin model. The culture medium was replaced every 3 days while incubation continued for 6 days. The tissue specimen were stained, and then fixed in 10% formaldehyde, followed by preparation of cross-sections.

#### (4) Skin Smoothing

The effect of YUZU SEED EXTRACT on skin texture was examined using oral preparation on 8 healthy female subjects:

Number of subjects: 8

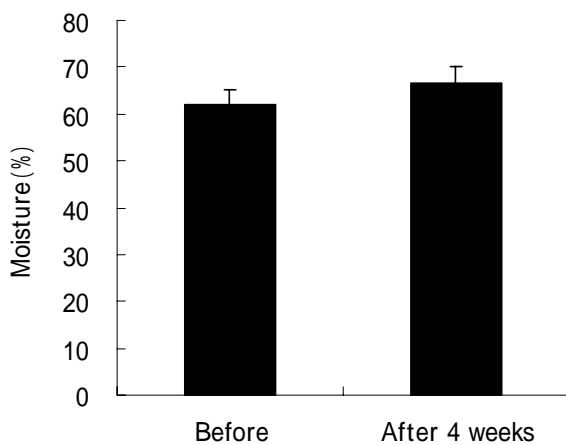
Age: 23 – 41

Dosage: 200 mg/day of YUZU SEED EXTRACT-P

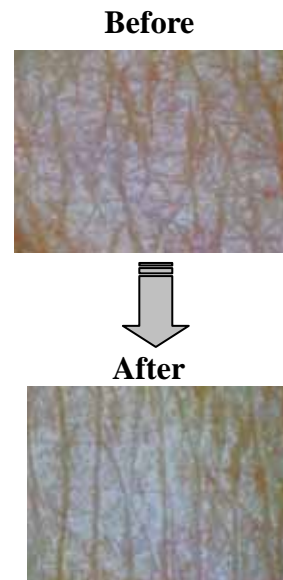
Duration: 4 weeks

Parameter: Moisture of skin around region below the left eye using a moisture meter.

As illustrated in Figure 6, the skin moisture content increased from 62% to 66% with oral preparation of YUZU SEED EXTRACT. A survey was conducted on test subjects on skin texture to assess the subjective comments after the test. Survey revealed that YUZU SEED EXTRACT improved overall skin texture, as shown in Table 1. It was confirmed that skin suppleness was greatly improved with oral YUZU SEED EXTRACT at the tested dosage. Improvement on skin texture was observed and confirmed by microscopic images, as illustrated in Figure 7. YUZU SEED EXTRACT possesses excellent skin revitalizing and rejuvenating effects.



**Figure 6 . Changes in skin moisture with Oral Preparation of YUZU SEED EXTRACT**



Subject : Female aged 24

**Figure 7 .Microscopic Illustration of Skin Texture after Oral Treatment of YUZU SEED EXTRACT**



**Table 1. Survey (Subjective) on Skin Texture after Oral Treatment of YUZU SEED EXTRACT after 4 Weeks**

Item	Rate of Improvement		
Cosmetic rash	Improved: 3	No change: 5	Worsened: 0
Dryness of the face	Improved: 4	No change: 4	Worsened: 0
Flushing of the face	Improved: 2	No change: 6	Worsened: 0
Fitness in makeup	Improved: 4	No change: 4	Worsened: 0
Smoothness of the skin	Improved: 3	No change: 5	Worsened: 0
Feeling of wetness	Improved: 4	No change: 4	Worsened: 0
Skin elasticity	Improved: 2	No change: 6	Worsened: 0
Degree of dryness	Improved: 6	No change: 2	Worsened: 0
Itching	Improved: 2	No change: 6	Worsened: 0
Skin roughness	Improved: 4	No change: 4	Worsened: 0
Improvement in crease	Improved: 2	No change: 6	Worsened: 0
Dullness of the skin	Improved: 2	No change: 6	Worsened: 0

