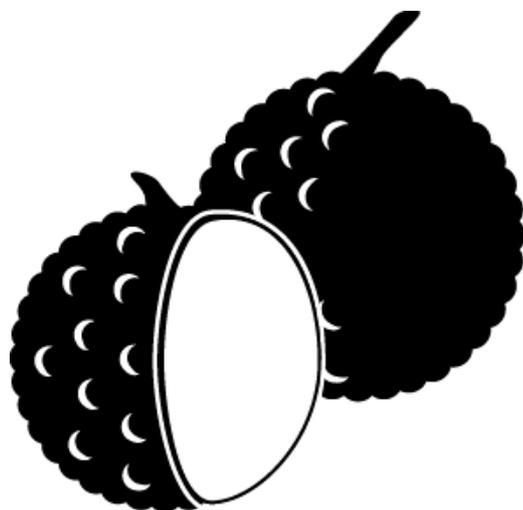


LITCHI SEED EXTRACT

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-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”. “*広西中薬 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

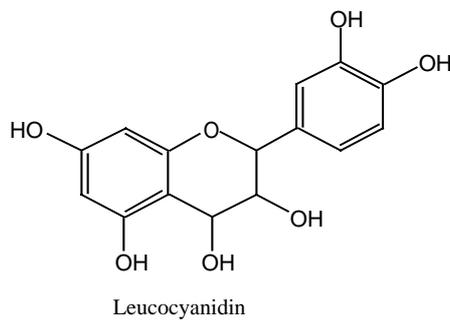


Litchi seed

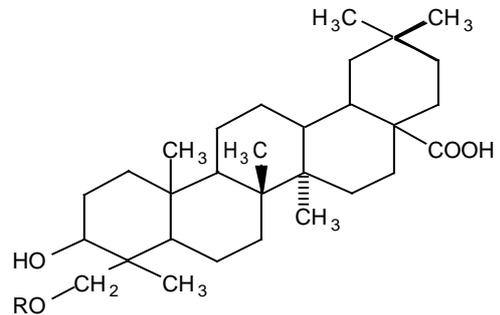
3. Components of LITCHI SEED EXTRACT

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

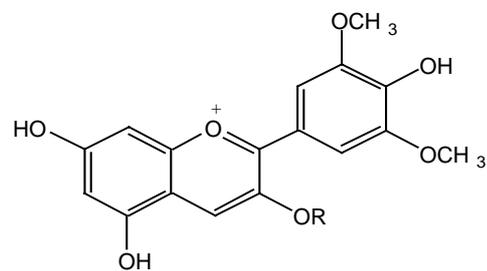
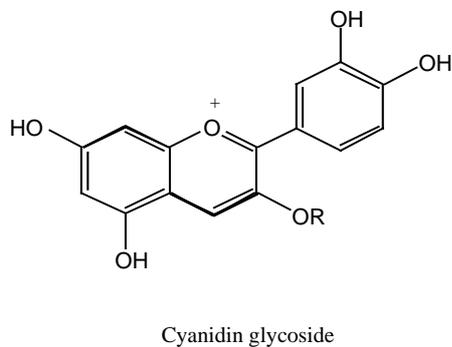
Flavonoid



Saponin



Anthocyanin



R = Sugar

Fig.1 Major Components of LITCHI SEED EXTRACT

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 to inhibit the degradation of collagen.

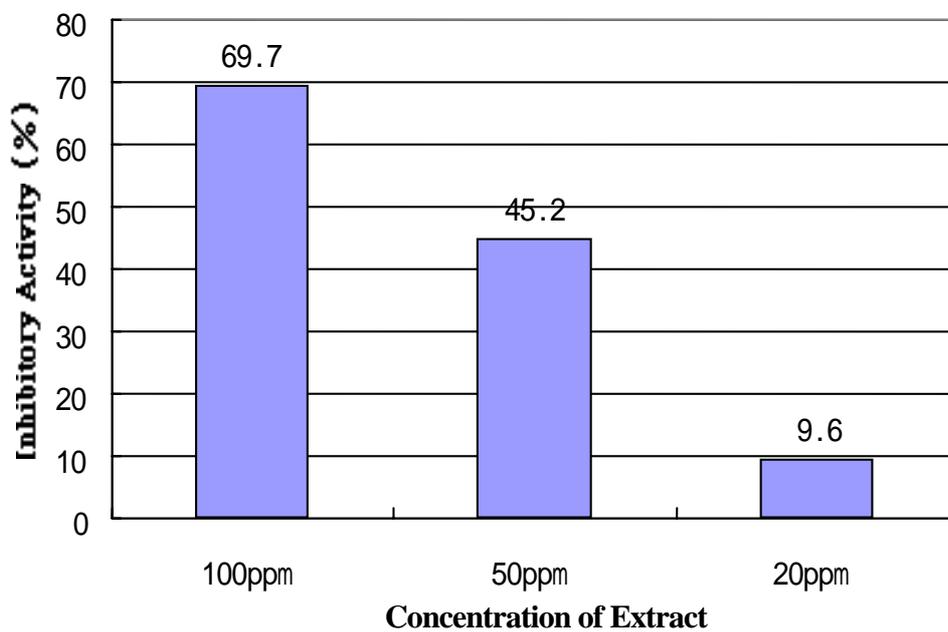


Fig. 2 Inhibition of Collagenase by LITCHI SEED EXTRACT.

	Inhibition Activity (%)					IC ₅₀
	20 ppm	50 ppm	100 ppm	500 ppm	1000 ppm	
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

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

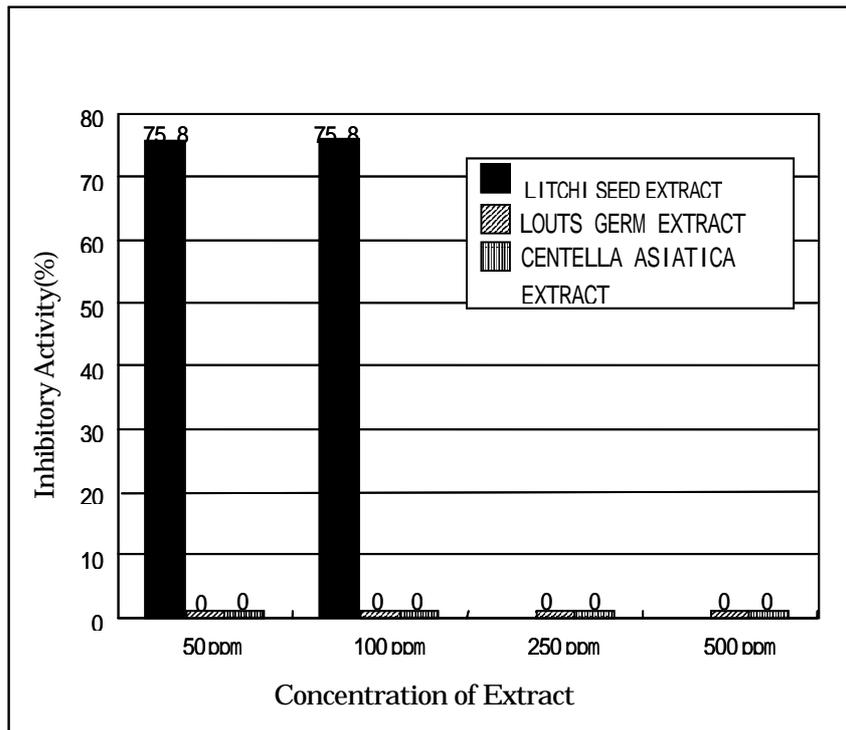


Fig. 3 Collagenase Inhibitory Effect of LITCHI SEEDS EXTRACT and Other Plant Extracts.

