



ORYZA OIL & FAT CHEMICAL CO., LTD.

PURPLE RICE EXTRACT

Cosmeceutical Food Supplement

Anti-Diabetic Supplement

Prevention of eye disease

PURPLE RICE EXTRACT-P

PURPLE RICE EXTRACT-PC

PURPLE RICE EXTRACT-LC0.1



ORYZA OIL & FAT CHEMICAL CO., LTD.

ver. 4.0 SJ/JT

Beauty/health/diet food materials

PURPLE RICE EXTRACT

1. Introduction

Many beauty foods have been developed for skin beauty. There are various types of beauty foods, such as ceramide and collagen that are skin components, and LITCHI SEED EXTRACT that regulates skin function for skin beauty. Considering body beauty, foods that have diet effects while maintaining health are desired to make a slender beautiful body.

To restore health, foods with nourishing and tonic effects are useful. Nourishment/tonification means the strengthening of vitality, and the recovery and maintenance of a healthy body.

Polyphenols are food components that promote health. Foods containing polyphenols have anti-oxidation action. Polyphenols are considered to have not only anti-aging effects but also effects on lifestyle-related disorders caused by oxidative damage, such as cancer and cardiovascular diseases. Anthocyanins contained in red wine have been also reported to have anti-oxidation effects. Anthocyanins reduce fatigue of the eyes and are effective for improving visual acuity.

Cataract impairs eye health. Senile cataract is closely associated with aging and oxidative stress. In the U.S., intake of anti-oxidative substances such as vitamin C and lutein has been recommended to inhibit the progression of cataract.

Skin health depends on maintaining normal intercellular tissue, which is mainly composed of collagen, ceramide, elastin, and hyaluronic acid. The skin tissue repeatedly undergoes degradation and regeneration, resulting in epidermal turnover. With age, intercellular tissue such as collagen, ceramide, elastin, and hyaluronic acid decrease, which reduces skin tension, elasticity, and water retention, resulting in dry skin. With an increase in active oxygen in the body, vitamin C is consumed, and collagen synthesis decreases. Vitamin C promotes collagen reproduction. Ingestion of substances that promote the effects of vitamin C enhance its efficacy. Thus, to make the body healthy and beautiful, beauty/health foods with multiple functions that improve various conditions are in demand.

PURPLE RICE EXTRACT we developed is expected to have various beneficial effects. We produce water-soluble PURPLE RICE EXTRACT with only slight odor by our novel method.

2. What is purple rice?

Purple rice is the seed of *Oryza sativa* Linne (*Gramineae*), and is a type of ancient rice originated in Hanzhong, Shanxi in China and is also called purple-black rice or black rice. Unpolished purple rice has a hard surface and a white inner area, and contains purple black pigments (anthocyanine) in the unpolished seed coat.

In ancient times, purple rice was considered to be effective for perennial youth and long life, and have nourishing/tonic effects, and effects on anemia. Since it is said that Chang Chien discovered this rice and rose to a high position in the Han period in China, purple rice has been considered to be “luck-success rice”. In those days, purple rice was precious and eaten only by the emperor. Subsequently, this rice was commonly eaten as a court dish. It is said that Yang-kuei-fei also loved purple rice as a beauty food.

In China, Li Shi Zhen, a medical herb cuisine scholar in the Ming period, described in “Ben cao gang mu” that purple rice promotes vitality, strengthens the spleen, liver, stomach, and intestine, and enhances pulmonary function by its hematopoietic effects. Purple rice is called “hematopoietic rice” and “medical rice” and has been used in medical herb cuisine such as “blood-tonifying purple rice porridge”. Tradition says that purple rice has nourishing/tonic effects in patients with chronic diseases, in the recovery stage, infants, and weak people, and constant consumption of purple rice makes the skin smooth and blackens hair, resulting in rejuvenation.

The pigments of purple rice (anthocyanine) promote anti-oxidation effects, scavenging active oxygen generated in the body, are useful for preventing carcinogenesis and aging, and promote recovery of asthenopia. Anthocyanine pigments are also contained in blueberries, and strengthen the capillaries and improve blood flow, being effective against eye fatigue, watery eye, and bleary eye. In addition, their preventive effects arteriosclerosis have been also reported.

Purple rice is used as a material for edible vinegar and alcohol and added to flour and kneaded to make bread, fine noodles, and noodles.

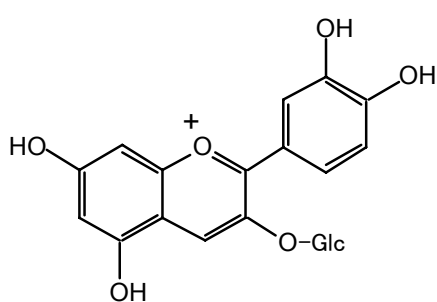
Purple rice seed

Purple rice

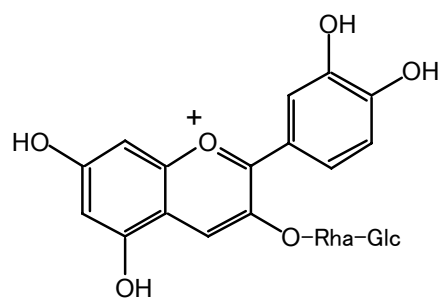
3. Components of purple rice

Purple rice contains anthocyanine glycosides such as chrysanthemin (Cyanidin-3-glucoside) that are not contained in other types of rice.

