

# ***LITCHI SEED EXTRACT***

## **Cosmeceutical Food Supplement**

### **■ LITCHI SEED EXTRACT-P**

(Powder, Food Grade)

### **■ LITCHI SEED EXTRACT-WSP**

(Water-soluble Powder, Food Grade)

### **■ LITCHI SEED EXTRACT-PC**

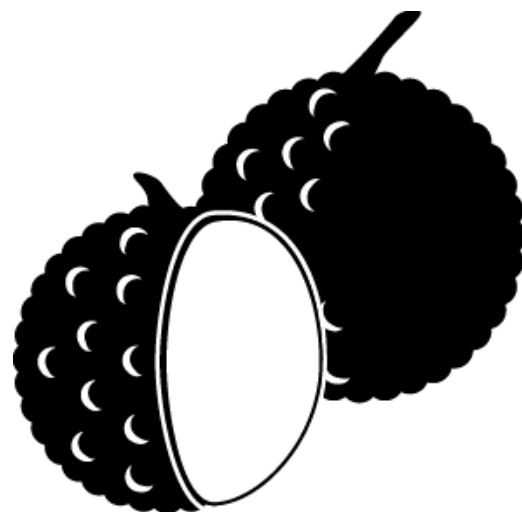
(Powder, Cosmetic Grade)

### **■ LITCHI SEED EXTRACT-WSPC**

(Water-soluble Powder, Cosmetic Grade)

### **■ LITCHI SEED EXTRACT-LC**

(Liquid, Cosmetic Grade)



Cosmeceutical Food Supplement  
**LITCHI SEED EXTRACT**

## 1. Introduction

With the females' increasing participation in social activities and the aging of society, the desire for a more beautiful healthy life has been increasing. However, with age, various signs of aging begin to be observed in the human body. The skin, which is particularly important for females, loses elasticity and sags with age.

The skin tissue consists of cells and intercellular macromolecular aggregates called matrix. The extracellular matrix is composed of fibrous proteins, such as collagen, elastin and hyaluronic acid and proteoglycan filling the space.

Hyaluronic acid is a high-molecular between cells weight mucopolysaccharide secreted from cells and widely distributed in tissue. Hyaluronic acid is involved in the immune system for biological defense and the control of electrolyte balance, and a major component of the dermal substrate of the skin. A decrease in hyaluronic acid is associated with the aging of the skin, and decreases in its elasticity and plasticity.

Quantitative decrease and qualitative changes (such as crosslinking and changes due to active oxygen after UV exposure) of collagen would lead to deep wrinkles.

LITCHI SEED EXTRACT, which was developed as a cosmeceutical food, is expected to inhibit enzymes involving in the degradation of collagen, elastin and hyaluronic acid, thus, to inhibit decreases in collagen, elastin and hyaluronic acid, and maintain the tension and moisture of the skin, preventing wrinkles.

LITCHI SEED EXTRACT also eliminates active oxygen (superoxide) and other molecular radicals, which would cause wrinkles, skin aging, and carcinogenesis, by acting like superoxide dismutase (SOD), a radical scavenger.

In addition, we newly discovered an amazing function in LITCHI SEED EXTRACT, that induces apoptosis in human stomach cancer cells.

Whitening is important from the cosmetic aspect. Dark skin, pigmentation, and spots are caused by melanin. An amino acid, tyrosine, is converted *in vivo* into dopa by an enzyme tyrosinase, and subsequently into dopa quinone, from which melanin is produced by oxidation. LITCHI SEED EXTRACT inhibits tyrosinase, thus, inhibiting melanin formation, so it is expected to promote whitening.

Collagen is present not only in skin, nails, and hair but also in bone stiffness, which has been reported to be useful for preventing osteoporosis, cartilage wear, and rheumatoid arthritis and preventing and improving osteoarthritis. These disorders are more frequently seen in females, and therefore, we recommend LITCHI SEED EXTRACT as food with multiple functions particularly for females.

## 2. What Is Litchi?

*Litchi chinensis* Sonn. [Litchi]

Litchis, whose origin is the southern China, have been cultivated for over 3,000 years. As is observed in historical tales of Yang-kuei-fei, litchis were particularly treasured in ancient times when it was difficult to keep freshness, litchis are evergreen subtropical trees. The flowers are small and pale white–light yellow. The fruits are roundish stone fruits with a thin solid pericarp and a torous surface, and turn from vivid red to dark red when ripe. The seeds are short oval, and isolated and covered with a white flesh juicy aril. This aril is delicious. The aril is one of the five major fine fruits in tropical and subtropical zones and has been loved by people in China. Litchi trees, which are about 5-15 m tall, are widely cultivated as fruit trees. Litchis bloom in February–March and ripe in June–July. Litch is generally eaten raw. Fresh fruits are used for fruit cocktails and salads, and canned ones are for dessert and flavoring of Chinese tempura. Litchis are sweet and have been treasured as a tonic in China or decocted as a cough medicine. The seeds were made into ointments and used for skin disease. The trees live long, and even 200-year-old trees bear fruits.

Litchi seeds are called litchi cores and used as a Chinese medicine. “Ben Cao gang mu” states, “Sweet, warming, astringent, and non-poisonous”. “*广西中藥 Journal*” states, “Slightly sweet, bitter, and astringent, no heating or cooling effects, non-poisonous. [Drug efficacy and indications] Litchis warm bodies from inside, regulate qui, and relieve pain, and are useful for gastric pain, lumbago pain, and blood- and qui-stimulating pain in females.”

Litchi is associated with “立子” (having a child) and symbolizes in China as a good wife-husband or male-female relationship or birth of offspring. Litchi fruits were used as signals showing the depth of love. Litchis are often compared to “状元”, the top successful applicant for keju, the traditional bureaucrats appointment test. In the past, in Beijing, litchis were thrown into the tub for newborn’s first bath 3 days after birth.

Yang-kuei-fei was very fond of litchis, and there are many pieces of episode of her and Litch. One says that she ordered officers servants to deliver Litch to the capital Choan in north-west from southern areas more than 10 thousand miles away. Dispatched messengers on a post horse were obliged to the order, because Litch is easy to decay. Another says beauty of Yang-kuei-fei, which was further increased by litchi, made the emperor Xuan zang neglected his state affairs.

Yang-kuei-fei, who was fond of litchi very much, may have not only maintained her beauty but also become more beautiful by Litch.

Were litchis the secret of the beauty of Yang-kuei-fei?



Litchi fruit

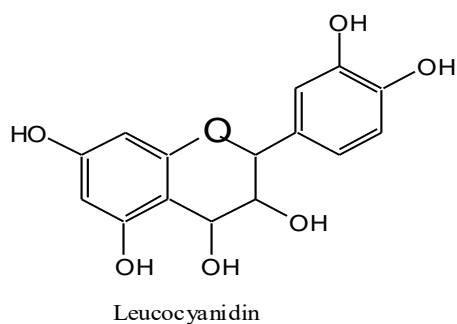


Litchi seed

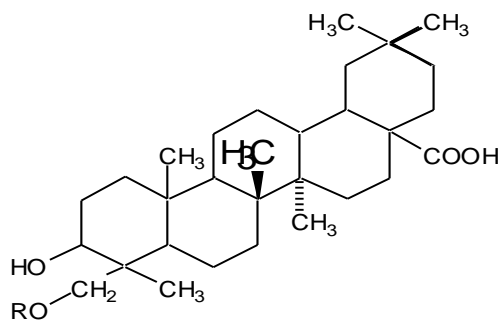
### 3. Components of LITCHI SEED EXTRACT

LITCHI SEED EXTRACT contains Saponin, Tannin, Leucocyanidin (Flavonoid), Anthocyanin, etc.

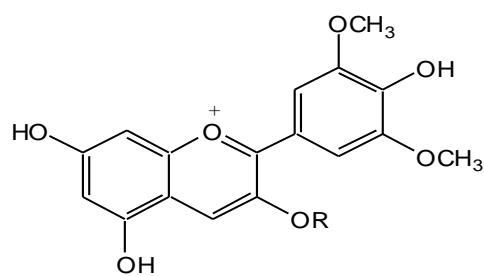
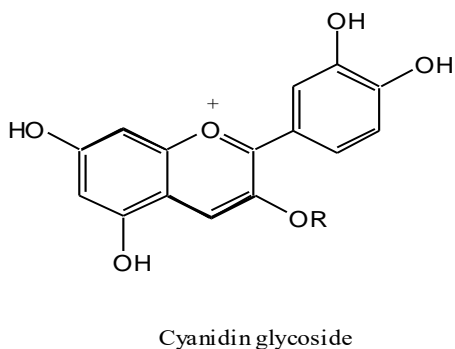
#### Flavonoid



#### Saponin



#### Anthocyanin



R= Sugar

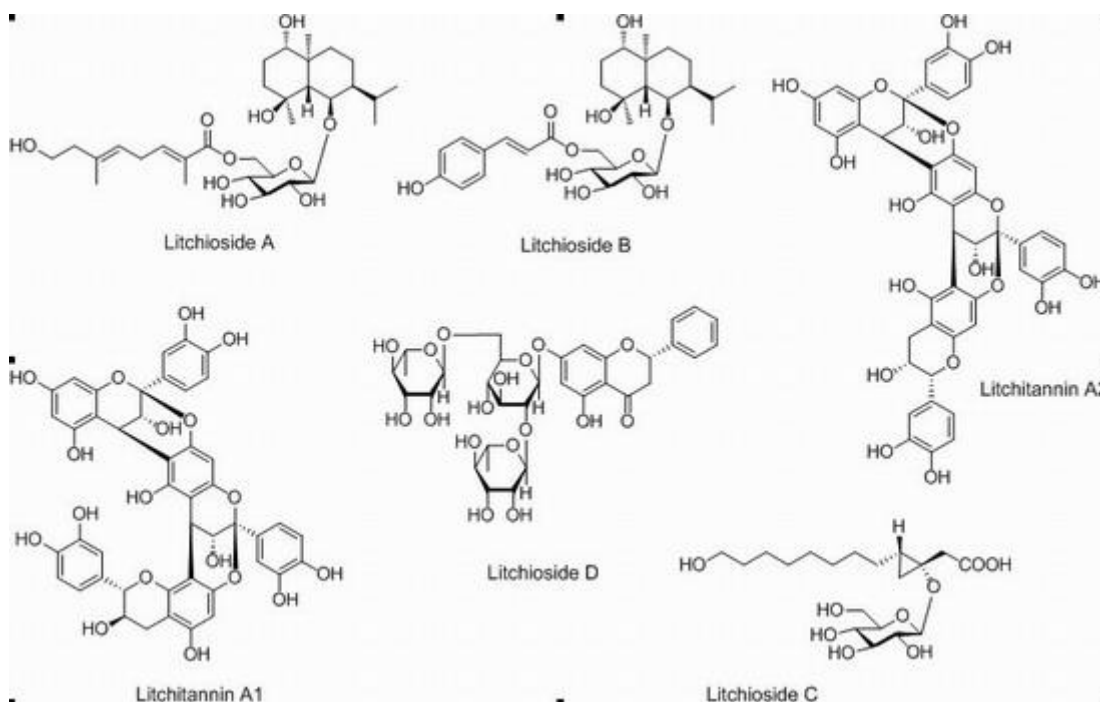


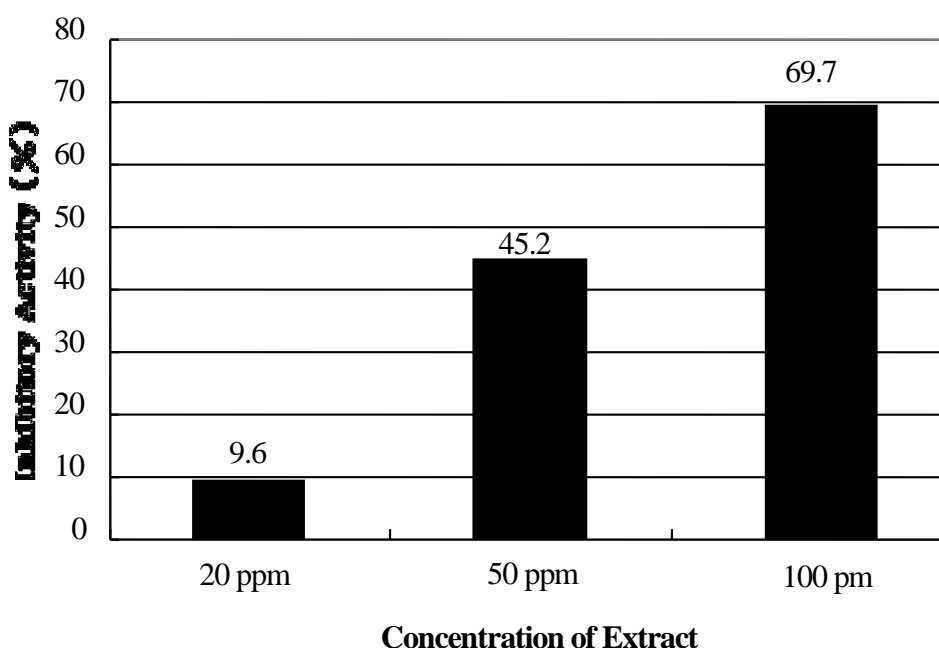
Fig.1 Compounds contained in litchi seeds

## 4. Functions of LITCHI SEED EXTRACT

### (1) Inhibition of Collagenase

Collagen is distributed in the entire dermis of the skin, constituting 90% of the dermis. Collagen maintains appropriate elasticity and strength of the skin. When collagenase, an enzyme, is activated, and collagen is degraded, wrinkles and sagging as aging phenomena of the skin develop. Collagen produced in the body is degraded by collagenase.