(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).

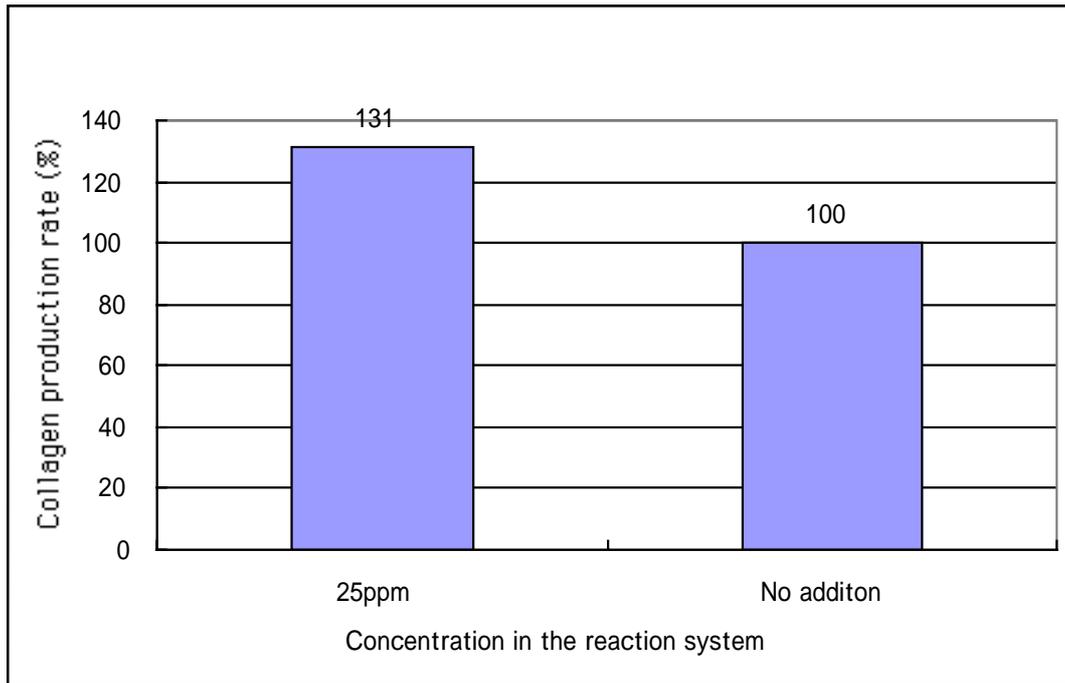


Fig.4. Effects of LITCHI SEED EXTRACT on Collagen Production.

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.

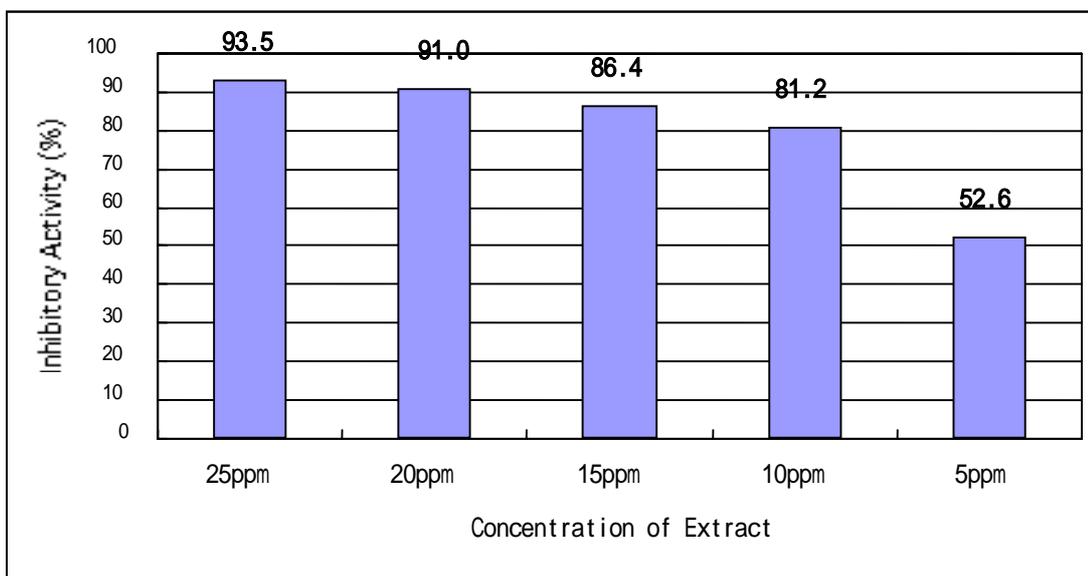


Fig. 5 Inhibition of Elastase by LITCHI SEED EXTRACT

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

	Inhibition Activity (%)							
	5 ppm	10ppm	15 ppm	20 ppm	25 ppm	50 ppm	500ppm	IC ₅₀
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.

