ORYZA OIL & FAT CHEMICAL CO., LTD.

LycoLift™

Tomato Seed Extract

Extracellular matrix production cycle enhancement

Skin wasted product removal

■**LycoLift™-P**
（Water-soluble powder、Food Grade）

■**LycoLift™-PC**
（Water-soluble powder、Cosmetic Grade）

■**LycoLift™-LC**
（Liquid, Cosmetic Grade）

ORYZA OIL & FAT CHEMICAL CO., LTD.

ver. 4.0ST

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Extracellular matrix production cycle enhancement
Skin wasted product removal

1. Introduction

Tomato（*Lycopersicon esculentum*）is commonly known as edible plant all over the world. The species originated in Andes highlands, South America (Fig. 1). Tomato fruits contains rich functional components such as vitamin C, lycopene and GABA. Because of these knowledge, tomato is used as a raw material of functional foods and juice. In terms of the function of tomato, cholesterol lowering effect and blood pressure lowering effect have been reported. Moreover, it was reported that novel saponin compound, escleoside is identified from fully ripe tomato fruits in 2004[1,2] and one of escleoside family, escleoside A has the prevention of arteriosclerosis effect in the mice[3]. However, these are knowledge about tomato fruits and there are not any functional reports about tomato seed.

Fig. 1 Tomato and its seed

Oryza Oil & Fat Chemical Co., Ltd. has found that tomato seed contains two unique saponin compounds, lycoperoside A and H as main component and developed the powder type product which standardized these components (LycoLift™). The result of functional research about LycoLift™, we found that LycoLift™ and its components have collagen and elastin production promotive effect and collagen and elastin degradation products absorption promotive effect. LycoLift™ contributes to anti-wrinkle and anti-sagging as a functional ingredients of processed food and cosmetics. Furthermore, LycoLift™ also available for the products in hope of synergistic effect with collagen and elastin.

1) Fujiwara S. et al., *Tetrahedron*, 60, 4915-4920 (2004).
2) Ono M. et al., *Chem. Pharm. Bull*., 54, 237-239 (2006).
3) Nohara T., *J. Trad. Med*., 27, 217-224 (2010).

2. Components of tomato seed

While there are many reports about components which are included in tomato fruits, research about components included in only tomato seed was not studied. Therefore, we researched components of tomato seed and **identified two main saponin compounds, lycoperoside A and H** (Fig.2). This research was co-worked with Kyoto Pharmaceutical University. It is novel knowledge that lycoperoside A and H are included in tomato seed as main components (Fig. 3). Moreover, These compounds do not have cell toxicity that observed in many kind of saponin compounds.

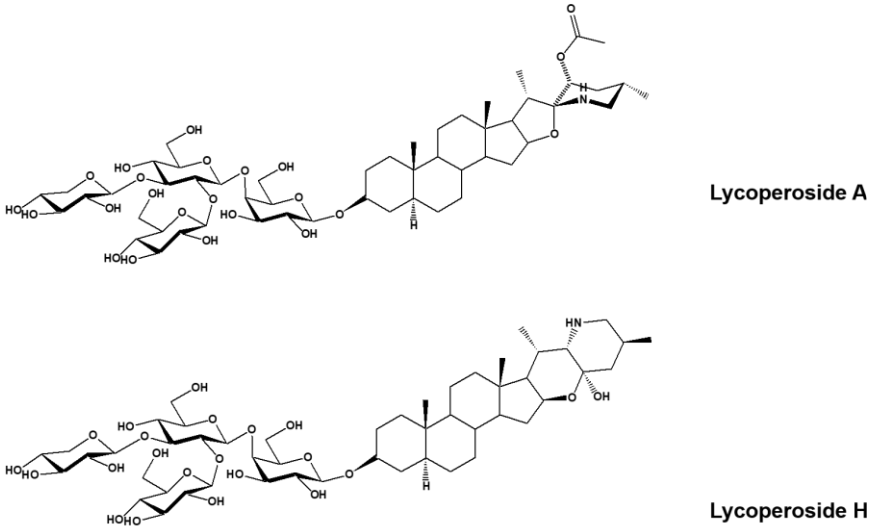

Fig. 2 Chemical structure of lycoperoside A and H

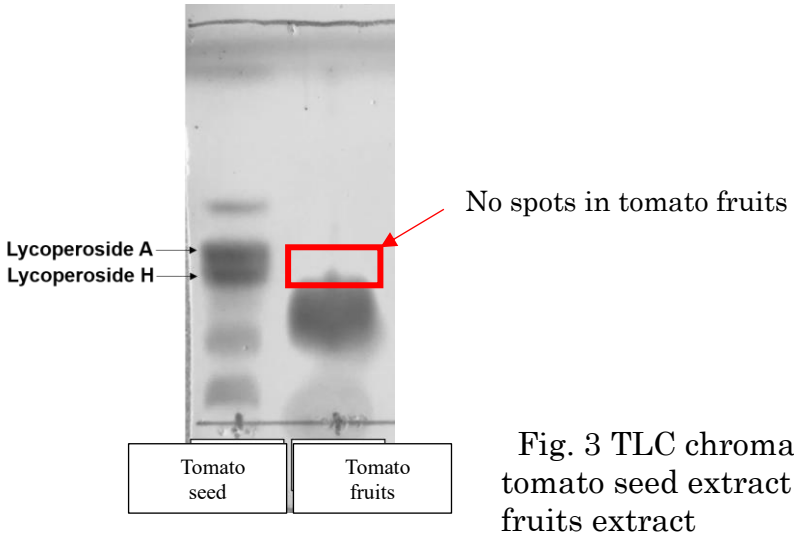

Fig. 3 TLC chromatogram of tomato seed extract and tomato fruits extract

3. Effect of Tomato Seed Extract on extracellular matrix

(1) Extracellular matrix and skin condition

Extracellular matrix (ECM) is located in dermis layer in skin and contributes to skin elasticity. ECM is consisted with collagen and elastin. Collagen and elastin are produced by fibroblast. After production, collagen and elastin are broken down and absorbed into fibroblast again (Fig. 4)[4,5]. We named this production and reduction cycle **"ECM Recycle System (ERS)"**. Skin condition is maintained freshly by ERS. Therefore, disorder of ERS causes change of cell shape and oxidative stress by accumulation of degradation products[6]. Accordingly, **it is very important maintain ERS normally to keep skin condition freshly.**

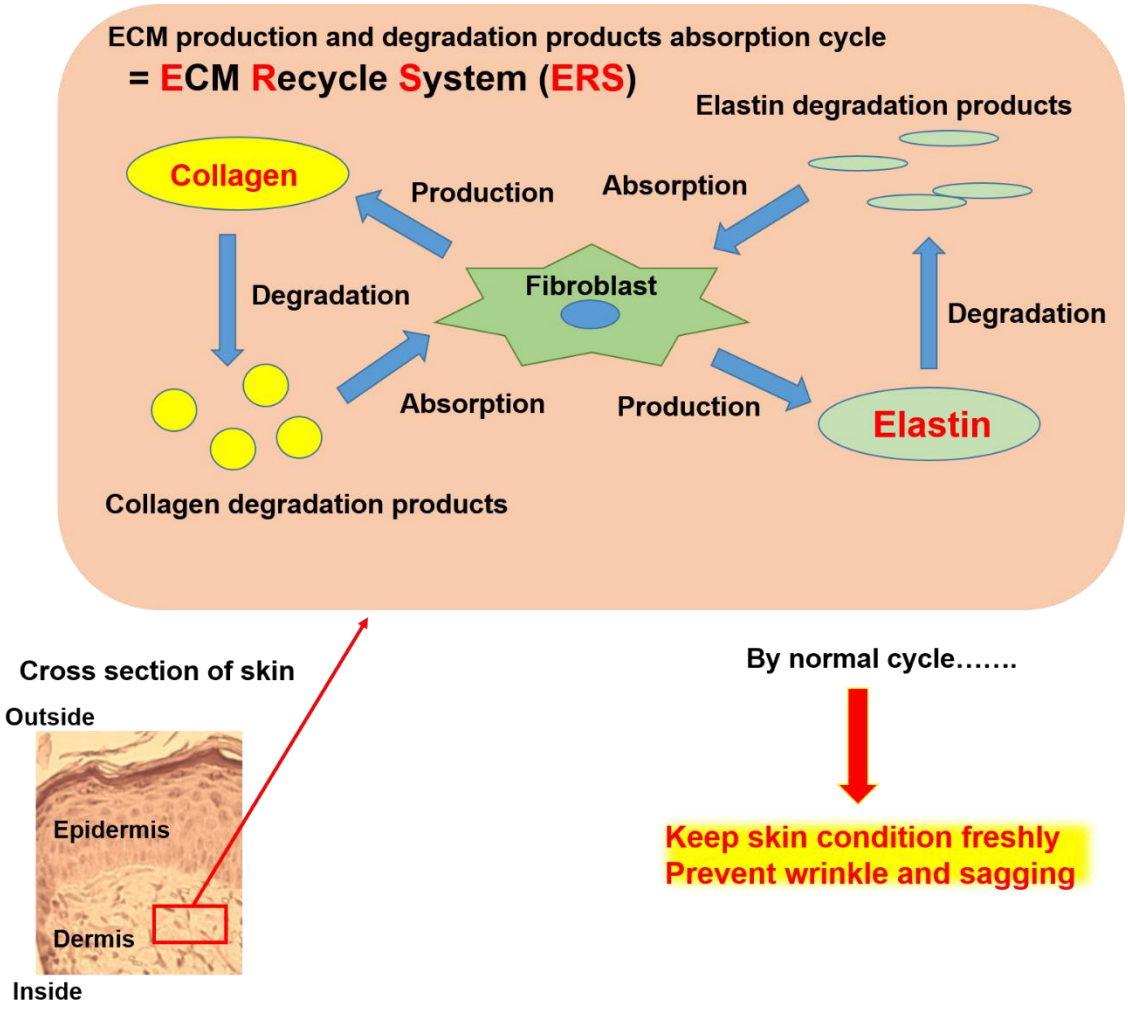

Fig. 4 ECM Recycle System (ERS) and its role in skin

4) Lars HE. Et al., *Front. Biosci*., 14, 2103-2114 (2009).
5) Amandine S. et al., *Front. Fharmacol*., 7, 1-10 (2016).
6) Fisher GJ. et al., *Am. J. Pathol.*, 174, 101-114 (2009).

(2) Effect of Tomato Seed Extract on collagen and elastin production

There are not any reports about function of lycoperoside A and H which are main components of Tomato Seed Extract. While many saponin compounds have cell toxicity, it is reported that some saponin compounds which do not have cell toxicity shows collagen production promote effect[7]. Therefore, we evaluated the effect of Tomato Seed Extract and lycoperoside on collagen production by fibroblast.

Tomato Seed Extract (1, 3, and 10 μg/mL), lycoperoside A or H (0.1, 0.3, and 1 μg/mL) were added to human skin fibroblast (TIG-103) and after cultivated 1 day, amount of collagen in cell were determined. As a result, Tomato seed Extract (3 μg/mL) and lycoperoside A (0.3 and 1 μg/mL) significantly increased amount of collagen production (Fig. 5).

From above result, we evaluated the effect on production of elastin which is main constitution factor of ECM same as collagen. Tomato Seed Extract (1, 3, and 10 μg/mL), lycoperoside A or H (1, 3, and 10 μM) were added to TIG-103 and after cultivated 1 day, amount of elastin in cell were determined. As a result, Tomato Seed Extract (10 μg/mL), lycoperoside A (3 and 10 μM) and lycoperoside H (1 μM) significantly increased amount of elastin production (Fig. 6). From these result, it is revealed that **Tomato Seed Extract and its main components, lycoperoside A increase collagen and elastin in skin fibroblast**.

7) Morikawa T. et al., *Phytochemistry,* 116, 203-212 (2015).