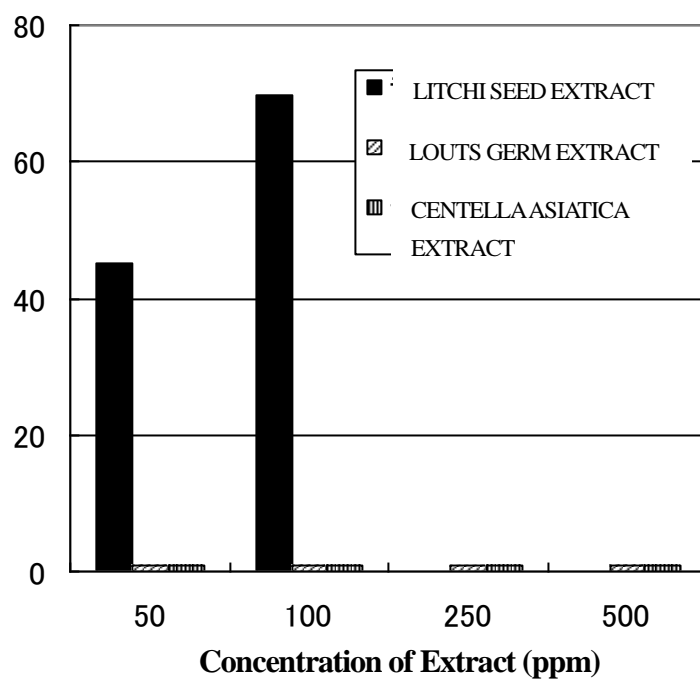
LITCHI SEED EXTRACT inhibited collagenase at a low concentration, thus, LITCHI SEED EXTRACT suggested inhibiting the degradation of collagen.



**Fig. 2 Inhibition of Collagenase by LITCHI SEED EXTRACT.**

**Table 1 Collagenase Inhibitory Effect of LITCHI Extracts from Various Parts.**

	Inhibition Activity (%)					
	20 ppm	50 ppm	100 ppm	500 ppm	1000 ppm	IC <sub>50</sub>
Seeds	9.6	45.2	69.7	—	—	59 ppm
Whole	—	—	0	0	0	>1000 ppm
Flesh	—	—	0	0	0	>1000 ppm
Peel	—	—	0	0	0	>1000 ppm

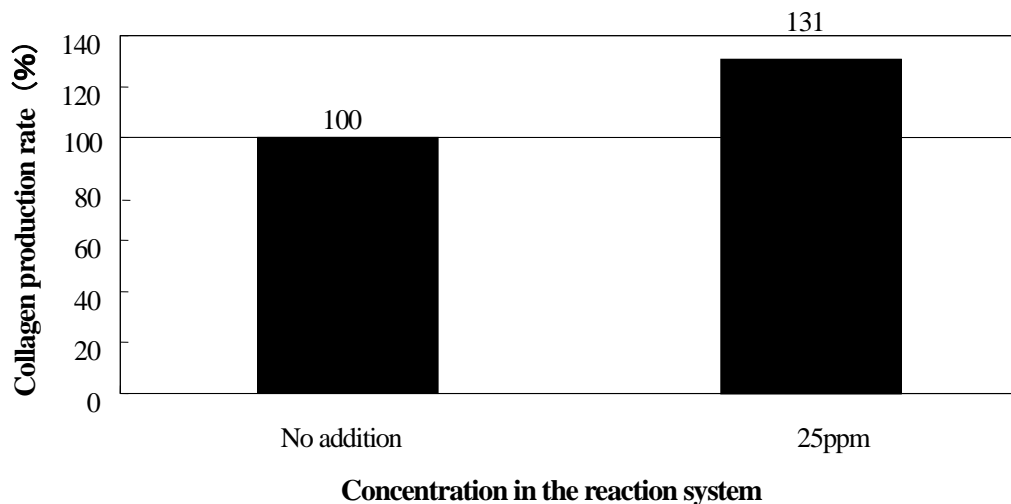


**Fig. 3 Collagenase Inhibitory Effect of LITCHI SEEDS EXTRACT and Other Plant Extracts.**

## (2) Collagen Productivity in Normal Human Fibroblasts

It is said that productivities of collagen in human skin starts to decline at around age of 20. It may be possible to maintain elastic and smooth skin if productivities of collagen is recovered.

LITCHI SEED EXTRACT increased collagen production in normal human fibroblasts (cells equivalent to those in humans at the ages when the collagen productivity starts declining).



**Fig.4 Effects of LITCHI SEED EXTRACT on Collagen Production.**

Twenty-five ppm of LITCHI SEED EXTRACT was added to normal human fibroblasts, and incubated for 3 days. Procollagen I C-terminal peptide (PIP) in the culture media was quantified by ELISA.

**Table 2 Collagen Productive Effect of LITCHI Extracts from Various Source (% of control\*)**

	25 ppm	100 ppm
Seeds	131	—
Whole	91	104
Flesh	82	77
Peel	95	98

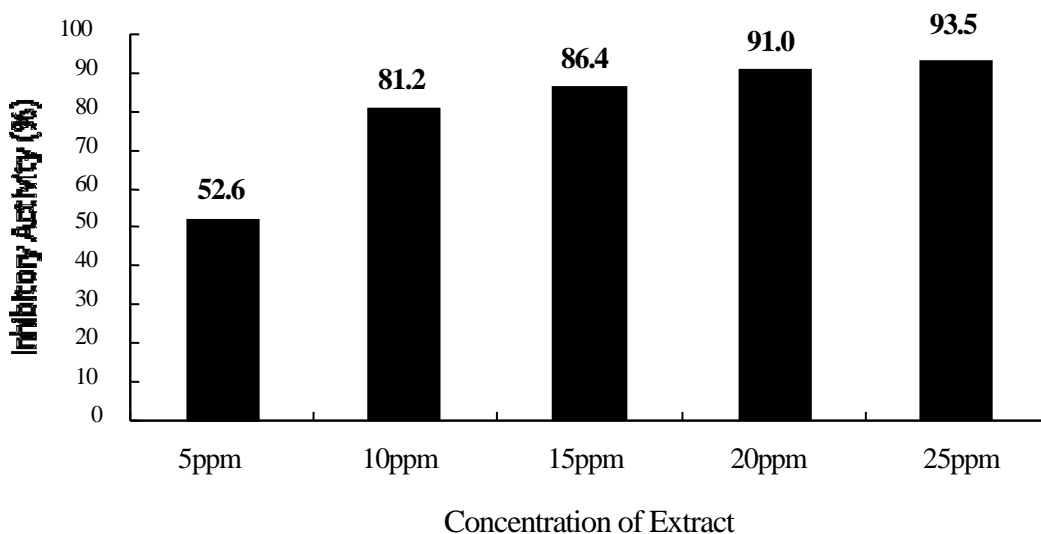
\*In control, no extract was added



### (3) Inhibition of Elastase

Elastin is distributed in the entire dermis of skin. Tension and elasticity of skin are kept by moderate balance of collagen and elastin and hyaluronic acid. Elastin maintains appropriate elasticity and strength of the skin. When elastase, an enzyme, is activated, and elastin is degraded, wrinkles and sags grow as aging phenomena of skin. Elastin produced in the body is degraded by elastase.

LITCHI SEED EXTRACT inhibited elastase at a low concentration, thus, it decreases the degradation of elastin effectively.



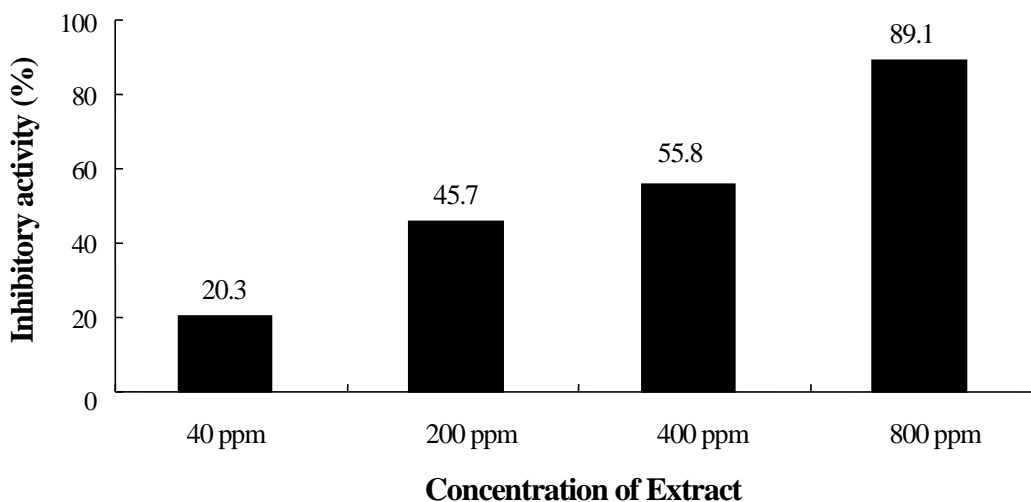
**Fig. 5 Inhibition of Elastase by LITCHI SEED EXTRACT**

**Table 3 Elastase Inhibitory Effect of LITCHI Extracts from Various Source**

	Inhibition Activity (%)							IC <sub>50</sub>
	5 ppm	10ppm	15 ppm	20 ppm	25 ppm	50 ppm	500ppm	
Seeds	52.6	81.2	86.4	91.0	93.5	—	—	4.5 ppm
Whole	—	—	—	—	20.4	40.4	91.5	74 ppm
Flesh	—	—	—	—	0	0	0	>500 ppm
Peel	—	—	—	—	11.3	17.6	56.5	320 ppm

#### (4) Inhibition of Hyaluronidase

Hyaluronic acid is widely distributed in tissues such as skin, synovial fluid, vitreous body, and ligaments. Hyaluronic acid is involved in the adhesion and protection of cells, formation of skin tissue, and the maintenance of the moisture and flexibility of tissue. With a decrease in hyaluronic acid, the skin loses moisture and tension, and develops wrinkles and sagging. Hyaluronic acid is degraded by the enzyme, hyaluronidase. LITCHI SEED EXTRACT inhibited hyaluronidase, thus, it inhibits the degradation of hyaluronic acid.

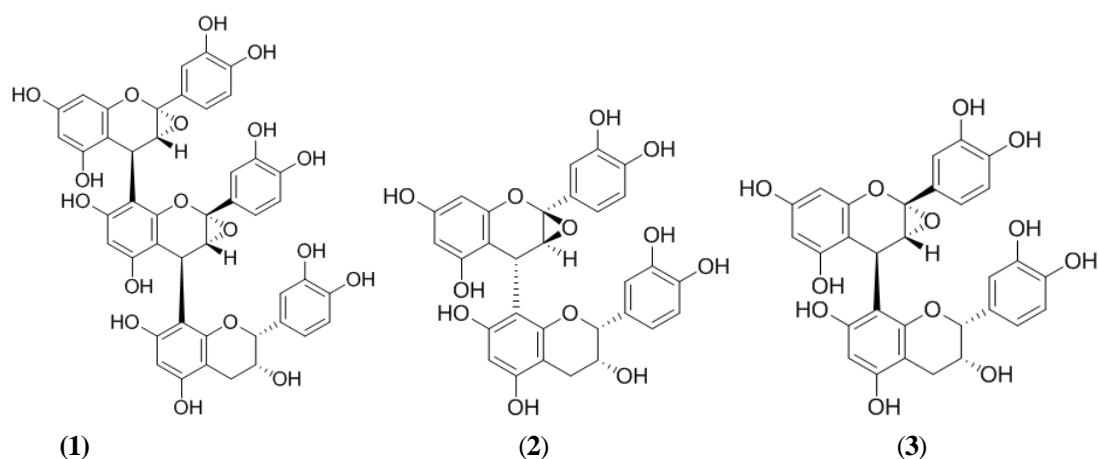


**Fig. 6 Inhibition of Hyaluronidase by LITCHI SEED EXTRACT**

**Table 4 Hyaluronidase Inhibitory Effect of LITCHI Extracts from Various Source**

	Inhibition Activity (%)					IC <sub>50</sub>
	40 ppm	200ppm	400 ppm	800 ppm	2500ppm	
Seeds	20.3	45.7	55.8	89.1	—	290 ppm
Whole	—	—	—	0	3.4	>2500 ppm
Flesh	—	—	—	0	0	>2500 ppm
Peel	—	—	—	4.1	9.2	>2500 ppm

We made a search of the active ingredient of litchi seeds inhibit hyaluronidase activity as an index. We ask the structure determination for Faculty of Agriculture, Biological and Environmental Laboratory of Kinki University. As a result, as shown in Figure 7, 2 and 3 of procyanidin dimer in maternal-epicatechin, flavan skeleton were obtained. These has a hyaluronidase activity, most do not contain ingredients berry.