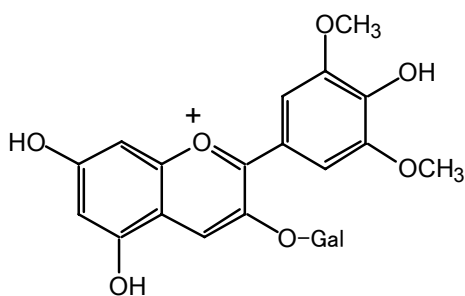
Anthocyanin



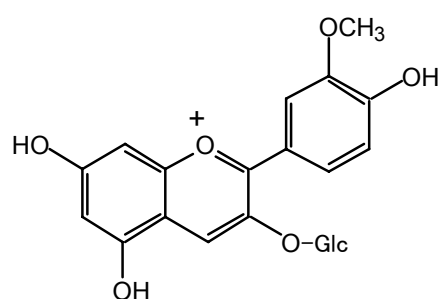
Cyanidin-3-Glucoside



Cyanidin-3-Rhamnoglucoside



Malvidin-3-Galactoside



Peonidin-3-Glucoside

Fig. 1 Major components of PURPLE RICE EXTRACT

4. Mechanism of the development of diabetes mellitus

Diabetes mellitus is chronic hyperglycemia due to insulin insufficiency. This disorder is considered to be caused by the combination of some genetic factors and lifestyle factors. In particular, in insulin non-dependent diabetes mellitus (type 2), which accounts for more than 90% of all cases of diabetes mellitus, blood glucose is increased by the combination of decreased insulin secretion from pancreatic β cells and aging or lifestyle factors that decrease insulin sensitivity, such as overeating, high fat diet, insufficient exercise, and obesity. Obesity is the main risk factor of diabetes mellitus. These causes, excluding insufficient exercise, can be prevented only by dietary control.

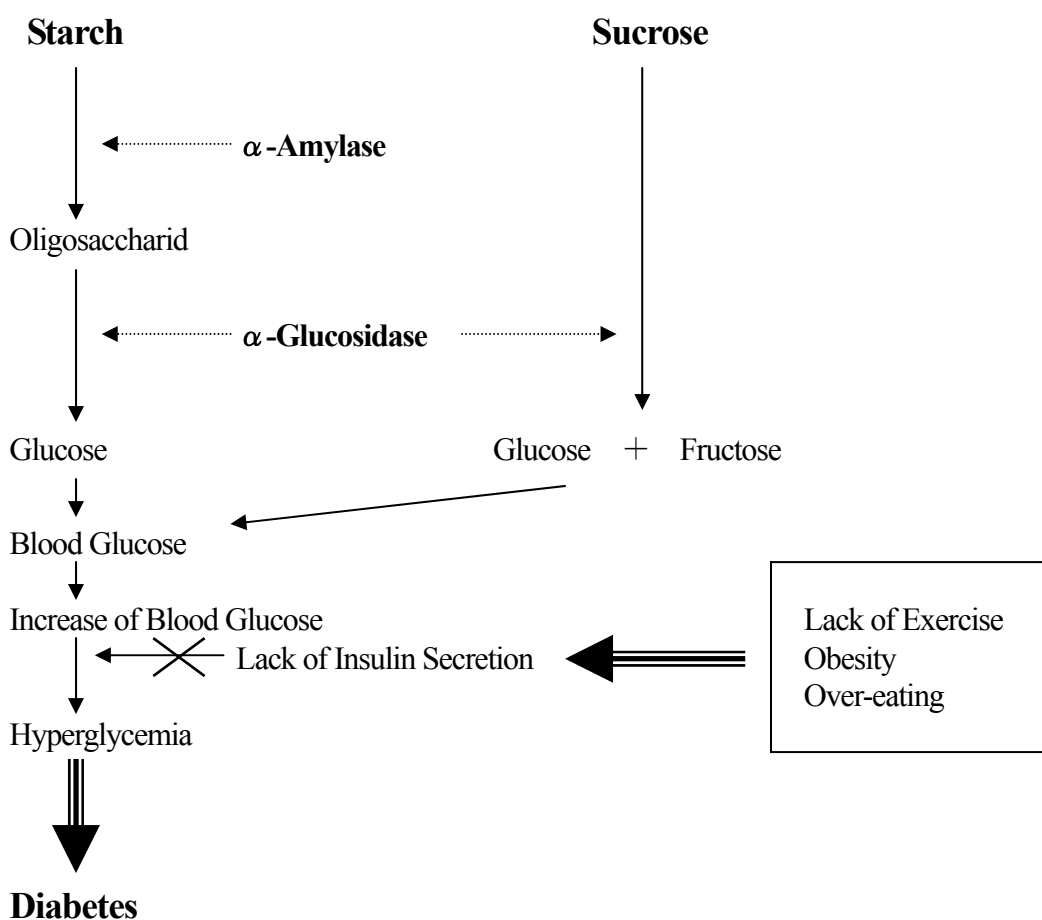


Fig. 2 Association between digestion/absorption of carbohydrates and type 2 diabetes mellitus

5. Function of PURPLE RICE EXTRACT

(1) Inhibition of carbohydrate absorption

1) Inhibition of carbohydrate absorption (in vitro)

The inhibitory activity of PURPLE RICE EXTRACT against carbohydrate degradation enzymes was evaluated using α -amylase derived from the swine pancreas and α -glucosidase derived from the rat intestine. PURPLE RICE EXTRACT inhibited the activity of α -amylase as an amylolytic enzyme, and markedly inhibited the activity of α -glucosidase as a disaccharidase.