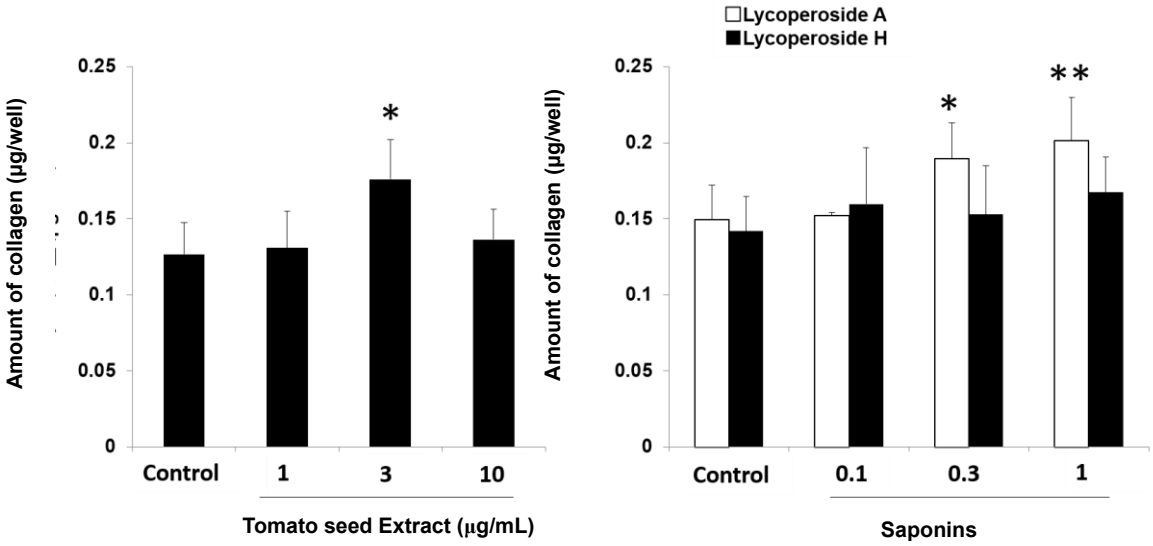

Fig. 5 Effect of Tomato Seed Extract and saponins on collagen production
n = 4, Mean±SE, *; $P < 0.05$, **$P < 0.01$ vs Control

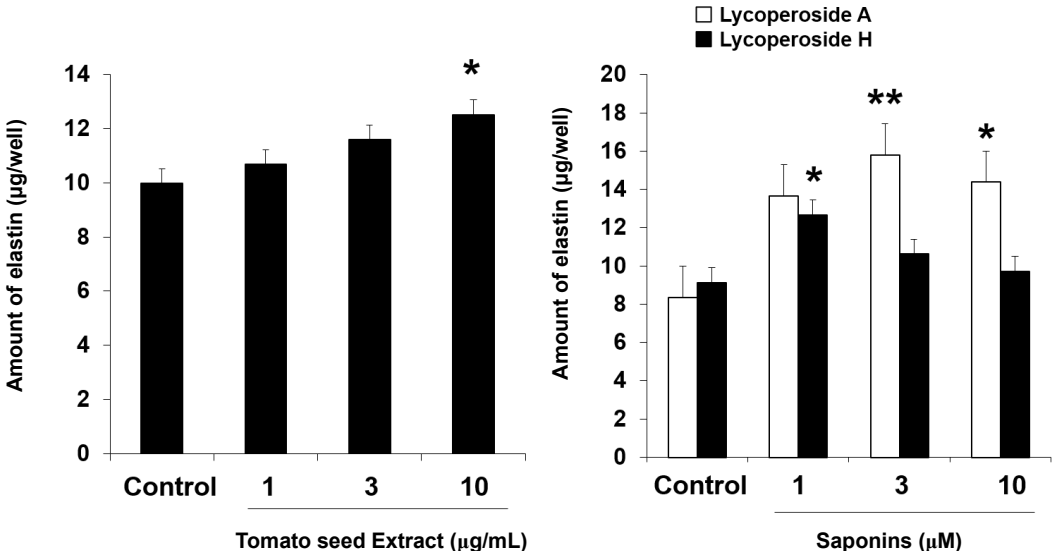

Fig. 6 Effect of Tomato Seed Extract and saponins on elastin production
n = 6, Mean±SE, *; $P < 0.05$, **$P < 0.01$ vs Control

(3) Effect on gene expression involved in collagen and elastin production/absorption cycle

As described above, ECM is maintained freshly by collagen and elastin production/absorption cycle. This cycle is regulated by several factors which involved in collagen and elastin production and its degradation products absorption. As shown in Fig. 7, collagen is produced by synthetic signal emitted from ①Smad and collagen degradation product is absorbed into fibroblast by ②Endo180. On the other hand, elastin is formulated by cohesion of ③Fibulin and ④Neuraminidase-1 is involved in absorption of elastin degradation products. Therefore, we evaluated effect of Tomato Seed Extract on gene expression of these factors.

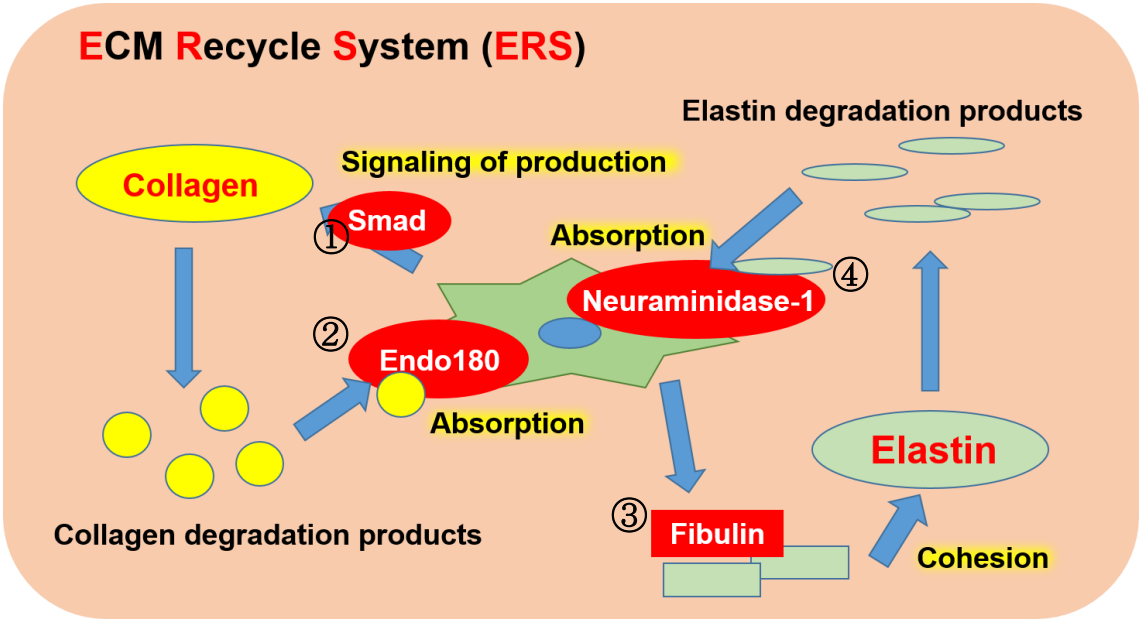

Fig. 7 Genes involved in collagen and elastin production/absorption cycle

Tomato Seed Extract (1, 3, and 10 μg/mL), lycoperoside A or H (1, 3, and 10 μM) were added to human skin fibroblast (TIG-103) and after cultivated 2 day, gene expression was determined. As a result, lycoperoside A (3 and 10 μM) significantly upregulates expression of Smad gene which involved in collagen production (Fig. 8) and Tomato Seed Extract (1 μg/mL) and lycoperoside A (3 μM) significantly upregulates expression of Endo180 gene which involved in absorption of collagen degradation products (Fig. 9).

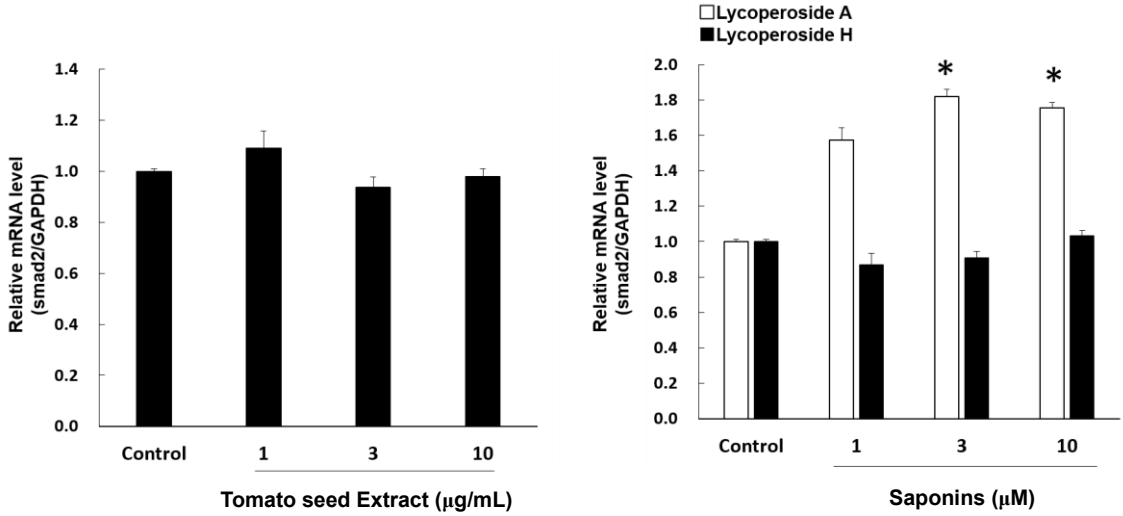

Fig. 8 Effect of Tomato Seed Extract and saponis on Smad gene expression
n = 3-6, Mean±SE, *; $P < 0.05$ vs Control

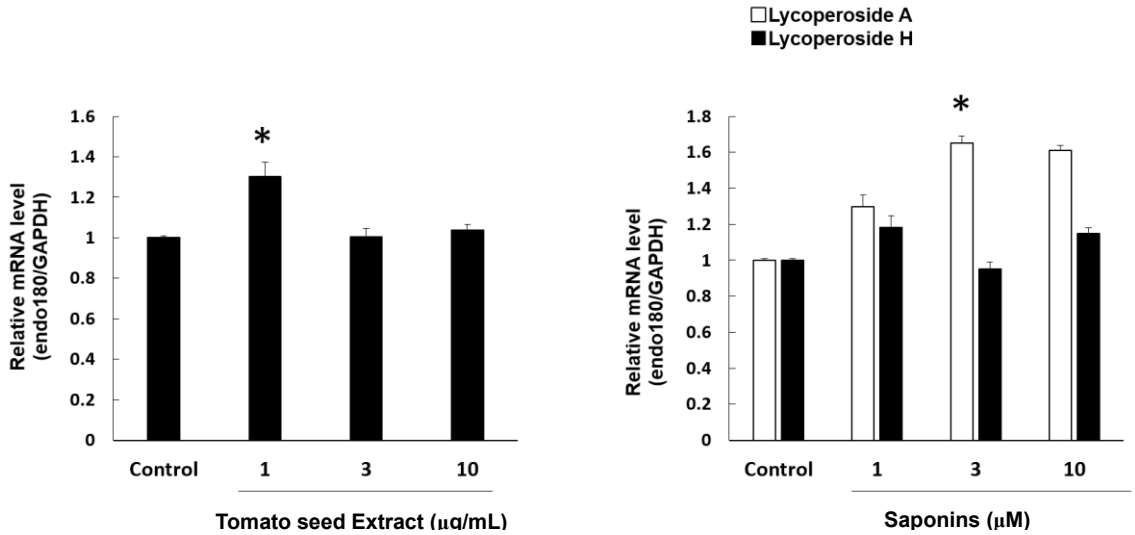

Fig. 9 Effect of Tomato Seed Extract and saponis on Endo180 gene expression n = 4-6, Mean±SE, *; $P < 0.05$ vs Control

On the other hand, lycoperoside A (10 μM) significantly upregulates expression of Fibulin gene which involved in elastin production (Fig. 10) and lycoperoside A (3 μM) also significantly upregulates expression of Neuraminidase-1 gene which involved in absorption of elastin degradation products (Fig. 11).