**Fig. 7 Inhibition of Hyaluronidase by LITCHI SEED EXTRACT components**

di [2  $\alpha$  , 3  $\alpha$  -epoxy-5,7,3',4'-tetrahydroxyflavan-(4  $\beta$  -8)]-epicatechin (1), 200 ppm (inhibitory activity: 55%)

2  $\beta$  , 3  $\beta$  -epoxy-5,7,3',4'-tetrahydroxyflavan-(4  $\alpha$  -8)-epicatechin (2), 200 ppm (inhibitory activity: 19%)

2  $\alpha$  , 3  $\alpha$  -epoxy-5,7,3',4'-tetrahydroxyflavan-(4  $\beta$  -8)-epicatechin (3), 200 ppm (inhibitory activity: 45%)

## (5) Usefulness of LITCHI SEED EXTRACT as A Skin Beautifier

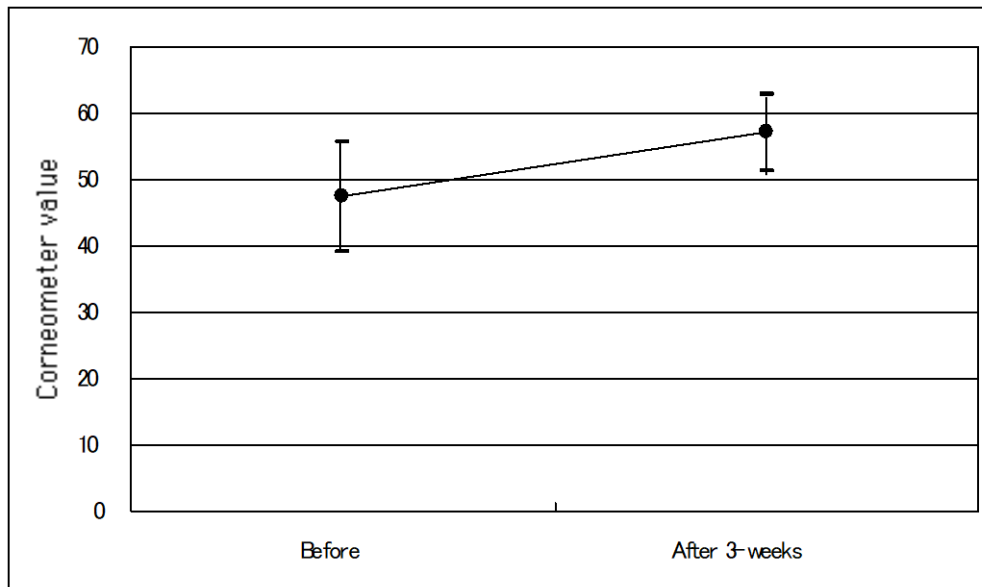
### [Protocol]

Test methods:	Double blind test.
Materials:	Caramel hard capsules containing 150 mg LITCHI SEED EXTRACT-P, 4 mg silicon dioxide and 66 mg corn starch (total, 220 mg) were prepared. As the placebo, caramel hard capsules containing 150 mg dextrin, 4 mg silicon dioxide and 66 mg corn starch (total, 220 mg) were produced.
Subjects:	Twenty healthy subjects without regular use of drugs.
Administration	
dose:	Three hundred mg/day (2 capsules) in the LITCHI SEED EXTRACT-P group (11 subjects), and 0 mg/day (2 dextrin capsules) in the placebo group (9 subjects).
Administration period:	Three weeks.
Examination methods:	The moisture of the skin was measured using a CORNEOMETER SM825 (CK electric GmbH), pH of the skin was measured using a SKIN-pH-METERPH900 (CK electric GmbH), and the amount of skin sebum was measured using a SEBUMETER SM810 (CK electric GmbH).
Examination regions:	Face and medial brachium.
Examination conditions:	Temperature, of 22 degrees; relative humidity, of 55%.

### 1. Improvement in The Moisture of Skin

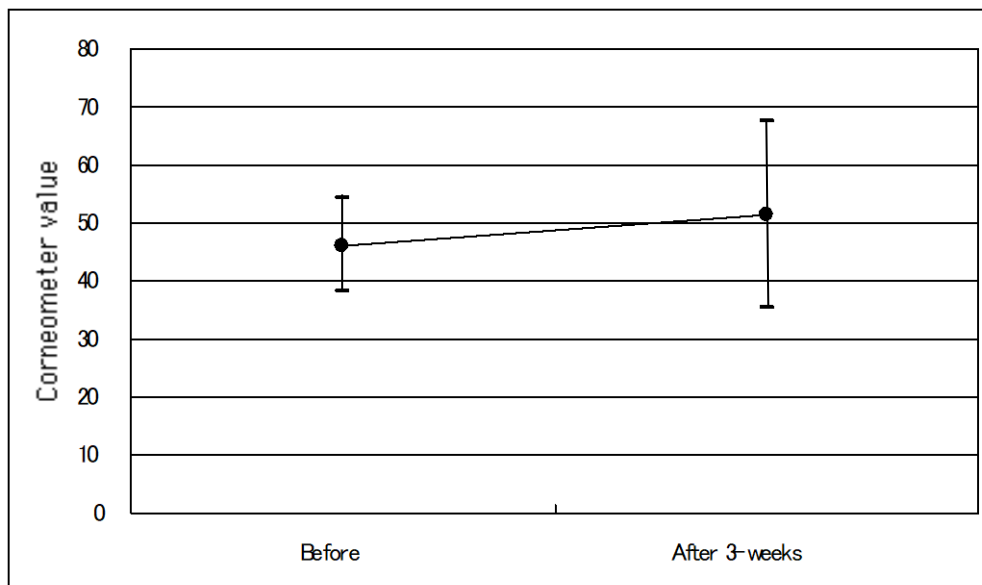
The moisture of the skin was measured around the outer corner of the eye as the test area in all subjects using a CORNEOMETER SM825 before and after 3-week administration of 2 capsules containing LITCHI SEED EXTRACT-P or dextrin per day (one capsule at a time, twice per day). The moisture of the skin was significantly higher after the 3-week administration in the LITCHI SEED EXTRACT-P group (Fig. 8) than in the placebo group (Fig.9) (*t*-test,  $p=0.04811$ ). As shown in Fig. 10, if examined individually, the moisture of the skin was improved in almost all subjects.

These results indicated that administration of LITCHI SEED EXTRACT-P was useful for increasing the moisture of the skin.



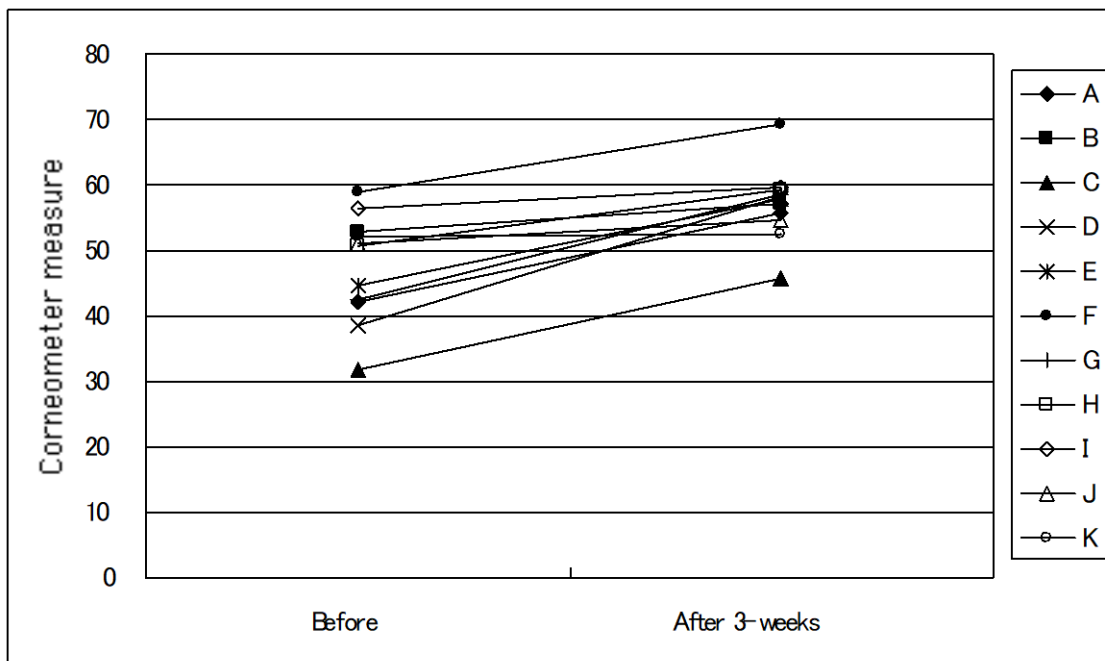
**Fig. 8 Moisturization Effect of LITCHI SEED EXTRACT**

The moisture of the skin measured using a commeter significantly increased by the 3-weeks administration of LITCHI SEED EXTRACT-P. This indicated that LITCHI SEED EXTRACT-P was useful for making the skin moist.



**Fig. 9 Moisturization Effect of Placebo (dextrin)**

In the placebo (dextrin) group, the moisture of the skin increased, but not greatly.



**Fig. 10 Improvement of Moisturization by LITCHI SEED EXTRACT**

In the LITCHI SEED EXTRACT-P group, the moisture of skin increased in almost all subjects, indicating that LITCHI SEED EXTRACT-P was useful for increasing the moisture of skin and effective in making skin beautiful.

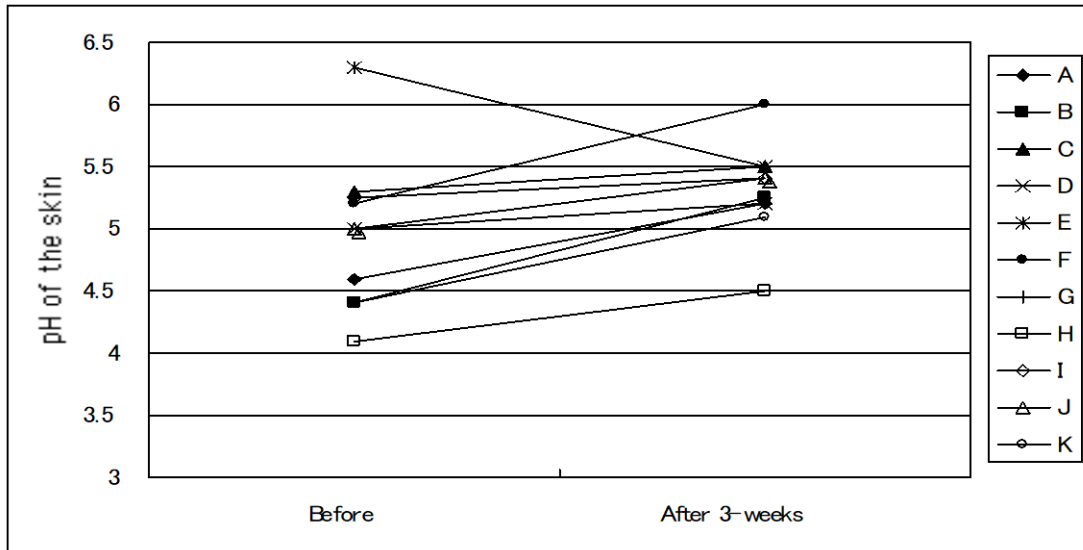
## 2. Improvement in pH of Skin

Skin pH was measured on the medial brachium as the test area in all subjects using a SKIN-pH-METER PH900 before and after 3-week administration of 2 capsules, containing LITCHI SEED EXTRACT-P or dextrin per day (one capsule at a time, twice per day).

In the placebo group, the pH of the skin increased with 3-week administration, but the degree was not large (Fig. 12). However, in the LITCHI SEED EXTRACT-P group, the pH of the skin converged within a range of 4.5-6.0, which is the range of pH for healthy skin, within the 3-weeks period of the administration. As shown in Fig. 11, almost all subjects showed ideal skin pH between 5.0 and 5.5 after the 3-week administration of the LITCHI SEED EXTRACT-P.

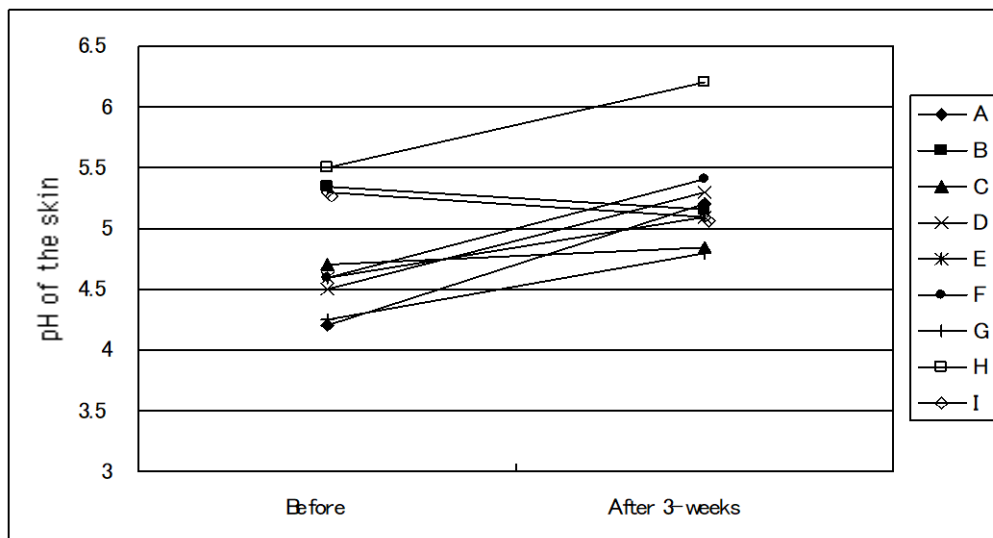
These results indicated that LITCHI SEED EXTRACT-P was useful for improving skin to normal condition by moderating the pH of the skin to a healthy level.

The pH of healthy skin is maintained at a weak acidic level (pH4.5-6.0), and the ideal pH is considered to be around the center of the range (pH5.0-5.5).



**Fig. 11 Improvement of pH by LITCHI SEED EXTRACT**

In the LITCHI SEED EXTRACT-P group, pH of before administration was widely scattered from 4.0 to 6.5, but it converged to 4.5-6.0, which is the range for normal skin, with 3-week administration. In 9 of 11 subjects, the pH became 5.0-5.5, which is the range of ideal skin.



**Fig. 12 Influence for pH of placebo (dextrin)**

In the placebo group, the of pH before administration was 4.0-5.5 (range, 1.5), and it increased with 3-weeks administration of dextrin, but the pH range was unchanged. These results suggested that the administration of dextrin was not effective.

### 3. Effects on The Amount of Skin Sebum

The amount of skin sebum was measured around the outer corner of an eye as the test area in all subjects using a SEBUMETER SM810 before and after 3-week administration of 2 capsules containing LITCHI SEED EXTRACT-P or dextrin per day (one capsule at a time, twice per day).

In both the LITCHI SEED EXTRACT group (Fig. 13) and the placebo group (Fig. 14), an increase or decrease in the amount of skin sebum was observed depending on individual subjects. There was no difference in the changes in the amount of skin sebum between the 2 groups. These results indicated that the changes in the amount of skin sebum largely varied with individual subjects.