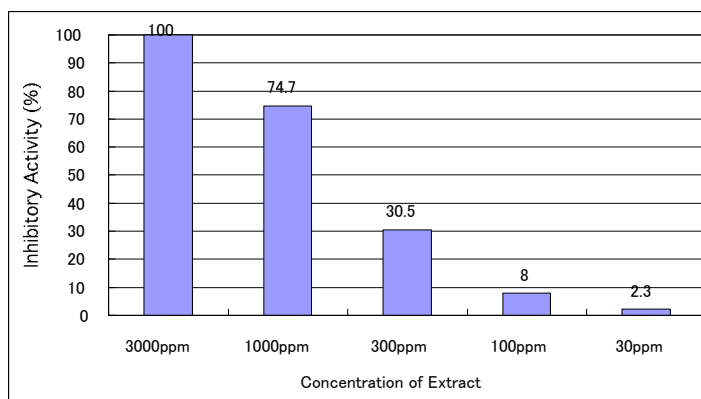


Fig. 3 Inhibition of α -Amylase by PURPLE RICE EXTRACT

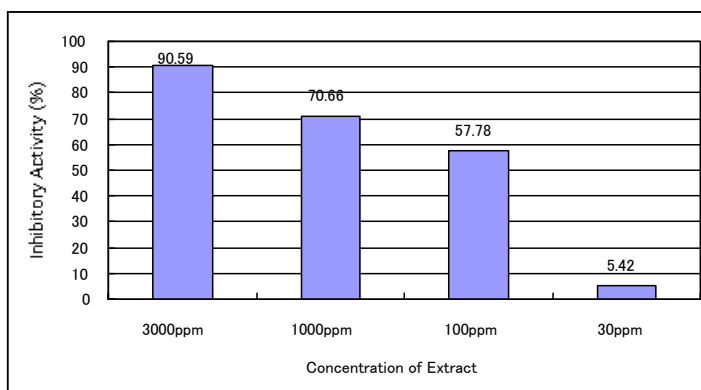


Fig. 4 Inhibition of α -Glucosidase by PURPLE RICE EXTRACT

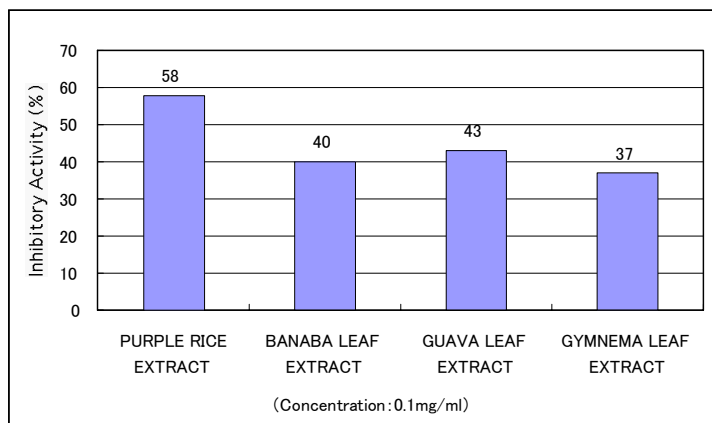


Fig. 5 Inhibition of α -Glucosidase by PURPLE RICE EXTRACT and Several Plant Extracts

2) Inhibition of increases in blood glucose in rats (in vivo)

The in vivo inhibitory effects of PURPLE RICE EXTRACT, which inhibited the activity of carbohydrate degradation enzymes, on increases in blood glucose after glucose loading were evaluated in normal rats. PURPLE RICE EXTRACT inhibited increases in the blood glucose level 30 minutes after administration of starch or sucrose compared with the control.

PURPLE RICE EXTRACT inhibited the activity of α -amylase and α -glucosidase, inhibiting absorption of carbohydrates (starch, sucrose). PURPLE RICE EXTRACT was more marked inhibitory effects on sucrose than starch absorption in glucose loading tests, which may reflect its more marked inhibitory effects on α -glucosidase than on α -amylase.

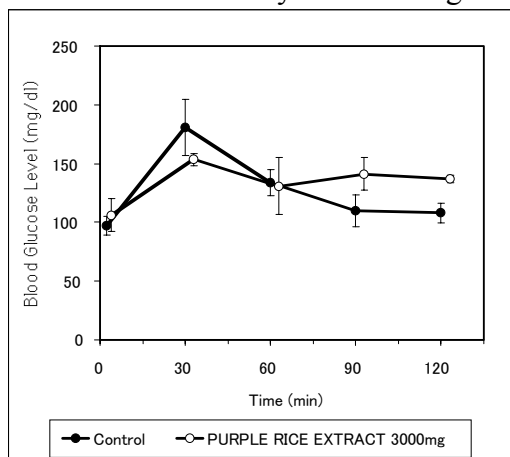


Fig. 6 Sugar Tolerance Test (Starch)

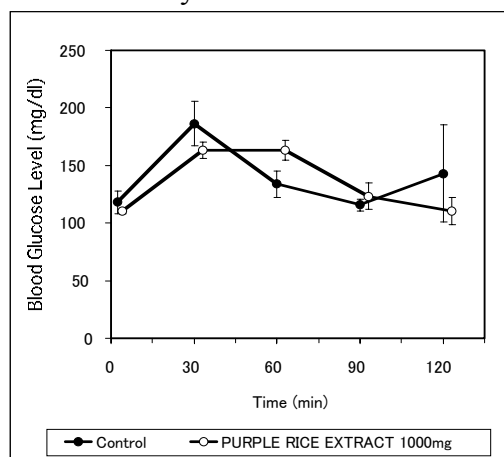


Fig. 7 Sugar Tolerance Test (Sucrose)

3) Inhibitory effects on postprandial increases in blood glucose in normal subjects

In diet loading tests in 4 healthy males and 3 healthy females, significant inhibition of postprandial increases in blood glucose was observed in the PURPLE RICE EXTRACT group compared with the placebo group. PURPLE RICE EXTRACT inhibited the total glucose absorption until 120 minutes after the meal.