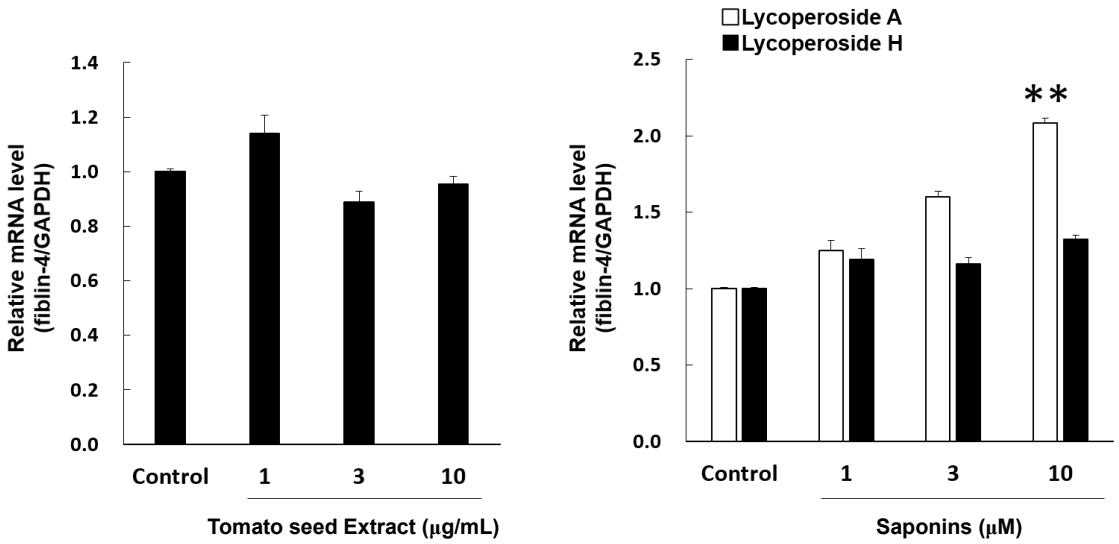

Fig. 10 Effect of Tomato Seed Extract and saponis on Fibulin gene expression n = 5-6, Mean±SE, $^{**}P < 0.01$ vs Control

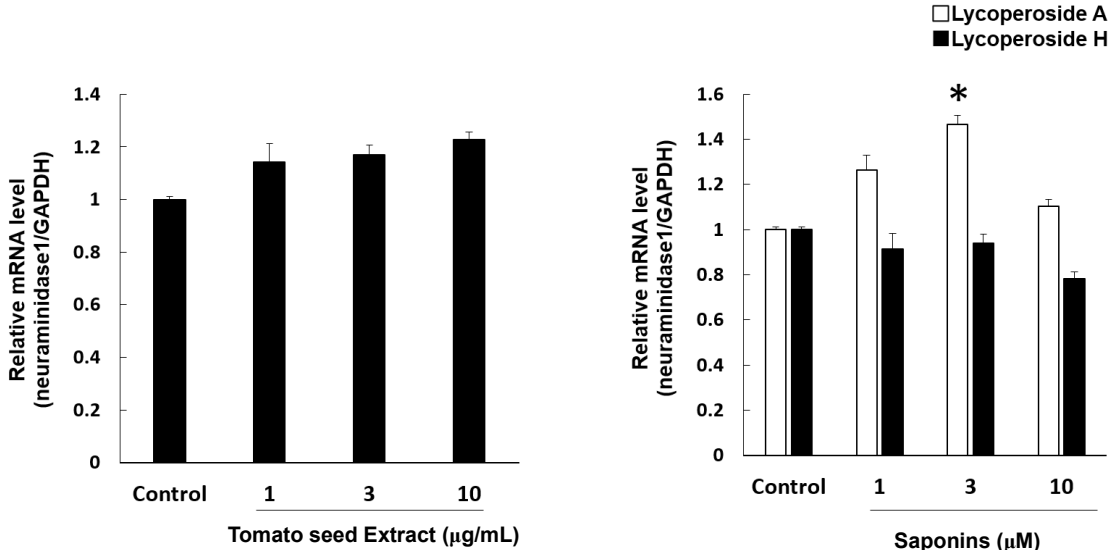

Fig. 11 Effect of Tomato Seed Extract and saponis on Neuraminidase-1 gene expression
n = 4-6, Mean±SE, $^{*}P < 0.05$ vs Control

From these results, **lycoperoside A is involved in collagen and elastin production promotive effect of Tomato Seed Extract**. Moreover, **Tomato Seed Extract significantly upregulated Endo180 gene and shows the tendency of upregulation of Neuraminidase-1 gene expression**. Lycoperoside A is also involved in this effect.

(4) Degradation products absorption enhancement effect on formulated ECM

From the results described above, it is suggested that Tomato Seed extract and its main components have the effect of ERS cycle enhancements. Accordingly, we evaluated the effect on cell sheet which formulates ECM. In usual cell test, cells do not adhere with each other tightly and do not formulate ECM. By preparing the cell sheet, cells adhere tightly and formulate ECM as a skin tissue model (Fig 12).

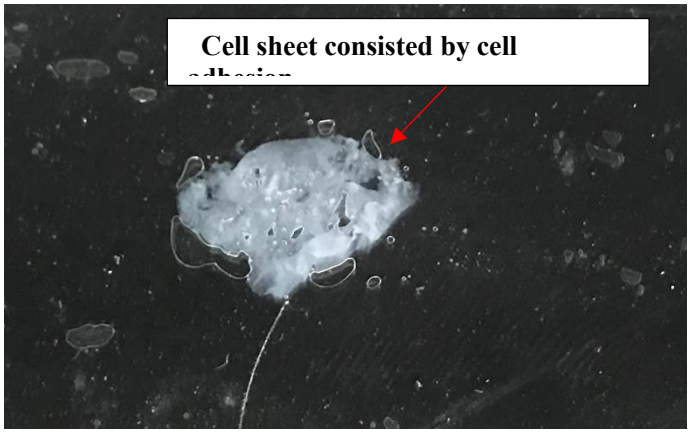

Fig. 12. Cell sheet consisted with human skin fibroblast

Firstly, H-Gly-Pro-Hyp-OH (collagen tripeptide) was added to cell sheet of human skin fibroblast (TIG-103) to duplicate accumulation of degradation products. After accumulation, Tomato Seed Extract (10 μg/mL), lycoperoside A and H (10 μM) added to cell sheet and after cultivated 1 day, degradation products were determined by immunofluorescence staining. Z stacking which is technique that series of images captured at different focus distance to create 3D image was applied to get stained degradation products in cell ECM. As a result of analysis, Amount of degradation products were removed by Tomato seed extract and saponis (Fig. 13). Namely, degradation products stained red color in Tomato Seed Extract group and lycoperoside group was decreased compere with control group. From this result, Tomato Seed Extract and lycoperosides promote absorption of degradation products in duplicate ECM.

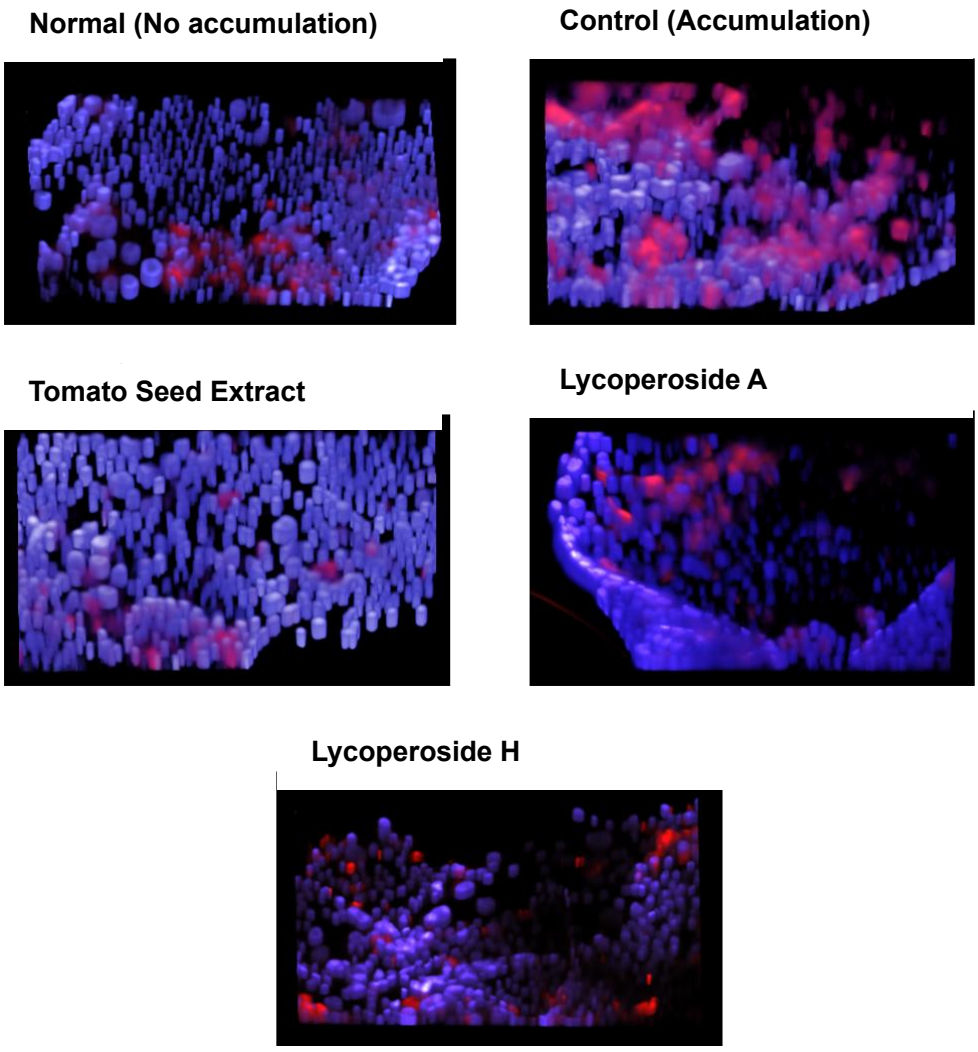

Fig. 13 Immunofluorescence staining images of ECM degradation products accumulated in cell sheet (3D images)
Red：ECM degradation products, Blue：Cell nucleus

(5) Upregulation of genes involved in absorption of degradation products on formulated ECM

In the next, we evaluated the gene expression involved in absorption of degradation products upregulation effect of Tomato Seed Extract and saponins on formulated ECM. Tomato Seed Extract (1, 3 and 10 μg/mL), lycoperoside A and H (1, 3 and 10 μM) added to cell sheet and after cultivated 1day, gene expression of Endo180 and Neuraminidase-1 were determined. As a result, gene expression of Endo180 and Neuraminidase-1 were significantly decreased by accumulation of degradation products. However, Tomato Seed Extract (10 μg/mL), lycoperoside A (1 and 3 μM) and lycoperoside H (1 μM) significantly recovered Endo180 expression (Fig. 14). Similarly, Tomato Seed Extract (10 μg/mL), lycoperoside A (1, 3 and 10 μM) and lycoperoside H

(10 μM) significantly recovered Neuraminidase-1 expression (Fig. 15). From these results, **Tomato Seed Extract and lycoperosides also upregulates gene expression involved in absorption of degradation products in formulated ECM**.

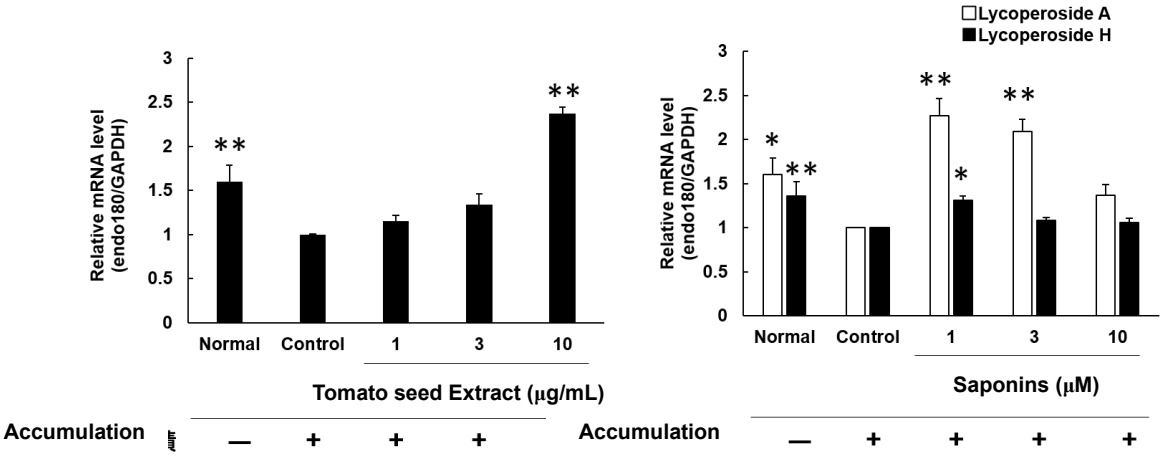

Fig. 14 Upregulation of Endo180 expression on formulated ECM
n =4-6, Mean±SE, *; $P < 0.05$, **$P < 0.01$ vs Control
Normal means no accumulation of ECM degradation products.
Accumulation was prepared in Control and sample group