**(5) Relaxation**

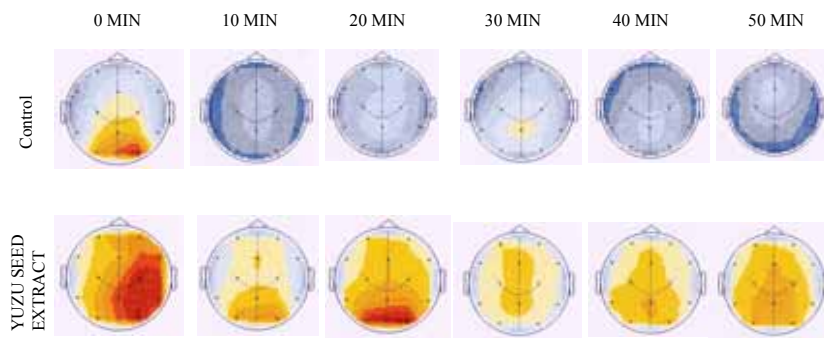
**A.  $\alpha$ -wave Propagation**

Yuzu traditionally has been used for relaxation. Relaxation effects of YUZU SEED EXTRACT were assessed by observation on brain  $\alpha$ -wave (a relaxation index) of subjects using topography.

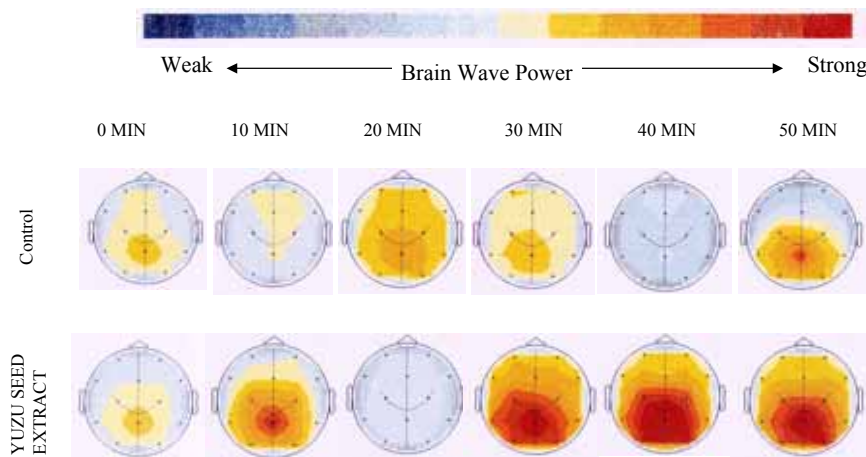
Subjects were acclimatized in room temperature of 27 °C, relative humidity of 55% for 1 hour. Two hundred mg of YUZU SEED EXTRACT wrapped in oblate was given with 100 ml of water. Only water was given for control. After 20 minutes, stress (mental arithmetic) was loaded for 5 minutes and resting brain waves were then measured for 50 minutes.

In subject T.S., strong  $\alpha$ -wave was observed within 50 minutes after stress loading when YUZU SEED EXTRACT was given. Similarly, marked  $\alpha$ -wave was observed in subject M.A. from 30 to 50 minutes after stress loading when YUZU SEED EXTRACT was given. On the other hand, no  $\alpha$ -wave was detected in the control test.

Appearance of  $\alpha$ -wave strongly suggests that YUZU SEED EXTRACT possesses anti-stress and relaxation effects.



Subject : T.S.

