ORYZA OIL & FAT CHEMICAL CO., LTD.

KIWI SEED EXTRACT

Food and cosmetic ingredients with anti-acne, wrinkles and under eye darkness properties

- **KIWI SEED EXTRACT-P**
  (Powder、Food Grade)
- **KIWI SEED EXTRACT-WSP**
  (Water-soluble Powder、Food Grade)
- **KIWI SEED EXTRACT-PC**
  (Powder、Cosmetic Grade)
- **KIWI SEED EXTRACT-WSPC**
  (Water-soluble Powder、Cosmetic Grade)
- **KIWI SEED EXTRACT-LC**
  (Liquid、Cosmetic Grade)
- **KIWI FRUIT OIL**
  (Oil、Food・Cosmetic Grade)

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Ver. 6.1 YF

KIWI SEED EXTRACT

Health Ingredients with anti-acne, wrinkles and under eye darkness properties.

1. Introduction

Kiwi fruit (*Actinidia chinensis* Planch.) is originated from the central and southern regions of China. It was introduced to New Zealand in the early 20th century. New Zealand is now the main exporter of high quality Kiwi fruits of various species. The fruit was named “kiwi” after the national bird of New Zealand as the fruit resemble kiwi chick. In 1966, Kiwi fruit was introduced to Japan and cultivation started in late 1970’s. Japan is currently producing 40,000 tonnes of Kiwi fruits per annum.

Kiwi fruits are oval or global shaped berries (Fig. 1) which is harvested between August and October. Kiwi grows on slopes of mountains of forestry area or among low tree bushes. Kiwi has been used as crude drug in the ancient China and it is described in *Chinese Medica Materia* as "acidic, sweet, cooling and non-toxic” with anti-arthritic and anti-haemorrhoidal properties. In addition, Kiwi fruits is also known to darken grey hair. Traditional Chinese Herbal Guidelines described that Kiwi fruist quench thirst, relieve feverish and gonococcal complaints. Mixture of juices from Kiwi fruits and ginger is effective against stomach upset due to gastric fever. On the other hand, Kiwi seeds has been widely used for oil extraction for cosmetics applications.

The physiological effects of Kiwi seeds extract was researched and experimented. Results revealed that Kiwi seeds extract is preventive against the development of acne by preventing the generation of dihydrotestosterone and bacterium lipase from *Propionibacterium acnes*. Research also confirmed its anti-wrinkle and anti-under eye darkness properties. Nevertheless, Kiwi seeds oil is rich in α-linolenic acid, an omega-3 essential fatty acids and natural amount of tocotrienols, a potent antioxidant. Oryza Oil & Fat Chemical Co., Ltd. has successfully commission the extraction and production of Kiwi Seed Extract as a new generation health ingredient suitable for food, health & cosmetics applications.

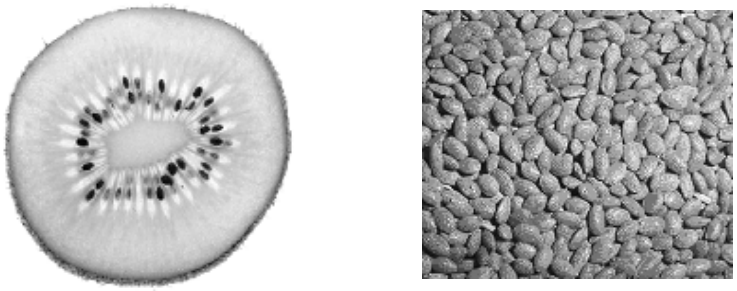


Fig1. Kiwi fruit and seeds

2. Functional Components of Kiwi Seeds

Analysis confirmed that Kiwi Seed Oil is rich in the omega-3 essential fatty acid, α-linolenic acid with renowned anti-allergenic properties. Besides, it is found to consist of naturally occurring amount of tocotrienols, a potent antioxidant with cholesterol lowering effects.

Secondarily, polyphenols content of different parts of Kiwi fruit was compared and Kiwi seeds are loaded with the highest amount of polyphenols (Fig. 2). Further research was prompted where polyphenols of Kiwi seeds were isolated and purified. Flavonol glycosides, namely, **quercitrin** and **kaempfenol 3-*O*-rhamnoside** (Fig. 3) identified as the main components of Kiwi Seed Extract. Study revealed that these components aid in the symptomatic relief of liver diseases[1]. On the other hand, Hanamura *et al*. & Jeong *et al*. confirmed the inhibitory effect quercitrin on advanced glycation end-product (AGE) formation[2] and tyrosinase[3] activity respectively. Quercitrin exert its anti-inflammatory effect by acting as precursor of quercetin[4] in the body, thus inhibiting the expression of NF-κB and iNOS[5]. Life Science magazines revealed the effect of quercitrin on the early hapten induced colonic inflammation in rats[6,7]. Nevertheless, studies also revealed that quercitrin is inhibitory against len aldose reductase activity[8,9] which is associated with complications of diabetes mellitus[10]. Kaempferol 3-*O*-rhamnoside having structural similarity to quercitrin is believed to exert similar physiological effects. Experimental results suggested that seed extract is highly versatile upon comparison with other parts of the fruits.

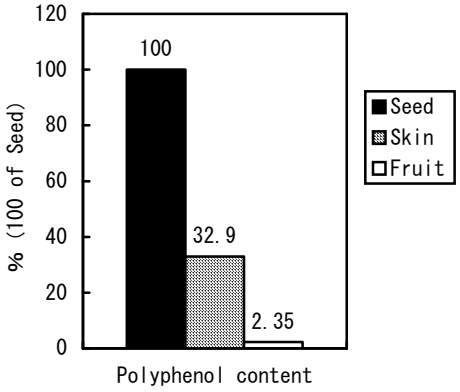
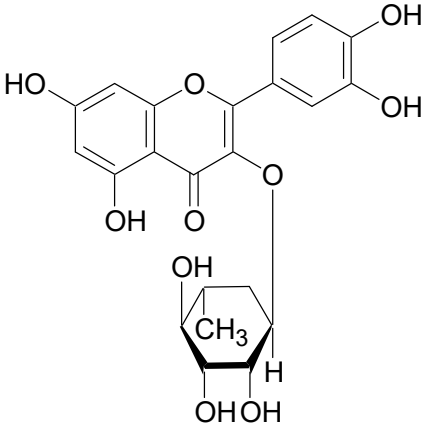
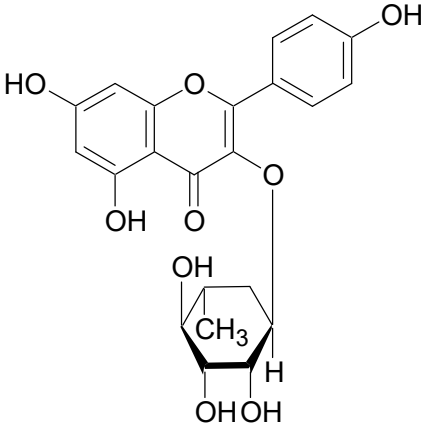


Fig 2. Polyphenol Content of Kiwi seed, skin and fruit



quercitrin
(quercetin 3-*O*-rhamnoside)



kaempferol 3-*O*-rhamnoside

Fig 3. Components of Kiwi Seed Extract

References

1) Matsuda H., Ninomiya K., Shimoda H., Yoshikawa M. Hepatoprotective principles from the flowers of *Tilia argentea* (Linden) :Structure requirements of tiliroside and mechanisms of action. *Bioorg. Med. Chem*. **10**, 707-712, (2002).

2) Hanamura T., Hagiwara T., Kawagishi H. Structural and functional characterization of polyphenols isolated from acerola (*Malpighia emarginata* DC.) fruit. *Biosci. Biotechnol. Biochem*. **69**, 280-286, (2005).

3) Jeong CH, Shim KH. Tyrosinase inhibitor isolated from the leaves of Zanthoxylum piperitum. *Biosci Biotechnol Biochem*. **68**, 1984-7,(2004).

4) Comalada M, Camuesco D, Sierra S, Ballester I, Xaus J, Galvez J, Zarzuelo A. In vivo quercitrin anti-inflammatory effect involves release of quercetin, whichinhibits inflammation through down-regulation of the NF-kappaB pathway. *Eur J Immunol*. **35**, 584-92, (2005).

5) Camuesco D, Comalada M, Rodriguez-Cabezas ME, Nieto A, Lorente MD, Concha A, Zarzuelo A, Galvez J. The intestinal anti-inflammatory effect of quercitrin is associated with aninhibition in iNOS expression. *Br J Pharmacol*. **143**, 908-18, (2004).

6) Sanchez de Medina F, Vera B, Galvez J, Zarzuelo A. Effect of quercitrin on the early stages of hapten induced colonic inflammation in the rat. *Life Sci*. **70**, 3097-108, (2002).

7) Sanchez de Medina F, Galvez J, Romero JA, Zarzuelo A. Effect of quercitrin on acute and chronic experimental colitis in the rat. *J Pharmacol Exp Ther*. **278**, 771-9, (1996).

8) Lee HS. Rat lens aldose reductase inhibitory activities of Coptis japonica root-derivedisoquinoline alkaloids. *J Agric Food Chem*. **50**, 7013-6, (2002).

9) Matsuda H, Morikawa T, Toguchida I, Yoshikawa M. Structural requirements of flavonoids and related compounds for aldose reductaseinhibitory activity. *Chem Pharm Bull (Tokyo)*. **50**, 788-95, (2002).

10) Galvez J, Crespo ME, Jimenez J, Suarez A, Zarzuelo A. Antidiarrhoeic activity of quercitrin in mice and rats. *J Pharm Pharmacol*. 45, 157-9, (1993).

3. Physiological Function of Kiwi Seed Extract

(1) Pathophysiology of Acne

a. Seabaceous gland hypersecretion

5α-reductase is the enzyme responsible for the conversion of testosterone to dihydrotestosterone (DHT) which stimulate the enlargement of sebaceous gland resulting in sebaceous hypersecretion. Sebum produced act as an important nutritional source for anaerobic *P. acnes* which activates the inflammatory pathway underneath the skin. Thus acne develops due to the hyperproduction of DHT.

b. Infectious *Propionibacterium Acnes* (*P. acnes*)

The growth of bacteria flora of the hair follicles, *P. acnes*, increases with sebum production. *P. acnes* produces bacterium lipase which breaksdown sebum triglyceride which travel as free fatty acids in the skin that activates the inflammatory cascade. Subsequently, inflammatory factors are released followed by leukocyte chemotaxis in the dermis leading to the formation of comedogenic acne on the epidermis (Fig. 4).

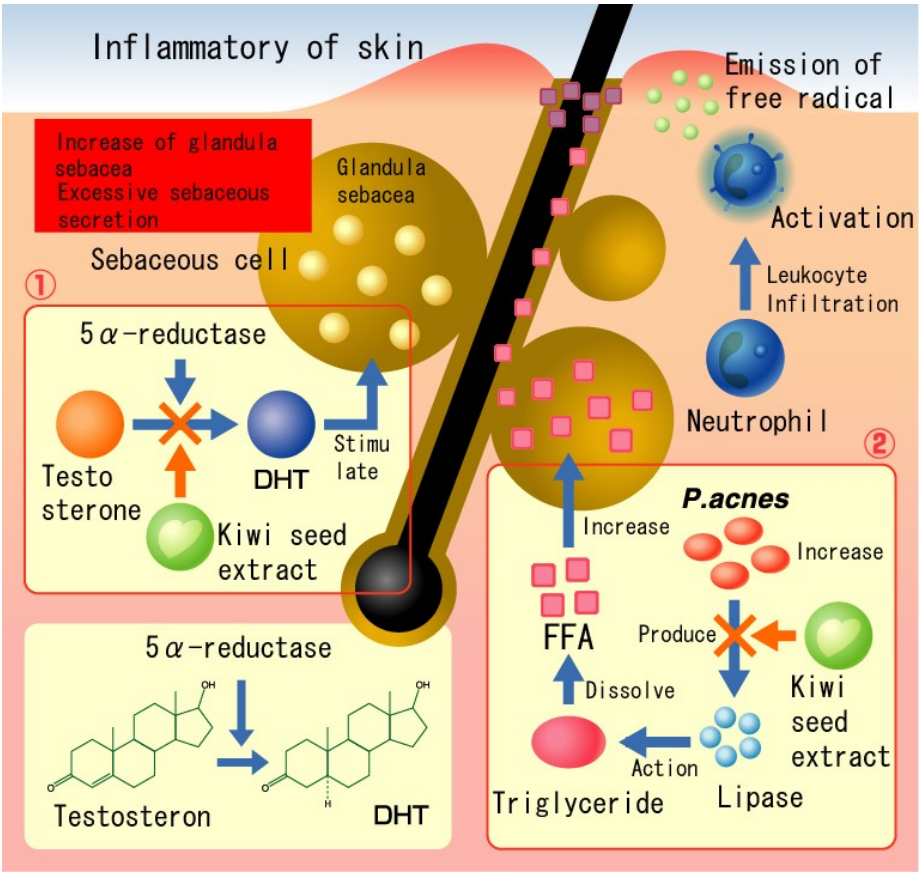


Fig 4. How Acne Occurs

1) Inhibition of 5α-reductase (*in vitro*)

Different concentration of Kiwi Seed Extract was added to testosterone containing enzyme 5α-reductase (S-9, Oriental Yeast) samples for reactions. The production of dihydrotestosterone (DHT) was analyzed using gas chromatography. Results confirmed that Kiwi Seed Extract prevents the production of DHT via its inhibition against 5α-reductase.