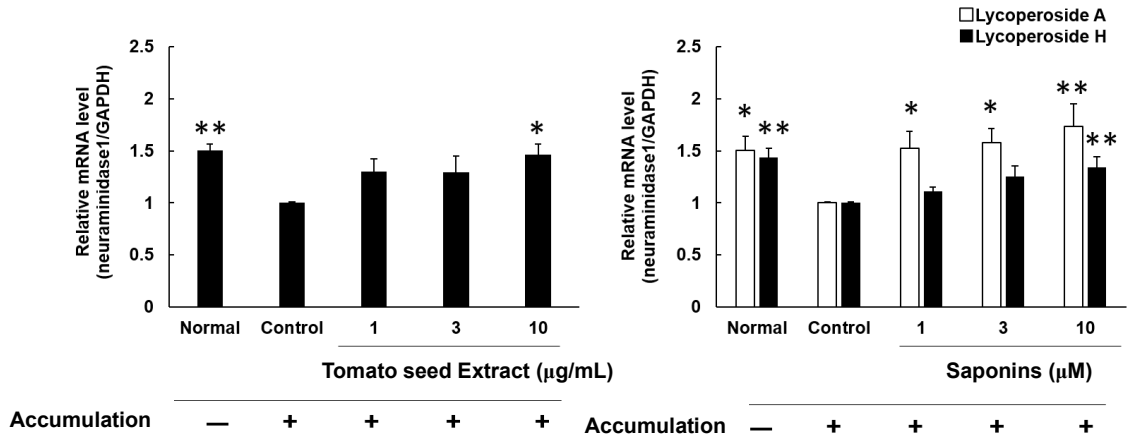

Fig. 15 Upregulation of Neuraminidase-1 expression on formulated ECM
n =4-6, Mean±SE, *; $P < 0.05$, **$P < 0.01$ vs Control
Normal means no accumulation of ECM degradation products.
Accumulation was prepared in Control and sample group

4. Effect on amount of intercellular lysosome (Wasted products removal)

As described above, it is suggested that Tomato Seed Extract and its main components, lycoperosides have collagen and elastin production/absorption cycle enhancement effect. Therefore, we evaluated the effect on amount of LAMP-1 (Lysosomal-associated membrane protein-1) which is lysosome involved in removal of wasted products not only collagen and elastin degradation products but also other degradation products in ECM. Similarly with collagen and elastin degradation products, it has been reported that accumulation of wasted products in ECM causes skin aging and deteriorating of skin condition[6].

Tomato Seed Extract (3 μg/mL), lycoperoside A and H (10 μM) added to human skin fibroblast (TIG-103) and after cultivated 2 days, LAMP-1 were determined by immunofluorescence staining. As a result, compare with control, amount of LAMP-1 was increased in fibroblast by Tomato Seed Extract and lycoperosides (Fig. 16). In addition, the effect of lycoperoside A was especially remarkable. From this result, **Tomato Seed Extract and lycoperosides have the removal of wasted products effect in ECM by increasing amount of lysosome involved in absorption and degradation of wasted products.**

6) Tashiro K. et al., *BBRC.*, 443, 167-172 (2014).

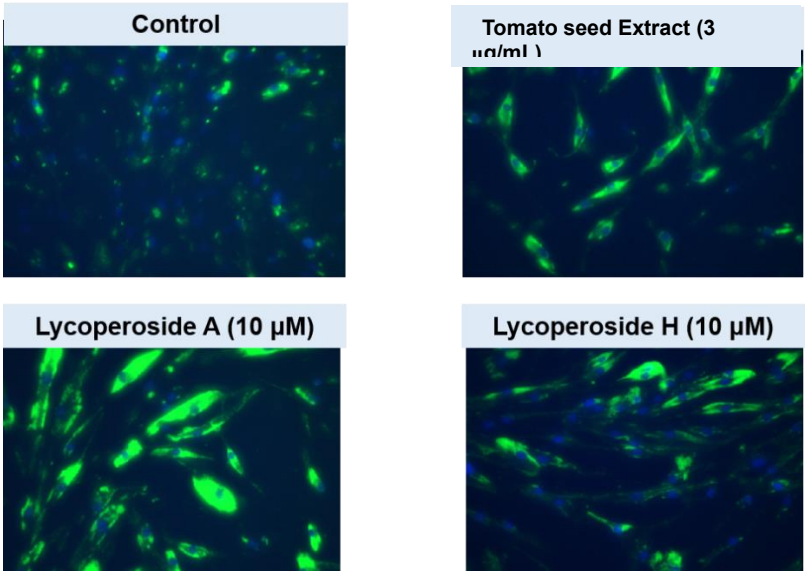

Fig. 16 Effect of Tomato Seed Extract and lycoperosides on amount of LAMP-1
Green：LAMP-1, Blue：Cell nucleus

5. Human clinical test (in-house)

We conducted the human clinical test (open trial) to evaluate the effect of LycoLift™-P on skin condition. Subjects were 18 healthy adult (Age: 36.8±12.2). Female were 12 (Age: 36.7±13.6) and male were 6 (Age: 37.0±10.0) among subjects. **All subjects ingested LycoLift™ –P by 200 mg/day for 4 weeks**. Collagen score and thickness of dermis in cheek and inside of upper arm were measured to evaluate the skin condition. In addition, questionnaire about skin condition was also performed. As a result, **collagen score in cheek and inside of upper arm were significantly increased** after ingestion and **thickness of dermis in inside of upper arm was also significantly increased.** Moreover, questionnaire items **"skin moisture", "Improvement of sagging", "Improvement of wrinkles" and "Elasticity" were significantly improved** after ingestion. From these results, Ingestion of LycoLift™ -P contributes improvement of wrinkle and sagging by increasing amount of collagen and thickness of dermis.

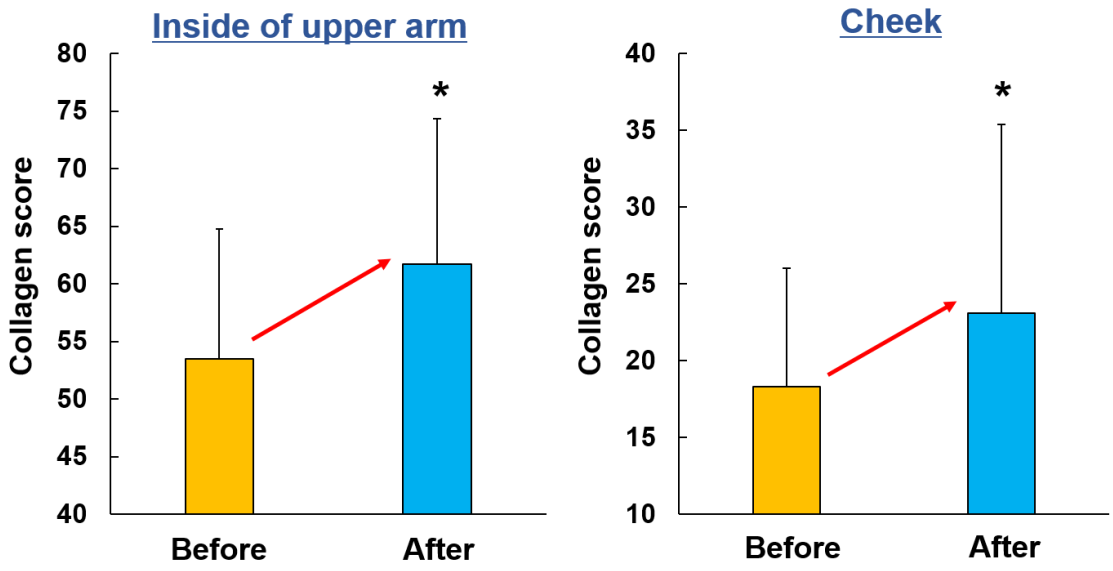

Fig. 17 Change in collagen score before and after ingestion
n = 18, Mean±SD, *; $P < 0.05$ vs before ingestion

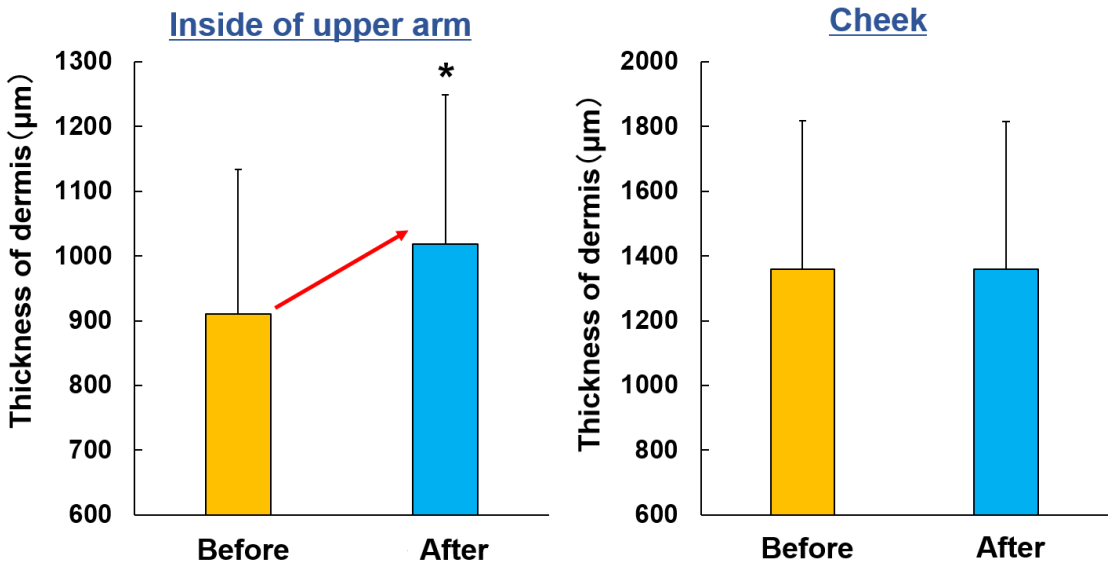

Fig. 18 Change in thickness of dermis before and after ingestion
n = 18, Mean±SD, *; $P < 0.05$ vs before ingestion

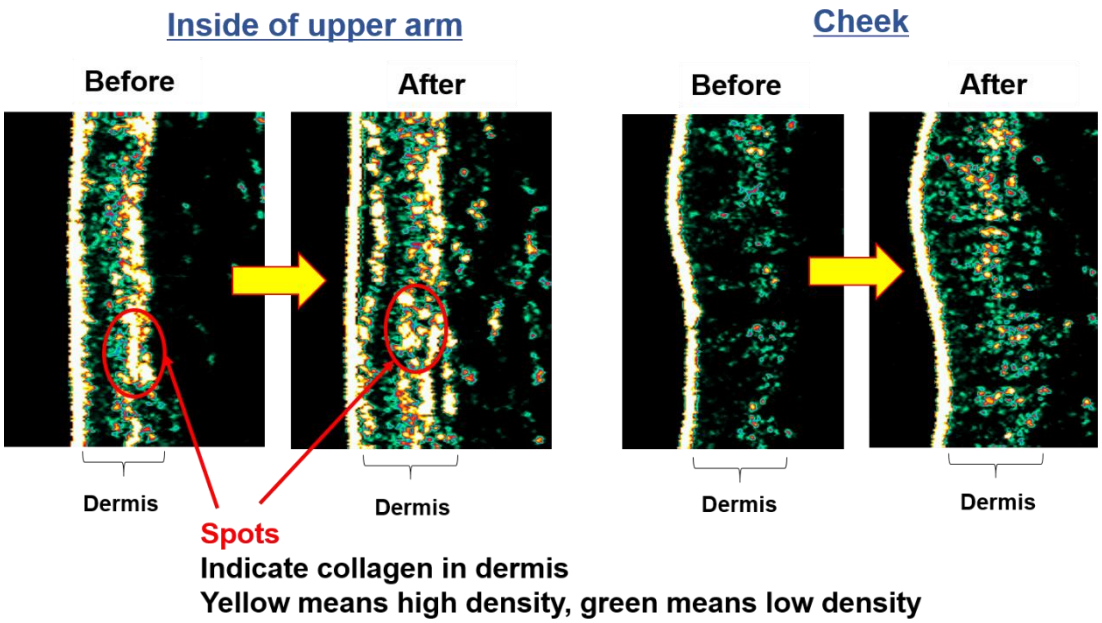

Fig. 19 Example of improvement

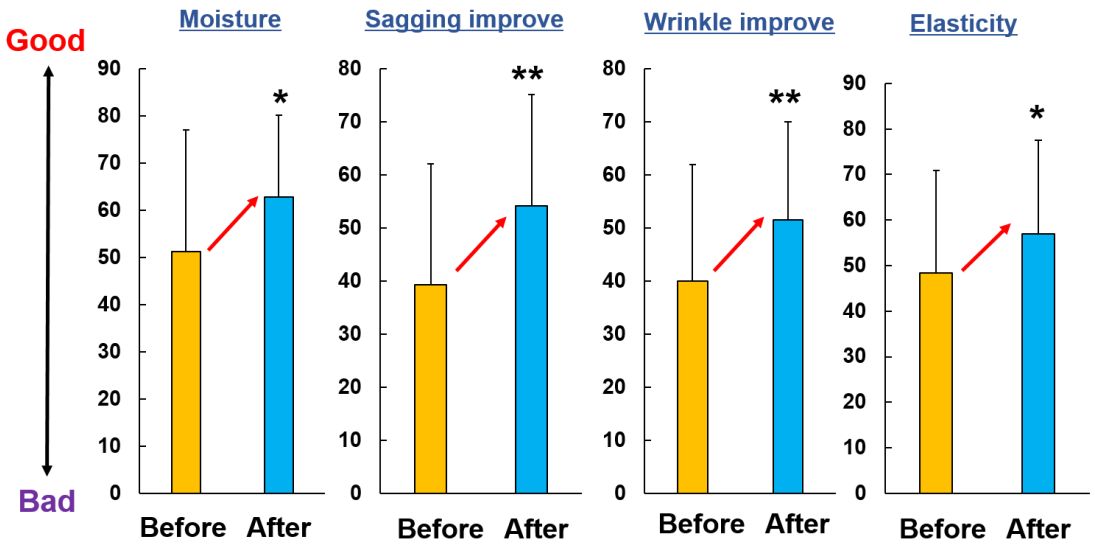

Fig. 20 Change in questionnaire before and after ingestion
n = 18, Mean±SD, *; $P < 0.05$, **; $P < 0.01$ vs before ingestion

6. Human clinical test (External CRO)

We evaluated the effects on skin elasticity in 44 healthy adult female subjects who consumed 200 mg of LycoLift™ –P per day for 8 weeks.