There was no difference in the increase in the mean amount of skin sebum between the LITCHI SEED EXTRACT and placebo groups. These results suggested that the LITCHI SEED EXTRACT had little effect on the amount of skin sebum.

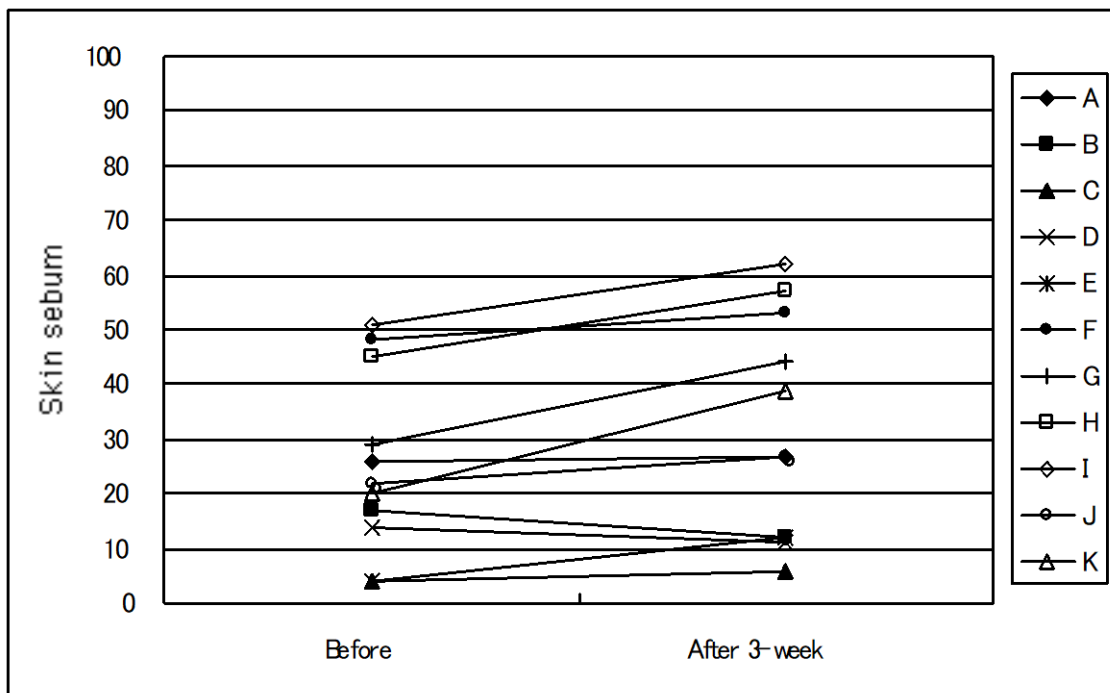
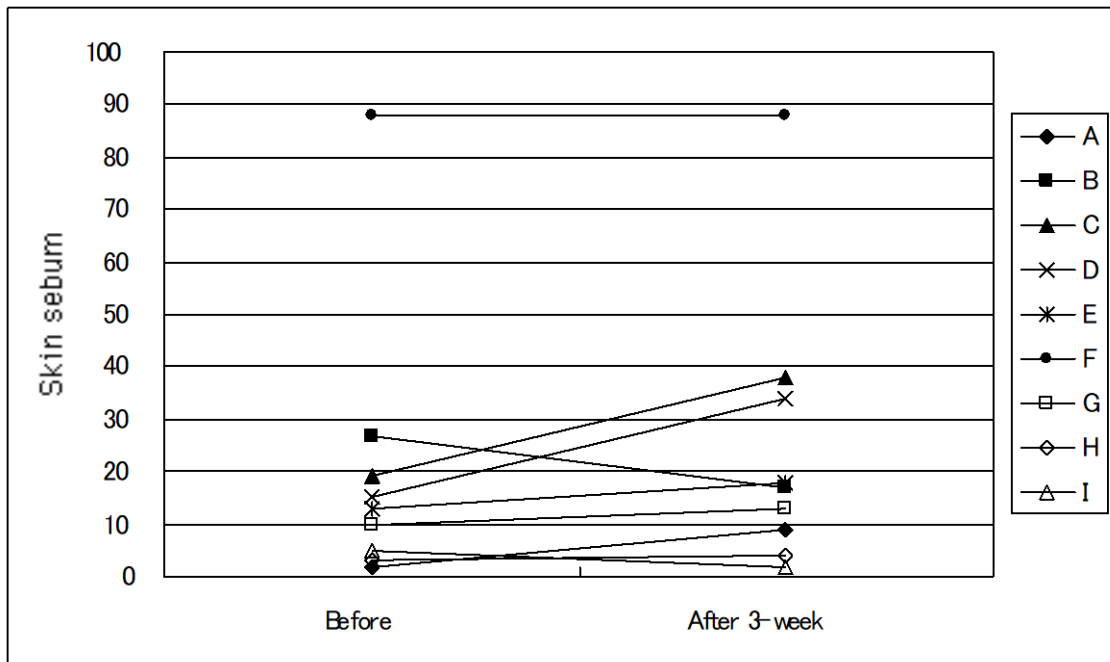


Fig. 13 Quantity of Sebum Change by LITCHI SEED EXTRACT





**Fig. 14 Quantity of Sebum Change by Placebo (dextrin)**

Since there were large differences in the amount of skin sebum between individuals, the mean was not considered to be very important, but similar increases were observed in the LITCHI SEED EXTRACT and placebo groups. LITCHI SEED EXTRACT did not affect the amount of skin sebum.

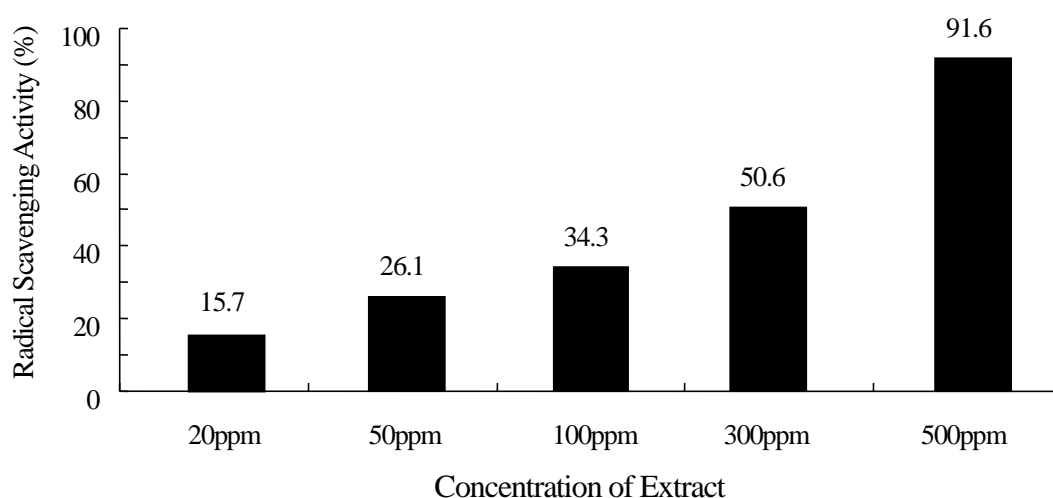
We found that LITCHI SEED EXTRACT was very useful in making skin not greasy but beautiful and healthy.

## 6 Antioxidative Activity

In the human body, the presence of active oxygen species ( $O_2^-$  radicals) causes cellular damage, which induces cancer and inflammation, and promotes aging. In particular, in the skin, active oxygen is considered to be a cause of spots, freckles, and wrinkles.

### 1. SOD-like Activity

LITCHI SEED EXTRACT showed SOD-like activity (elimination of active oxygen) and eliminated radicals. LITCHI SEED EXTRACT is expected to prevent lifestyle-related diseases caused by active oxygen.



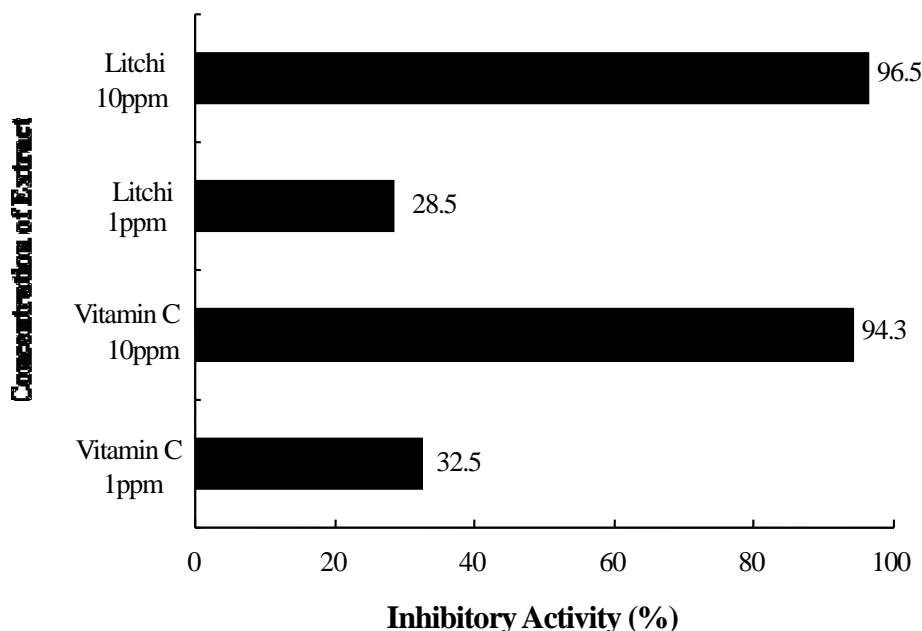
**Fig. 15 SOD-like Activity of LITCHI SEED EXTRACT**

**Table 5 SOD-like Activity of LITCHI Extracts of Various Sources**

	Radical Scavenging Activity (%)				
	50 ppm	100ppm	300 ppm	500 ppm	1000ppm
Seeds	26.1	34.3	50.6	91.6	—
Whole	—	—	—	11.7	26.5
Flesh	—	—	—	5.8	13.1
Peel	—	—	—	22.5	35.1

## 2. DPPH Radical Quinching Activity

The radical scavenging activity of LITCHI SEED EXTRACT was comparable to that of vitamin C.



**Fig. 16 DPPH Radical Scavenging Activity of LITCHI SEED EXTRACT**

**Table 6. DPPH Radical Quinching Activity of LITCHI Extracts of Various Sources**

	0.1 ppm	1 ppm	10 ppm	50 ppm	250 ppm	500 ppm
Seeds	13.5	28.5	96.5	—	—	—
Whole	—	—	—	9.9	44.3	71.1
Flesh	—	—	—	0	3.6	6.6
Peel	—	—	—	7.1	28.6	58.4

## 3. Effects of LITCHI SEED EXTRACT on The Removal of Active Oxygen in The Body

### [Protocol]

**Materials:** Caramel hard capsules containing 150 mg LITCHI SEED EXTRACT-P, 4mg silicon dioxide and 66 mg corn starch (total, 220 mg) were produced.

**Subjects:** Nine healthy subjects without regular use of drugs.

Administration

dose: Three hundred mg/day (2 capsules) in the LITCHI SEED EXTRACT group (9 subjects).

Administration

period: 3 weeks.

Examination

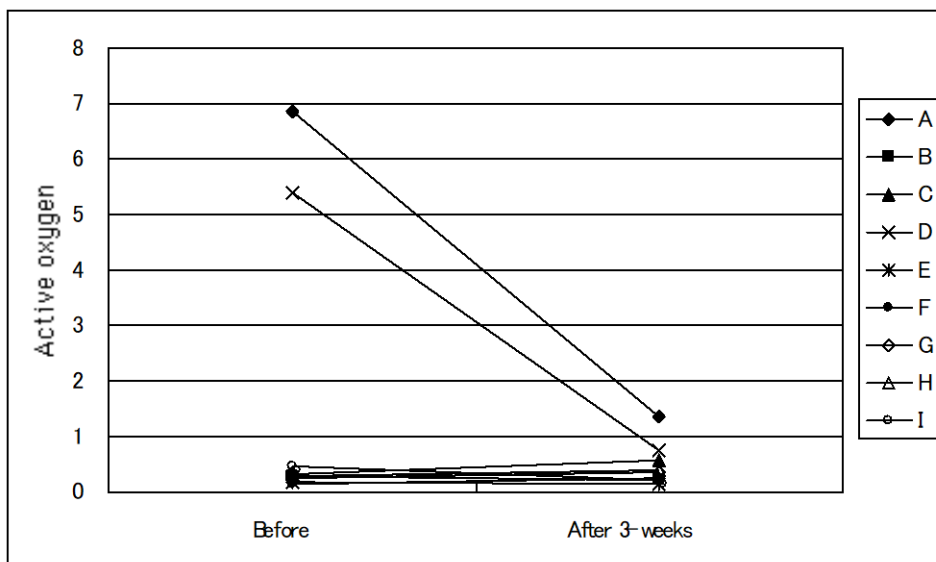
methods: The concentration of active oxygen in the body was measured by the concentration of urine malondialdehyde using an active oxygen test kit (Free Radical Test (FRT), Nihon Shokuyo Kagaku).

Materials: Urine samples.

[Results]

The concentration of malondialdehyde was measured in urine samples collected before and after 3-week administration of 2 capsules containing LITCHI SEED EXTRACT per day (one capsule at a time twice per day) using active oxygen test kits.

Fig. 17 shows that the concentration of active oxygen in the body was reduced by the 3-week administration of LITCHI SEED EXTRACT to a normal level in 2 subjects in whom it had been very high, and that the concentration of active oxygen was not increased in the other subjects in whom it had been normal. These results suggested that LITCHI SEED EXTRACT reduced the concentration of active oxygen in the body if it was abnormally high, and did not affect if it was normal.