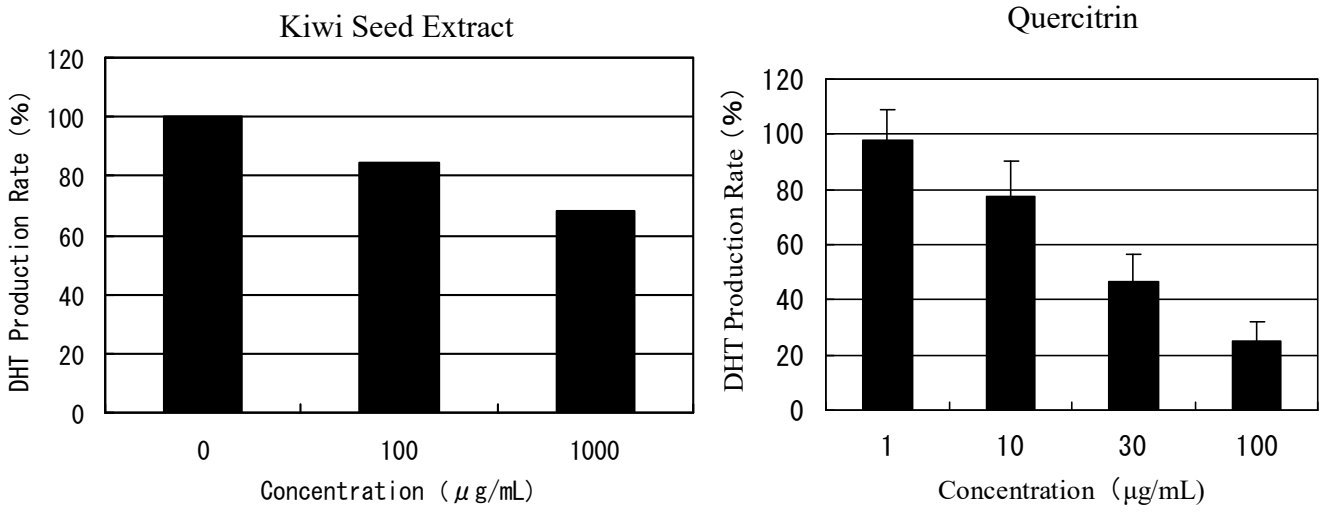


Fig 5. 5α-reductase Inhibitory Activity (Left: Kiwi Seed Extract, Right: Quercitrin)

【Method of experiment】

Testosterone (3.0 μmol) was dissolved in propylene glycol and addition of 5mMTris-HCl buffer solution (pH 7.2) to produce a 5 ml solution. Then, Kiwi Seed Extract and NADPH (5 mg) was added followed by heating at 37 °C. Last, addition of the enzyme solution (S-9, Oriental Yeast) and incubation for 1 hour. Reaction system is halted by adding methylene chloride (5 ml). After removal of methylene chloride, the solution was dried under reduced pressure followed by dissolution with MeOH. Quantity of DHT produced in the reaction system was analysed using GC (column: DB-17, J&W, carrier: He, 2 ml/min., column temperature: 250 °C).

2) Inhibition of lipase of *P. acnes* (*in vitro*)

P. acnes was cultured in GAM medium to obtain the enzyme lipase. Kiwi Seed Extract of different concentration was added to evaluate its effect on lipase. Results demonstrated a dose-dependent inhibition against lipase of *P. acnes*.

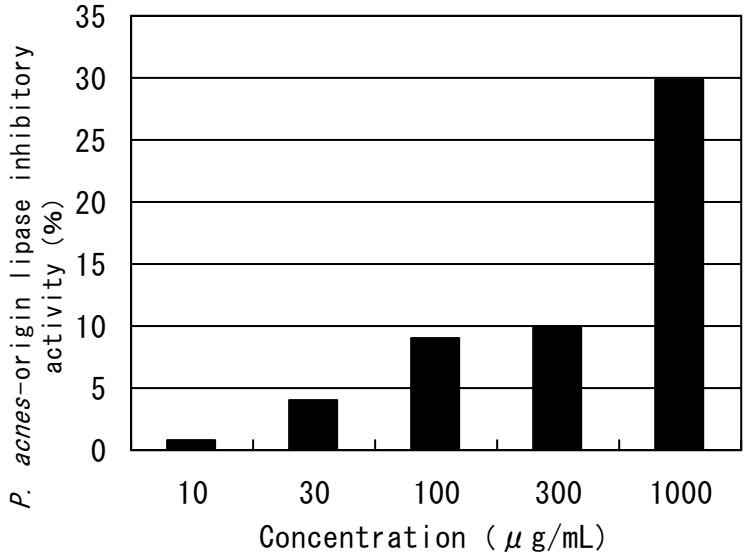


Fig 6. *P. acnes*-origin Lipase Inhibitory Activity of Kiwi Seed Extract

【Method of experiment】

P. acnes was cultured in GAM medium and centrifuged at 3000rpm for 10 min. Fungus bodies are collected and PBS was added followed by crushing with ultrasound and second centrifugation. Supernatant liquid was collected and dialyzed with PBS for 3 days at 4°C followed by freeze-drying to obtain lipase of *P. acnes*. Lipase Kit S (Dainippon Pharmaceutical) was used to measure the lipase inhibitory action. Colouring agent (390 μl), Kiwi Seed Extract of different concentrations (25 μl), 25 μl of lipase (50 mg/ml) and esterase inhibitory agent (10 μl) was added in a test tube and heated in a thermostatic chamber at 30°C for 5 min in dark. A matrix solution (50 μl) was added for reaction at 30°C for 30 mins. Reaction stopper solution (500 μl) was added upon completion of reaction and absorbance was measured at wavelength 415 nm.

3) The Effect of Kiwi Seed Extract on sebum production of normal healthy adults (topical & oral applications)

a. Topical Application

The effect of Kiwi Seed Extract on human sebum production was studied. Kiwi Seed Extract-LC was used where sebum production of specific area of the forehead was measured prior to and after the application. As illustrated in Fig. 7, sebum production significantly decreases while acneic area subsided after 2-week application.

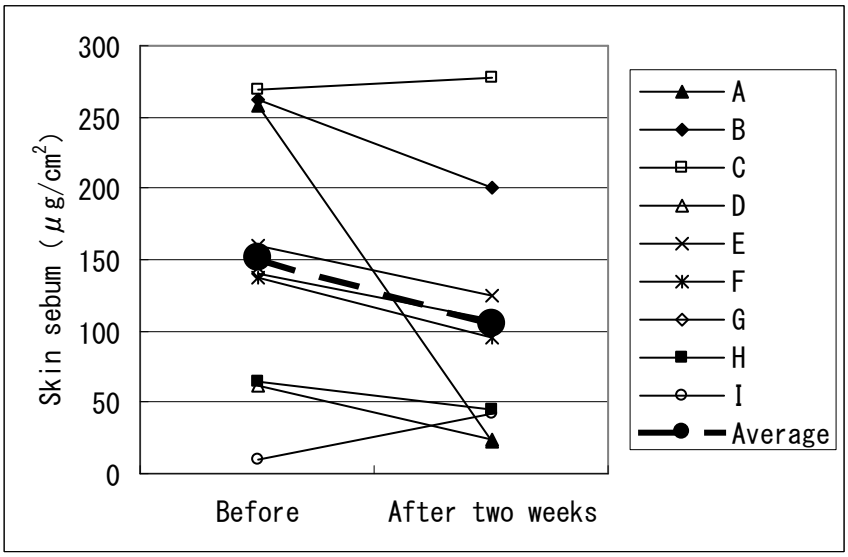
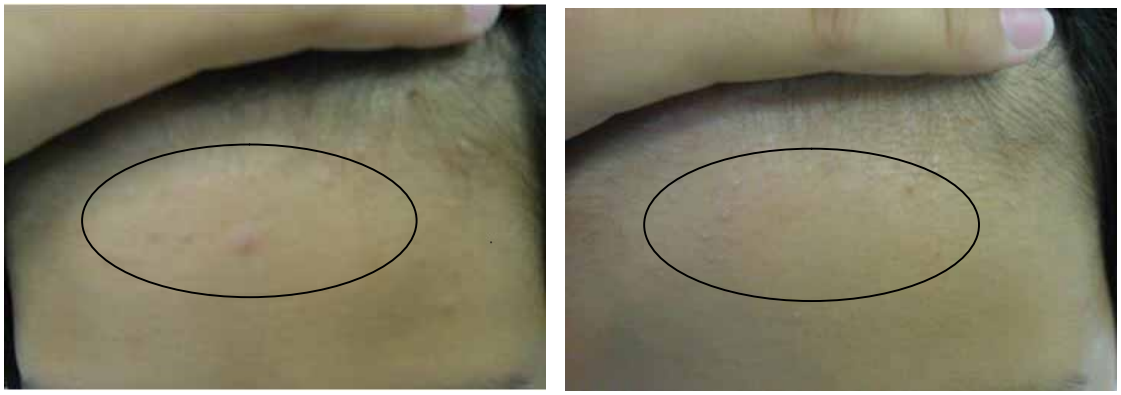


Fig 7. The Effect of Kiwi Seed Extract –LC on human sebum production

—Images of forehead—



Before After two weeks

Inflammation of acneic forehead reduced after 2-week application of Kiwi Seed Extract-LC.

Fig 8. Anti-acne property of Kiwi Seed Extract-LC

b. Oral Application

Further experiment was prompted to examine the effect of Kiwi Seed Extract on human sebum production *in vivo*. The sebum production on area around eye corner was measured prior to and after oral administration of Kiwi Seed Extract-P 50 mg/day for 2-week. Similarly, as illustrated in Fig. 9, sebun production reduces after 2-week oral administration of Kiwi Seed Extract.

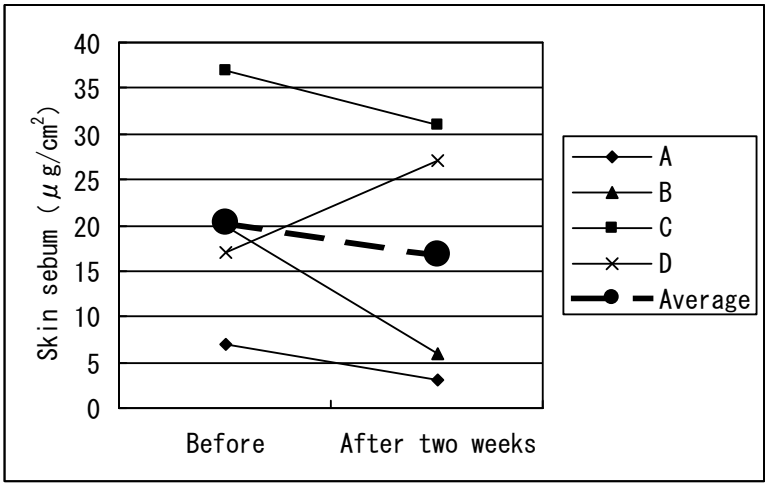


Fig 9. The effect of Kiwi Seed Extract (oral) in human sebum production

【Method of Experiment】

a. Topical Application

The topical effect of Kiwi Seed Extract-LC on human sebum production was assessed on 9 healthy subjects (6 males & 3 females aged between 14-37). Kiwi Seed Extract-LC (containing Kiwi Seed Extract 1%) was applied topically to the forehead of subjects daily for 2 weeks. Sebum content of forehead was measured prior to and after application of Kiwi Seed Extract-LC using SEBUMETER SM810. Similarly, images of foreheads prior to and after application of Kiwi Seed Extract-LC were recorded.

b. Oral Administration

The effect of Kiwi Seed Extract on human sebum production *in vivo* was examined among the females employees of the company. 4 female subjects (aged 24-52) were given Kiwi Seed Extract-P 50 mg/day orally for 2 weeks. Sebum production around eye area prior to and after the oral administration of Kiwi Seed Extract was measured using SEBUMETER SM810.