The results showed significant effects of tomato seed extract intake on internationally recognized elasticity parameters such as total elasticity (R2)[*1], net elasticity (R5)[*2], and recovery elasticity (R7)[*3] (Fig. 21). These three values have been reported to decrease with aging, and are widely used as elasticity indicators of healthy skin. Furthermore, a significant decrease in blood pentosidine level, one of the biomarkers of glycation stress, was observed after 8 weeks of intake of LycoLift™ –P (Fig. 22). It is known that skin aging occurs when skin glycation progresses, as collagen fibers are destroyed, skin elasticity is lost, and turnover becomes difficult to occur. These results indicate that continuous intake of LycoLift™ –P maintains skin elasticity by reducing glycation stress as a mechanism.

※1. Gross elasticity (R2) : Ratio of the instantaneous return of the skin to its maximum elongation after being pinched and pulled.
※2. Net elasticity (R5) : Ratio of the value of the skin returned to its original height to the instantaneous elongation value.
※3. Recovery elasticity (R7) : Ratio of the instantaneous return value to the instantaneous elongation value.

These clinical results are published from below scientific report.

Izumi T., Yamamoto K., Suzuki N., Yamashita S., Iio S., Noguchi H., Kakinuma T., Baba A., Takeda S., Yamada W. and Shimoda H. Tomato Seed Extract Containing Lycoperoside H Improves Skin Elasticity in Japanese Female Subjects: A Randomized, Placebo-Controlled, Double-Blind Trial. Journal of Cosmetics, Dermatological Sciences and Applications, 11, 217-236 (2021)

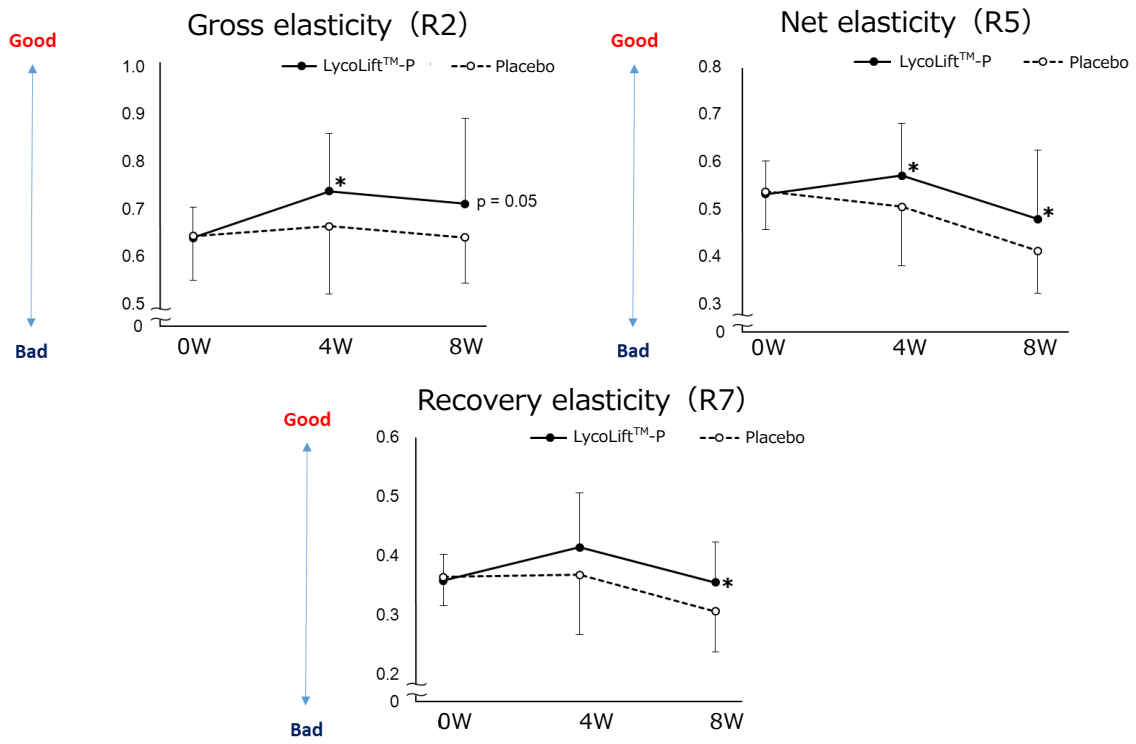

Fig. 21. Intake of LycoLift™-P improves skin elasticity
Mean±SE（n = 20-22）, *; P<0.05 vs placebo

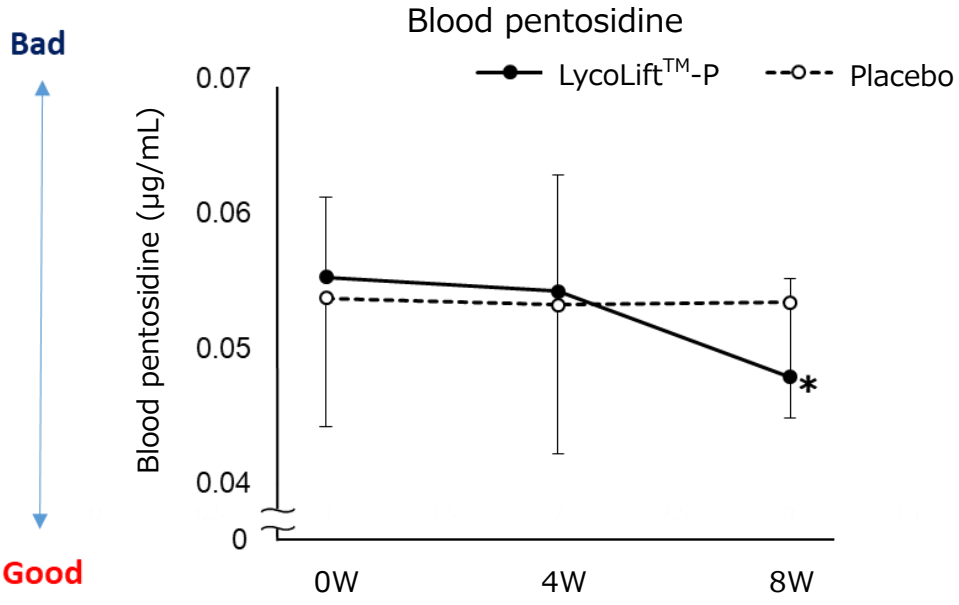

Fig. 22. Intake of LycoLift™-P reduces blood pentosidine
Mean±SE（n = 20-22）, *; P<0.05 vs placebo

7. Effect on Epidermis

As described above, LycoLift™ has the effect of supporting skin elasticity by acting on the extracellular matrix in the dermis layer of the skin (inside). On the other hand, LycoLift™ also has the effect of improving dry skin and allergic dermatitis by working on the epidermis (outer layer) of the skin.

While the dermis is the inner layer of the skin, the epidermis is the outer layer of the skin and serves as a barrier that blocks foreign substances such as antigens from the outside while preventing the skin from losing moisture. However, if the epidermis is damaged or its function deteriorates with age, not only does moisture easily escape from the skin, resulting in dry skin, but it also causes allergic dermatitis symptoms due to foreign substances entering the skin. Therefore, it is important to maintain the moisture retention and barrier function by taking care of the epidermis.

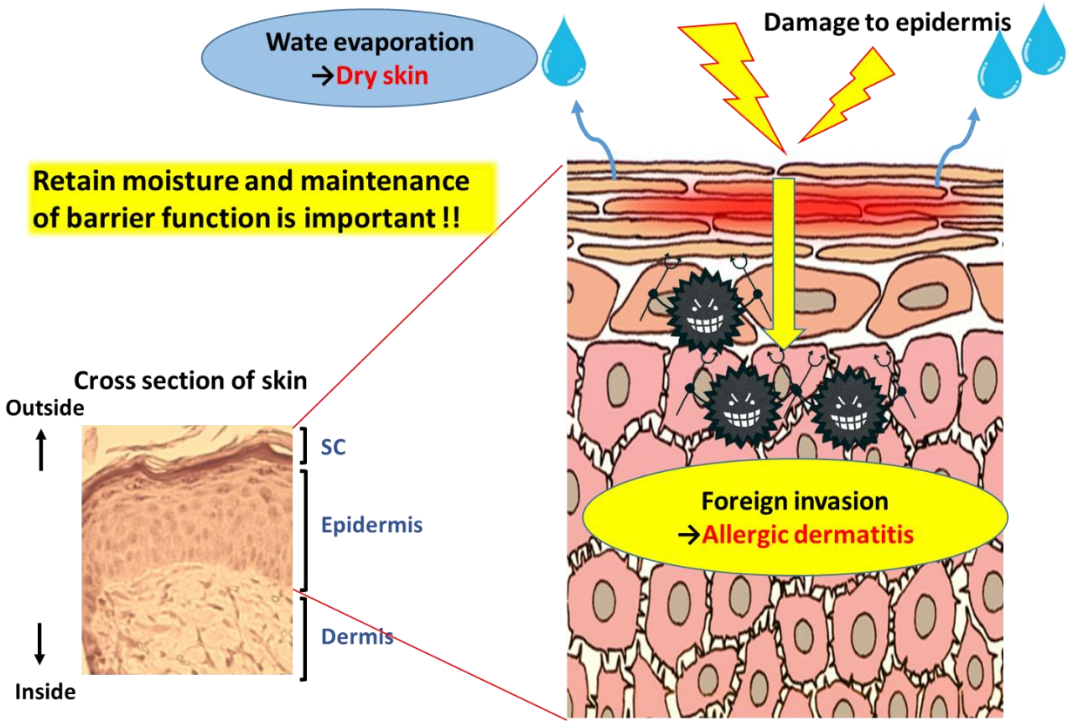

Fig. 23 Image of skin condition deterioration due to epidermal damage

(1) Increased expression of moisturizing genes

We quantified the expression levels of filaggrin gene and ceramide synthase-3 gene, which are involved in moisturizing, after culturing human epidermal 3D cells with tomato seed extract or lycoperoside H. Filaggrin plays an important role in skin moisturization as the source of natural moisturizing factor (NMF), and it is known that filaggrin is decreased in the skin of patients with atopic dermatitis. Ceramide synthase-3 is involved in the synthesis of ceramide, which contributes greatly to moisture retention in the stratum corneum.

Experimental results showed that filaggrin and ceramide synthase-3 genes in human epidermal 3D cells were significantly increased by tomato seed extract and lycoperoside H (Fig. 24 and 25).