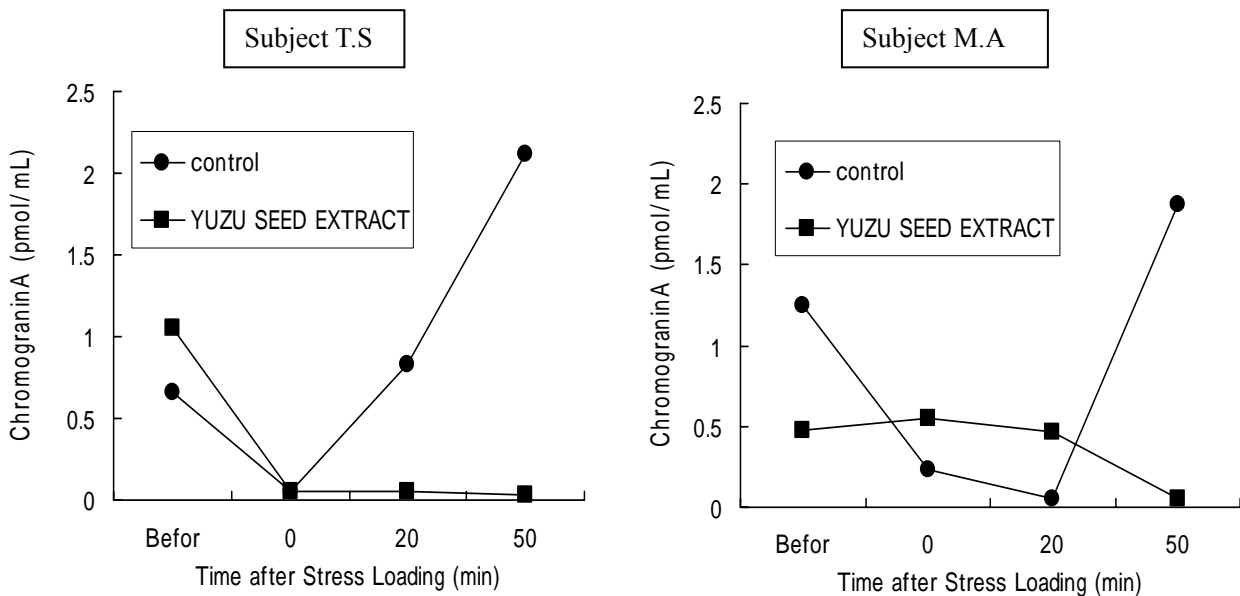


Subject : M.A.

**Figure 8. Brain  $\alpha$ -Wave Topography**

## B. Suppression of Chromogranin A Secretion in Saliva

ChromograninA (CgA) is known to increase with stress loading, so its secretion is authorized as an index which represents mental stress. In this examination, stress (mental arithmetic) was loaded to subjects for 5 minutes, and then CgA in saliva was measured. The time-course was illustrated in Figure 9. In subject T.S., increasing amount of CgA was observed 0 to 50 minutes after stress loading. In subject M.A., sheering amount of CgA was observed 20 to 50 minutes after stress loading. On the other hand, when YUZU SEED EXTRACT was given to subjects, no increase in the amount of CgA was observed, making drastic contrast with and without YUZU SEED EXTRACT. This examination demonstrated that YUZU SEED EXTRACT has an anti -stress functionality.