(2) Anti-wrinkle Property

1) Promotion of fibroblasts growth (*in vitro*)

Further experiment was prompted to evaluate the anti-wrinkle property of Kiwi Seed Extract using NB1RGB (neonatal human fibroblast cells). Results revealed that Kiwi Seed Extract demonstrated a dose-dependent promotion on NB1RGB cells growth (*in vitro*). Therefore, Kiwi Seed Extract promote growth of skin cells.

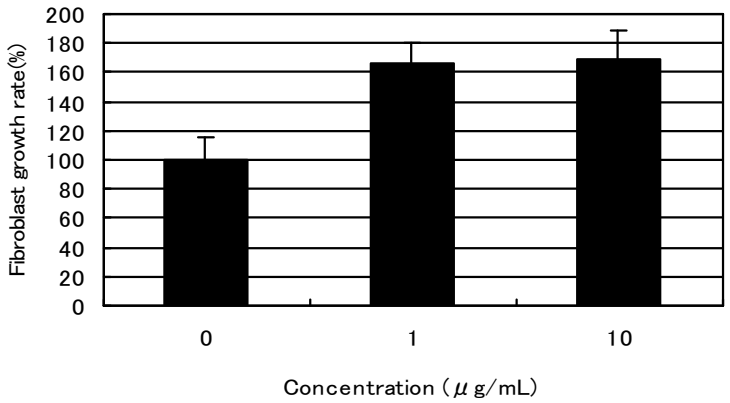


Fig 10. Effects of Kiwi Seed Extract on NB1RGB Fibroblast Growth (n=5,mean±S.E.)

【Method of Experiment】

NB1RGB fibroblasts were suspended in α-MEM medium (containing 10% FCS, 100 units/ml penicillin & 100 μg/ml streptomycin) ($2x10^5$ cells/ml) followed by dissemination on a 96-welled plates (each 100 μl). Kiwi Seed Extract of various concentrations (10 μl) was added and culture for 2 days. Fibroblast cells was measured by MTT Assay.

2) Anti-wrinkle property-Oral Trial

A human trial using Kiwi Seed Extract was conducted to evaluate its anti-wrinkle property. 10 healthy female subjects (aged 20-43) were give Kiwi Seed Extract-P 50 mg/day orally for 4 weeks. Replica of skin was taken prior to and after the oral administration of Kiwi Seed Extract. Meanwhile, moisture content, pH of skin on the inner forearm was measured.

① Anti-wrinkle property

As illustrated in Fig. 12, NIH image confirmed that the total wrinkling area reduced after 4-week administration of oral Kiwi Seed Extract.

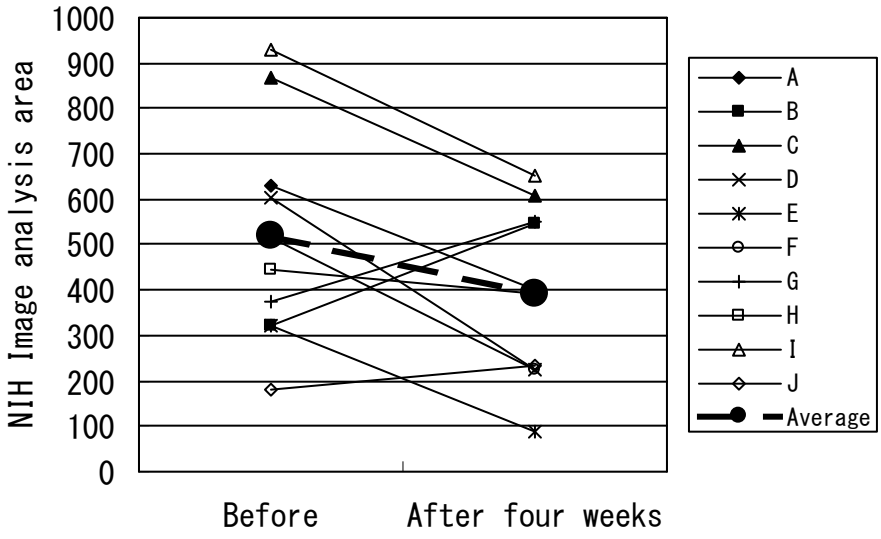
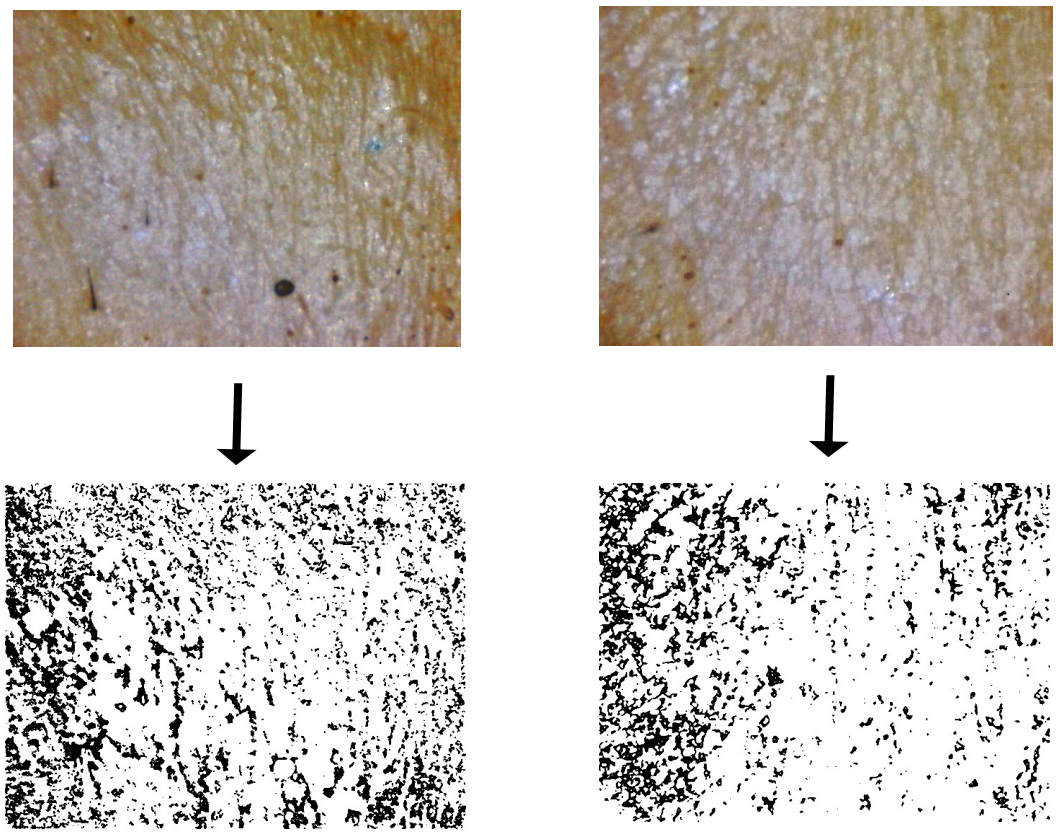


Fig 11. The effect of Kiwi Seed Extract on Wrinkling Area



Before（binary image）　　After four weeks（binary image）

Fig 12. The effect of Kiwi Seed Extract on Wrinkling Skin

② The effect of Kiwi Seed Extract on skin moisture and pH

As illustrated in Fig. 13, skin moisture (as measured using Corneometer) remained unchanged after 4-week oral administration of Kiwi Seed Extract. Hence, Kiwi Seed Extract regulated the sebum production without altering the skin moisture content keeping skin soft and supple.

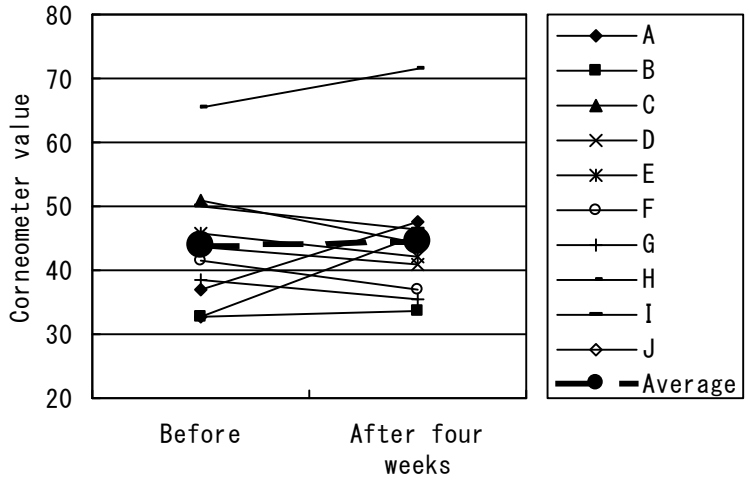


Fig 13. Moisturizing Effect of Kiwi Seed Extract

Similarly, Kiwi Seed Extract normalizes skin pH to ideal skin pH range, i.e. pH5.0-5.5 (as shown in Fig. 14).

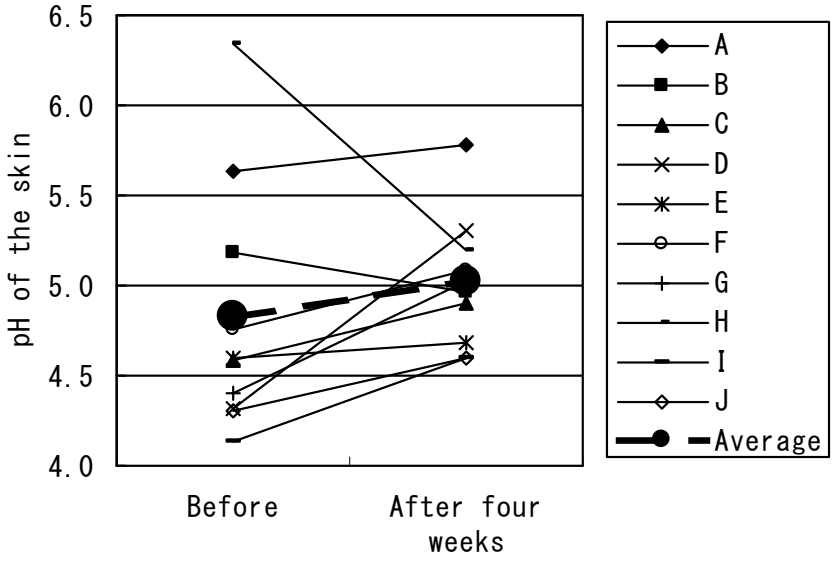


Fig 14. The effect of Kiwi Seed Extract on skin pH

【Method of Experiment】

① Anti-wrinkle property －Oral Trial

10 healthy female subjects (aged 20-43) were given oral Kiwi Seed Extract-P 50 mg/day for 4 weeks. Skin around eye corner was replicated prior to and after oral administration of Kiwi Seed Extract using skin cast agent. Images of skin replica were examined under USB Microscope M2 and binary images were produced for NIH Image analysis. The total area of wrinkles were measured and compared.

② The effect of Kiwi Seed Extract on skin moisture and pH

CORNEOMETER SM825 and SKIN-pH-METER PH900 were used to determine skin moisture content and pH value respectively.

(3) Anti-under eye darkness－a skin lightening effect

1) Inhibition of tyrosinase (*in vitro*)

Tyrosinase is the enzyme responsible for skin hyperpigmentation in the production of dopa-quinone pathway. *In vitro* studies confirmed that Kiwi Seed Extract demonstrated a dose-dependent inhibitory effect against tyrosinase (Fig. 15).