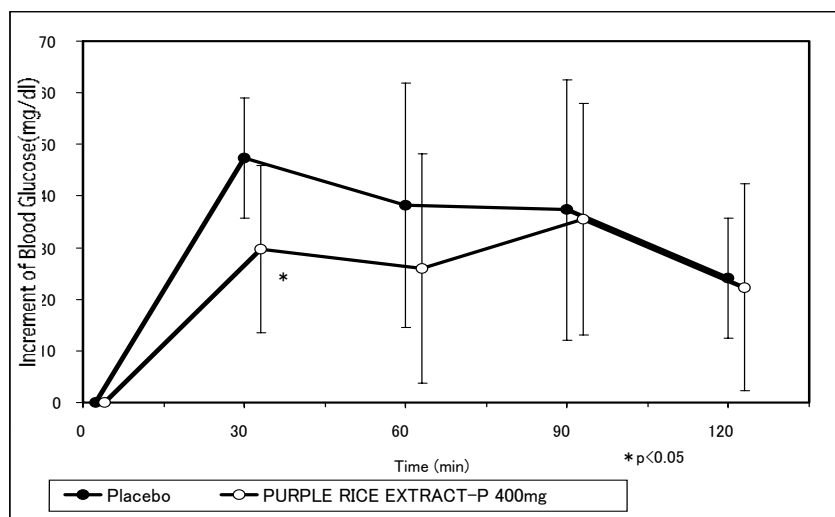


Fig. 8 Sugar Tolerance Test in Normal human Subjects (Rice)

Our results showed that PURPLE RICE EXTRACT inhibits the acute increase in blood glucose after meals.

4) Inhibitory effects on increases in blood glucose after sucrose intake in normal subjects

In sucrose loading tests in 2 healthy males and 7 healthy females, significant inhibition of increases in blood glucose was observed 30 minutes after loading in the PURPLE RICE EXTRACT group compared with the placebo group. PURPLE RICE EXTRACT inhibited the total absorption 120 minutes after the loading.

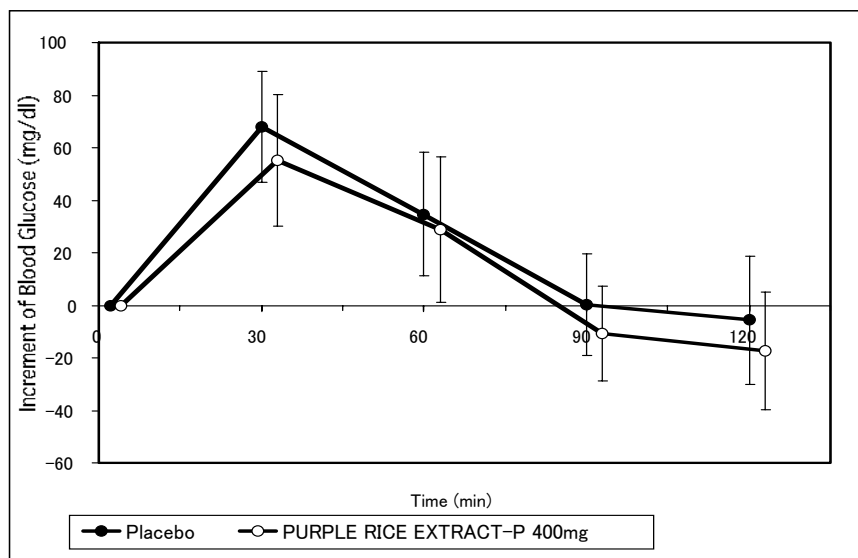


Fig. 9 Sugar Tolerance Test in Normal human Subjects (Sucrose)

Our results showed that PURPLE RICE EXTRACT inhibits the increase in blood glucose sucrose absorption after sucrose loading.

(2) Inhibition on elastase by PURPLE RICE EXTRACT

Elastin is a major protein component of the skin, and is present in large amounts in tissue with high elasticity such as the skin. Elastin is involved in skin elasticity, and its degradation reduces skin elasticity, resulting in the formation of wrinkles and sags. Elastin formed in the body is degraded by elastase. PURPLE RICE EXTRACT inhibited elastase, inhibiting elastin degradation. This extract may inhibit elastin degradation and maintain the amount of elastin.

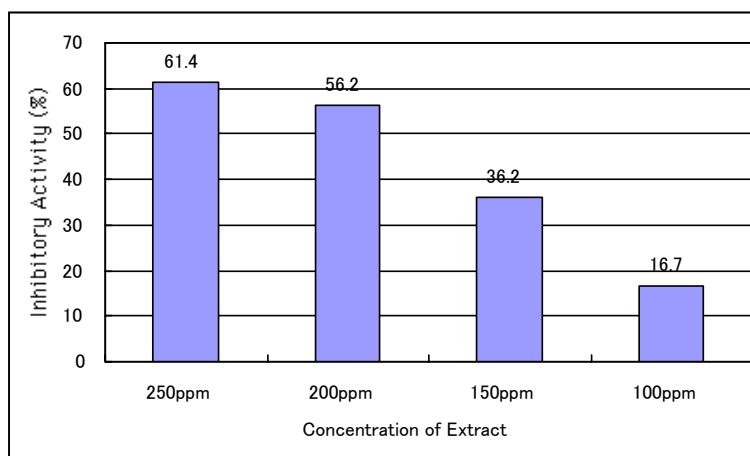


Fig. 10 Inhibition of elastase by PURPLE RICE EXTRACT

(3) Inhibition on collagenase by PURPLE RICE EXTRACT

Collagen accounts for 90% of the dermis of the skin, and is distributed in all layers of the dermis. Collagen is involved in the maintenance of appropriate elasticity and strength. Collagen degradation by collagenase induces wrinkles and sags as aging phenomena of the skin. Collagen formed in the body is degraded by collagenase.

PURPLE RICE EXTRACT inhibited collagenase, inhibiting collagen degradation. This extract may inhibit the degradation of produced collagen and maintain the amount of collagen.