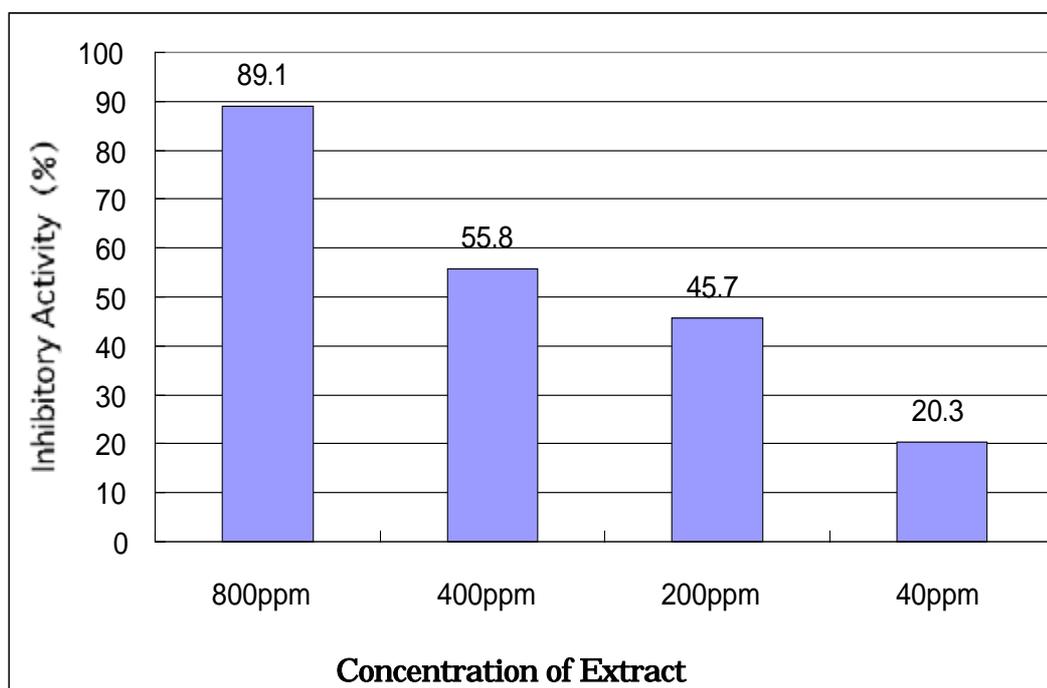


Fig. 6 Inhibition of Hyaluronidase by LITCHI SEED EXTRACT

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

	Inhibition Activity (%)					IC ₅₀
	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

(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).

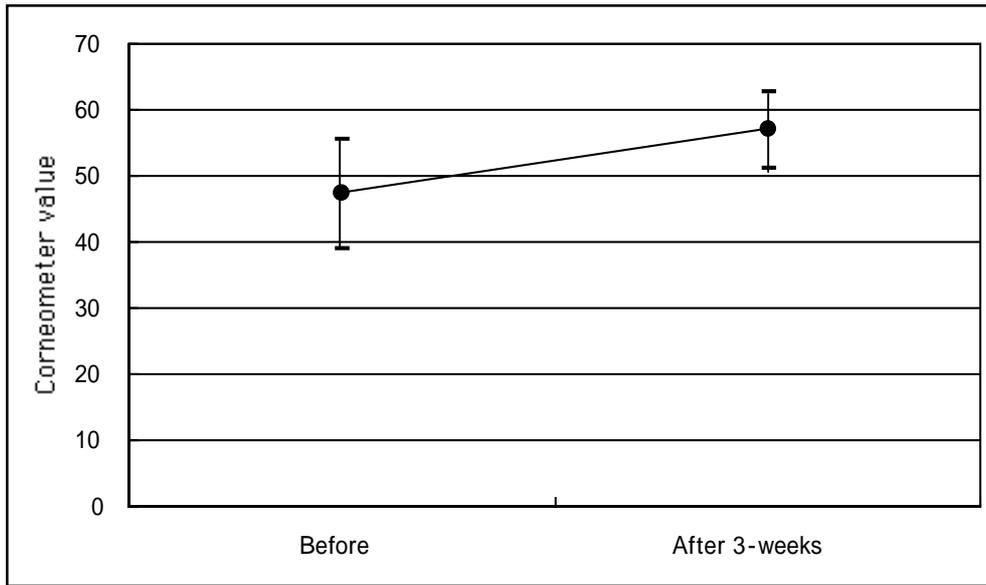
Examination regions: Face and medial brachium.

Examination conditions: Temperature, of 22 degrees; relative humidity, of 55%.

Improvement in The Moisture of Skin

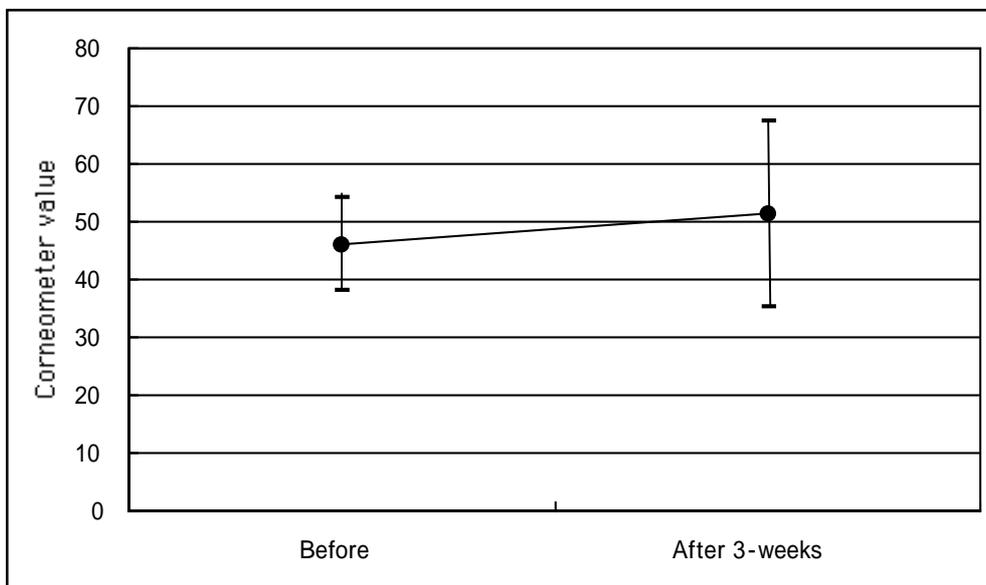
The moisture of the skin was measured around the outer corner of the eye as the test area in all subjects using a CORNEOMETER SM825 before and after 3-week administration of 2 capsules containing LITCHI SEED EXTRACT-P or dextrin per day (one capsule at a time, twice per day). The moisture of the skin was significantly higher after the 3-week administration in the LITCHI SEED EXTRACT-P group (Fig. 7) than in the placebo group (Fig.8)(*t*-test, $p=0.04811$). As shown in Fig. 9, 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.



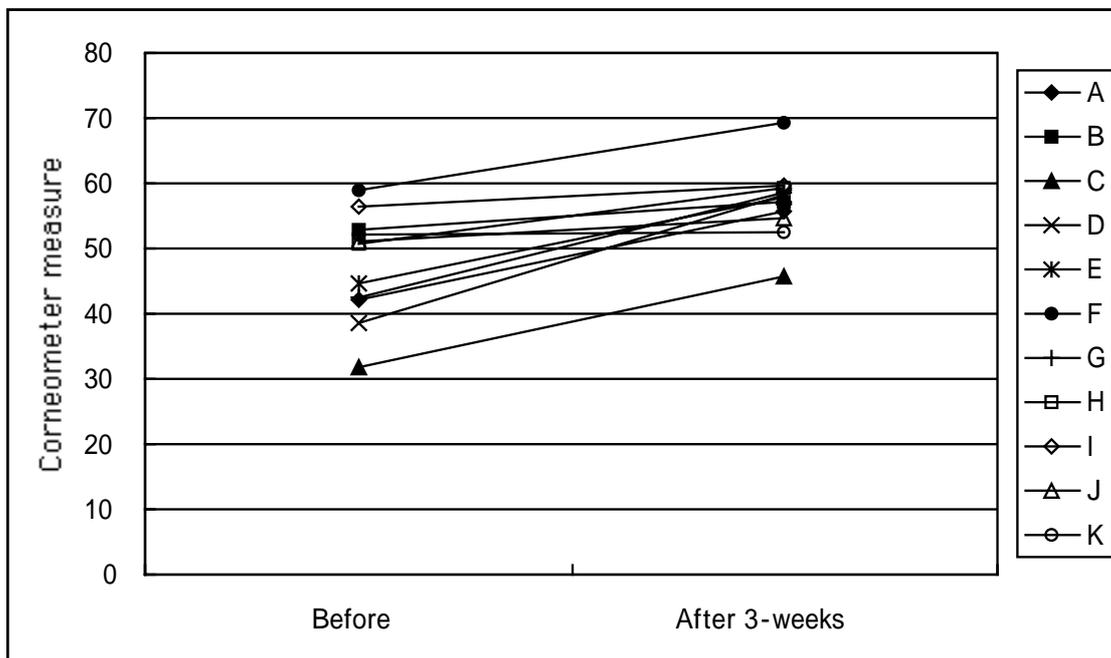
The moisture of the skin measured using a cornmeter significantly increased by the 3-weeks administration of LITCHI SEED EXTRACT-P. This indicated that LITCHI SEED EXTRACT-P was useful for making the skin moist.