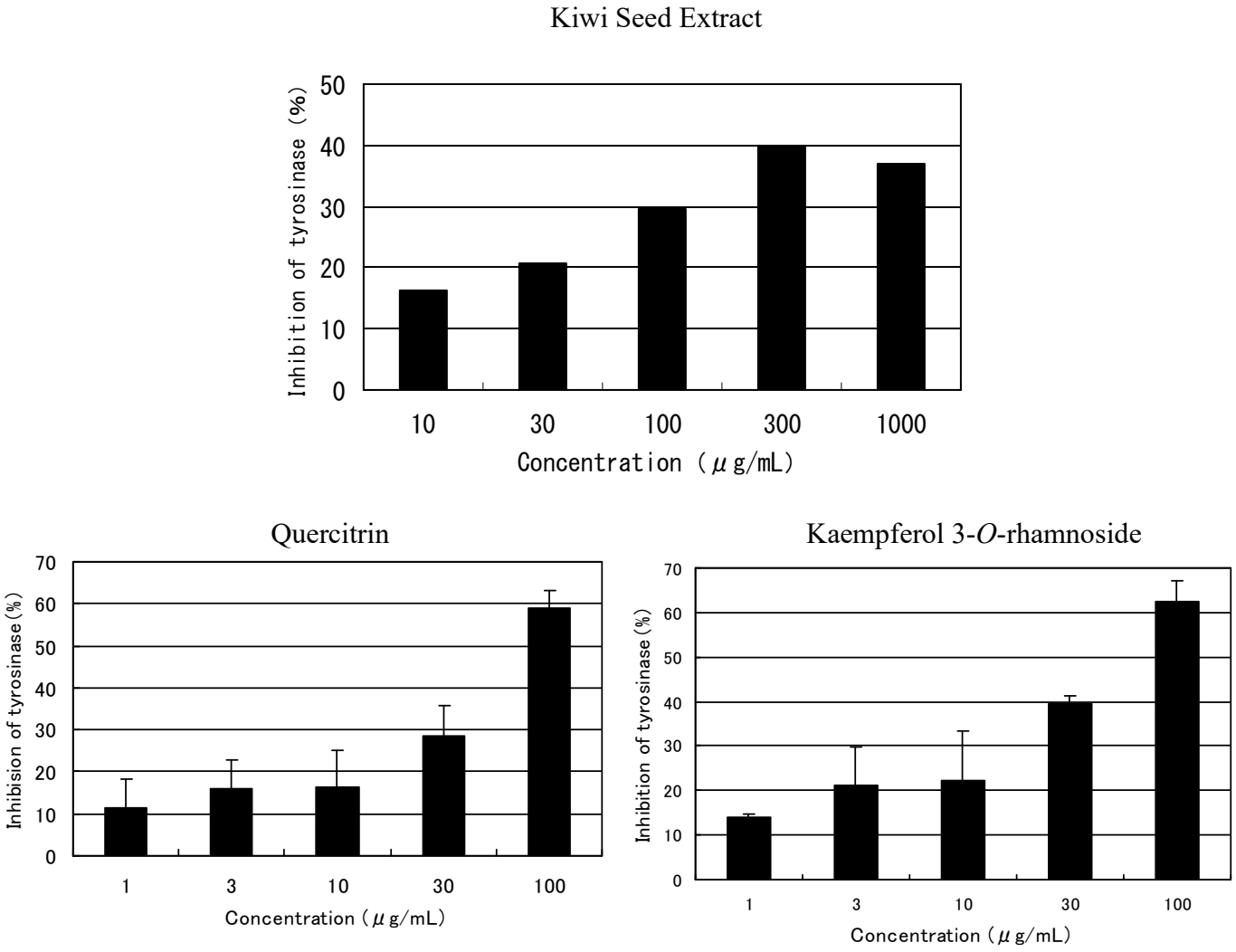


Fig 15. The Effect of Kiwi Seed Extract on Tyrosinase

【Method of Experiment】

Kiwi Seed Extract of different concentrations was added to L-DOPA 0.3% (70 μL/well) followed by heating at 37°C for 5 mins. Tyrosinase (mushroom origin, 1.6 units/mL) was added (70 μL/well) for reaction at 37°C for 5 mins. Upon completion of reaction, absorbance at 492nm was measured using a microplate reader.

2) Inhibition on B16 melanoma cells

Further experiment was prompted using B16 melanoma cells to evaluate the skin lightening effect of Kiwi Seed Extract. As shown in Fig. 16, Kiwi Seed Extract demonstrated a dose-dependent inhibitory effect against B16 melanoma cells production. Therefore, Kiwi Seed Extract is preventive against skin hyperpigmentation.

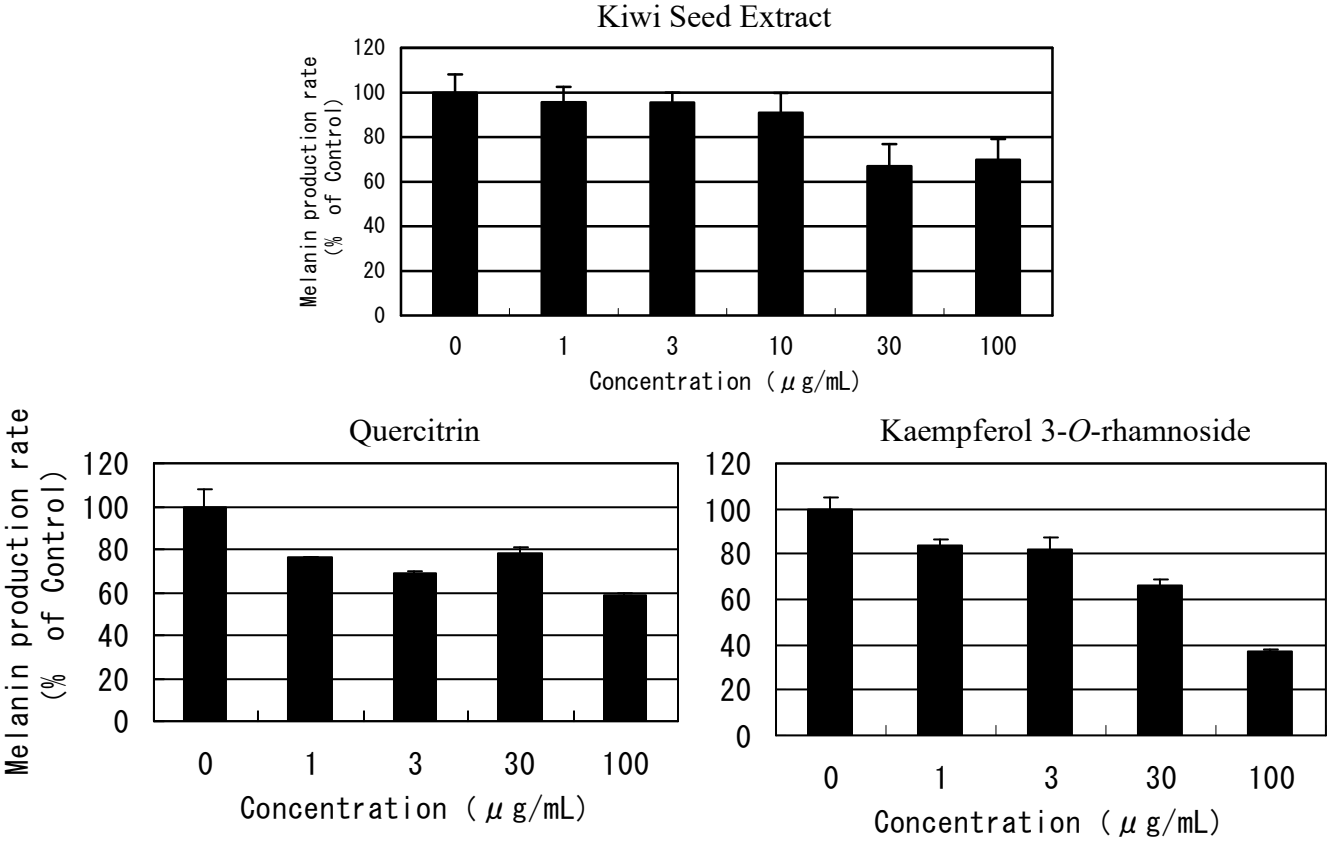


Fig16. The Effect of Kiwi Seed Extract on Melanocyte Growth (n=3-6, mean±S.E.)

【Method of Experiment】

B16 melanoma cells were suspended on theophylline-containing α-MEM medium ($5x10^4$ cells/ml) and 200 μl of the suspension was disseminated on a 48-hole plates. Kiwi Seed Extract was added and the medium was cultured for 3 days. Lastly, cells were crushed by ultrasound and absorbance was measured at wavelength 415nm.

3) Prevention against Hyperpigmentation (*in vivo*)

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

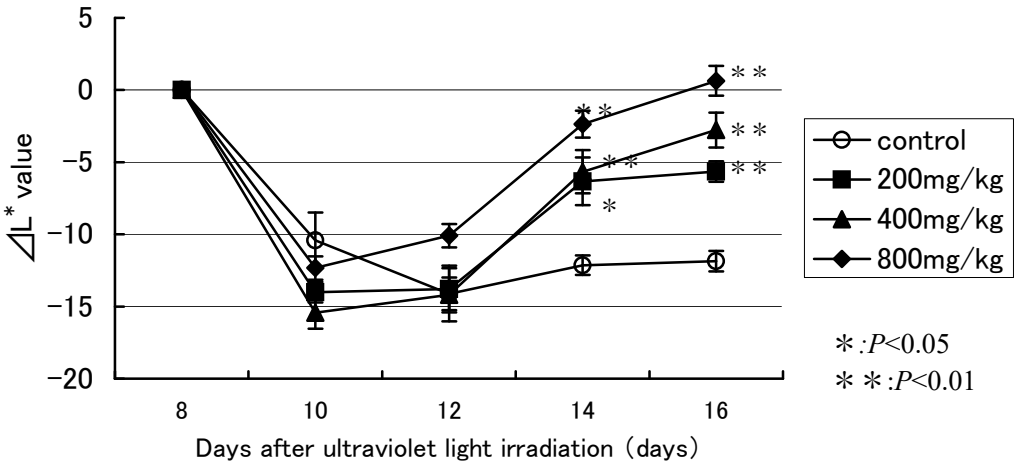


Fig 17. The Effect of Kiwi Seed Extract on UV induced skin hyperpigmentation (n=4, mean±S.E.)