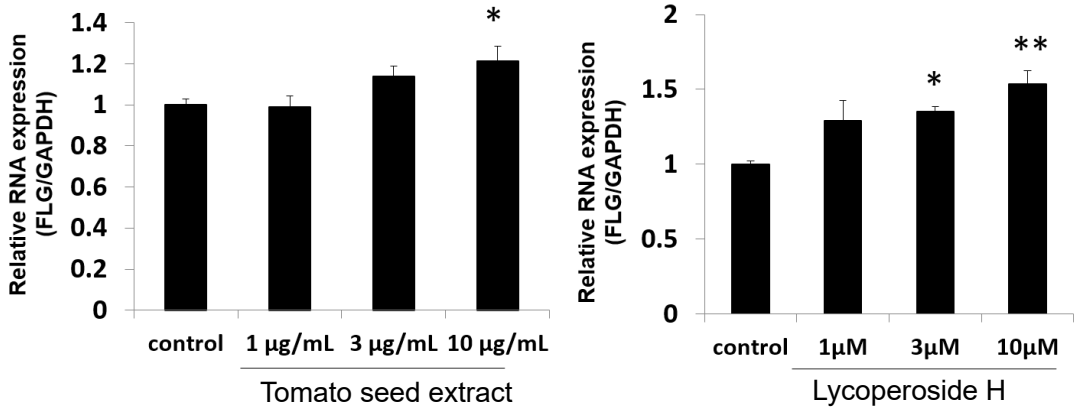

Fig 24. Effects of tomato seed extract and lycopeloside H on filaggrin gene expression in human epidermal 3D cells.
n =3-4, Mean±SE, *; $P < 0.05$, **$P < 0.01$ vs Control

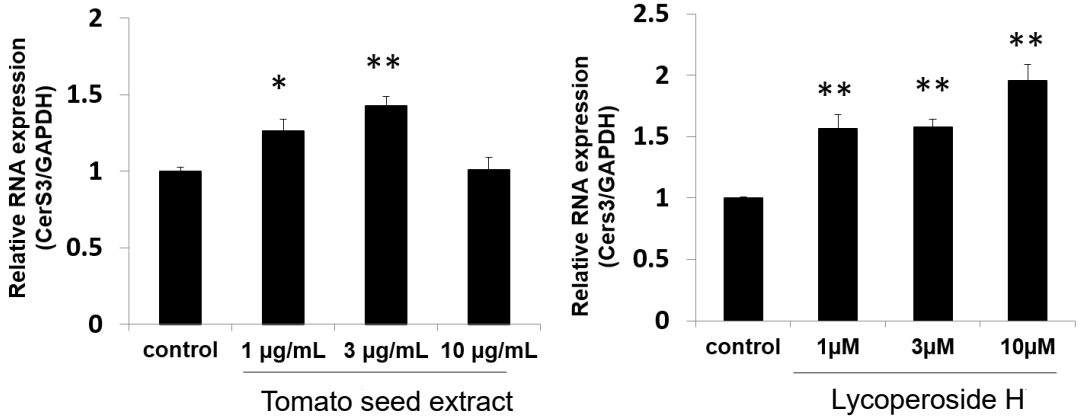

Fig 25. Effects of tomato seed extract and lycopeloside H on ceramide synthase gene expression in human epidermal 3D cells.
n =3-4, Mean±SE, *; $P < 0.05$, **$P < 0.01$ vs Control

(2) Improvement of trans-epidermal water loss (TEWL)

Based on the results of the experiment described in the previous section, which showed that tomato seed extract and lycoperoside H promoted the expression of moisture retention-related genes in human epidermal 3D cells, we evaluated the moisturizing effects of tomato seed extract and lycoperoside H by measuring TEWL, a commonly used method for evaluating moisturizing ability. The results showed that TEWL decreased (i.e., moisturizing ability improved) as the number of days of culture increased in human epidermal 3D cells cultured with tomato seed extract and lycoperoside H (Fig. 26).

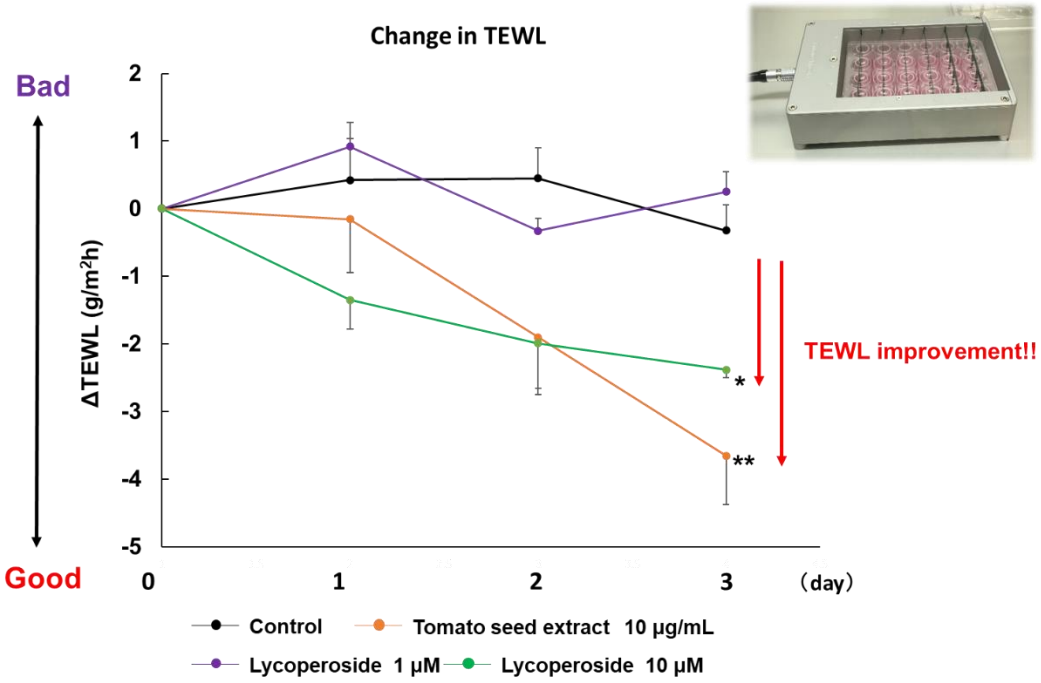

Fig. 26. Effects of Tomato Seed Extract and Lycopeloside H on TEWL in Human Epidermal 3D Cells

n =3-4, Mean±SE, *; $P < 0.05$, **$P < 0.01$ vs Control

(3) Inhibition of scratching behavior in a mouse scratching model

The inhibitory effects of tomato seed extract and lycoperoside H on allergy-induced itching were evaluated in a compound 48/80-induced mouse scratching model. After oral administration of tomato seed extract or lycoperocide H, a 3% saline solution of compound 48/80 was subcutaneously administered to the back of the neck of mice to induce scratching behavior due to itching, and the number of scratches over 30 minutes was measured. The results showed that administration of tomato seed extract and lycopersoside H tended to decrease the number of scratching compared to the control group.

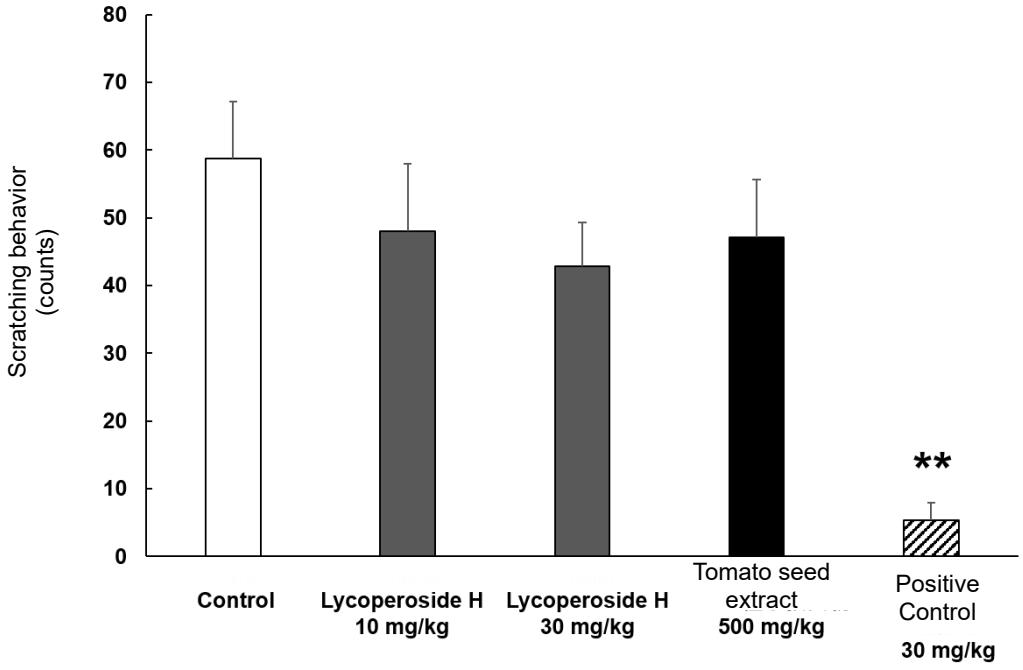

Fig. 27. Effects of Tomato Seed Extract and Lycopeloside H on mouse scratching model
n =5-7, Mean±SE, **; $P < 0.01$ vs Control
Positive control: Diphenhydramine hydrochloride

(4) Inhibition of immediate allergic reaction

The effects of tomato seed extract and lycoperoside H on immediate allergic reactions were evaluated using the mouse auricular PCA reaction. This evaluation system is a well-known model for evaluating the effects on immediate allergic reactions. Mice were sensitized by intradermal administration of saline-diluted anti-DNP-IgE into both auricles, followed 2 days later by oral administration of tomato seed extract or ricoperoside H. Two hours later, a saline solution of DNP-BSA and Evans blue was administered via the tail vein to elicit an antigen-antibody reaction. After 30 minutes, the auricles of mice lethally killed by cervical dislocation were cut, and the dye (blue) leaking into the auricles was extracted and its absorbance at 620 nm was measured. The results of the experiment showed that the amount of pigment leakage was significantly reduced in the groups treated with tomato seed extract and lycopersoside H (10 mg/kg). These results indicate that tomato seed extract and lycoperoside H have an inhibitory effect on immediate-type allergy.

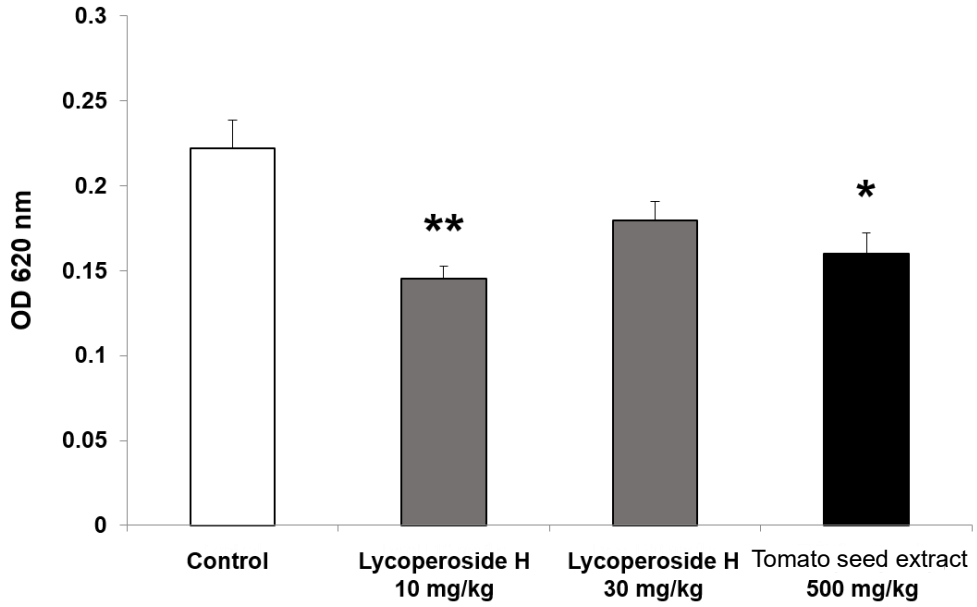

Fig. 28. Effects of Tomato Seed Extract and Lycopeloside H on on PCA Reaction in Mouse Ear
n =10-14, Mean±SE, *; $P < 0.05$, **; $P < 0.01$ vs control

5) Effects on atopic dermatitis model mice

As described in (3) and (4), the inhibitory effects of tomato seed extract and lycoperoside H on allergies were confirmed, so we evaluated their effects on alleviating dermatitis symptoms in a mouse model of atopic dermatitis, a typical form of allergic dermatitis. Experiments were conducted using IL33-Tg mice, a model mouse that spontaneously develops atopic dermatitis. Tomato seed extract (500 mg/kg/day) or lycopersoside H (10 mg/kg/day) was orally administered to the mice for 14 weeks before the onset of symptoms. After confirming the onset of dermatitis symptoms, we first measured limb inflammation scores, locomotor activity by the wheel test, and TEWL. The results showed that the tomato seed extract group showed a tendency to suppress skin inflammation in the limb inflammation score, while the lycopersoside H group significantly suppressed inflammation. In the wheel test, locomotion, which had been decreased due to stress caused by inflammation, was significantly restored by treatment with tomato seed extract and lycoperocide H. TEWL was also significantly improved by treatment with tomato seed extract and lycoperocide H.