Fig. 7 Moisturization Effect of LITCHI SEED EXTRACT



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

Fig.8 Moisturization Effect of Placebo (dextrin)



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.

Fig.9 Improvement of Moisturization by LITCHI SEED EXTRACT

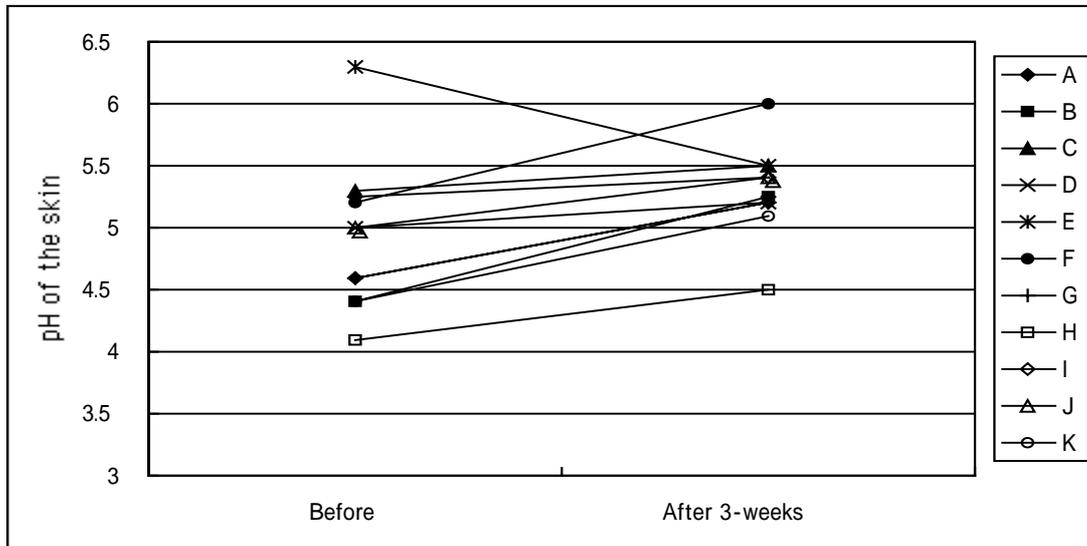
Improvement in pH of Skin

pH of skin 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. 11). 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. 10, 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).



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.10 Improvement of pH by LITCHI SEED EXTRACT

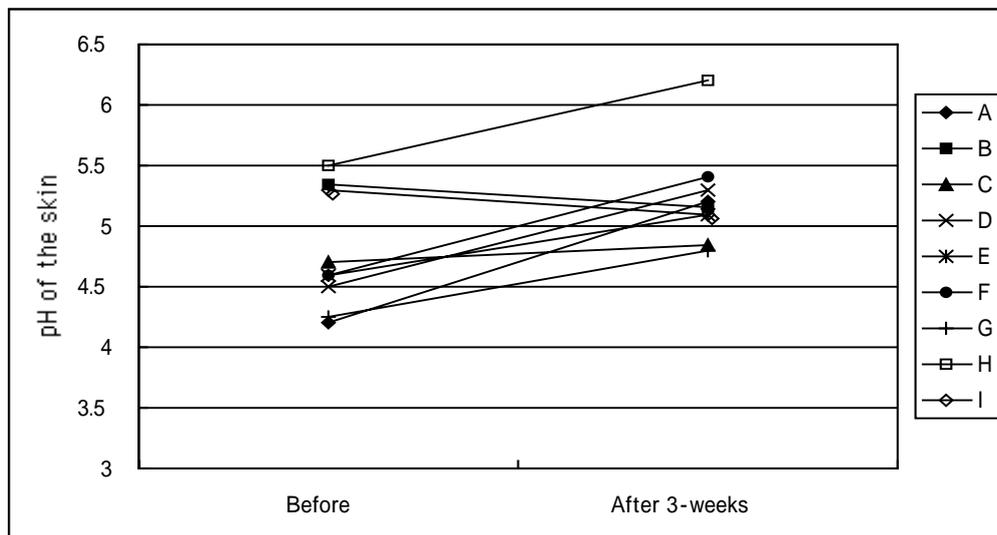


Fig.11 Influence for pH of placebo(dextrin)

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.

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. 12) and the placebo group (Fig. 13), 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.