**Fig. 17 Change in Density of Active Oxygen by LITCHI SEED EXTRACT**

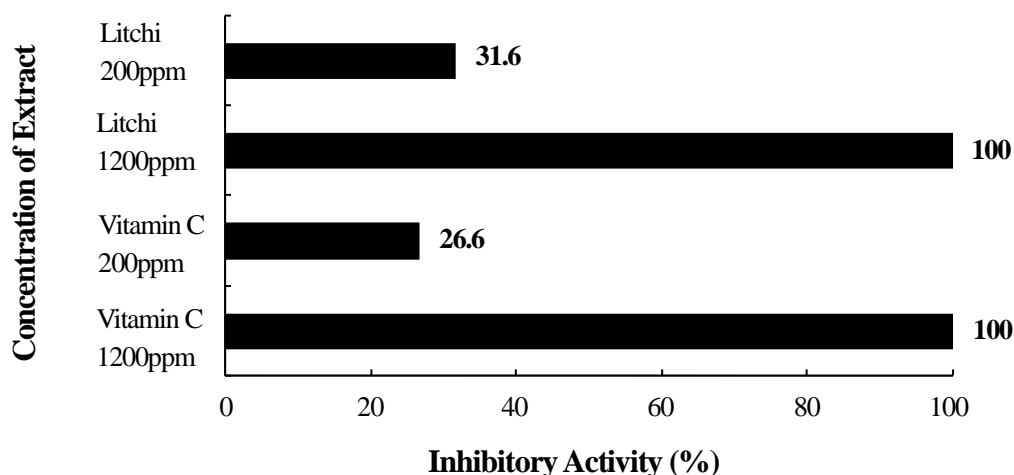
The 3-week administration of LITCHI SEED EXTRACT-P (300 mg/day) reduced the concentration of active oxygen to a level close to the normal (a level below 1) in subjects A and D in whom it was high before administration.

## (7) Skin-Whitening Effect

### 1. Inhibition of Tyrosinase

Dullness, darkness, and spots of the skin are caused by melanin. Melanin is formed from dopa quinone that is converted from tyrosine by tyrosinase. LITCHI SEED EXTRACT inhibited tyrosinase activity and appears to be applicable to foods for whitening.

This tyrosinase inhibitory activity of LITCHI SEED EXTRACT was comparable to that of vitamin C.



**Fig. 18 Inhibition of Tyrosinase Activity by LITCHI Extracts and Vitamin C**

**Table 7 Tyrosinase Inhibitory Effect of Various Parts of LITCHI Extracts**

	100 ppm	200 ppm	400 ppm	1200 ppm	2000 ppm	3000 ppm	IC <sub>50</sub>
Seeds	17.9	31.6	83.9	100	—	—	250 ppm
Whole	—	—	—	46.9	68.4	—	1100 ppm
Flesh	—	—	—	0	36.6	73.2	2300 ppm
Peel	—	—	—	31.0	—	—	>1200 ppm

### 2. Effect on Melanin Formation

LITCHI SEED EXTRACT was added to cultured B16 melanoma cells. It was found that 100 ppm of the extract suppressed melanin production by 33 percent. It was also found that the extract of seed showed the strongest suppression than the ones of other parts of litchi fruit.

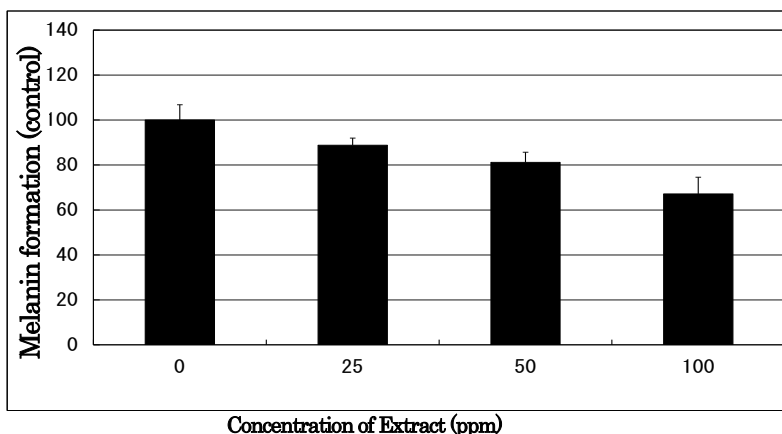


Fig. 19 Effect of LITCHI SEED EXTRACT on Melanocyte (B16) Growth

### 3. Prevention against Hyperpigmentation (*in vivo*)

Further study was prompted to examine the effect of Litchi Seed Extract on UV induced skin hyperpigmentation. Skin transparency value, L\* value of skin was measured as pigmentation index. Results showed that Litchi Seed Extract increases L\* value of skin (increases transparency of skin) significantly as compared to control group in topical and oral (Fig. 20, 21). Litchi Seed Extract is proven to possess skin lightening effect that prevent against skin hyperpigmentation.

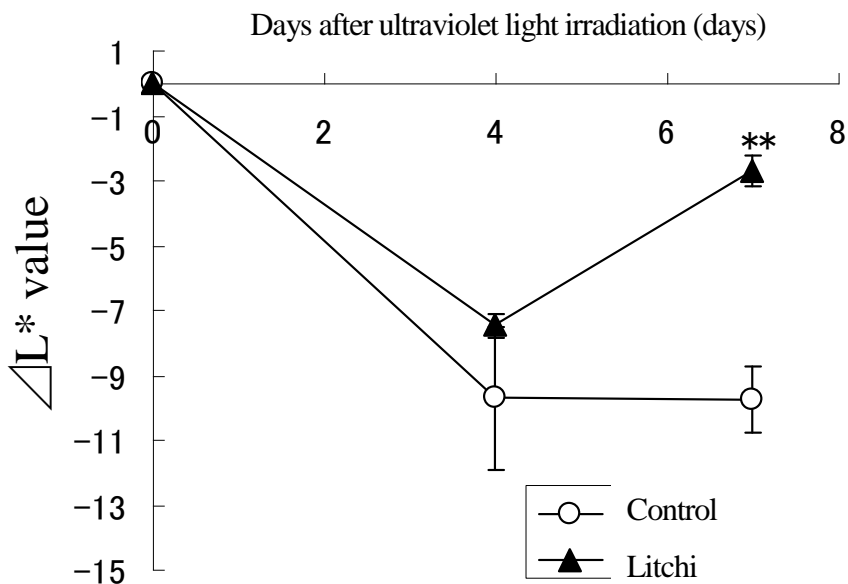
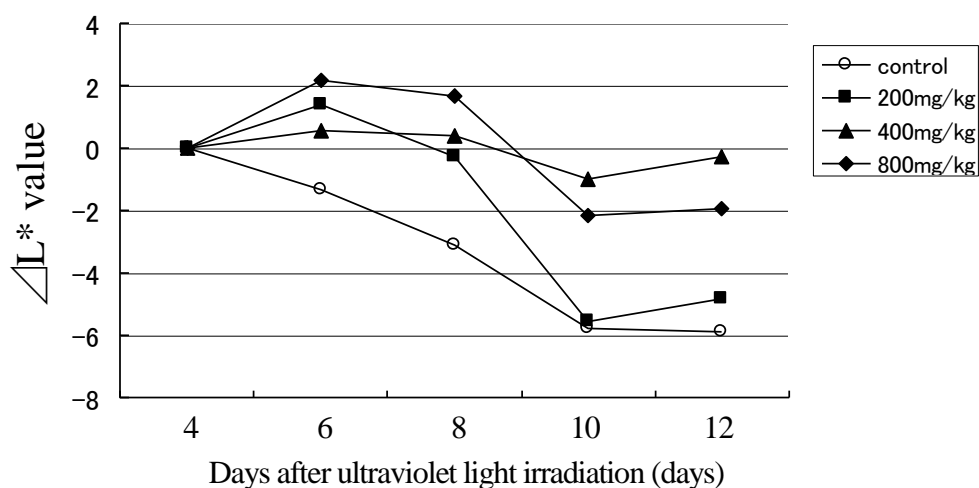
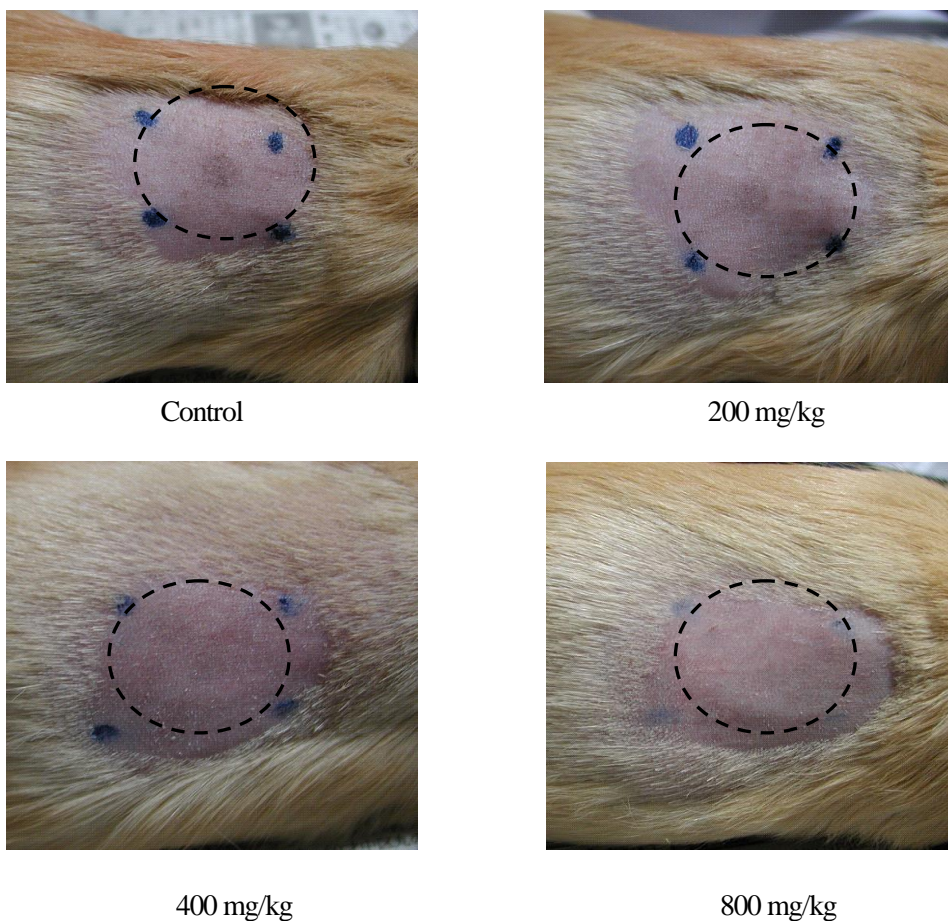


Fig. 20 The Effect of Litchi Seed Extract on UV induced skin hyperpigmentation (Topical, n=4, mean±S.E.)



**Fig. 21 The Effect of Litchi Seed Extract on UV induced skin hyperpigmentation (Oral)**



**Fig. 22 The Effect of Kiwi Seed Extract on UV induced area on day 12<sup>th</sup> (Oral)**  
**(Black circle indicates the area exposed to UV induction)**

### (8) Moisture Retention Test

After direct topical application of samples to skin, moisture retention ability was measured. When only distilled water was applied, the water content returned to the pre-application level after about 25 minutes. On the other hand, moisture was retained for more than 60 minutes after application of LITCHI SEED EXTRACT.

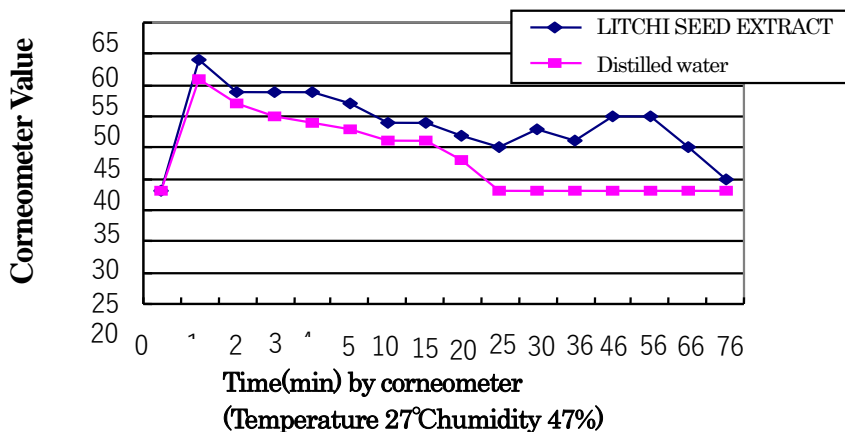
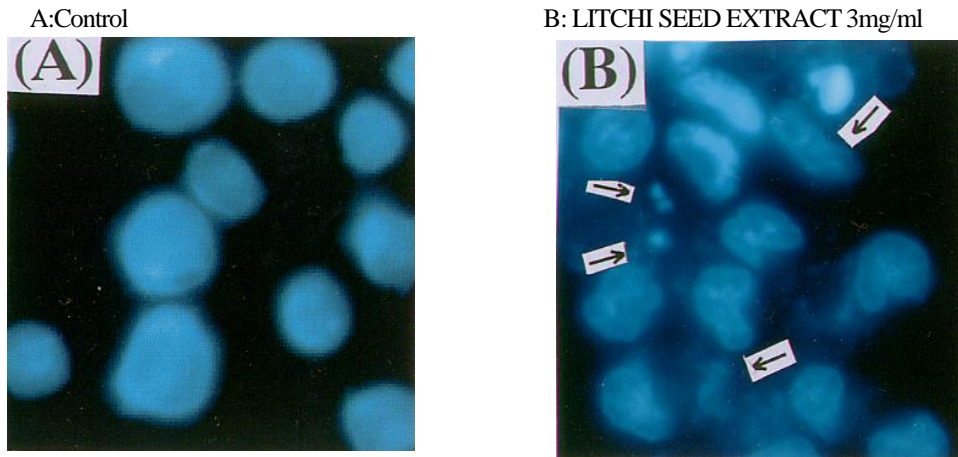