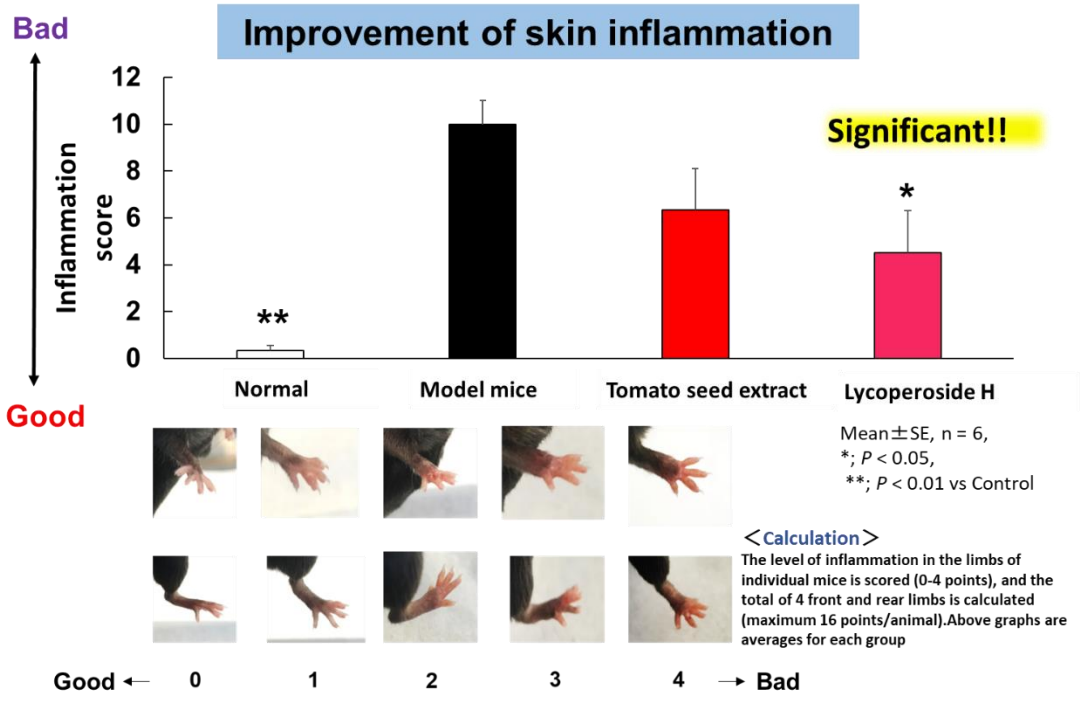

Fig. 29. Effects of Tomato Seed Extract and Lycopeloside H on Skin Inflammation

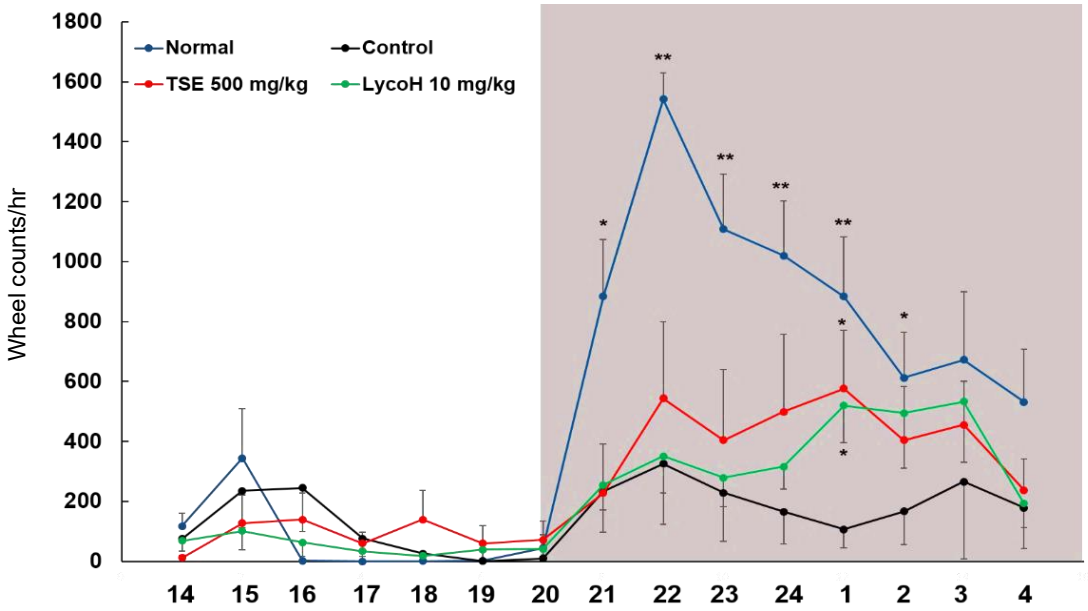

Fig. 30. Effects of Tomato Seed Extract and Lycopeloside H on locomotor activity by the wheel test.
n =6, Mean±SE, *; $P < 0.05$, **; $P < 0.01$ vs control

Control: Model mice
TSE: Tomato seed extract
LycoH: Lycoperoside H

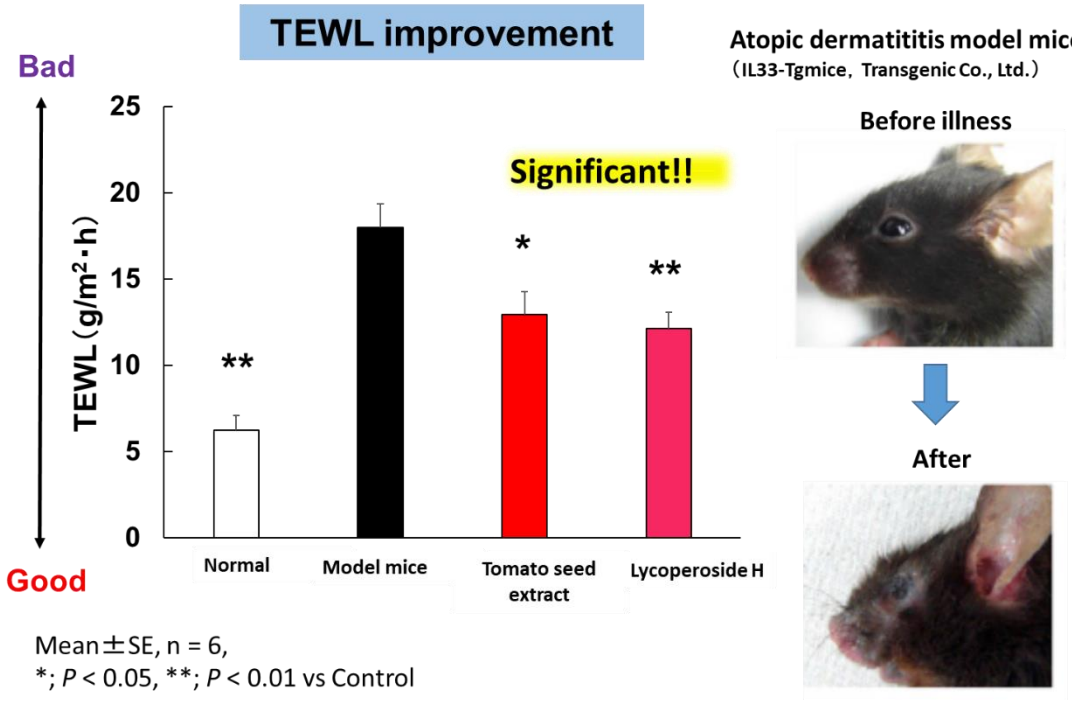

Fig. 31. Effects of Tomato Seed Extract and Lycopeloside H on TEWL

Next, serum IgE and skin Th1/Th2 cytokines (IL-2, IFN-γ, IL-4, and IL-5), which are biomarkers of allergic reactions, were quantified, and skin pathology specimens were prepared for microscopic observation. The results showed that the total IgE levels in the serum were significantly decreased by the administration of tomato seed extract and lycoperoside H. The cytokine balance in the skin was also improved from the Th2 cytokine predominant type observed in allergic dermatitis to a normal balance. Furthermore, microscopic observation of pathological specimens showed that epidermal thickening and eosinophilic wetting (black granular cells), which are seen in atopic dermatitis skin, were observed in the untreated control group, whereas these symptoms were restored in the tomato seed extract and lycopersidone H.

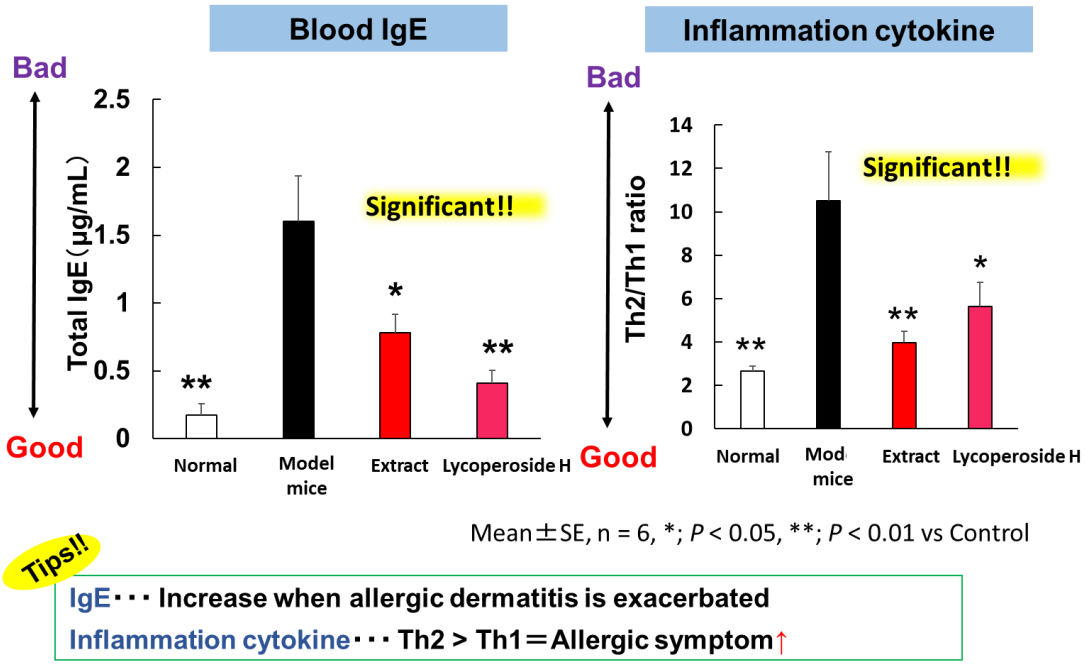

Fig. 32. Effects of Tomato Seed Extract and Lycopeloside H on blood IgE and inflammation cytokines

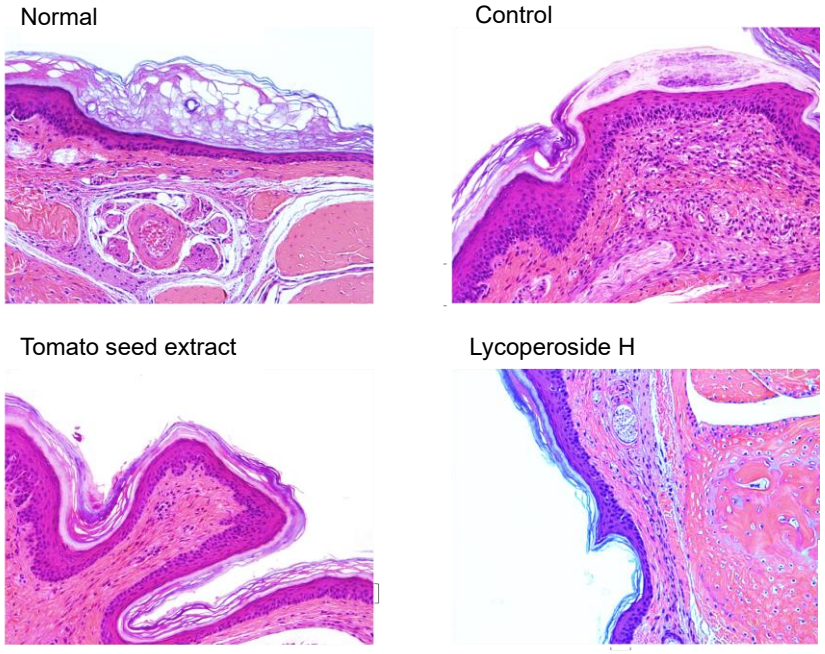

Fig. 33. Pathological Specimens

These results suggest that tomato seed extract and lycoperoside H are effective in alleviating atopic dermatitis symptoms, a typical example of allergic dermatitis.