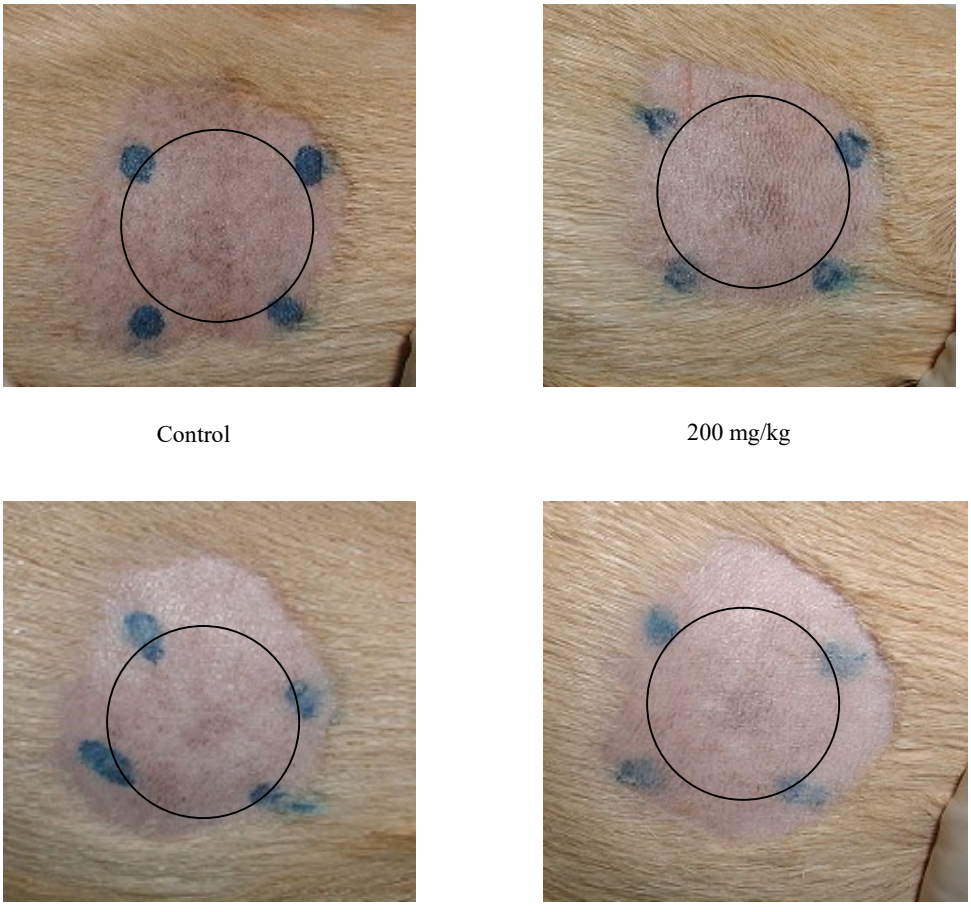


Fig 18. The Effect of Kiwi Seed Extract on UV induced area on day 16th (blue circle indicates the area exposed to UV induction)

【Method of Experiment】

Brown Weiser-Malpes guinea pigs, male, aged 4-week old were fed orally Kiwi Seed Extract of different concentration 7 days (i.e. day -7) prior to UV light induction. UV-B induction was introduced for 7 days (from day 0 – 6) to guinea pigs at 2000mJ/cm^2 using a UV-ray radiator (solar simulator by Ushio, Inc). Feeding of Kiwi Seed Extract continued during UV induction period towards end of protocol period (day 0 – 16). Transparency of skin, L* value was measured using spectro-color difference meter (by Nippon Denshoku Industries Co., Ltd.) prior to and on day 4, 6, 8, 10, 12, 14 and 16 after UV induction.

4) Anti-under eye darkness - Oral Trial on Healthy Female subjects

The effect of Oral Kiwi Seed Extract on under eye darkness was studied. The brightness under eye area of test subjects was measured prior to and after oral administration of Kiwi Seed Extract for 4 weeks. Results showed that the brightness under eye area improved after 4-week oral treatment of Kiwi Seed Extract, hence preventive against under eye dark circles.

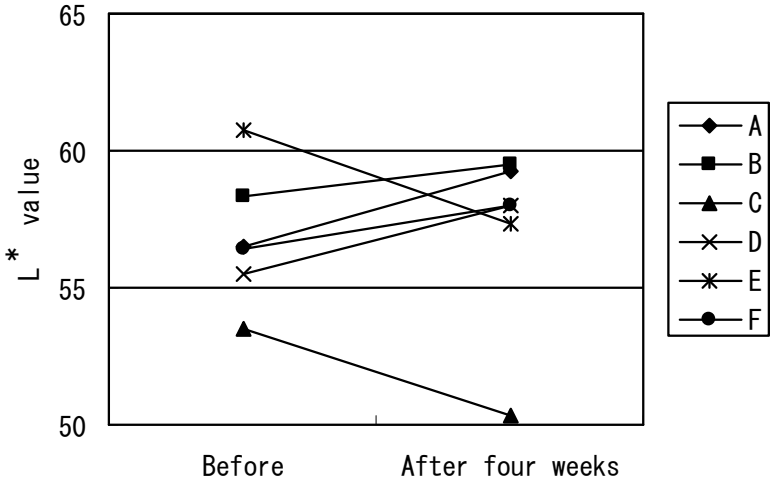


Fig 19. The Effect of Kiwi Seed Extract on Under Eye Darkness

【Method of Experiment】

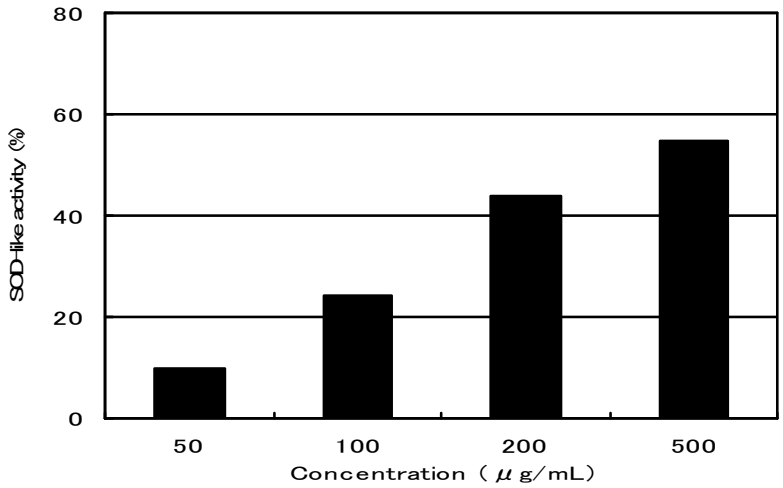
6 healthy females aged between 25-41 were given oral Kiwi Seed Extract-P 50mg/day for 4 weeks. The colour difference under eye areas was measured prior to and after oral administration of Kiwi Seed Extract using Spectro Color Meter SE2000.

(4) Kiwi Seed Extract - Antioxidant Activities

Free radicals generate in our body in response to various endogenous metabolic reactions (e.g. stress & medications). Free radicals such as reactive oxygen species (ROS) activates series of cells oxidation process leading to cells death and various degenerative diseases. Meanwhile, ageing process is accelerated with the increased in endogenous free radicals.

The antioxidative effect of Kiwi Seed Extract is evaluated using superoxide dismutase (SOD) model and 1,1-diphenyl 2-picryl-hyrazil (DPPH) radical scavenging model. As illustrated in Fig. 20, Kiwi Seed Extract with high content of plant polyphenols demonstrated a dose-dependent antioxidative effect on SOD & DPPH radical scavenging models.

①SOD-like Activity



②DPPH Radical Scavenger Activity

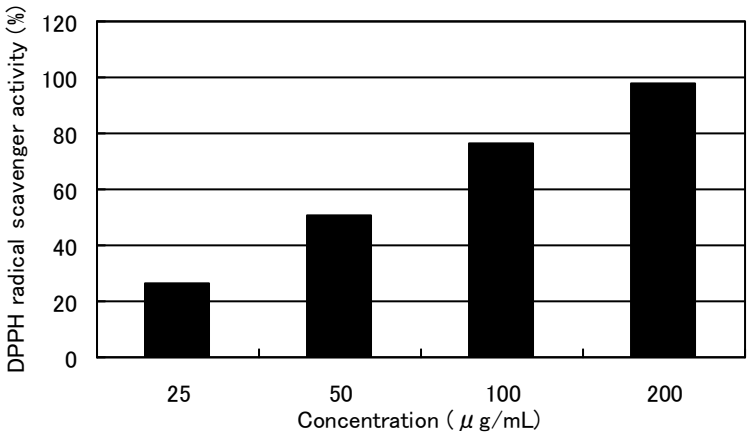


Fig 20. Antioxidative Activity of Kiwi Seed Extract

(5) Kiwi Seed Extract - Moisturizing Effect (topical use)

The moisturizing effect of Kiwi Seed Extract upon topical usage was evaluated and compared with distilled water. Fig. 21 revealed that Kiwi Seed Extract increased and maintained skin moisture up to 120 minutes while maintenance of distilled water lasted only for 40 minutes.

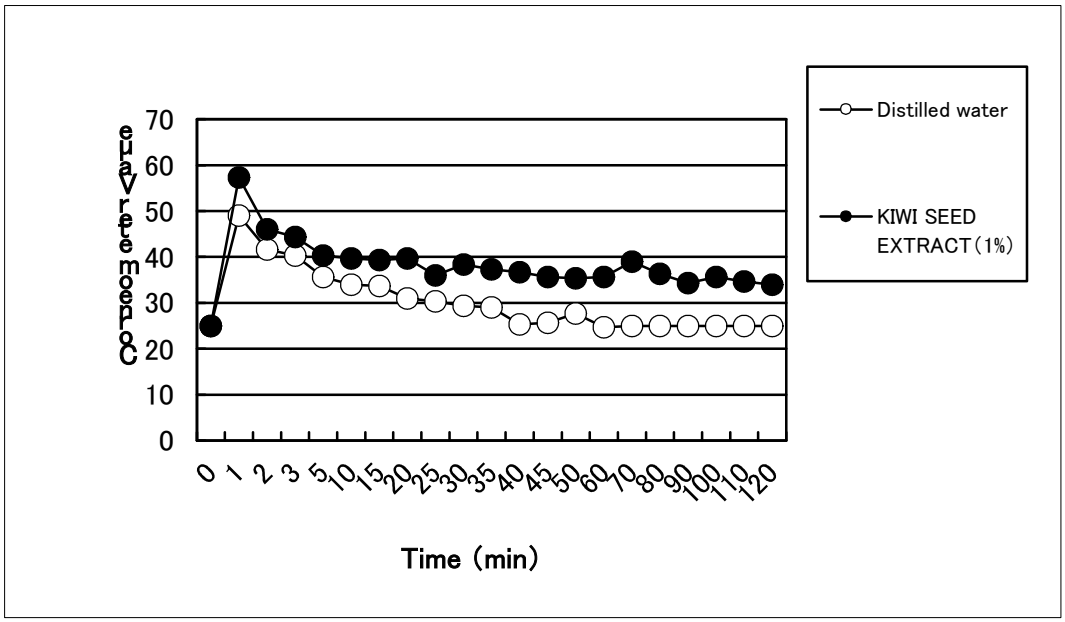


Fig 21. Moisturizing Effect of Kiwi Seed Extract（topical use）

【Method of Experiment】

The moisture content of the skin was measured prior to the evaluation. Kiwi Seed Extract is dissolved in distilled water to produce a concentration of Kiwi Seed Extract 1% solution which was applied on a 2x2cm area on the inside left forearm of test subjects. Moisture content of skin was measured again after absorption of Kiwi Seed Extract in approximately 1 minute. Corneometer SM825 was used for the above measurement at 27°C with 47% humidity.

(6) Anti- inflammatory effect

1) Inhibition of prostaglandins E_2 (PGE_2) production (*in vitro*)

Further experiment was prompted to study the effect of Kiwi Seed Extract on PG production. Cultured macrophage cells (RAW264.7) were stimulated by lipopolysaccharide (LPS) to produce prostaglandins (PG). Results showed that Kiwi Seed Extract significantly suppressed the production of PGE_2 from cells RAW264.7 at concentration of 1-100 μg/mL (Fig. 22). Meanwhile, no cytotoxicity occurred at these concentrations.