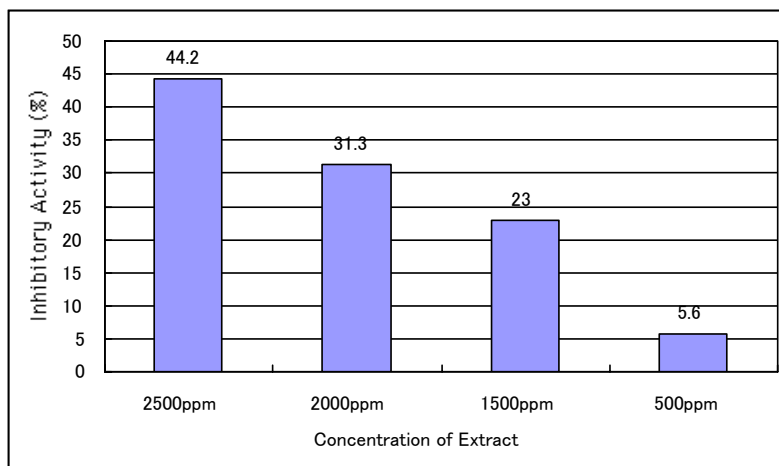


Fig. 11 Inhibition of collagenase by PURPLE RICE EXTRACT

(4) Inhibition of hyaluronidase by PURPLE RICE EXTRACT

Hyaluronic acid is widely distributed in the skin, synovial fluid, vitreous body, and ligaments. Hyaluronic acid is involved in cell adhesion and protection, formation of skin tissue, water retention in tissue, and the maintenance of flexibility in the skin. A decrease in hyaluronic acid reduces the moisture and tension of the skin, causing spots and sags. Hyaluronic acid formed in the body is degraded by hyaluronidase in a short time.

PURPLE RICE EXTRACT inhibited hyaluronidase, inhibiting the degradation of hyaluronic acid. This extract may inhibit the degradation of hyaluronic acid by hyaluronidase and maintain the amount of hyaluronic acid.

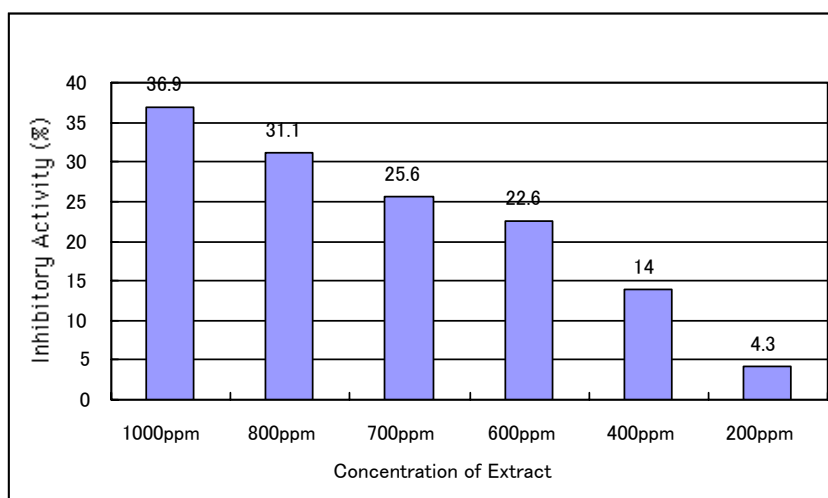


Fig. 12 Inhibition of hyaluronidase by PURPLE RICE EXTRACT

(5) Inhibition of tyrosinase (potentiation of the effects of vitamin C)

The dull or dark color of the skin is caused by melanin. In the body, tyrosine is converted to dopa quinone by the action of tyrosinase. After subsequent oxidation reactions, melanin forms. Vitamin C is a well-known inhibitor of tyrosinase, and used in various functional foods and cosmetics. Addition of a small amount of PURPLE RICE EXTRACT with vitamin C enhances the effects of vitamin C, increasing its tyrosinase inhibitory activity. The tyrosinase inhibitory activity of vitamin C increased from 61.0% to 85.6% after addition of PURPLE RICE EXTRACT at a dose of 0.5 mg/ml (1/10 of the vitamin C dose).

Thus, PURPLE RICE EXTRACT increases the tyrosinase inhibitory activity of vitamin C. Addition of this extract with vitamin C to foods and cosmetics for whitening is expected to enhance whitening effects and the effects of vitamin C.

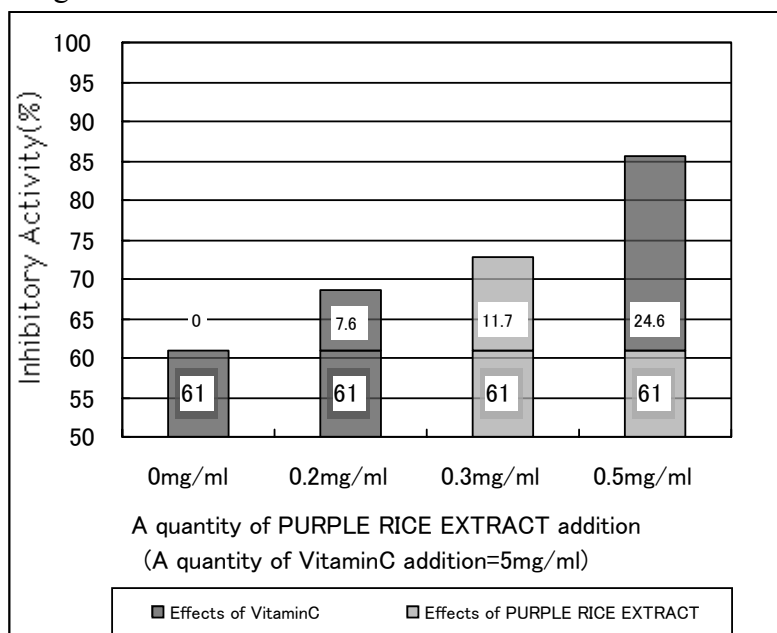


Fig. 13 Tyrosinase inhibition action reinforcement effect of PURPLE RICE EXTRACT

(6) Inhibition of melanin formation by PURPLE RICE EXTRACT

To measure the whitening effects of PURPLE RICE EXTRACT, its inhibitory effects on melanin formation were measured in B16 melanoma 4A5 cells. In the culture system containing PURPLE RICE EXTRACT, melanin formation was inhibited. PURPLE RICE EXTRACT may produce skin-whitening effects by inhibiting melanin formation.