**Figure 9. Suppression of ChromograninA Secretion in Saliva**

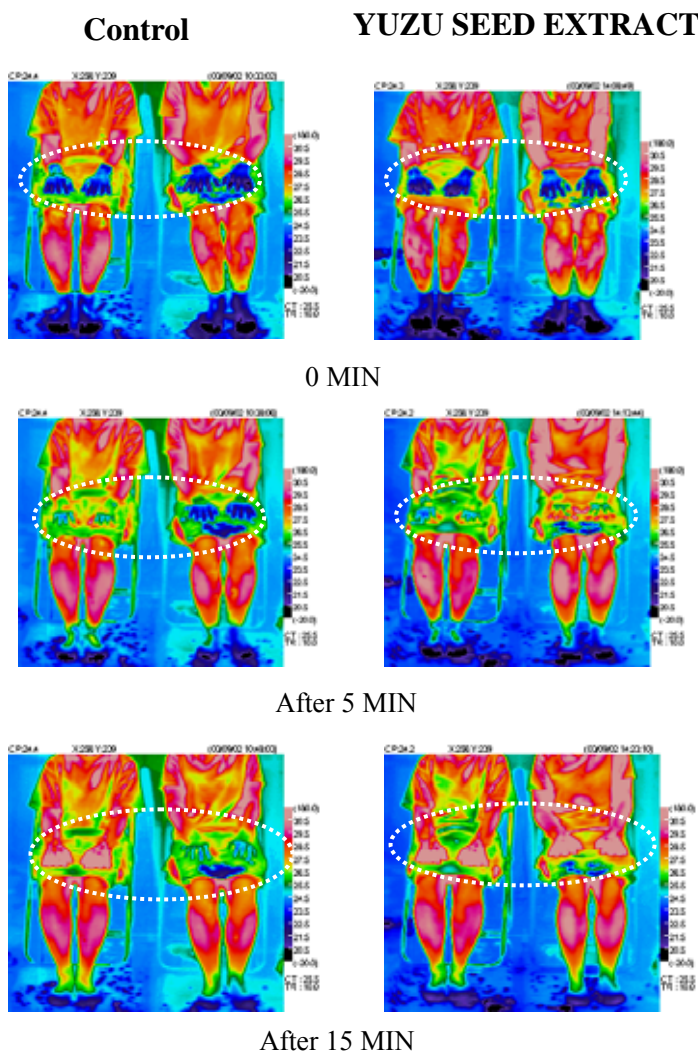
**(6) Improvement of Blood Circulation**

The effect of YUZU SEED EXTRACT on blood circulation was examined in a single-dose oral administration clinical trial using thermograph.

Subjects were acclimatized to room temperature of 25°C, relative humidity of 50%. Subjects were given 100 ml of water, 2 to 3 hours after breakfast. The upper and lower limbs of subjects were immersed into cold water of 14°C for 1 minute. Changes in skin temperature were determined using thermographic illustration for 30 minutes immediately after immersion.

Two hours after lunch, 200 mg of YUZU SEED EXTRACT wrapped in oblate was given to subjects with 100 ml of water and their limbs were immersed into cold water of 14°C for 1 minute. Changes in skin temperature were determined using thermographic illustration for 30 minutes immediately after immersion.

The thermographs clearly showed the improvement of blood circulation of subjects, as illustrated in Figure 10. Temperature of limbs recovered faster when YUZU SEED EXTRACT was given.

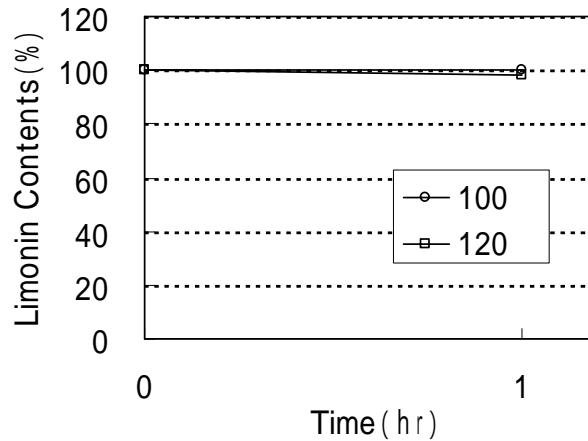


**Figure 10 . Thermographs Taken after Low-Temperature Loading**

## 4. Stability

### (1) Thermostability

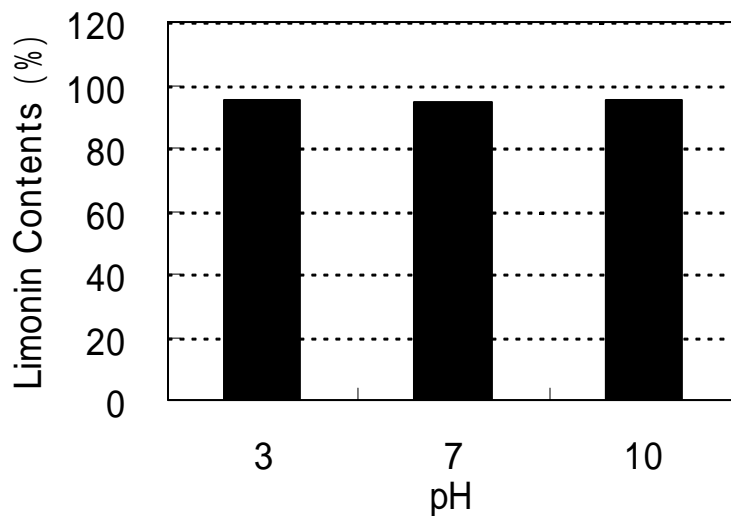
The thermostability of YUZU SEED EXTRACT was examined. The limonin content of YUZU SEED EXTRACT remain stable after heating at either 100 and 120 for 1 hour. YUZU SEED EXTRACT is highly stable at food processing temperatures.