Fig. 23 Moisture Retention Test of LITCHI SEED EXTRACT

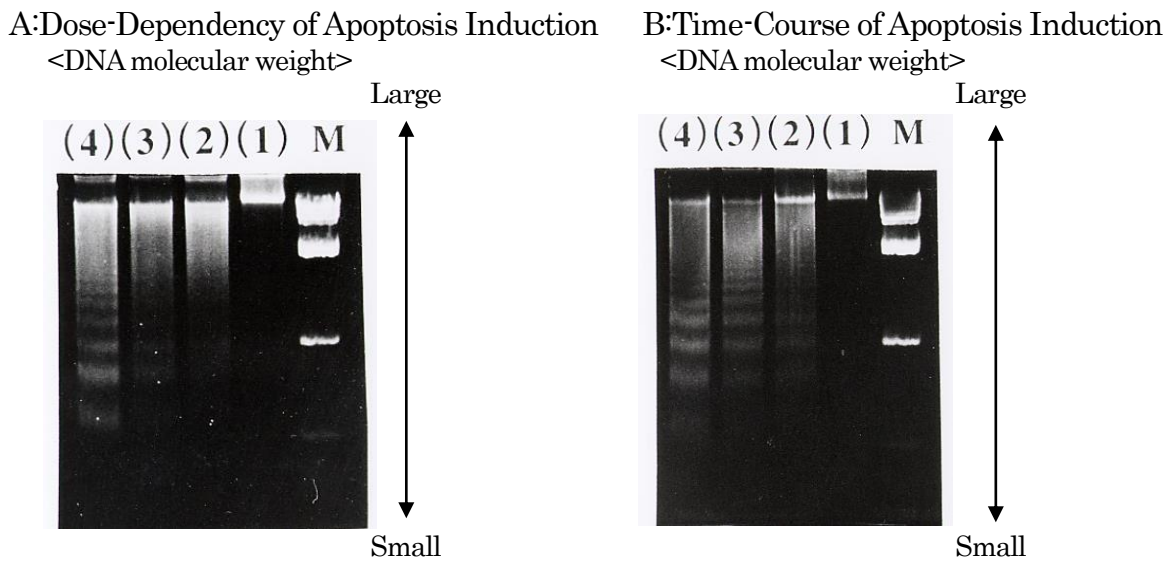
### (9) Induction of Apoptosis in Human Stomach Cancer Cells by LITCHI SEED EXTRACT

Effect of LITCHI SEED EXTRACT on induction of Apoptosis was examined in human stomach cancer cells *in vitro*. Nuclei of stomach cancer cells were stained by DAPI, and observed under fluorescent microscope equipped with a CCD camera. As a result, any change was not found in controls (Fig. 24). On the other hand, DNA fragmentation was found by LITCHI SEED EXTRACT (arrows). DNA fragmentation was confirmed in electrophoresis as well (Fig. 25). The result suggests that LITCHI SEED EXTRACT induced DNA fragmentation on cell in a dose-dependent manner.





**Fig. 24 Induction of Apoptosis by LITCHI SEED EXTRACT in Human Stomach Cancer Cells**



**Fig. 25 DNA Fragmentation by LITCHI SEED EXTRACT in Human Stomach Cancer Cells**

M: DNA marker

- (1) Control
- (2) LITCHI SEED EXTRACT (1mg/ml)
- (3) LITCHI SEED EXTRACT (2mg/ml)
- (4) LITCHI SEED EXTRACT (3mg/ml)

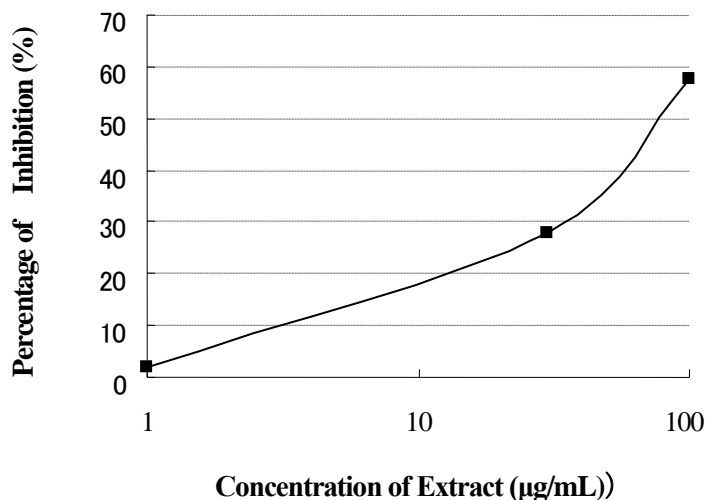
M: DNA marker

- (1) Control
- (2) LITCHI SEED EXTRACT (3mg/ml) 1day
- (3) LITCHI SEED EXTRACT (3mg/ml) 2days
- (4) LITCHI SEED EXTRACT (3mg/ml) 3days

**(10) Inhibition of Aldose Reductase**

Aldose reductase exists in various tissues where diabetes complications were found, such as in crystal lenses, retinas, peripheral nerves, kidneys, and blood vessels. Aldose reductase is involved in the occurrence and clinical conditions of diabetes. Inhibitory effect of LITCHI SEED EXTRACT on aldose reductase was examined using purified aldose reductase.

As shown in Fig. 26, 100 µg/mL of LITCHI SEED EXTRACT suppressed (57.5%) aldose reductase activity. As we previously mentioned, LITCHI SEED EXTRACT showed anti-oxidative activity in internal body. Therefore, LITCHI SEED EXTRACT is possibly prevents complications that would occur as diabetes progresses via aldose reductase inhibition.



**Fig. 26 Inhibitory Effect of LITCHI SEED EXTRACT on Aldose Reductase Activity**

**(11) Inhibitory effect on the onset of diabetes (Joint research with Professor Tanaka of Siebold University of Nagasaki)**

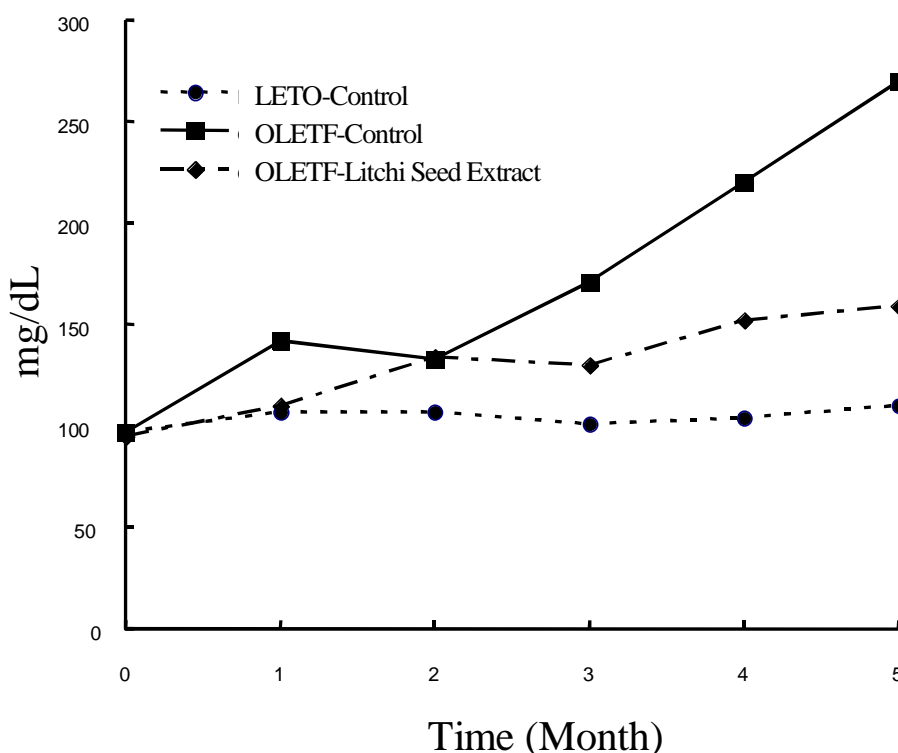
In collaboration with Professor Tanaka of Siebold University of Nagasaki, we studied effects of litchi seed extract on inhibition of the onset of diabetes using rats.

As a result, as shown in Figure 27, blood glucose levels of LETO rats (not develop diabetes) was a low value throughout the feeding period. On the other hand, blood glucose levels of OLETF rats (develop diabetes) increased with time, and the onset of diabetes. Litchi seed extract was significantly lower than blood glucose value of OLETF rats Therefore, litchi seed extract has the effect of suppressing rise in blood glucose level.

In addition, litchi seeds also have been reported that suppressing of blood glucose level in mouse by subcutaneous injection<sup>1)</sup>.

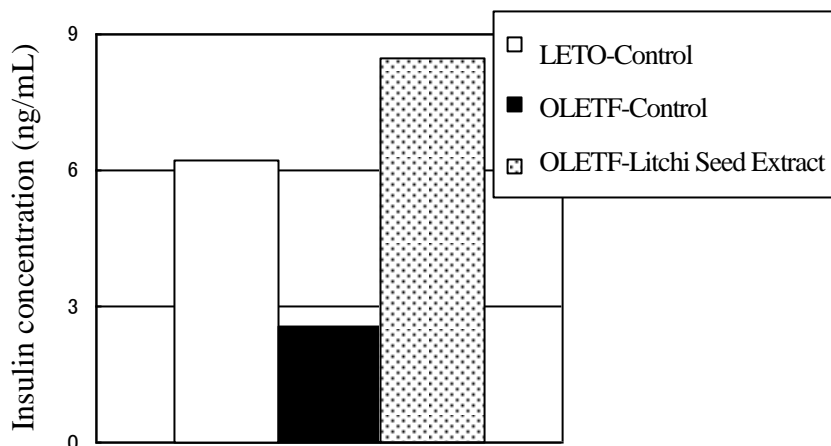
References;

- 1) D. O. Gray, L. Fowden.  $\alpha$ -(Methylenecyclopropyl)glycine from *Litchi* Seeds. *Biochem. J.* **82**, 385, (1962).



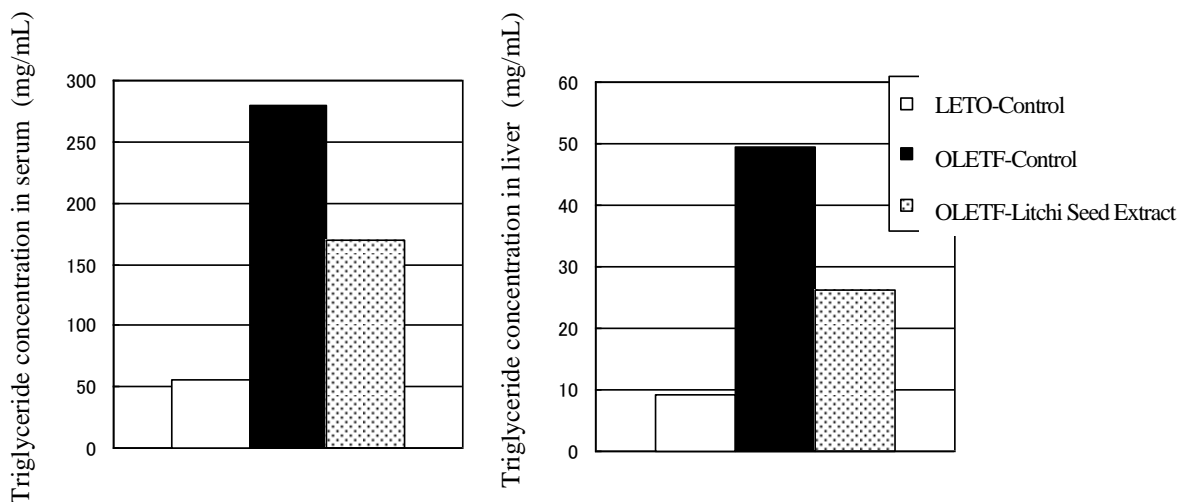
**Fig. 27 Inhibitory effect of LITCHI SEED EXTRACT on blood glucose level**

Serum insulin concentration of OLETF rats was reduced after 5 months. Insulin levels of OLETF rats fed litchi seed extract is a high value for the same level as the LETO (Fig. 28). Therefore, litchi seed extract is to inhibit the function of  $\beta$ -cell pancreatic islet injury due to diabetes, to suppress the decrease in insulin secretion has been observed.



**Fig. 28 Effect of LITCHI SEED EXTRACT on insulin concentration**

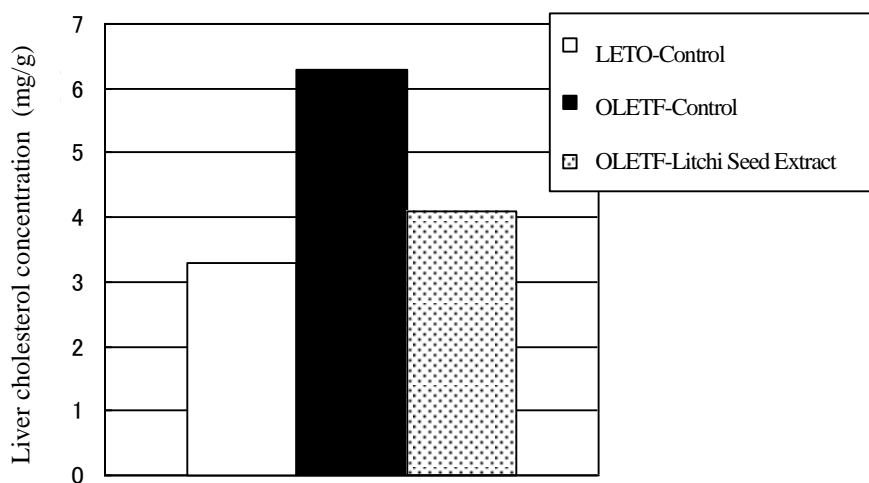
Triglyceride concentration in serum and liver of OLETF rat was significantly elevated for the LETO rats, litchi seed extract was effectively reduces the concentration of serum and liver triglyceride in OLETF rat (Fig. 29).