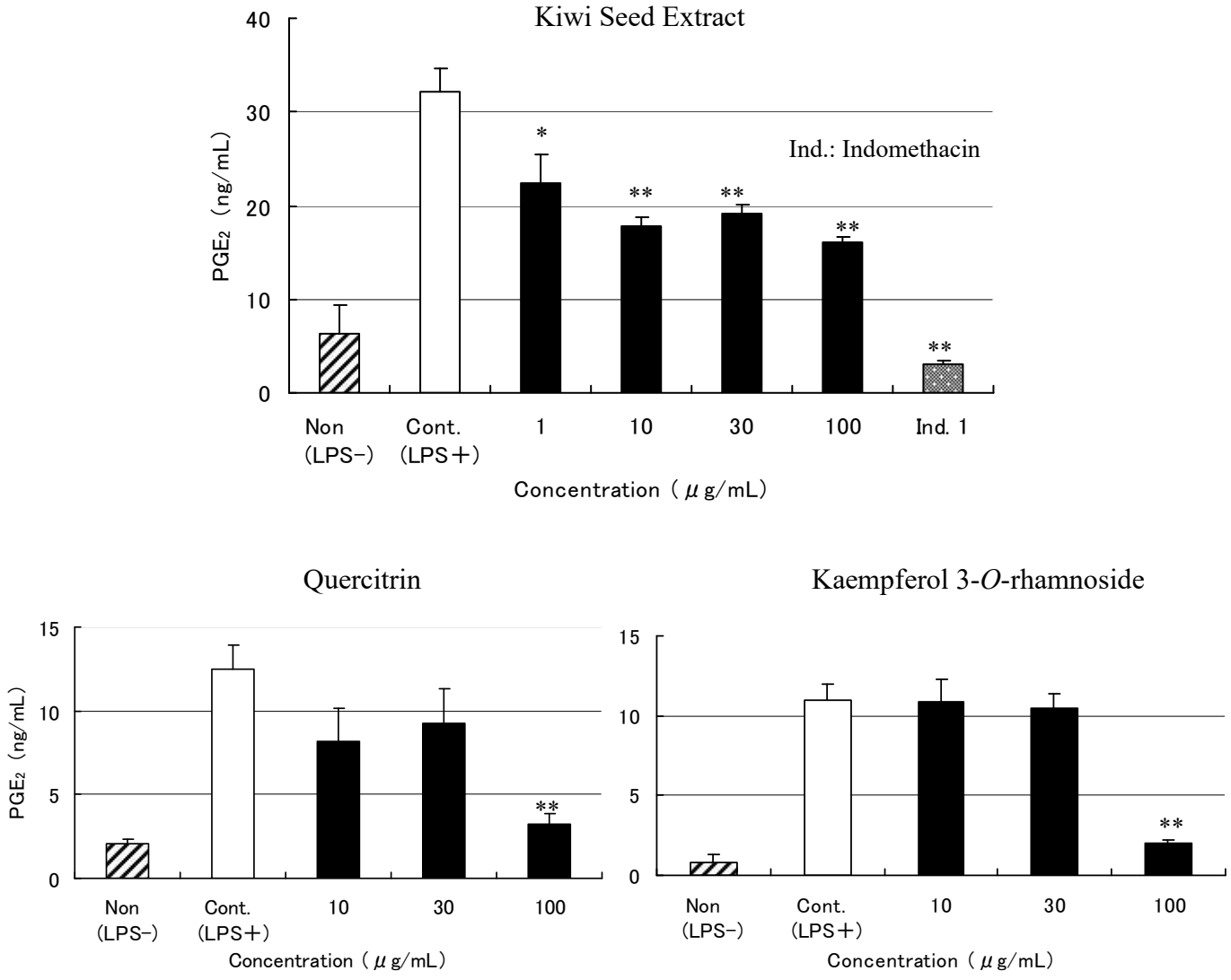


Fig. 22 The effect of Kiwi Seed Extract on PGE_2 production (*: $p<0.05$, **: $p<0.01$)

[Method]

RAW264.7 cells were suspended in MEM medium containing 0.1mM non-essential amino acids, 10% fetal bovine serum, penicillin (100units/mL) and streptomycin (100μg/mL) at concentration of $1x10^6$ cells/mL. 200μl of the suspension was cultured in a 48-welled plate for 24 hours followed by rinsing in serum-free medium (200μl). Later, serum-free medium (170μl) was added to each well followed by the addition of 10μl LPS (200μg/ml; *E.coli* serotype 0127:B8, Sigma) solution and continue culture for 20 hours. Supernatant layer was collected upon completion of culture for the measurement of PGE_2 with Prostaglandin E_2 EIA Kit Monoclonal (Cayman Chemical Co.)

2) Pain relief in acute inflammation －Acetic Acid-Induced Abdominal Inflammation in Mice－

The effect of Kiwi Seed Extract on acute inflammation was experimented on an acetic acid-induced abdominal inflammation model in mice. The analgesic effect of Kiwi Seed Extract was determined by Writhing counts.
As illustrated in Fig. 23, oral administration of Kiwi Seed Extract (100 – 400 mg/kg) demonstrated a reduction in Writhing counts in a dose-dependent manner, suggesting a pain relief effect of Kiwi Seed Extract. Similarly, significant reduction in the dye leakage was observed at doses of 100-400 mg/kg in a dose-dependent manner, suggesting suppression of inflammation in the abdominal area. In addition, comparison with Licorice Extract as anti-inflammatory material demonstrated equivalent effect with Licorice Extract.

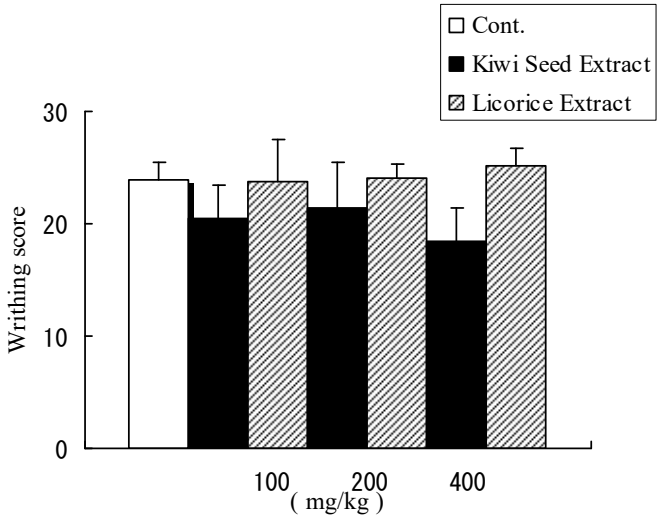


Fig. 23 The effect of Kiwi Seed Extract on acetic acid induced abdominal inflammation

in mice (The Analgesic Effect of Kiwi Seed Extract on Writhing counts).

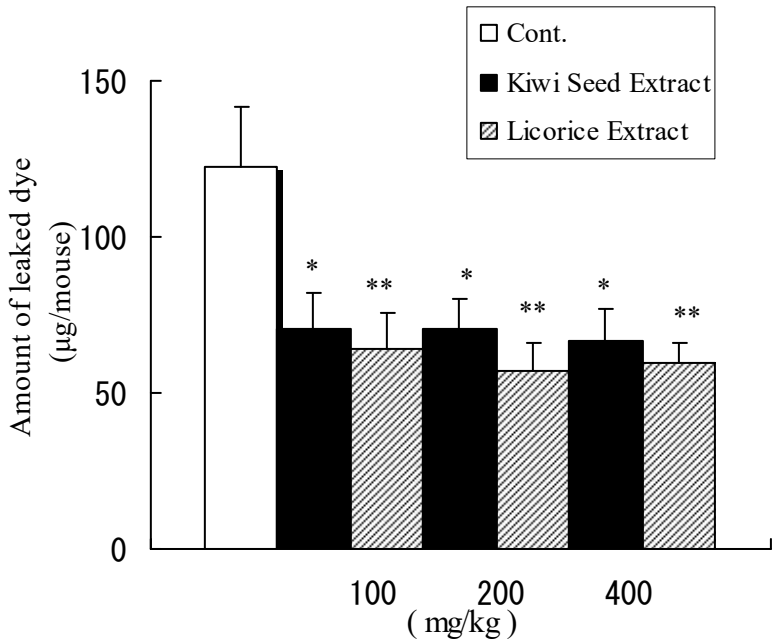


Fig. 24 The effect of Kiwi Seed Extract on acetic acid induced abdominal inflammation in mice (The Anti-inflammatory Effect of Kiwi Seed Extract, *: $p<0.05$, **: $p<0.01$).

[Method]

Kiwi Seed Extract was given orally to fasting mice (ddy, male, 5-wk old) followed by I.V. injection of Pontamin Sky Blue 2% (10mL/kg), an indicator of inflammation 55min later. Inflammation was induced 5min later by acetic acid 1% (10mL/kg) via intraperitoneal injection. Frequency of Writhing events was counted for 15min followed by dissection of abdominal cavity under ether anesthesia. Abdominal cavity containing dye of Pontamin Sky Blue was flushed with saline (8mL). Leaked dye was measured and calibrated to 10mL for absorbance measurement at wavelength 590nm.

【Reference】

Tanaka, J., Shan, S., Kasajima, N. and Shimoda, H. Suppressive effect of defatted kiwi fruit seed extract on acute inflammation and skin pigmentation. *Food Sci. Technol. Res.*, **13** (4), 310-314, 2007.

(7) Clinical Test of ORYZA CERAMIDE® with Kiwi Seed Extract

ORYZA CERAMIDE® exhibits moisturizing effect and activation of fibroblasts. On the other hand Kiwi seed extract suppresses activities of 5α-reductase and lipase involved in acne. We evaluated clinical effect of the oral co-treatment of ORYZA CERAMIDE® and Kiwi seed extract. As a result, reduction of sebum and improvement of acne and skin condition were observed.

[Method]

Japanese female aged 18 to 34 years old with acne were nominated as subjects. The food sample containing Kiwi seed extract (50 mg) and ORYZA CERAMIDE® (20 mg) were given for 4 weeks. After 4-week ingestion, the changes of facial factors were evaluated.

[**Result**]

1) Gross diagnosis

Acne is classified into comed, papule, pustule, abscess, and nodule. The acne score was set as comed: 1, papule: 2, pustule: 3, abscess: 4, and nodule: 5 and acne symptoms were evaluated. After 4- week ingestion of the sample, significant improvement of the score was observed. (Fig. 26).

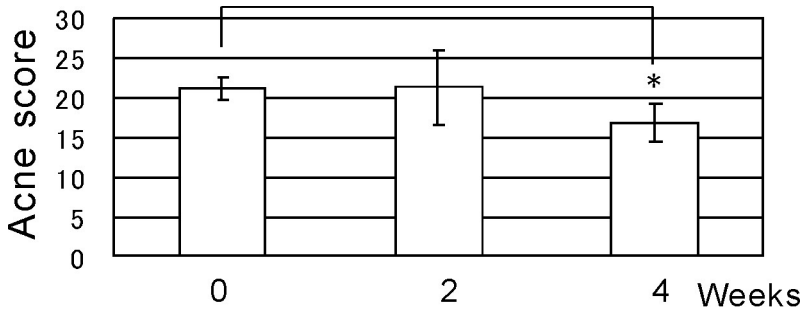


Fig. 26. Change in Acne Score (*: $p<0.05$)

2) Porphyrin

Porphyrin is typical metabolite form *P. acne* and reveal the existence of *P. acne*. As shown in Fig. 27, The number of porphyrin sites in the face were decreased toward ingestion term. Hence, the number of P. acne was found to be decreased by the ingestion of the sample.

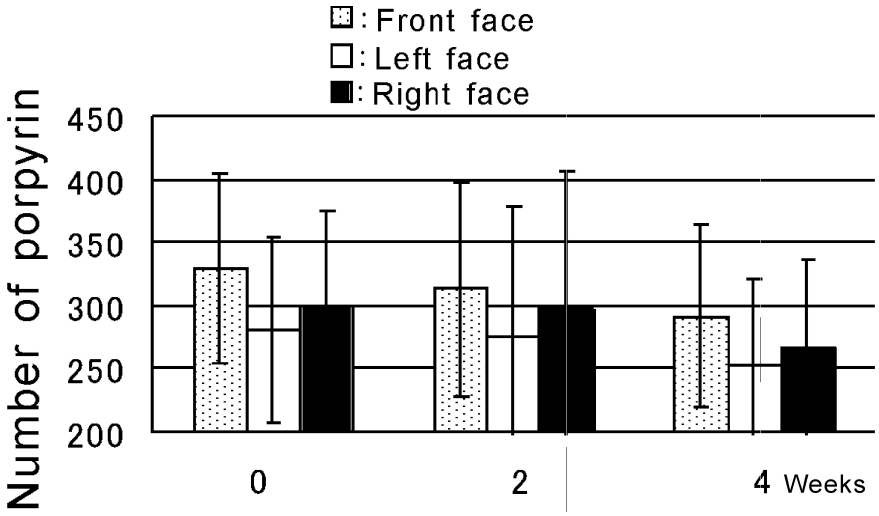


Fig.27. Change in porphyrin sites

3) Sebum

The amount of sebum was decreased in both points of central forehead and the top of left cheek. Significant difference was observed on the top of left cheek at 2 weeks (Fig. 28).