**Figure11 . Thermal Resistance of YUZU SEED EXTRACT ( 100% of The Initial Value )**

### (2) pH Stability

The effect of pH on aqueous solutions of YUZU SEED EXTRACT was examined at room temperature for 1 week. The content of limonin remained totally. YUZU SEED EXTRACT is highly stable at pH of 3 - 10.



**Figure12 . pH Stability ( 100% of The Initial Value )**

### (3) Stability of Liquid Formulation

Water-soluble YUZU SEED EXTRACT (product name: YUZU SEED EXTRACT-WSP), was prepared in 0.7% aqueous solution (pH 3.5), and stored either at room temperature, 40°C or 5°C for 4 weeks under light protection. Neither sedimentation nor colour changes were observed visually. YUZU SEED EXTRACT -WSP is highly stable in acidic conditions.

## 5. Nutritional Information (in non-excipient form)

	Values in 100 g	Method
Water	1.3 g	heat drying method under ordinal pressure
Protein <sup>*1</sup>	9.4 g	kieldahl method
Fat	4.1 g	acid fat dissolution method
Ash	5.7 g	direct ashing method
Carbohydrate <sup>*2</sup>	79.5 g	
Energy <sup>*3</sup>	393 kcal	
Dietary Fiber	0.3 g	prosky method
Sodium	13 mg	atomic absorption spectrophotometry

\*1 ) N=6.25

\*2 ) 100 - (moisture + protein + fat + ash)

\*3 ) Factors for calculating the energy value : protein, 4; fat, 9; carbohydrate, 4; dietary fiber, 2  
Test trustee : SRL, Inc.

Date of issue of the test result report : September 2, 2004

Research result issue number : No. 200408200016

## 6. Safety Profile (in non-excipient form)

### (1) Residual Agricultural Chemicals

Assayed Items	Results	Detection Limits	Assay Method
Ditianon	Not Detected	0.5 ppm	gas chromatography

Test trustee : Kyusai analysis institute Co., LTD.

Date of issue of the test result report : April 17, 2004

Research result issue number : No. 20040902-32

The Yuzu seeds are in compliance with the standards stipulated in the Food Sanitation Law by The Ministry of Health, Labour & Welfare. The 213 agricultural chemicals were measured and they were all below the criteria.

Assay Method : Determined by GC-ECD/NPD, confirmed by GC-MS.

Test Trustee: R J Hill Laboratories Ltd., New Zealand.

Date of analysis: December 30, 2004

Reference No.: 363372

The YUZU SEED EXTRACT (with no diluents added) was again examined for 525 residual agricultural chemical compounds following the provisions of the Food Hygiene Law and pesticide legislation. As a result, contents of all compounds were confirmed to be below the standard values (measurable limits).

Test trustee: Food Safety Evaluation and Analysis Center, Masis Co., Ltd.

Date of test report issued: April 28, 2010

Report No. 38068

## **(2) Acute Eye Irritation Test**

Eye irritation test was performed on YUZU SEED EXTRACT-PC by using an alternative *in vitro* method of bovine corneal opacity and permeability assay (BCOP). The result showed that YUZU SEED EXTRACT-PC might be considered a mild eye irritant. Therefore, the safety of YUZU SEED EXTRACT-PC was confirmed.