**Fig. 29 Effect of LITCHI SEED EXTRACT on triglyceride concentration in serum and liver**

Liver cholesterol concentration of OLETF rat was significantly elevated for the LETO rats, litchi seed extract was effectively reduces the concentration of liver cholesterol concentration in OLETF rat (Fig. 30).

From the above results, litchi seed extract, it is to effectively suppress the onset of diabetes. It has also been suggested that improvement has the effect of lipid metabolism in animals with a predisposition to type 2 diabetes in genetically lowering the lipid concentration.



**Fig. 30 Effect of LITCHI SEED EXTRACT on liver cholesterol concentration**

## 5. Stability of LITCHI SEED EXTRACT

### 1. Thermal Resistance

No pyrolysis of LITCHI SEED EXTRACT occurred at normal food processing temperatures for 60 minutes.

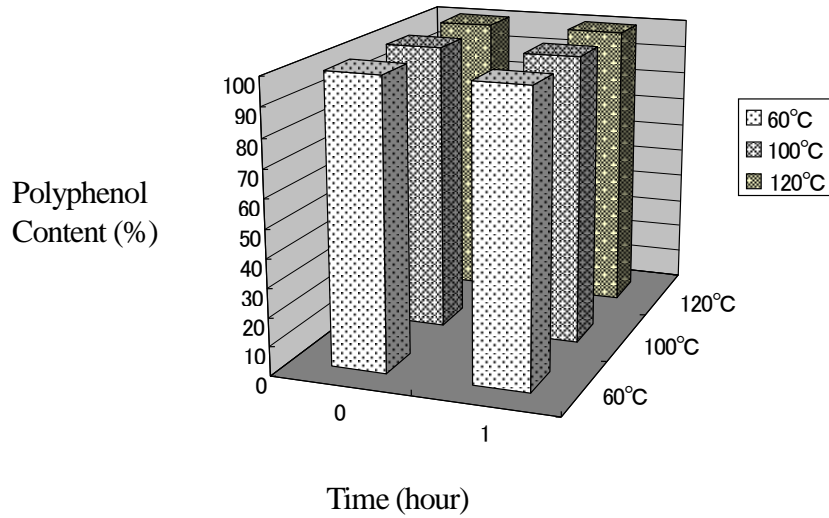


Fig. 31 Heat Resistance of LITCHI SEED EXTRACT

### 2. pH Stability

Polyphenols in LITCHI SEED EXTRACT remains stable especially less than pH 8.

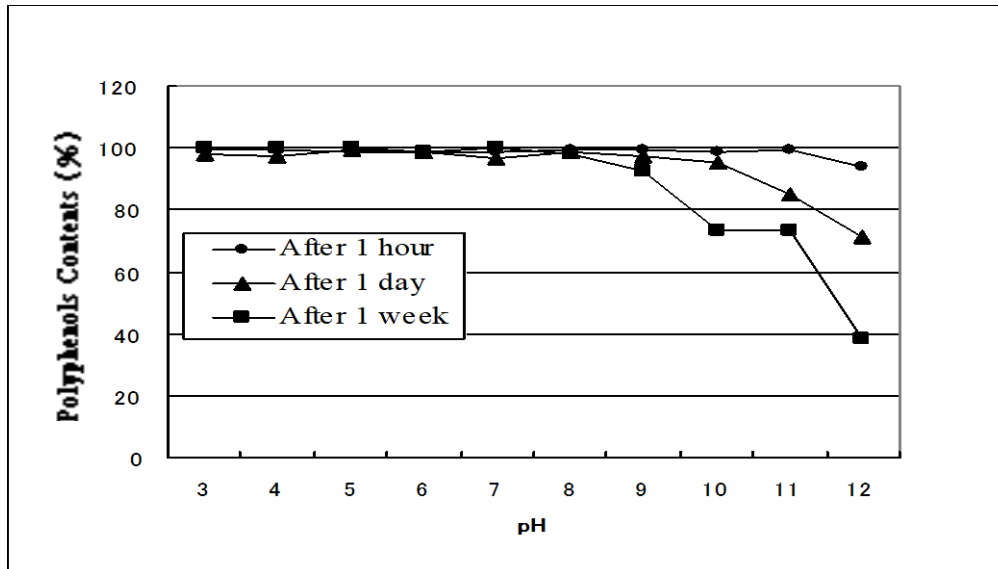


Fig. 32 pH Stability of Polyphenols Contents

## 6. Daily Recommended Dosage

We recommend to take more than 150~300 mg/day of LITCHI SEED EXTRACT-P.

## 7. Nutrition Information (Litchi Seed Extract-P)

Items Analyzed	Result
Water	5.0g/100g
Protein* <sup>1</sup>	2.6g/100g
Fat	0.8g/100g
Ash	2.3g/100g
Available carbohydrate* <sup>2</sup>	88.8g/100g
Energy* <sup>3</sup>	373kcal/100g
Dietary Fiber	0.5g/100g
Sodium	11.2mg/100g

\*1 N×6.25

\*2 100-(Moisture + Protein + Fat + Ash + Dietary fiber)

\*3 Factors for the energy value: Protein - 4, Fat - 9,

Available carbohydrate - 4

Tested institute: Japan Food Research Center Foundation

Research result issue number: 301080295-001

## 8. Safety

### (1) Residual Agricultural Chemicals

LITCHI SEED EXTRACT is conformed to regulation stipulated for 447 residual agricultural chemical compounds. No residual agricultural chemicals were detected as confirmed by test trustee.

Test trustee : Masis Co. Ltd.

Data : October 26, 2006

Report No. : 7912

### (2) Acute Toxicity

Five weeks old mice were given LITCHI SEED EXTRACT (5000 mg/kg) orally and then fed a laboratory chow for 2 weeks. No toxicity was observed, thus the LD50 in mice was estimated more than 5000 mg/kg

### **(3) Sub-acute Toxicity**

LITCHI SEED EXTRACT was orally administered to male and female rats at 75-300 mg/kg and kept for 28 days. No abnormalities and fatal event were observed at 75-300 mg/kg. Upon autopsy no abnormalities were observed.

### **(4) Acute Eye Irritation Study in Rabbit**

The solution of LITCHI SEED EXTRACT (0.1 ml) was applied into the conjunctival sac of the left eye of 3 rabbits. The conjunctival of iris and corneal lesions were observed approximately 1, 24, 48 and 72 hours after instillation.

Under the experimental condition, LITCHI SEED EXTRACT was found to be non-irritant for eyes of the rabbits.

### **(5) Acute Skin Irritation Study in Rabbit**

The solution of LITCHI SEED EXTRACT (0.5 ml) was applied on the skin of 3 rabbits for 4 hours. The treated lesions were observed approximately 1, 24, 48 and 72 hours after removal of the dressing.

Under the experimental conditions, LITCHI SEED EXTRACT was found to be non-irritant for skin of rabbits.

### **(6) Skin Sensitisation Study in Guinea Pig**

The examination was performed according to the technique of Magnusson-Kligman (1969) and Guillot and Coll. (1983). The sensitivity and the reliability of the experimental method are verified using dinitrochlorobenzene (DNCB) as a positive control.

Under the experimental condition, the test substance showed only minimal allergic sensitivity. According to the terminology, it was considered that LITCHI SEED EXTRACT is free of any sensitising capacity in the guinea-pig.

### **(7) Mutagenicity Test**

Ames test was performed with/without S9mix using *Salmonella* strains of TA1535, TA1537, TA98, TA100 and *E. coli* strain WP2uvrA. LITCHI SEED EXTRACT showed no mutagenicity at concentrations of 50 to 5,000 µg/plate.

### **(8) Chromosomal Aberration Test**

The clastogenic property of LITCHI SEED EXTRACT was examined using CHL (Chinese hamster lung) cells. LITCHI SEED EXTRACT-LC did not cause abnormal cells in any conditions examined; short-time (6 hr) test (3.9 to 62.5 µg/ml) without S9mix, short-time (6 hr.) test (31.25 to 1,000 µg/ml) with S9mix, and long-time (24 hr.) test (3.9 to 46.9 µg/ml) without S9mix, long-time (24 hr) test (3.9 to 46.9 µg/ml) with S9mix.



### (9) Phototoxicity Test

Following the guideline, phototoxicity test was performed using mouse fibroblast (Balb/c 3T3 A31). LITCHI SEED EXTRACT-LC (40.0 to 100.0 µg/ml) was added to cells of under confluency. Cells were incubated for 1 hour, then exposed to UVA/visible light (5 J/cm<sup>2</sup>). The control group was not exposed to light. After the exposure, culture medium was changed, then cells were cultured over night. Viability was measured by an MTT method. The viability became lower in neither group, proving that LITCHI SEED EXTRACT-LC has no phototoxicity.

### (10) Photosensitization Test

Photosensitization was examined using male guinea pigs with complete Freund's adjuvant. Three weeks after sensitization, LITCHI SEED EXTRACT-LC (0.25 ml) was applied to topical areas and then the UV light was exposed to the area. We visually checked the areas 24 and 48 hours after the exposure to light. No sensitization on local skin of guinea pigs were found.

### (11) Patch Test

0.025 ml of LITCHI SEED EXTRACT -LC was spread over film in a circle of 1-cm diameter. The film was patched on 13 women aged between 22 and 61, and 7 men aged between 22 and 54 for 48 hours. No irritation on skin of human were found.

## 9. Ecocert registration

LITCHI SEED EXTRACT-PC (Litchi Chinensis Seed Extract, Dextrin) is registered Ecocert (organic product certification body based in France).

## 10. Applications

Applications	Examples
Confectionery	Candies, Gum, Cookies, Pudding, Jelly, Yogurt, Chocolate, etc...
Cosmetics	Base cosmetics (Lotion, Milk, Cream, and so on) Body cosmetics (Body lotion, Body cream, and so on) Cleansing cosmetics (Soap, and so on) Makeup cosmetics (Lipstick, Foundation, and so on)
Others	Functional foods, Nutraceutical foods, and Health foods

## 11. Packaging

**LITCHI SEED EXTRACT-P, WSP** (Powder, Food Grade)

**LITCHI SEED EXTRACT-PC, WSPC** (Powder, Cosmetic Grade)

5kg Interior packaging : aluminum-coated plastic bag

Exterior packaging : cardboard box

**LITCHI SEED EXTRACT-LC** (Liquid, Cosmetic Grade)

5kg Interior packaging : cubic polyethylene container

Exterior packaging : cardboard box

## 12. Storage

Store in cool, dry place. Avoid humidity.

## 13. Expression of LITCHI SEED EXTRACT

<Food>

LITCHI SEED EXTRACT-P, WSP

Example: LITCHI SEED EXTRACT

<Cosmetic>

	LITCHI SEED EXTRACT-PC	LITCHI SEED EXTRACT-LC
INCI Name	Litchi Chinensis Seed Extract Dextrin	Water Butylene Glycol Litchi Chinensis Seed Extract

\*Please refer to your nation's standard.

## 14. Patent of LITCHI SEED EXTRACT

Patent number	Title of invention
3027427	Cosmetic (Although this is a patent of other company, we have obtained the permission to use.)
3027428	Cosmetic (Although this is a patent of other company, we have obtained the permission to use.)
3708036	Composition for beautiful skin
3953387	Elastase inhibitor
4982052	Composition for beautiful skin

## Test Methods

### **Fig. 2, 3 Inhibition of Collagenase by LITCHI SEED EXTRACT**

LITCHI SEED EXTRACT was dissolved in distilled water, and the amount of the severance of PZ-peptides produced by collagenase was measured. The absorbance of the ethyl acetate layer was measured.

### **Fig. 4 Effects of LITCHI SEED EXTRACT on Collagen Productivity**

Human fibroblast was grown in EAGLE at 37 °C under humidified 5%CO<sub>2</sub>. Twenty-five ppm of extracts from Litchi Seed Extract was added to normal human fibroblasts, and incubated for 3 days. Pro-collagen I C-terminal peptide (PIP) in culture media was quantified by ELISA.

### **Fig. 5 Inhibition of Elastase by LITCHI SEED EXTRACT**

LITCHI SEED EXTRACT was dissolved in distilled water. Elastase was added to elastin and incubated. Then, fluorescence was measured with excitation at 485 nm and emission at 530 nm.

### **Fig. 6 Inhibition of Hyaluronidase by LITCHI SEED EXTRACT**

LITCHI SEED EXTRACT was dissolved in distilled water, and hyaluronic acid was hydrolyzed by hyaluronidase. After reaction with *p*-dimethylamino- benzaldehyde, absorbance was measured.

### **Fig.7, 8, 9 Moisturization Effect of LITCHI SEED EXTRACT**

Twenty healthy man and woman were involved in examination. Moisture of skin was measured around the outer corner of an eye as the test area in all subjects using a CORNEOMETER SM825 (CK electric GmbH) before and after 3-week administration of 2 capsules containing LITCHI SEED EXTRACT-P or dextrin as placebo per day (one capsule at a time, twice per day).

### **Fig.10, 11 The pH Measurement of Skin**

Twenty healthy man and woman were involved in examination. pH of skin was measured on medial brachium as the test area in all subjects using a SKIN-pH-METER PH900(CK electric GmbH) before and after 3-week administration of 2 capsules containing LITCHI SEED EXTRACT-P or dextrin as placebo per day (one capsule at a time, twice per day).

### **Fig.12,13 The measurement of Quantity of Skin Sebum**

Twenty healthy man and woman were involved in examination. The amount of skin sebum was measured around the outer corner of the eye as the test area in all subjects using a SEBUMETER SM810 (CK electric GmbH) before and after 3-week administration of 2 capsules containing LITCHI SEED EXTRACT-P or dextrin as placebo per day (one capsule at a time, twice per day).