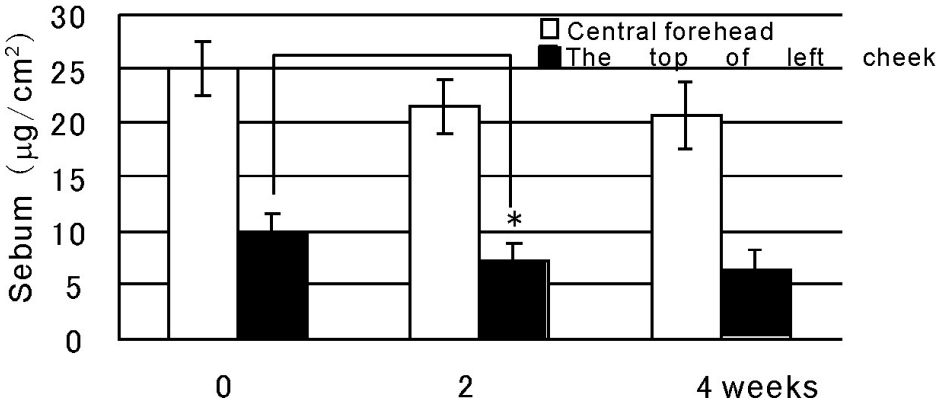


Fig.28. Change in sebum

4) Smoothness

Smoothness at left and right faces was slightly increased (Fig. 29).

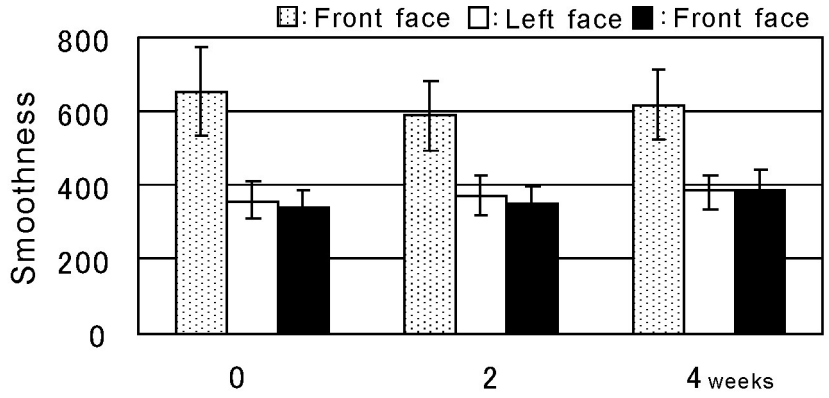


Fig. 29. Change in smoothness

5) Change in skin disease specific QOL by Skindex-16

Skindex-16 is a scale of QOL in skin and consists of 16 question belongs to the fields of symptom, emotion and function. Each field is evaluated 0 to 100 points and maximu total score is 300. Increase in the score reveals low QOL. As a result, Emotion and total score were decreased. Significant decrease was observed at 2 weeks in total score (Fig. 30). This result suggests that the sample improved QOL of the subject with acne.

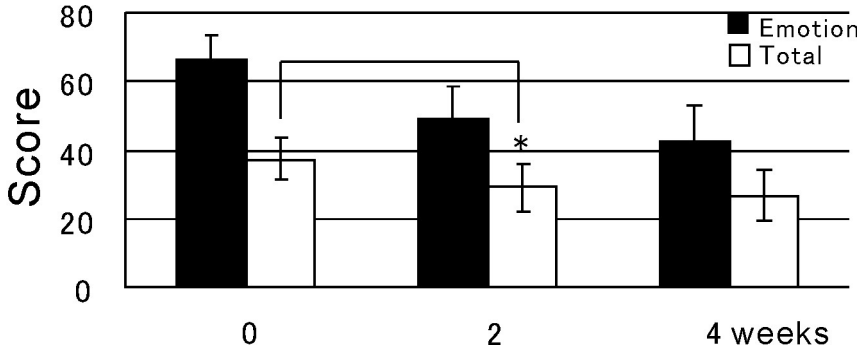


Fig. 30. Change in QOL (*: p<0.05)

4. Functionalities of Kiwi Fruit Oil

Research and development revealed that Kiwi Fruit Oil is loaded with omega-3 essential fatty acid, α-linolenic acid, similar to that of Perilla Seed Oil. In addition, Kiwi Fruit Oil was found to contain naturally occurring amount of tocotrienols & tocopherol. Hence, Kiwi Fruit Oil is suitable to be incorporate into food and cosmetics applications.

(1) Fatty Acid Composition

	Kiwi Fruit Oil	Perilla Seed Oil
Palmitic Acid	6.2%	6.7%
Stearic Acid	2.3%	1.8%
Oleic Acid	15.6%	19.6%
Linoleic Acid	15.8%	12.0%
α-linolenic Acid	59.8%	59.9%

(2) Health Benefits of α-linolenic Acid

α-linolenic acid is an omega-3 essential fatty acid which cannot be synthesized within our bodies.

α-linolenic acid has been documented to reduce allergy[1)], prevent against breast and colon cancers[2,3)]. In mice model, α-linolenic acid was reported to promote longevity[4)].

(3) Health Benefits of tocotrienols

Tocotrienols belongs to the family of vitamin E with 3 double bond at the side chain. It is a potent antioxidant (40-60x more potent than tocopherol as an antioxidant) with cholesterol lowering effects, prevention against hardening of arteries[5)], and potent free radical scavenger[6)].

More information on tocotrienols is available on product brochure for Oryza Tocotrienol.

Books for reference

1) Harumi Okuyama “Therapeutics”, **25**, 47 (1991).
2) Ikumi Yonekura, Akio Sato “Igaku no Ayumi”, **150**, 233 (1989).
3) Tomio Narisawa, Yukio Nishizawa, Mieko Koban, Yoshihiro Isoda, Jiro Hirano “Igaku no Ayumi”, **145**, 911 (1988).
4) Hiramitsu Suzuki, Shizuko Arai, Junji Terao, Teruhiko Nagao, Megumi Yamazaki, Hideki Saito, Shun Wada “Executive Summary of the 44th Japan Association of Nutrition and Food Meeting”, 2E-20P, (1989).
5) Tomeo A. C. *et al., lipids*, **30**, 1179-83 (1995).
6) Kamat J. P. *et al., Neurosci. Lett*., **195**, 179-82 (1995).

5. Stability of Kiwi Seed Extract

(1) Thermostability

As illustrated in Fig. 25, polyphenols content of Kiwi Seed Extract (no added diluent) is highly stable at 100°C and 120°C for 1 hour. It is stable at temperatures for processing food.

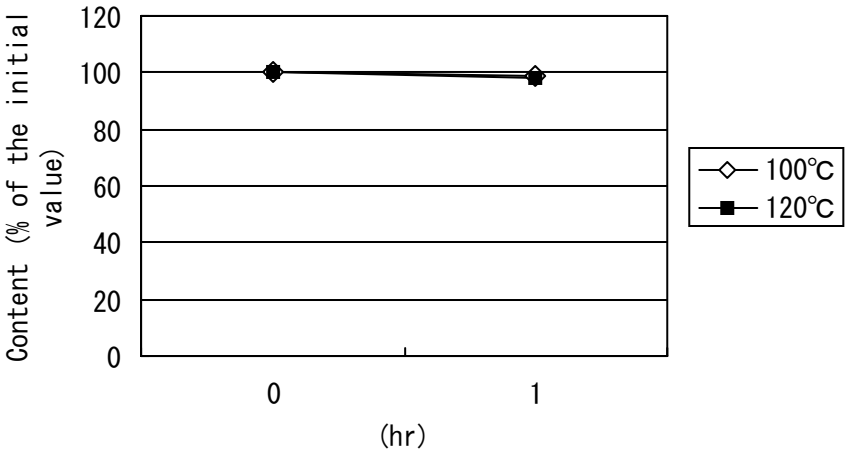


Fig. 25 Thermostability of Kiwi Seed Extract

(2) pH stability

Kiwi Seed Extract (no added diluent) was dissolved in 40% ethanol, adjusted to its pH and stored at room temperature for 1 day and 1 week respectively. Polyphenols content of Kiwi Seed Extract was measured and results showed (Fig. 26) that polyphenols content remained stable at acidic condition but reduced by 15% and 30% in alkaline condition after 1 hour and 1 week respectively.

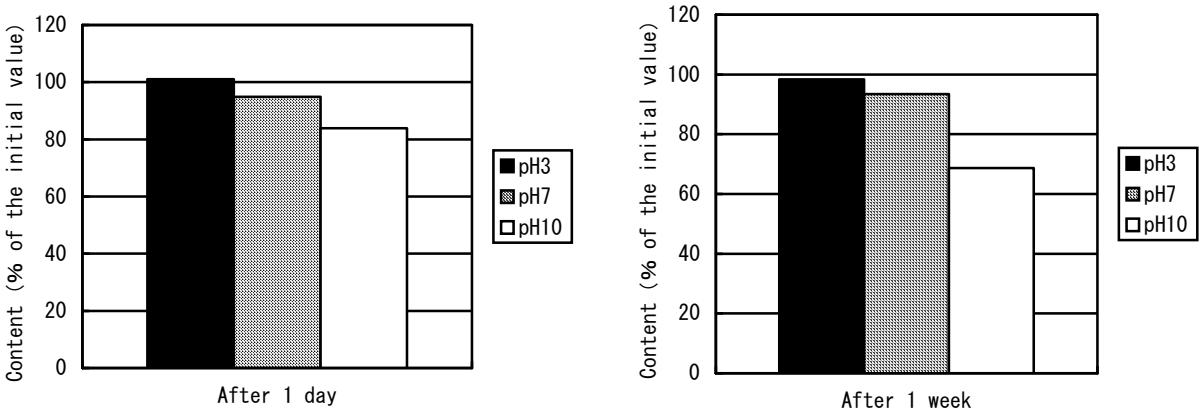


Fig. 26 pH stability of Kiwi Seed Extract（100% as initial value）

(3) Stability in Aqueous Solution

A 0.4% solution (pH 3.5) of Kiwi Seed Extract-WSP (powder, water soluble) was prepared and stored at room temperature (with and without light), 40°C (without light) & 5°C (without light) for 2 weeks. Visual observation on precipitation, turbidity and color change was conducted. As tabulated below, Kiwi Seed Extract-WSP is highly in aqueous condition.

	Liquid stability (0.4% solution、pH 3.5)			
	Room temperature (light shielding)	25℃ (without light shielding)	40℃ (without light shielding)	5℃ (without light shielding)
Precipitation, turbidity	Negative	Negative	Negative	Negative
Color changes	Negative	Negative	Negative	Negative

6. Nutrition Information (KIWI SEED EXTRACT-P)

Description	Amount	Note	Analytical Method
Moisture	1.0g/50g		Heat-drying at atmospheric pressure
Protein	0.8g/50g	1	Kjeldahl Method
Fat	0.4g/50g		Acid degradation
Ash	0.8g/50g		Direct Incineration
Carbohydrate	47.0g/50g	2	
Energy	195kcal/50g	3	Atwater Method (Revised)
Dietary fiber	0.03g/50g		Prosky Method
Sodium	6.5mg/50g		Atomic absorption spectrophotometory
Sodium	0.03g/50g		Sodium Equiv. value

1. Nitrogen, protein conversion factor: 6.25
2. Carbohydrate expression standard (Ministry of Health and Welfare's announcement No. 176)
   Calculation: 50 – (water + protein + fat + ash)
3. Energy expression standard (Ministry of Health and Welfare's announcement No. 176)
   Conversion factor: Protein 4, fat 9, sugar 4; dietary fiber 2

Test trustee: SRL, Inc
Date of analysis: August 18, 2005
Test No.: 200508050029

7. Kiwi Seed Extract (in non-excipient form) – Product Safety Profile

(1) Residual Agricultural Chemicals

Kiwi Seed Extract (without binder) is conformed to regulation stipulated for 447 residual agricultural chemical compounds. No residual agricultural chemicals detected as confirm by test trustee.

Test trustee：Masis Co. Ltd.
Data：March 2, 2007
Report No.：10735

(2) Acute Toxicity (LD_{50})

Acute Toxicity test was conducted accordingly to the Guidelines for Single-Dose Toxicity Tests for Pharmaceutical Products. Kiwi Seed Extract was orally administered to male and female ICR mice (aged 5 weeks old) at 2,000 mg/kg and kept for 14 days. No abnormalities and fatal event observed at 2,000 mg/kg. Upon autopsy performance, no abnormalities observed under macroscopic examinations. Thus, LD_{50} of Kiwi Seed Extract is deduced to be >2,000 mg/kg in both male and female mice.

Furthermore, LD_{50} of Kiwi Fruit Oil is deduced to be >5,000 mg/kg in both male and female mice.

(3) Mutagenicity

Ames test was conducted and finding was Negative (TA97a, TA98, TA100, TA102 and TA1535). Kiwi Seed Extract is non-mutagenic.