## **(3) Repeated Insult Patch Test (RIPT)**

Repeated insult patch test using YUZU SEED EXTRACT-PC was conducted among 30 panelists of either sex, without visible skin diseases or known hypersensitivity.

The test substance was applied to the skin of the panelist via an occlusive patch at a suitable concentration. The patch limited contact of the panelist's skin with the test substance to a local area and exposure was exaggerated due to the occlusive conditions. The skin was checked 3 times within 7 weeks each time 24, 48 and 72 hours after patch application.

As a result, YUZU SEED EXTRACT-PC was considered the rating of very good. This product did not lead to toxic-irritative intolerance reactions in repeated patch testing carried out in accordance with international guidelines.

## **(4) Mutagenicity Test**

Ames test was performed with/without S9mix using *Salmonella* strains of TA1535, TA1537, TA98, TA100 and *E. coli* strain WP2uvrA. YUZU SEED EXTRACT-PC showed no mutagenicity at concentrations from 33 to 5000 µg/plate.

## **7. Recommended Daily Dosage**

Recommend daily dosage of YUZU SEED EXTRACT is 200 – 300 mg. The Ministry of Health, Labour and Welfare approved the products of YUZU SEED EXTRACT as foodstuffs.

## 8. Applications

Applications	Examples
Foods	drinks (beverage, juice, <i>etc.</i> ), soft gel capsule, hard capsule, tablet, candy, chewing gum, cookies, chocolate, jelly, <i>etc.</i>
Cosmetics	base cosmetics (lotion, milk, cream, <i>etc.</i> ) body cosmetics (body lotion, body cream, <i>etc.</i> ) cleansing cosmetics (soap, <i>etc.</i> ) makeup cosmetics (lipstick, foundation, <i>etc.</i> ) <i>etc.</i>

YUZU SEED EXTRACT-WSP is highly soluble in water, so it is suitable for wide range of water-soluble preparation in food and cosmetic industries, for example, beverages or conditioners and lotions.

## 9. Packaging

YUZU SEED EXTRACT – P (Powder, for food)

YUZU SEED EXTRACT – WSP ( Powder, water-soluble, for food )

5kg Interior packaging : aluminum-coated plastic bag

Exterior packaging : cardboard box

YUZU SEED EXTRACT – PC ( Powder, for Cosmetic )

5kg Interior packaging : aluminum-coated plastic bag

Exterior packaging : cardboard box

YUZU SEED EXTRACT – LC ( liquid, for Cosmetic )

5kg Interior packaging : cubic polyethylene container

Exterior packaging : cardboard box

## 10. Storage

Store in cool and dry place. Avoid direct sunlight and humidity.

## 11. Expression

YUZU SEED EXTRACT-P

YUZU SEED EXTRACT -WSP

Expression: YUZU SEED EXTRACT

YUZU EXTRACT

YUZU SEED EXTRACT-PC

INCI name: Dextrin



Citrus Junos Seed Extract

YUZU SEED EXTRACT-LC

INCI name: Butylene Glycol

Water

Citrus Junos Seed Extract

PRODUCT STANDARD

PRODUCT NAME

**YUZU SEED EXTRACT-P**

(FOOD)

This product is extracted from Yuzu (*Citrus junos*, Rutaceae) seeds with aqueous ethanol. It guarantees minimum of 0.5% limonin.

1.Appearance Slightly yellow-brown powder with slight unique smell.

2.Content of Limonin Min. 0.5% (HPLC)

3.Certificaion Test

Nomilin

After methanol (HPLC grade) is added to 0.1 g of this product in a 10 ml volume flask, and the flask is treated with ultrasonic wave for 1 minutes. The solution is filtered through a 0.45 μm PTFE filter after addition of methanol (HPLC grade) to adequate volume. For preparation of standard solution, methanol (HPLC grade) is added to nomilin, and the concentration is prepared 0.2 mg/ml (standard solution). HPLC analysis is performed according to the following conditions for 5 μl of test solution and standard solution. The peak of nomilin is found in the HPLC chromatogram of test solution.