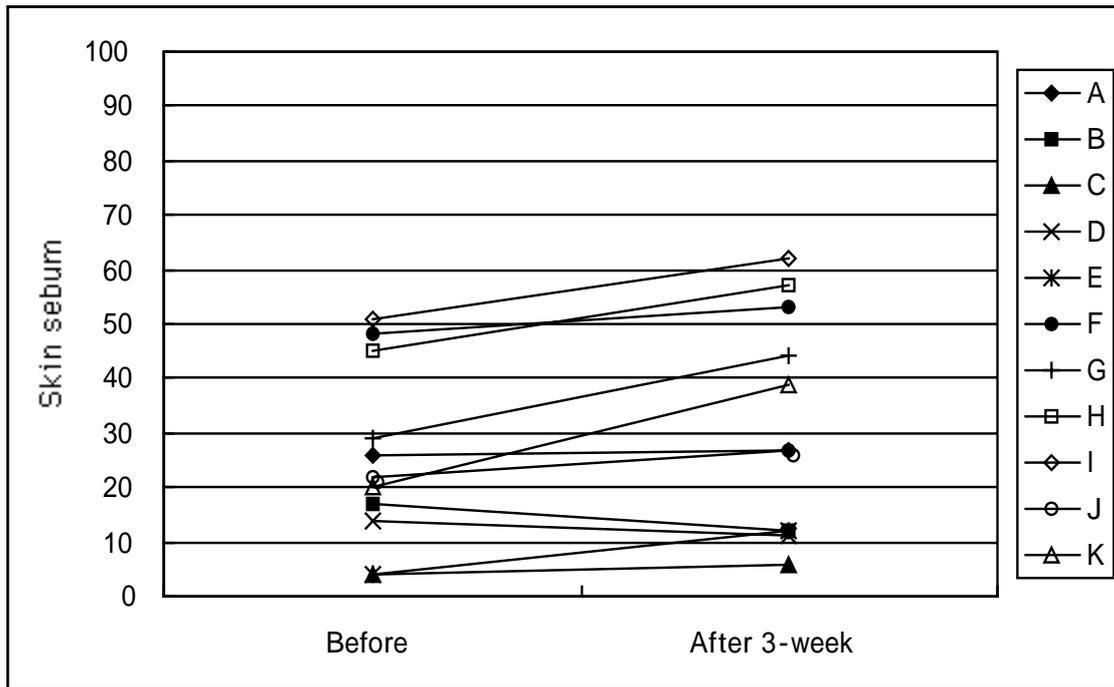


Fig.12 Quantity of Sebum Change by LITCHI SEED EXTRACT

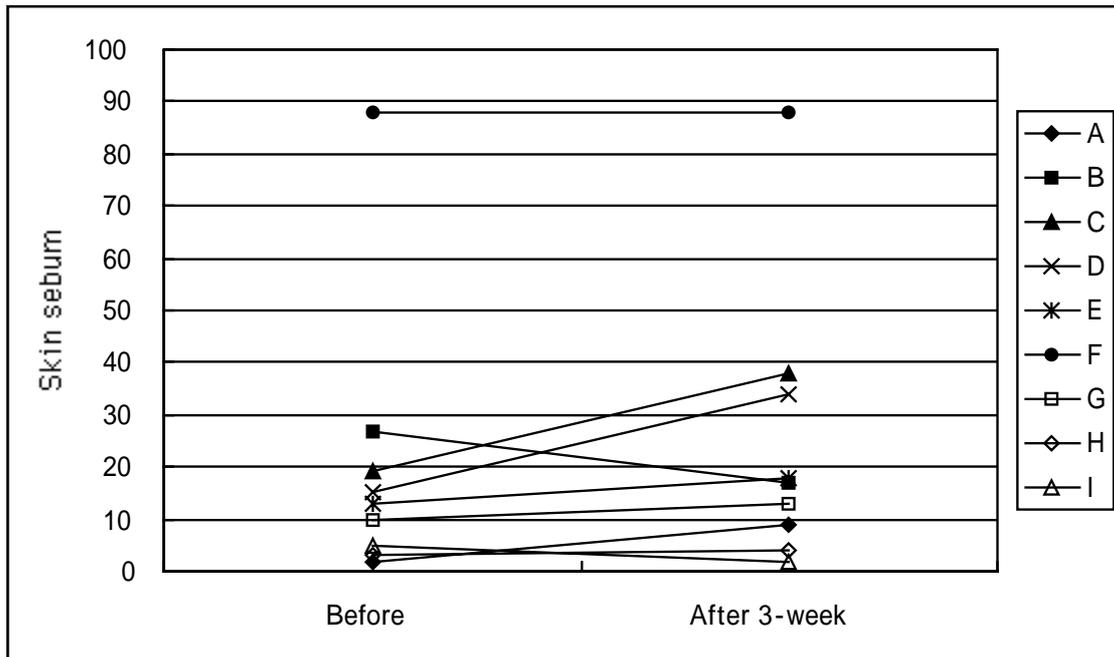


Fig. 13 Quantity of Sebum Change by Placebo (dextrin)

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.

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.

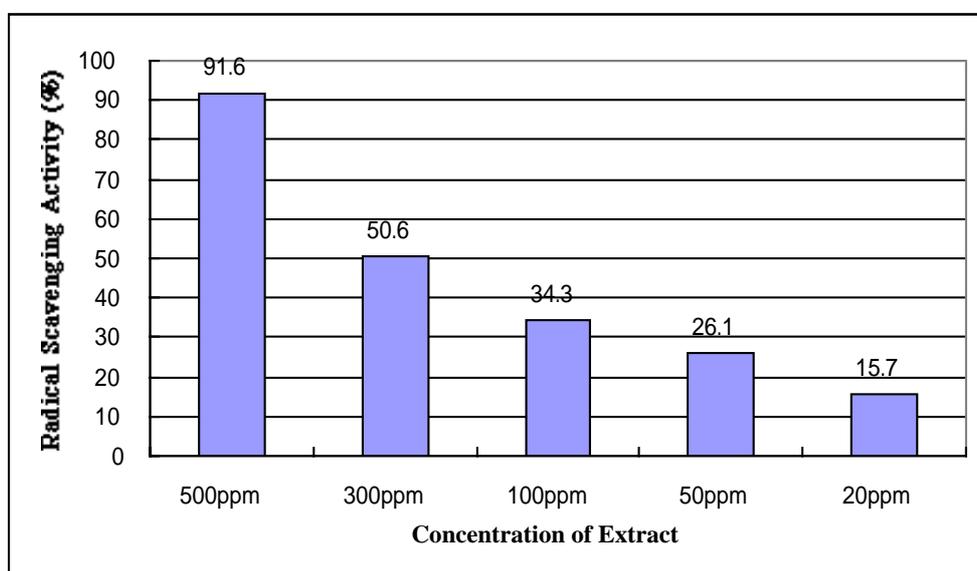


Fig.14 SOD-like Activity of LITCHI SEED EXTRACT

	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

Table5 SOD-like Activity of LITCHI Extracts of Various Sources

DPPH Radical Quinching Activity

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

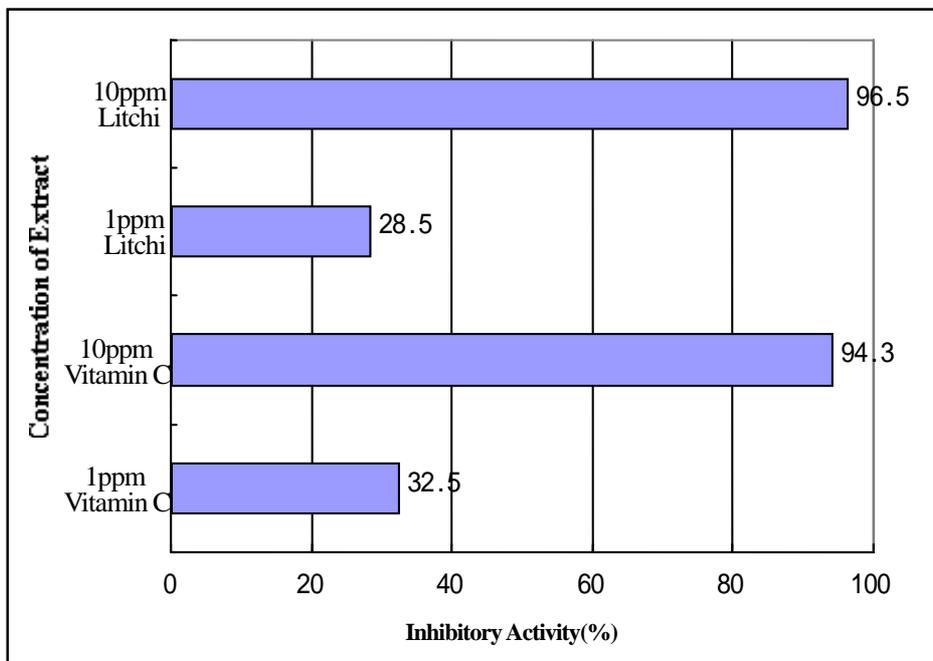


Fig. 15 DPPH Radical Scavenging Activity of LITCHI SEED EXTRACT

	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

Table6. DPPH Radical Quinching Activity of LITCHI Extracts of Various Sources

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. 16 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.