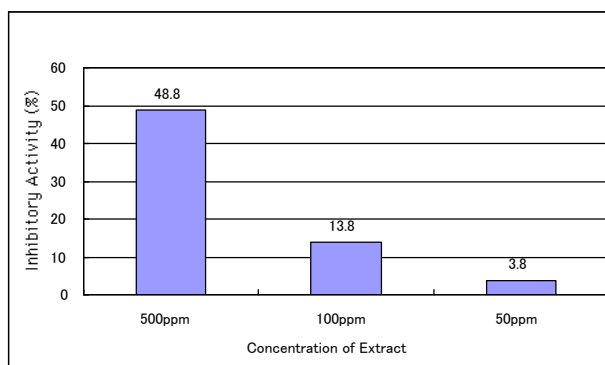


Fig. 14 Inhibition of melanin formation by PURPLE RICE EXTRACT

(7) Hair dyeing effect of Purple Rice Extract

The hair dyeing effect of Purple Rice Extract was investigated using three fundamental prescriptions showing in table 1. The test coloring agents were prepared according to the above prescriptions and then applied to the human white hair. The hair with the coloring agents was heated at 40°C for 15 min and returned to room temperature for 5 min, and finally, the hair was washed with tap water and dried with a hairdryer as dyed samples for evaluation by the following items.

- 1) Effect of dyeing : The dyeing effect was evaluated by visual judgement.
- 2) Stability and endurance : The dyed hair samples were dipped into 10% sodium lauryl sulfate solution for 15 min. After washing with tap water, the dyed hair samples were dried with a hairdryer and the stability and endurance of the color were evaluated by visual judgement.

Table 1. Prescriptions for hair dyeing agents

Composition	Function	Prescription 1	Prescription 2	Prescription 3
		Percentage (%)		
hydroxyethyl cellulose	gel excipient	2	2	2
benzyl alcohol	permeation promotor	5	5	5
ethyl alcohol	solvent	20	20	20
citric acid	pH adjustment	0.5	0.5	1.5
Purple Rice Extract	dye	0.5	1	1
distilled water	solvent	72	71.5	70.5
pH value		4.8	4.8	3.7

The thickness of the color of the dyeing agents prepared according to the above prescriptions varied with the amount of Purple Rice Extract-PBC prescribed. The more Purple Rice Extract-PBC was added, the thicker the color of the dyeing agents was (Fig.15).



Fig.15 Dyeing agents prepared according to prescription1 and 2.

Fig.16 shows the state of the human white hair after being dipped into the dyeing agents.

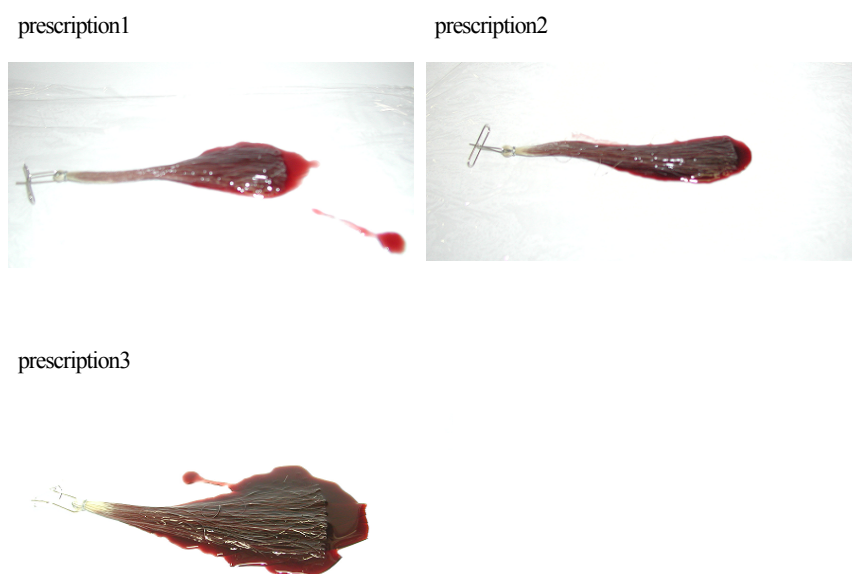


Fig.16 Human white hair after being dipped into the dyeing agents

After treatment by the dyeing agents prepared with Purple Rice Extract-PBC according to the above-mentioned prescriptions, the human white hair was dyed and changed to brown color as showing in Fig.17. The color of the human white hair dyed by the 1% Purple Rice Extract-PBC containing dyeing agent was a little thicker than that by the 0.5% Purple Rice Extract-PBC containing one (Fig.17).



Fig.17 The dyeing effect of Purple Rice Extract-PBC and the stability and endurance of the dyed color in human hair.

0 : the original human white hair. 1, 2, 3 : human white hair dyed by dyeing agents prepared according to prescription 1, 2, 3, respectively. 4 : dyed hair treated by 10% sodium lauryl sulfate solution.

Although the dyed color seemed to fade a little after treatment by 10% sodium lauryl sulfate solution, an anionic surfactant, it still can be concluded that the dyed color has a good stability and endurance against washing by surfactant.

From the above results, it is concluded that Purple Rice Extract-PBC can be used as an ingredient of hair dye in the cosmetic field. Furthermore, it is considered that the dyed color of the human hair could be adjusted according to the amount of Purple Rice Extract-PBC used in the prescription.

(8) Anti-oxidation activity

The generation of active oxygen species (superoxide, hydroxy radical) induces cell damage, causing carcinogenesis and inflammation, and promoting aging. In the skin, active oxygen is considered to cause spots, freckles, and wrinkles.