(4) Acute Skin Irritation Study

Kiwi Seed Extract-PC (0.5g) was applied on the skin of 3 rabbits for 4 hours. The treated lesions were observed approximately 1, 24, 48 and 72 hours, 7, 10 and 14 days after removal of the dressing. Under the experimental conditions, Kiwi Seed Extract-PC was found to be non-irritant for skin of rabbits.

(5) Eye Irritation Test

This *in vitro* study was performed to assess the corneal irritation and damage potential of Kiwi Seed Extract-PC by means of the BCOP (Bovine Corneal Opacity and Permeability) assay using fresh bovine cornea. It can be stated that in this study and under the experimental conditions reported, the test item Kiwi Seed Extract-PC is not considered to be an eye irritant.

(6) Human Patch Test

The Kiwi Seed Extract-PC (5 mg/5 μ L) was patched on 21 women aged between 18 and 71, and 9 men aged between 18 and 78 for 24 hours. No irritation on skin of human ware found.

(7) Repeated Human Patch Test

The Kiwi Seed Extract-PC (5 mg/15 μ L) was patched on 18 women aged between 22 and 76, and 12 men aged between 20 and 77 for 24, 48 and 72 hours. No irritation on skin of human ware found.

8. Kiwi Seed Extract – Recommended Daily Dosage

The recommended daily dosage for Kiwi Seed Extract – P, – WSP is 50-100 mg/day

9. Applications

	Applications	Claims	Examples
Foods	Beauty food Skin whitening food	1. Anti-acne 2. Anti-wrinkles 3. Anti-dark cerkles 4. Skin whitening	Beverages, hard & soft capsules, tablets, candies, chewing gums, chocolates, wafers, jellies etc
Cosmetics	Beauty cosmetic		Body lotions, body gel etc.

10. Packaging

KIWI SEED EXTRACT-P（Powder, for food）
KIWI SEED EXTRACT-WSP（Water-solbue , for food）
KIWI SEED EXTRACT-WSPC（Water-solbue , for cosmetic）
KIWI SEED EXTRACT-PC（Powder, for cosmetic）
5kg Interior packaging：Aluminium bag
Exterior packaging：Cardboard

KIWI SEED EXTRACT-LC（Liquid, for cosmetic）
5kg Interior packaging：Cubic polyethylene container
Exterior packaging：Cardboard

KIWI FRUIT OIL（Oil, for food and cosmetic）
16kg Interior packaging：Tin can
Exterior packaging：Cardboard

11. Storage

Store in cool, dry dark place.

12. Expression

KIWI SEED EXTRACT-P、KIWI SEED EXTRACT-WSP
Expression： Kiwi Seed Extract

KIWI FRUIT OIL
Expression： Kiwi Fruit Oil、Kiwi Oil、Kiwi Seed Oil

KIWI SEED EXTRACT-PC
INCI Name: Dextrin and Actinidia Chinensis (Kiwi) Seed Extract

KIWI SEED EXTRACT-WSPC
INCI Name: Dextrin and Actinidia Chinensis (Kiwi) Seed Extract

KIWI SEED EXTRACT-LC
INCI Name: Butylene Glycol and Water and Actinidia Chinensis (Kiwi) Seed Extract

PRODUCT STANDARD
PRODUCT NAME

KIWI SEED EXTRACT-P

FOOD

This product is extracted from kiwi (*Actinidia chinensis* Planch.) seeds with aqueous ethanol. It guarantees minimum of 5.0 mg/100g quercitrin and 2.0 % polyphenols.

Appearance	Yellow powder with light unique smell.	
Quercitrin	Min. 5.0 mg/100g	(HPLC)
Polyphenols	Min. 2.0 %	(Folin-Denis method)
Loss on Drying	Max. 10.0 %	(Analysis for Hygienic Chemists, 1 g, 105℃, 2 h)
Purity Test		
(1)Heavy Metals	Max. 10 ppm	(The Japanese Standards for Food Additives)
(2)Arsenic	Max. 1 ppm	(Standard Methods of Analysis in Food Safety Regulation)
Standard Plate Counts	Max. 1×10^3 cfu/g	(Analysis for Hygienic Chemists)
Moulds and Yeasts	Max. 1×10^2 cfu/g	(Analysis for Hygienic Chemists)
Coliforms	Negative	(Analysis for Hygienic Chemists)

Composition

Ingredients	Contents
Kiwi Seed Extract	25 %
Dextrin	75 %
Total	100 %

PRODUCT STANDARD
PRODUCT NAME

KIWI SEED EXTRACT-WSP

FOOD

This product is extracted from kiwi (*Actinidia chinensis* Planch.) seeds with aqueous ethanol. It guarantees minimum of 5.0 mg/100g quercitrin and 2.0 % polyphenols.
This product is water-soluble.

Appearance	Yellow powder with light unique smell.	
Quercitrin	Min. 5.0 mg/100g	(HPLC)
Polyphenols	Min. 2.0 %	(Folin-Denis method)
Loss on Drying	Max. 10.0 %	(Analysis for Hygienic Chemists, 1 g, 105℃, 2 h)
Purity Test		
(1)Heavy Metals	Max. 10 ppm	(The Japanese Standards for Food Additives)
(2)Arsenic	Max. 1 ppm	(Standard Methods of Analysis in Food Safety Regulation)
Standard Plate Counts	Max. 1×10^3 cfu/g	(Analysis for Hygienic Chemists)
Moulds and Yeasts	Max. 1×10^2 cfu/g	(Analysis for Hygienic Chemists)
Coliforms	Negative	(Analysis for Hygienic Chemists)

Composition

Ingredients	Contents
Kiwi Seed Extract	25 %
Dextrin	75 %
Total	100 %

PRODUCT STANDARD
PRODUCT NAME

KIWI SEED EXTRACT-PC

COSMETIC

This product is extracted from kiwi (Actinidia chinensis Planch.) seeds with aqueous ethanol. It guarantees minimum of 5.0 mg/100g quercitrin and 2.0 % polyphenols.

Appearance	Yellow powder with light unique smell.	
Quercitrin	Min. 5.0 mg/100g	(HPLC)
Polyphenols	Min. 2.0 %	(Folin-Denis method)
Loss on Drying	Max. 10.0 %	(1 g, 105℃, 2 h)
Purity Test		
(1)Heavy Metals	Max. 10 ppm	(The Second Method)
(2)Arsenic	Max. 1 ppm	(The Third Method)
Standard Plate Counts	Max. 1×10^2 cfu/g	(Analysis for Hygienic Chemists)
Moulds and Yeasts	Max. 1×10^2 cfu/g	(Analysis for Hygienic Chemists)
Coliforms	Negative	(Analysis for Hygienic Chemists)

Composition

Ingredients	Contents
Dextrin	75 %
Actinidia Chinensis (Kiwi) Seed Extract	25 %
Total	100 %

Ref: The Japanese Standards of Quasi-Drug Ingredients.

PRODUCT STANDARD
PRODUCT NAME

KIWI SEED EXTRACT-WSPC

COSMETIC

This product is extracted from kiwi (*Actinidia chinensis* Planch.) seeds with aqueous ethanol. It guarantees minimum of 5.0 mg/100g quercitrin and 2.0 % polyphenols.
This product is water-soluble.

Appearance	Yellow powder with light unique smell.	
Quercitrin	Min. 5.0 mg/100g	(HPLC)
Polyphenols	Min. 2.0 %	(Folin-Denis method)
Loss on Drying	Max. 10.0 %	(Analysis for Hygienic Chemists, 1 g, 105℃, 2 h)

Purity Test

(1)Heavy Metals	Max. 10 ppm	(The Japanese Standards for Food Additives)
(2)Arsenic	Max. 1 ppm	(Standard Methods of Analysis in Food Safety Regulation)
Standard Plate Counts	Max. 1×10^2 cfu/g	(Analysis for Hygienic Chemists)
Moulds and Yeasts	Max. 1×10^2 cfu/g	(Analysis for Hygienic Chemists)
Coliforms	Negative	(Analysis for Hygienic Chemists)

Composition

Ingredients	Contents
Actinidia chinensis (kiwi) seed extract	25 %
Dextrin	75 %
Total	100 %

PRODUCT STANDARD
PRODUCT NAME

KIWI SEED EXTRACT-LC

COSMETIC

This product is extracted from kiwi (*Actinidia chinensis* Planch.) seeds with aqueous 1, 3-butylene glycol.

Appearance Brown liquid. Odorless or light unique smell.

Certification Test
Polyphenols — Dissolve 30 μl of this product in 3.5 ml water. Add 0.2 ml Folin-Denis reagent into the solution followed by 0.4 ml saturated Na_2CO_3. The solution will turn into blue color.

Purity Test

(1)Heavy Metals	Max. 10 ppm	(The Second Method)
(2)Arsenic	Max. 1 ppm	(The Third Method)

Standard Plate Counts Max. 1×10^2 cfu/g (Analysis for Hygienic Chemists)

Moulds and Yeasts Max. 1×10^2 cfu/g (Analysis for Hygienic Chemists)

Coliforms Negative (Analysis for Hygienic Chemists)

Composition

Ingredients	Contents
Butylene Glycol	69 %
Water	30 %
Actinidia Chinensis (Kiwi) Seed Extract	1 %
Total	100 %

Ref: The Japanese Standards of Quasi-Drug Ingredients.

PRODUCT STANDARD
PRODUCT NAME

KIWI FRUIT OIL

FOOD

This oil is extracted and refined from kiwi (*Actinidia chinensis* Planch.) seeds.

Appearance	Light yellowish liquid oil with light unique smell.	
Acid Value	Max. 0.50	
Color	Max. (Y+10R) 50	(Lovibond, 133.4mm cell)
α-Linolenic Acid	Min. 55.0 %	(GC)
Tocotrienols	A peak is detectable.	(HPLC)

Purity Test

(1)Heavy Metals	Max. 10 ppm	(Sodium Sulfide Colorimetric Method))
(2)Arsenic	Max. 1 ppm	Standard Methods of Analysis in Food Safety Regulation, The Third Method,Apparatus B)
Standard Plate Counts	Max. 1×10^2 cfu/g	(Analysis for Hygienic Chemists)
Moulds and Yeasts	Negative	(Analysis for Hygienic Chemists)
Coliforms	Negative	(Analysis for Hygienic Chemists)

Composition

Ingredient	Content
Kiwi Seed Oil	99.90 %
Mixed Tocopherol	0.05 %
L-Ascorbic Acid Palmitate	0.05 %
TOTAL	100%

100

Expiry date 2 years from date of manufacturing

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.

PRODUCT STANDARD
PRODUCT NAME

KIWI FRUIT OIL

COSMETIC

This oil is extracted and refined from kiwi (*Actinidia chinensis* Planch.) seeds.

Appearance	Light yellowish liquid oil with light characteristic odor.	
Acid Value	Max. 0.50	(The First method, 10g)
Color	Max. (Y+10R) 50	(Lovibond, 133.4mm cell)
α-Linolenic Acid	Min. 55.0 %	(GC)
Tocotrienols	A peak is detectable.	(HPLC)

Purity Test

(1)Heavy Metals	Max. 10 ppm	((The Second Method of The Japanese Standards of Quasi-Drug Ingredients)
(2)Arsenic	Max. 1 ppm	(The Third Method of The Japanese Standards of Quasi-Drug Ingredients))
Standard Plate Counts	Max. 1×10^2 cfu/g	(Analysis for Hygienic Chemists)
Moulds and Yeasts	Negative	(Analysis for Hygienic Chemists)
Coliforms	Negative	(Analysis for Hygienic Chemists)

Composition

Ingredient	Content
Actinidia chinensis (kiwi) seed oil	99.90 %
Tocopherol	0.05 %
Ascorbyl Palmitate	0.05 %

Expiry date 2 years from date of manufacturing

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.

Total 100.00 %

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：

ORYZA OIL & FAT CHEMICAL CO., LTD.

Head quarter

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E-mail: tokyo@oryza.co.jp

***Correction**

- Add to Ames Test (Five strains) (P. 25)
- Add to Clinical Test of ORYZA CERAMIDE® with Kiwi Seed Extract (P.21-23)

Established Date: December 16, 2005
Revised Date: November 29, 2019

ORYZA OIL & FAT CHEMICAL CO., LTD.