### **Fig. 14 SOD-like Activity of LITCHI SEED EXTRACT**

LITCHI SEED EXTRACT was dissolved in distilled water. SOD-like activity was measured using an SOD test kit (Wako Pure Chemicals).

### **Fig. 15 DPPH Radical Scavenging Activity of LITCHI SEED EXTRACT**

LITCHI SEED EXTRACT was dissolved in 70% ethanol. LITCHI SEED EXTRACT was added to DPPH (1,1-diphenyl-2-picrylhydrazyl) solution, and the fading of the DPPH solution was measured in terms of absorbance.

### **. Fig.16 Active oxygen density change by recipe of LITCHI SEED EXTRACT**

We examined it for 9 healthy men. The concentration of malondialdehyde was measured in urine samples collected before and after 3-weeks administration of 2 capsules containing

LITCHI SEED EXTRACT-P per day (one capsule twice per day) using active oxygen test kits. This examination measured it using Free Radical Test (FRT, Nihon Shokuyo Kagaku).

**Fig. 17 Inhibition of Tyrosinase Activity by LITCHI SEED EXTRACT**

LITCHI SEED EXTRACT was dissolved in distilled water. After addition of the extract to mushroom-derived tyrosinase solution, the oxidative reaction from L-tyrosine to dopa quinone was measured in terms of the absorbance of dopa quinone.

**Fig. 18 Effect of LITCHI SEED EXTRACT on Melanocyte (B16) Growth**

Five hundred  $\mu\text{L}$  of B16 cells solution ( $1.8 \times 10^5$  cells/ml) were plated in 24-well plates in MEM medium (10% FBS, penicillin/streptomycin) and 2 mM theophylline. Sample solution (55  $\mu\text{L}$ ) was added to cells, and they cultured for three days. Then medium was removed, and cells were fractured by ultrasonication in 300  $\mu\text{L}$  of PBS. The absorbance (measurement wavelength: 415 nm, reference wavelength: 700 nm) was measured by a microplate reader. The prevention ratio (%) was calculated by the equation that [absorbance of the sample/absorbance of the control x 100].

**Fig. 19 Moisture Retention Test of LITCHI SEED EXTRACT**

The epidermal moisture content was measured. LITCHI SEED EXTRACT was dissolved in distilled water to obtain 1% solution. One drop of this solution was topically applied to the medial side of the left brachium. The drop was spread over a 2-cm<sup>2</sup> area and absorbed into the skin. After another 1 minute, the solution on the surface was absorbed using paper. After another 1 minute, measurement was initiated using a Corneometer CM825 (temperature, 27 °C; Relative humidity, 47%).

**Fig.20 Effects of Apoptosis Induction in Human Stomach Cancer Cells by LITCHI SEED EXTRACT**

Human stomach cancer cells were grown in RPMI1640 medium containing 10 % FBS at 37 °C under humidified 5%CO<sub>2</sub>. After cultivation for 3 days in the presence of vehicle, LITCHI SEED EXTRACT (3 mg/ml). The morphology of the cells was examined by a epifluorescence microscope equipped with a CCD camera digital imaging system

**Fig. 21 The Effect of Litchi Seed Extract on UV induced skin hyperpigmentation (Oral)**

Brown Weiser-Malpes guinea pigs, male, aged 4weeks were fed orally Litchi Seed Extract of different dosages 7 days (P.O. day -7) prior to UV-B irradiation. UV-B induction was introduced for 3 days (from day 0 – 2) to guinea pigs at 2000mJ/cm<sup>2</sup> using a USHIO Optical Modulex (Ushio, Inc Japan). Feeding of Litchi Seed Extract continued during UV induction period towards the end of the protocol period (day 0 – 12). Transparency of the skin, L\* value was measured using a spectro-color meter (Nippon Denshoku Industries Co., Ltd. Tokyo) on day 4, 6, 8, 10 and 12 after UV induction.

**Fig.25 DNA Fragmentation Effect in Human Stomach Cancer Cells by LITCHI SEED EXTRACT**

Exponentially growing human stomach cancer cells were placed at the initial density of 5X10<sup>5</sup>cells/ml in culture flasks. After cultivation for 3days in the presence of vehicle,1,2,3 mg/mL of LITCHI SEED EXTRACT.

After cultivation in the presence of LITCHI SEED EXTRACT for 1, 2, or 3 days, cells were pelleted by slow centrifugation. DNA was isolated from the cell pellets as described previously. Equivalent amounts of DNA were put into the well of 2% agarose gel and electrophoresed in 40mM Tris-acetic acid (PH 7.5) containing 2mM EDTA.

**Fig.26 Inhibition of Aldose Reductase**

To examine the inhibition of aldose reductase activity,0.18 M phosphate buffer solution (pH 7.0, 500  $\mu\text{L}$ ), 1.5 mM NADPH (100  $\mu\text{L}$ ), 100 mM DL-glyceraldehyde (100  $\mu\text{L}$ ), water (295

$\mu\text{L}$ ), and DMSO-dissolved sample (10  $\mu\text{L}$ ) were mixed and preheated at 30°C for five minutes. One unit/mL of aldose reductase (5  $\mu\text{L}$ , Wako Pure Chemical) was added, then incubated at 30°C for 30 min. Reaction was stopped by cooling on ice. The absorbance was measured at wavelength of 340nm.

**Figs. 31, 32 Polyphenol Content**

Samples prepared in distilled water, and their polyphenol were quantified by the Folin-Denis method described in the Food Function Study Method. Gallic acid was used as a standard.

PRODUCT STANDARD

PRODUCT NAME

**LITCHI SEED EXTRACT-P**

(FOOD)

This product is extracted from Litchi seed, the seeds of *Litchi Chinensis Sonn. (Sapindaceae)* with aqueous ethanol. It guarantees a minimum of 12.0% polyphenols.

<u>1. Appearance</u>	Red brown powder with lightly unique smell.	
<u>2. Polyphenols</u>	Min. 12.0%	(Folin-Denis method)
<u>3. Loss on Drying</u>	Max. 8.0%	(Analysis for Hygienic Chemists, 1g, 105 °C, 2h)
<u>4. Purity Test</u>		
(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)
<u>5. Standard Plate Counts</u>	Max. $1 \times 10^3$ cfu/g	(Analysis for Hygienic Chemists)
<u>6. Moulds and Yeasts</u>	Max. $1 \times 10^2$ cfu/g	(Analysis for Hygienic Chemists)
<u>7. Coliforms</u>	Negative	(Analysis for Hygienic Chemists)
<u>8. Composition</u>	Ingredients	Contents
	<hr/>	
	Litchi Seed Extract	50 %
	Dextrin	50 %
	<hr/>	
	Total	100 %
<u>9. Expiry date</u>	2 years from date of manufacturing	
<u>10. Storage</u>	Store it in a cool, dry, ventilated area with desiccant. Keep it away from high Temperature and sunlight, and store it in a closed container.	

PRODUCT STANDARD

PRODUCT NAME

**LITCHI SEED EXTRACT-WSP**  
(FOOD)

This product is extracted from Litchi seed, the seeds of *Litchi Chinensis Sonn. (Sapindaceae)* with aqueous ethanol. It guarantees a minimum of 12.0% polyphenols. This product is water-soluble.

<u>1. Appearance</u>	Red brown powder with lightly unique smell.	
<u>2. Polyphenols</u>	Min. 12.0%	(Folin-Denis method)
<u>3. Loss on Drying</u>	Max. 8.0%	(Analysis for Hygienic Chemists, 1g, 105 °C, 2h)
<u>4. Purity Test</u>		
(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)
<u>5. Standard Plate Counts</u>	Max. $1 \times 10^3$ cfu/g	(Analysis for Hygienic Chemists)
<u>6. Moulds and Yeasts</u>	Max. $1 \times 10^2$ cfu/g	(Analysis for Hygienic Chemists)
<u>7. Coliforms</u>	Negative	(Analysis for Hygienic Chemists)
<u>8. Composition</u>	Ingredients	Contents
	Litchi Seed Extract	60 %
	Dextrin	40 %
	Total	100 %
<u>9. Expiry date</u>	2 years from date of manufacturing	
<u>10. Storage</u>	Store it in a cool, dry, ventilated area with desiccant. Keep it away from high Temperature and sunlight, and store it in a closed container.	

PRODUCT STANDARD

PRODUCT NAME

**LITCHI SEED EXTRACT-PC**

(COSMETIC)

This product is extracted from Litchi seed, the seeds of *Litchi Chinensis Sonn. (Sapindaceae)* with aqueous ethanol. It guarantees a minimum of 12.0% polyphenols.

<u>1.Appearance</u>	Red brown powder with lightly unique smell.	
<u>2.Polyphenols</u>	Min. 12.0%	(Folin-Denis method)
<u>3.Loss on Drying</u>	Max. 8.0 %	(1g, 105 °C, 2 hours)
<u>4.Purity Test</u>		
(1)Heavy Metals	Max. 10 ppm	(The Second Method)
(2)Arsenic	Max. 1 ppm	(The Third Method)
<u>5.Standard Plate Counts</u>	Max. $1 \times 10^2$ cfu/g	(Analysis for Hygienic Chemists)
<u>6.Moulds and Yeasts</u>	Max. $1 \times 10^2$ cfu/g	(Analysis for Hygienic Chemists)
<u>7.Coliforms</u>	Negative	(Analysis for Hygienic Chemists)

<u>8.Composition</u>	Ingredients	Contents
	Litchi Chinensis Seed Extract	50 %
	Dextrin	50 %
	Total	100 %

9. Expiry date 2 years from date of manufacturing

10. Storage Store it in a cool, dry, ventilated area with desiccant. Keep it away from high Temperature and sunlight, and store it in a closed container.

Ref: The Japanese Standards of Quasi-Drug Ingredients.



PRODUCT STANDARD

PRODUCT NAME

**LITCHI SEED EXTRACT-WSPC**

(COSMETIC)

This product is extracted from Litchi seed, the seeds of *Litchi Chinensis Sonn. (Sapindaceae)* with aqueous ethanol. It guarantees a minimum of 12.0% polyphenols. This product is water-soluble.

<u>1. Appearance</u>	Red brown powder with lightly unique smell.	
<u>2. Polyphenols</u>	Min. 12.0%	(Folin-Denis method)
<u>3. Loss on Drying</u>	Max. 8.0%	(Analysis for Hygienic Chemists, 1g, 105 °C, 2h)
<u>4. Purity Test</u>		
(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)
<u>5. Standard Plate Counts</u>	Max. $1 \times 10^2$ cfu/g	(Analysis for Hygienic Chemists)
<u>6. Moulds and Yeasts</u>	Max. $1 \times 10^2$ cfu/g	(Analysis for Hygienic Chemists)
<u>7. Coliforms</u>	Negative	(Analysis for Hygienic Chemists)
<u>8. Composition</u>	<b>Ingredients</b>	<b>Contents</b>
	Litchi chinensis seed extract	60 %
	Dextrin	40 %
	<b>Total</b>	<b>100 %</b>
<u>9. Expiry date</u>	2 years from date of manufacturing	
<u>10. Storage</u>	Store it in a cool, dry, ventilated area with desiccant. Keep it away from high Temperature and sunlight, and store it in a closed container.	

PRODUCT STANDARD

PRODUCT NAME

**LITCHI SEED EXTRACT-LC**  
(COSMETIC)

This product is extracted from Litchi seed, the seeds of *Litchi Chinensis Sonn. (Sapindaceae)* with aqueous 1,3-butylene glycol. It guarantees polyphenols of anthocyanin and flavonoid.

<u>1.Appearance</u>	Yellow red brown liquid with no smell and lightly unique smell.	
<u>2.Certification Test</u>		
(1)Anthocyanin	The solution of 1drop in 5ml of methanol add 0.2ml of hydrochloric acid, heat to boiling, the color of the solution changes to red.	
(2)Flaboniod	Dissolve 1ml of the solution in methanol to make 50ml. To a solution of 2ml in 0.1g of magnesium (ribbon) add 1ml of hydrochloric acid, sonicate for 1 to 2 minutes, the color of the solution changes to red.	
(3) Saponin	The solution of 1drop in 5ml of acetic anhydride add calmly 1ml of sulfuric acid, the color of the solution changes to dark red brown.	
(4)Tannin	Add 1ml of the solution to 1-2drop of Iron (III) chloride anhydrous reagent(9 in 100), to black-green.	
<u>3.Polyphenols</u>	Min.0.20%	(Folin-Denis method)
<u>4.pH</u>	4.10 - 6.00	(10% Solution)
<u>5.Specific Gravity</u>	1.010 - 1.060	(25 °C)
<u>6.Purity Test</u>		
(1)Heavy Metals	Max. 10 ppm	(The Second Method)
(2)Arsenic	Max. 1 ppm	(The Third Method, Apparatus B)
<u>7.Standard Plate Counts</u>	Max. $1 \times 10^2$ cfu/g	(Analysis for Hygienic Chemists)
<u>8.Moulds and Yeasts</u>	Max. $1 \times 10^2$ cfu/g	(Analysis for Hygienic Chemists)
<u>9.Coliforms</u>	Negative	(Analysis for Hygienic Chemists)
<u>10.Composition</u>	Ingredients	Contents
	Water	50 %
	Butylene Glycol	49 %
	Litchi Chinensis Seed Extract	1 %
	Total	100 %

Ref: The Japanese Standards of Quasi-Drug Ingredients.

**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 :

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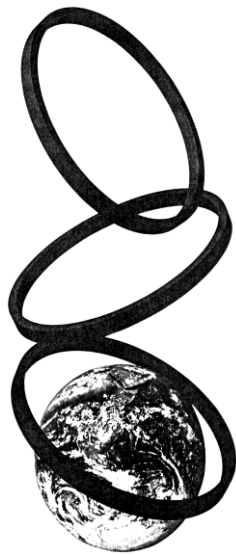


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