1) SOD-like activity of PURPLE RICE EXTRACT

Addition of PURPLE RICE EXTRACT to the SOD measurement reaction system resulted in SOD-like action. PURPLE RICE EXTRACT is expected to prevent lifestyle-related disorders caused by active oxygen.

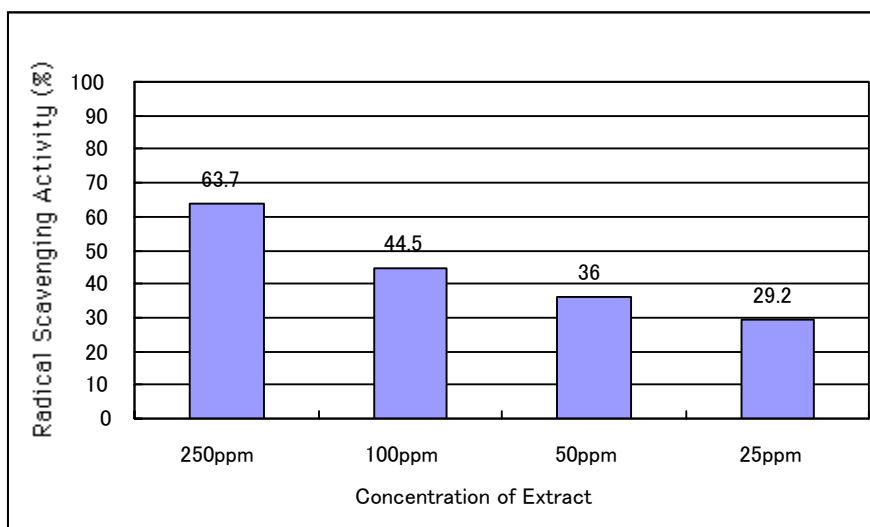


Fig. 18 SOD-like Activity of PURPLE RICE EXTRACT

2) DPPH radical scavenging activity of PURPLE RICE EXTRACT

As a parameter of anti-oxidation activity, radical scavenging ability was measured. After addition of PURPLE RICE EXTRACT, radical scavenging ability was observed. PURPLE RICE EXTRACT has not only SOD-like action but also radical scavenging ability. This extract is expected to prevent lifestyle-related disorders caused by active oxygen.

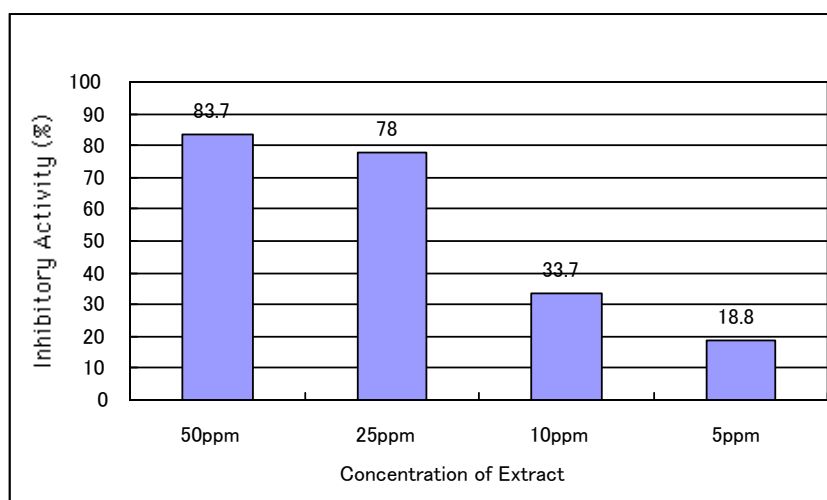
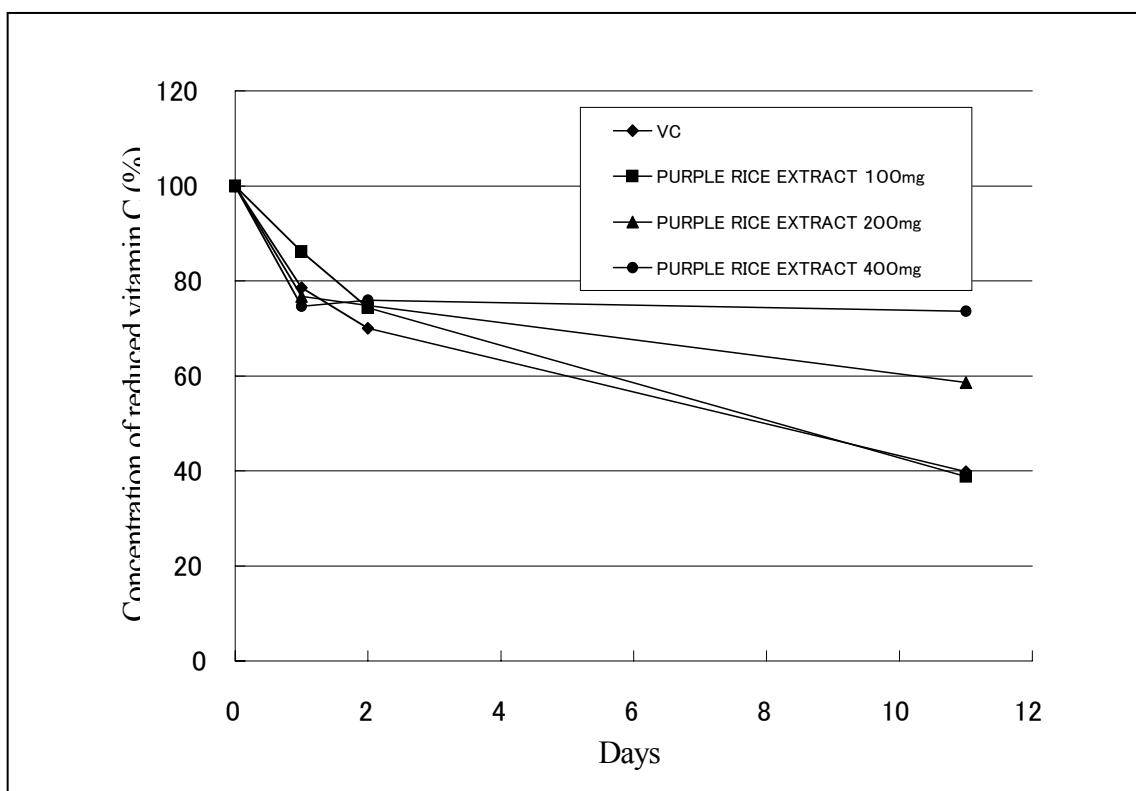