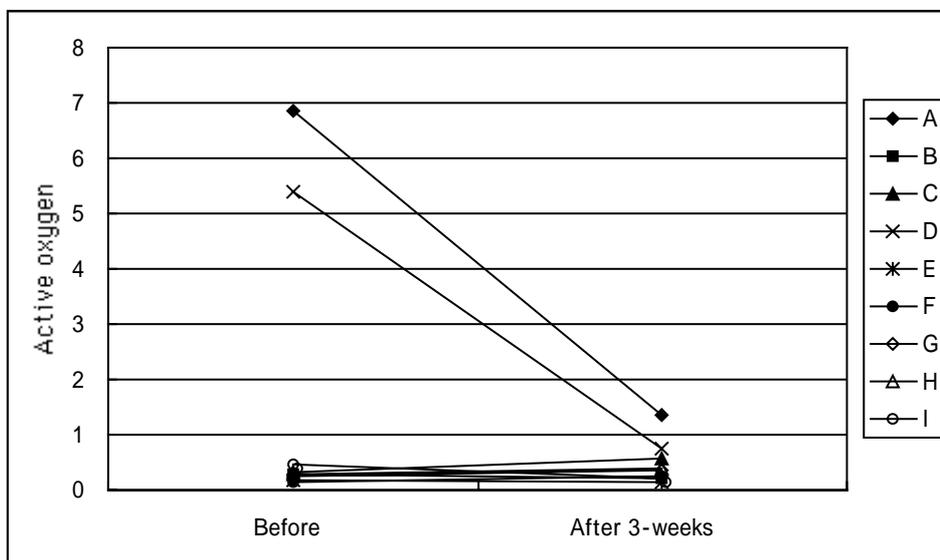


Fig.16 Change in Density of Active Oxygen by LITCHI SEED EXTRACT

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

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 (see page 1). 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.

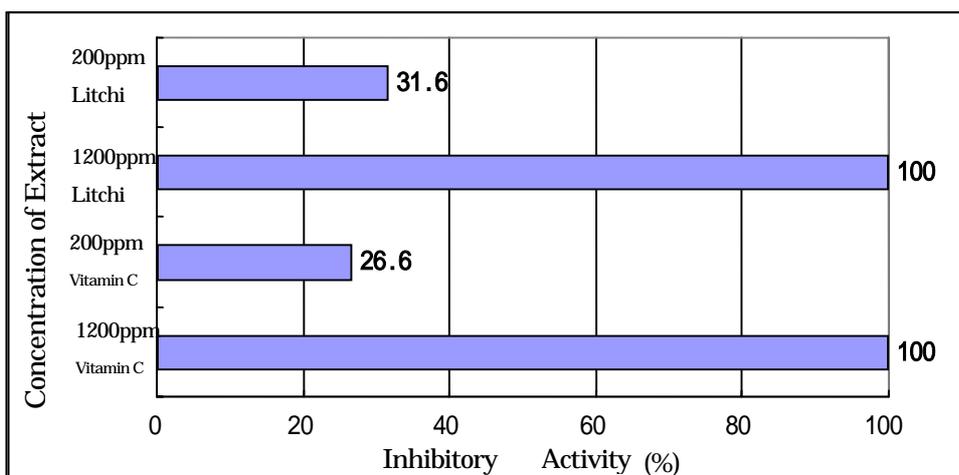


Fig. 17 Inhibitory of Tyrosinase Activity by LITCHI Extracts and Vitamin C

	100 ppm	200 ppm	400 ppm	1200 ppm	2000 ppm	3000 ppm	IC ₅₀
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

Table7 Tyrosinase Inhibitory Effect of Various Parts of LITCHI Extracts

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.

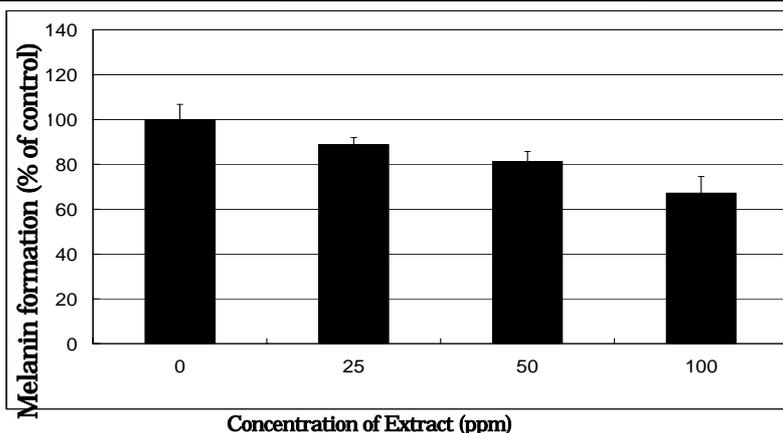


Fig. 18 Effect of LITCHI SEED EXTRACT on Melanocyte(B16) Growth

(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.

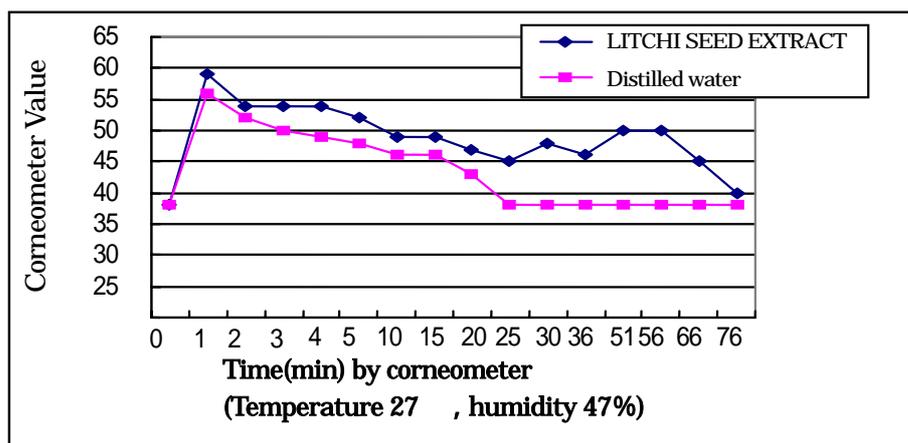


Fig. 19 Moisture Retention Test of LITCHI SEED EXTRACT

(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. 20). On the other hand, DNA fragmentation was found by LITCHI SEED EXTRACT (arrows). DNA fragmentation was confirmed in electrophoresis as well (Fig. 21). The result suggests that LITCHI SEED EXTRACT induced DNA fragmentation on cell in a dose-dependent manner.