8. Product stability of LycoLift™

(1) Heat stability

Heat stability of LycoLift™-P was examined by heating at 100℃ and 120℃ continuously for 1hour. As shown in Fig. 21, total lycoperosides contents were not decreased after heating. Therefore, Tomato Seed Extract-P is highly stable upon heating at normal food processing temperature.

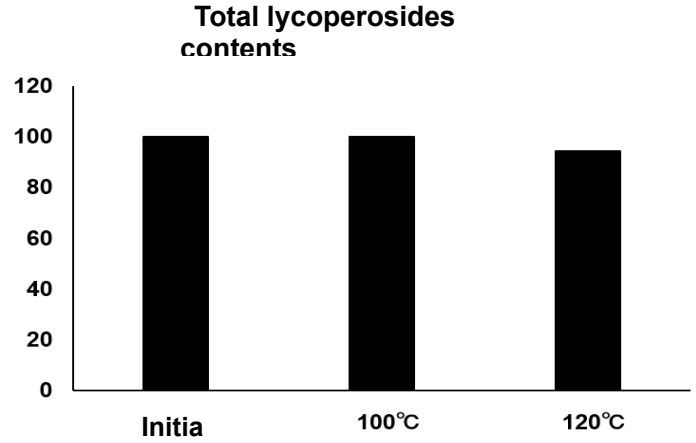

Fig. 34. Heat stability of LycoLift™-P
Heating for 1 hours at each temperature

(2) pH stability

The pH stability of LycoLift™-P was examined by stored at different pH value at room temperature and for 1 week. No alteration was observed neither in the color of extract nor in the total lycoperosides content.

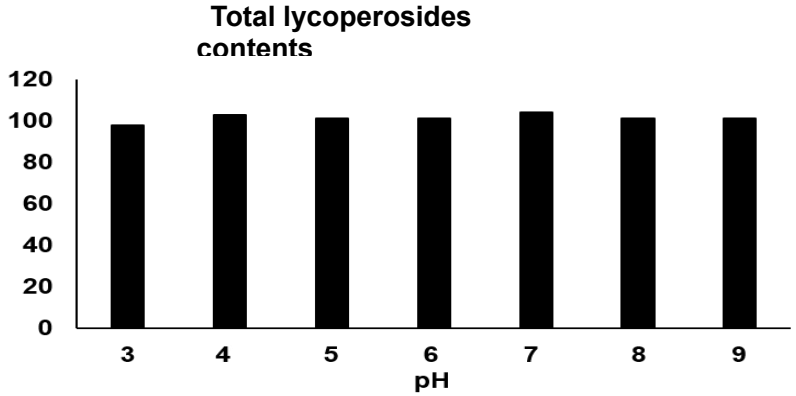

Fig. 35. pH stability of LycoLift™-P

7. Nutrition profiles

Analyzed item (/100g)	LycoLift™-P, PC	Method
Water (g)	3.2	Heating drying method under normal pressure
Protein (g)	0.8	Kjeldahl method, nitrogen protein conversion factor: 6.25
Fat (g)	0.5	Acid decomposition method
Ash (g)	0.3	Direct incineration method
Carbohydrate (g) －Sugar (g) －Fiber (g)	95.2 95.0 0.2	 Refer note 1 Prosky's method
Energy (kcal)	388	Refer note 2
Sodium (mg)	22.7	Atomic absorption spectrophotometory
Sodium chloride equivalent (g)	0.06	Refer note 3

The nutritional information of LycoLift™ was analyzed according to the standard in nutrition labeling (March 30, 2015; No 139 Eishin)

Note 1: Calculation: 100-(water + protein + fat + ash)

Note 2: Energy conversion factor: Protein 4, fat 9, sugar 4, dietary fiber 2

Note 3: In terms of sodium

Test trustee: SUNATECH/ Dte of analysis: April 18, 2019

Test No: 160804527-001-01

8. Safety profiles

（1） Residual agricultural chemicals

Tomato Seed Extract was screened and analyzed for residual agricultural chemicals (535 items) stipulated under the Food Sanitation Act and Pesticides Control Act, presence of the test items was lower than the allowed limits.

Test trustee: Masis Co., Ltd.; Center for Food Safety Evaluation and Analysis
Date: November 28, 2018
Report No: 123136

（2） Acute toxicity（LD_{50}）

Acute Toxicity test was conducted according to the Guidelines for Single-Dose Toxicity Tests for Pharmaceutical Products. Tomato Seed Extract 2000 mg/kg was orally given to fasted ICR mice (6 weeks old). After 14 days, no abnormalities and fatal event were observed at 2000 mg/kg. No abnormalities were observed under macroscopic examination upon autopsy. Thus, LD_{50} of Tomato Seed Extract is deduced to be > 2000 mg/kg.

（3） Mutagenicity（Ames test）

Ames test was conducted to evaluate the mutagenicity of Tomato Seed Extract using *Salmonella typhimurium* TA98, TA100, TA1535, TA1537 and *E. coil* WP2 at concentration 19.5-5,000 μg/plate. No mutagenicity was observed.

9. Recommended dosage

We recommend 200 mg/day of LycoLift™-P based on the result of human clinical trial.

10. Application

	Applications	Indication	Examples
Food	Nutritional supplement, Beauty food	-Extracellular matrix production cycle enhancement -Skin wasted product removal	Beverages, Hard and soft capsules, tablets, Candies, Chewing gums, Gummies, Cookies, Chocolates, Wafers, Jellies etc.
Cosmetic	Beauty		Toners, Lotions, Packs, Body gels etc.

11. Packing

LycoLift™-P（PC）

1 kg, 5kg　　Interior packing: Aluminium bag
Exterior packing: Cardboard box

12. Storage

Store in a dry, ventilated location. Keep away from high temperature and sun light, store in the closed containers.

13. Expression

<Food>

LycoLift™ -P

Maltodextrin, Tomato Seed Extract

<Cosmetic>

LycoLift™ -PC

Maltodextrin, Tomato Extract

INCI name: MALTODEXTRIN (AND) SOLANUM LYCOPERSICUM (TOMATO) EXTRACT

PRODUCT STANDARD

PRODUCT NAME : **LycoLift™ -P** (FOOD)

(TOMATO SEED EXTRACT)

This product is extracted with aqueous ethanol from the seed of tomato Lycopersicon esculentum Miller（＝Solanum lycopersicum Linne）(Solanaceae).
It contains a minimum of 0.5% total lycoperosides.

Appearance	Pale white to pale yellowish white powder with slightly characteristic odor.	
Total lycoperosides	Min. 0.5%	(HPLC)
Loss on Drying	Max. 10.0 %	(Analysis for Hygienic Chemists, 1 g, 105ºC, 2 hr)
Purity Test		
(1) Heavy Metals (as Pb)	Max. 20 ppm	(Sodium Sulfide Colorimetric Method)
(2) Arsenic (as As_2O_3)	Max. 1 ppm	(Standard Methods of Analysis in Food Safety Regulation, The Third Method, Apparatus B)
Standard Plate Counts	Max. 1×10^3 cfu/g	(Analysis for Hygienic Chemists)
Moulds and Yeasts	Max. 1×10^2 cfu/g	(Analysis for Hygienic Chemists)
Coliforms	Negative	(Analysis for Hygienic Chemists)

Composition

Ingredient	Content
Maltodextrin	95%
Tomato Seed Extract	5%
Total	100%

Expiry date 2 years from date of manufacturing.

Storage Store in a dry, ventilated location. Keep away from high temperature and sun light.

PRODUCT STANDARD

PRODUCT NAME : **LycoLift™ -PC** (COSMETIC)
(TOMATO SEED EXTRACT)

This product is extracted with aqueous ethanol from the seed of tomato (*Lycopersicon esculentum*). It contains a minimum of 0.5% total lycoperosides.

Appearance	Pale white to pale yellowish white powder with slightly characteristic odor.	
Total lycoperosides	Min. 0.5%	(HPLC)
Loss on Drying	Max. 10.0 %	(Analysis for Hygienic Chemists, 1 g, 105ºC, 2 hr)
Purity Test		
(1) Heavy Metals (as Pb)	Max. 20 ppm	(Sodium Sulfide Colorimetric Method)
(2) Arsenic (as As_2O_3)	Max. 1 ppm	(Standard Methods of Analysis in Food Safety Regulation, The Third Method, Apparatus B)
Standard Plate Counts	Max. 1×10^2 cfu/g	(Analysis for Hygienic Chemists)
Moulds and Yeasts	Max. 1×10^2 cfu/g	(Analysis for Hygienic Chemists)
Coliforms	Negative	(Analysis for Hygienic Chemists)

Composition

Ingredient	Content
Maltodextrin	95%
Tomato Seed Extract	5%
Total	100%

Expiry date	2 years from date of manufacturing.
Storage	Store in a dry, ventilated location. Keep away from high temperature and sun light.

This standards and test methods are referred to General Notices and General Tests, Processes and Apparatus of The Japanese Standards of Quasi-drug Ingredients, unless otherwise specified.

LycoLift™ -LC
(TOMATO SEED EXTRACT)

This product is a mixture of 1,3-butylene glycol and water-extract obtained from the defatted solution of the seeds of *Lycopersicon esculentum* Miller（＝*Solanum lycopersicum* Linne）(Solanaceae).

Manufacturing method
Extract with ethanol solution from the seeds of *Lycopersicon esculentum* Miller（＝*Solanum lycopersicum* Linne）(*Solanaceae*), extract and remove fat and oil substances from the filtrate with *n*-hexane, and evaporate *n*-hexane, ethanol and water, and obtain the solid. Dissolve solid substance to 1,3-butylene glycol water solution, and filter as the product.
Raw material: seed 100kg Product: approx. 400 – 500 kg

Description
This product is pale yellow to pale yellowish brown liquid, with slightly characteristic odor.

Identification
・ Saponin

To 0.3mL of this product, add 5mL of acetic anhydride, shake well, allow to stand for 2minutes, add gently 1mL of sulfuric acid; reddish brown color develops in contact zone.
・ Sugar

To 1mL of this product add 2 to 3 drops of α-naphthol・ethanol solution (1→20) and shake well, add gently 1 to 2mL of sulfuric acid; reddish purple color develops in contact zone.

Purity
・ Heavy metals

Take 1.0g of this product, determine heavy metals according to the method 2: the limit is not more than 20ppm. Use 1.0mL of standard lead solution as the control solution.
・ Arsenic

Take 1.0g of this product, prepare the test solution according to the method 3, and perform the test: the limit is not more than 2ppm.

Bacterial Count
Take 5g of this solution, make 50mL test solution with diluent and perform the bacterial count test, using standard agar medium according to Hygiene Test Method; the limit is not more than 1 ×102 cfu/g.

Fungus・Molds Count
Take 5g of this solution, make 50mL test solution with diluent and perform the fungus count test using potato dextrose agar medium added chloramphenicol according to Hygiene Test Method; the limit is not more than 1×10^2 cfu/g.

Coli form
Take 1mL of the solution which prepare the bacterial count test, and perform the coli form test using BGLB medium according to Hygiene Test Method; Negative / Not observe any colony.

These standards and test method are referred to General Notices and General Tests, Processes and Apparatus of The Japanese Standards of Quasi-drug Ingredients, unless otherwise specified.

ORYZA OIL & FAT CHEMICAL CO., LTD. striving for the development of the new functional food materials to promote health and general well-being.

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

Headquarters:
ORYZA OIL & FAT CHEMICAL CO., LTD.
No.1, Numata Kitagata-cho, Ichinomiya-city, Aichi-pref.,
493-8001 JAPAN
TEL : +81 (0) 586 86 5141
FAX : +81 (0) 586 86 6191
URL/http : //www.oryza.co.jp/
E-mail : info@oryza.co.jp

Tokyo sales office:
5F of Diamant Building, Kanndasuda-cho 1-5
Chiyoda-ku, Tokyo, 101-0041 Japan
TEL (03)5209-9150 FAX (03)5209-9151
E-mail: tokyo@oryza.co.jp

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ORYZA OIL & FAT CHEMICAL CO., LTD.