<HPLC condition>

Column : Chromatorex C18 (4.6 mm × 150 mm)

Column temperature: 40

Mobile phase : Acetonitrile : 0.1 % H<sub>3</sub>PO<sub>4</sub> = 35 : 65

Flow rate : 1.0 ml/min

Detector : UV 210 nm

4.Loss on Drying Max. 10.0 % (Analysis for Hygienic Chemists,1g,105 ,2h)

5.Purity Test

(1)Heavy Metals Max. 10 ppm (The Japanese Standards for Food Additives)

(2)Arsenic Max. 1 ppm (Standard Methods of Analysis in Food Safety Regulation)

6.Standard Plate Counts Max. 3 × 10<sup>3</sup> cfu/g (Analysis for Hygienic Chemists)

7.Moulds and Yeasts Max. 1 × 10<sup>3</sup> cfu/g (Analysis for Hygienic Chemists)

8.Coliforms Negative (Analysis for Hygienic Chemists)

9.Composition

Ingredients	Contents
Yuzu Seed Extract	40 %
Dextrin	60 %
Total	100 %

PRODUCT STANDARD

PRODUCT NAME

**YUZU SEED EXTRACT-WSP**

(FOOD)

This product is extracted from Yuzu (*Citrus junos*, Rutaceae) seeds with aqueous ethanol. This product is water-soluble.

1.Appearance                      White or slightly yellow-brown powder with slight unique smell.

2.Certificaion Test

Limonic

After methanol (HPLC grade) is added to 0.1 g of this product in a 10 ml volume flask, and the flask is treated with ultrasonic wave for 1 minutes. The solution is filtered through a 0.45 μm PTFE filter after addition of methanol (HPLC grade) to adequate volume. For preparation of standard solution, methanol (HPLC grade) is added to limonic, and the concentration is prepared 0.2 mg/ml (standard solution). HPLC analysis is performed according to the following conditions for 5 μl of test solution and standard solution. The peak of limonic is found in the HPLC chromatogram of test solution.

<HPLC condition>

Column                               : Chromatorex C18 (4.6 mm × 150 mm)  
 Column temperature           : 40  
 Mobile phase                       : Acetonitrile : 0.1 % H<sub>3</sub>PO<sub>4</sub> = 35 : 65  
 Flow rate                            : 1.0 ml/min  
 Detector                             : UV 210 nm

3.Loss on Drying                Max. 10.0 %                      (Analysis for Hygienic Chemists,1g,105 ,2h)

4.Purity Test

(1)Heavy Metals                    Max. 10 ppm                      (The Japanese Standards for Food Additives)

(2)Arsenic                            Max. 1 ppm                        (Standard Methods of Analysis in Food Safety Regulation)

5.Standard Plate Counts       Max. 3 × 10<sup>3</sup> cfu/g               (Analysis for Hygienic Chemists)

6.Moulds and Yeasts            Max. 1 × 10<sup>3</sup> cfu/g               (Analysis for Hygienic Chemists)

7.Coliforms                        Negative                            (Analysis for Hygienic Chemists)

8.Composition

Ingredients	Contents
Yuzu Seed Extrac	40 %
Dextrin	60 %
Total	100 %

PRODUCT STANDARD

PRODUCT NAME

**YUZU SEED EXTRACT-PC**

(COSMETIC)

This product is extracted from Yuzu (*Citrus junos*, Rutaceae) seeds with aqueous ethanol. It guarantees minimum of 0.5% limonin.