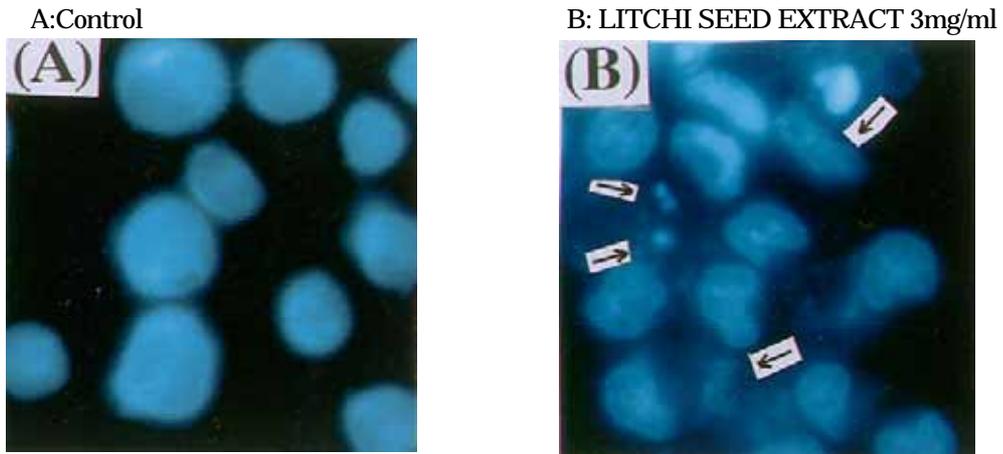


Fig.20 Induction of Apoptosis by LITCHI SEED EXTRACT in Human Stomach Cancer Cells

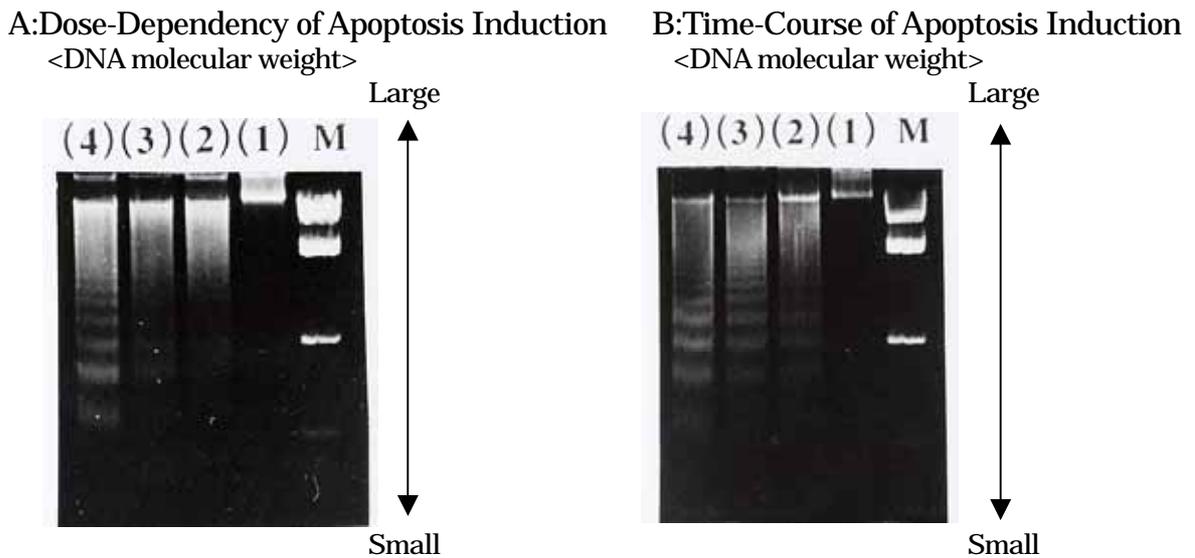


Fig.21 DNA Fragmentation by LITCHI SEED EXTRACT in Human Stomach Cancer Cells

M: DNA marker

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

M: DNA marker

- Control
- LITCHI SEED EXTRACT (3mg/ml) 1day
- LITCHI SEED EXTRACT (3mg/ml) 2days
- 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. 22, 100 $\mu\text{g/mL}$ 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.

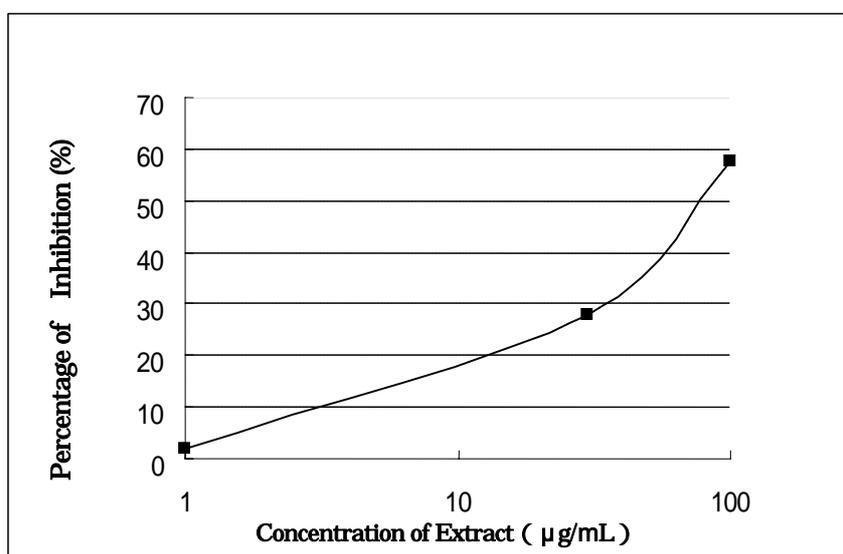


Fig.22 Inhibitory Effect of LITCHI SEED EXTRACT on Aldose Reductase Activity

5. Stability of LITCHI SEED EXTRACT

(1) Thermal Resistance

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

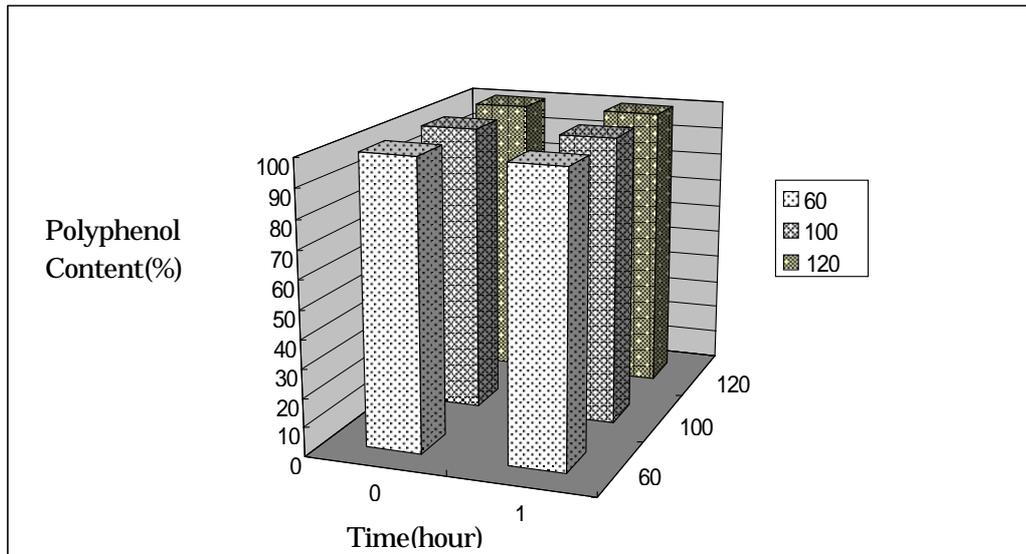


Fig. 23 Heat Resistance of LITCHI SEED EXTRACT

(2) pH Stability

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

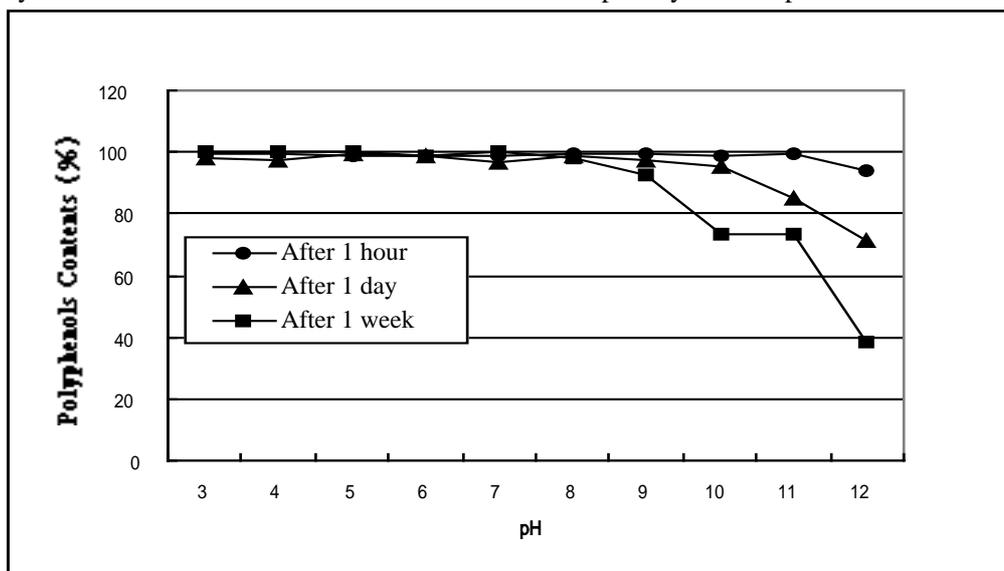


Fig. 24 pH Stability of Polyphenols Contents

6. Daily Recommended Dosage

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

7. Nutrition Information

Items Analyzed	Result
Water	8.0g/100g
Protein*1	5.1g/100g
Fat	1.6g/100g
Ash	4.6g/100g
Available carbohydrate*2	79.7g/100g
Energy*3	354kcal/100g
Dietary Fiber	1.0g/100g
Sodium	22.4mg/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

Assayed Items	Results	Detection Limits	Assay Method
BHC	Not Detected	0.02ppm	Gas Chromatography
DDT	Not Detected	0.02ppm	Gas Chromatography
Aldrin	Not Detected	0.01ppm	Gas Chromatography
Dieldrin	Not Detected	0.01ppm	Gas Chromatography
Endrin	Not Detected	0.01ppm	Gas Chromatography
Diazinon	Not Detected	0.05ppm	Gas Chromatography
Parathion	Not Detected	0.05ppm	Gas Chromatography
Cypermethrin	Not Detected	0.01ppm	Gas Chromatography
Methamidophos	Not Detected	0.05ppm	HPLC-MS

Tested institute: Japan Food Research Center Foundation

Research results issue number : 301080295-001

(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) 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.

(4) 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.

(5) 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.

(6) 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.

(7) 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.

(8) 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/cm²). 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.

(9) 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.

(10) 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. 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

10. Packaging

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

LITCHI SEED EXTRACT-PC (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

11. Storage

Store in cool, dry place. Avoid humidity.

12. Expression of LITCHI SEED EXTRACT

<Food >

LITCHI SEED EXTRACT-P, WSP

Example: LITCHI SEED EXTRACT

<Cosmetic >

	LITCHI SEED EXTRACT-P C	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.

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%CO₂. 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 μL of B16 cells solution (1.8×10^5 cells/ml) were plated in 24-well plates in MEM medium (10% FBS, penicillin/streptomycin) and 2 mM theophylline. Sample solution (55 μL) was added to cells, and they cultured for three days. Then medium was removed, and, cells were fractured by ultrasonication in 300 μL 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^2 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_2 . 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 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 5×10^5 cells/ml in culture flasks. After cultivation for 3 days 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.22 Inhibition of Aldose Reductase

To examine the inhibition of aldose reductase activity, 0.18 M phosphate buffer solution (pH 7.0, 500 μ L), 1.5 mM NADPH (100 μ L), 100 mM DL-glyceraldehyde (100 μ L), water (295 μ L), and DMSO-dissolved sample (10 μ L) were mixed and preheated at 30°C for five minutes. One unit/mL of aldose reductase (5 μ 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. 23, 24 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 , 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. 1×10^3 cfu/g	(Analysis for Hygienic Chemists)
<u>6.Moulds and Yeasts</u>	Max. 1×10^2 cfu/g	(Analysis for Hygienic Chemists)
<u>7.Coliforms</u>	Negative	(Analysis for Hygienic Chemists)
<u>8.Composition</u>	Ingredients	Contents
	Litchi Seed Extract	50 %
	Dextrin	50 %
	Total	100 %

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. 1×10^3 cfu/g	(Analysis for Hygienic Chemists)
<u>6.Moulds and Yeasts</u>	Max. 1×10^2 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 %

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. 9.0 %	(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. 1×10^2 cfu/g	(Analysis for Hygienic Chemists)
<u>6.Moulds and Yeasts</u>	Max. 1×10^2 cfu/g	(Analysis for Hygienic Chemists)
<u>7.Coliforms</u>	Negative	(Analysis for Hygienic Chemists)

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

Ref: The Japanese Standards of Quasi-Drug Ingredients.

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 () 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)
<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 × 10 ² cfu/g	(Analysis for Hygienic Chemists)
<u>8.Moulds and Yeasts</u>	Max. 1 × 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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