<u>1.Appearance</u>	Slightly yellow-brown powder with slight unique smell.									
<u>2.Content of Limonin</u>	Min. 0.5%	(HPLC)								
<u>3.Certificaion Test</u>										
Nomilin	<p>After methanol (HPLC grade) is added to 0.1 g of this product in a 10 ml volume flask, and the flask is treated with ultrasonic wave for 1 minutes. The solution is filtered through a 0.45 μm PTFE filter after addition of methanol (HPLC grade) to adequate volume. For preparation of standard solution, methanol (HPLC grade) is added to limonin, and the concentration is prepared 0.2 mg/ml (standard solution). HPLC analysis is performed according to the following conditions for 5 μl of test solution and standard solution. The peak of limonin is found in the HPLC chromatogram of test solution.</p> <p>&lt;HPLC condition&gt;</p> <p>Column : Chromatorex C18 (4.6 mm × 150 mm)</p> <p>Column temperature: 40</p> <p>Mobile phase : Acetonitrile : 0.1 % H<sub>3</sub>PO<sub>4</sub> = 35 : 65</p> <p>Flow rate : 1.0 ml/min</p> <p>Detector : UV 210 nm</p>									
<u>4.Loss on Drying</u>	Max. 10.0 %	(1g,105 °C,2h)								
<u>5.Purity Test</u>										
(1)Heavy Metals	Max. 10 ppm	(The Second Method)								
(2)Arsenic	Max.1ppm	(The Third Method, Apparatus B)								
<u>6.Standard Plate Counts</u>	Max. 3 × 10 <sup>3</sup> cfu/g	(Analysis for Hygienic Chemists)								
<u>7.Moulds and Yeasts</u>	Max. 1 × 10 <sup>3</sup> cfu/g	(Analysis for Hygienic Chemists)								
<u>8.Coliforms</u>	Negative	(Analysis for Hygienic Chemists)								
<u>9.Composition</u>	<table border="0" style="width: 100%; border-collapse: collapse;"> <thead> <tr> <th style="text-align: left; border-bottom: 1px solid black;">Ingredients</th> <th style="text-align: right; border-bottom: 1px solid black;">Contents</th> </tr> </thead> <tbody> <tr> <td>Dextrin</td> <td style="text-align: right;">60 %</td> </tr> <tr> <td>Citrus Junos Seed Extract</td> <td style="text-align: right;">40 %</td> </tr> <tr> <td style="border-top: 1px solid black;">Total</td> <td style="text-align: right; border-top: 1px solid black;">100 %</td> </tr> </tbody> </table>		Ingredients	Contents	Dextrin	60 %	Citrus Junos Seed Extract	40 %	Total	100 %
Ingredients	Contents									
Dextrin	60 %									
Citrus Junos Seed Extract	40 %									
Total	100 %									

Ref. Japanese Standards of Cosmetic Ingredients.

PRODUCT STANDARD  
PRODUCT NAME

**YUZU SEED EXTRACT-LC**

( COSMETIC )

This product is extracted from Yuzu (*Citrus junos*, Rutaceae) seed with aqueous 1,3-butylene glycol.

1.Appearance                      Slightly yellow color liquid with scentless or slight unique smell.

2.Certification Test  
Polyphenols                      Mix this product (0.5 ml) with water (2.0 ml), and Folin-Denis reagent (0.2 ml) and saturated Na<sub>2</sub>CO<sub>3</sub> solution (0.4 ml) are added. The solution reveals blue color.

3.Purity Test  
(1)Heavy Metals                      Max. 10 ppm                      (The Second Method)  
(2)Arsenic                              Max. 1 ppm                      (The Third Method, Apparatus B)

4.Standard Plate Counts Max. 1 × 10<sup>3</sup> cfu/g                      (Analysis for Hygienic Chemists)

5.Moulds and Yeasts                      Max. 1 × 10<sup>2</sup> cfu/g                      (Analysis for Hygienic Chemists)

6.Coliforms                              Negative                              (Analysis for Hygienic Chemists)

<u>7.Composition</u>	<u>Ingredients</u>	<u>Contents</u>
	Butylene Glycol	69 %
	Water	30 %
	Citrus Junos Seed Extract	1 %
	<b>Total</b>	<b>100 %</b>

Ref. Japanese Standards of Cosmetic Ingredients.

**ORYZA OIL & FAT CHEMICAL CO.,LTD.**, striving for the development of the new functional food materials to promote your health.

**From product planning to OEM** – For any additional information or assistance, please contact :

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