Fig. 19 DPPH Radical Scavenging Activity of PURPLE RICE EXTRACT

(9) Inhibitory effect on decrease in reduced vitamin C

There are reduced vitamin C (L-ascorbic acid) and oxidized vitamin C (L-dehydroascorbic acid) in naturally. Although reduced vitamin C has strong antioxidative activity, it is oxidized easily and is transformed to oxidized vitamin C without antioxidative activity.

Therefore, we examined whether PURPLE RICE EXTRACT with antioxidative activity inhibits decrease in reduced vitamin C in aqueous solution. As a result of the experiment, the reduced vitamin C was inhibited by addition of PURPLE RICE EXTRACT. Coexistence of PURPLE RICE EXTRACT and reduced vitamin C is expected to contribute to stabilization of reduced vitamin C in aqueous solution. Moreover enhancement in tyrosinase inhibitory activity and synergistic antioxidative activity has been represented by our findings.



**Fig.20 Inhibitory effect on decrease in reduced vitamin C
of PURPLE RICE EXTRACT**

(10) Prevention of eye disease

1) Purple rice extract and anthocyanidins of the constituents protect light-induced retinal damage *in vitro* and *in vivo*

We evaluated the protective effects of purple rice (*Oryza sativa* L.) bran extract (PRE) and/ or its major anthocyanidins against light-induced retinal damage *in vitro* and *in vivo*. In an *in vitro* experiment, cultured murine photoreceptor cells (661W) were damaged by a 24 h exposure to light. Cell viability was assessed by the tetrazolium salt (WST-8) cell-viability assay. In an *in vivo* experiment, we evaluated the effects of PRE on light-induced retinal damage in mice using hematoxylin-eosin staining. As a result, PRE, cyanidin, and peonidin significantly inhibited the cell death in 661W (Fig. 21). In histological analysis, intravitreal injection of PRE significantly suppressed the photoreceptor degeneration induced by exposure to light in mice (Fig. 22). These findings suggest that PRE and its anthocyanidins possess protective effects with antioxidation mechanism in *in vitro* and *in vivo* models of retinal diseases.

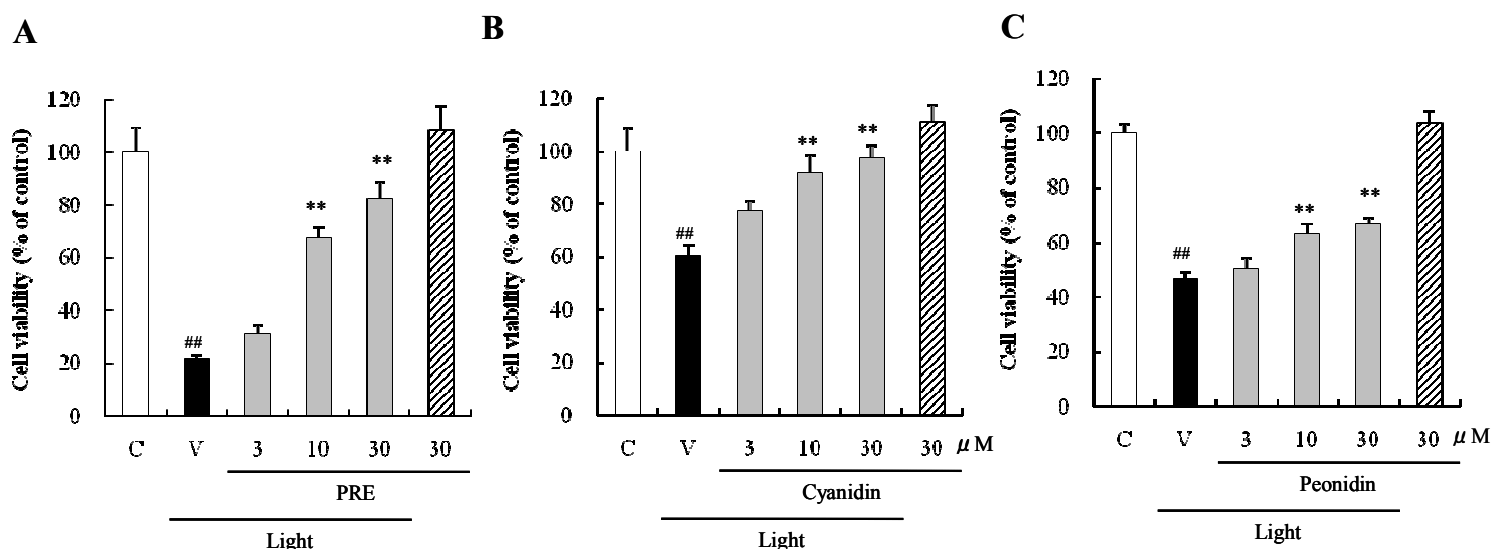


Fig. 21. Effects of PRE and its constituents on visible light-induced 661W cell death. Data are shown as mean \pm SEM ($n = 6$). C, control; V, vehicle. ##, $p < 0.01$ versus control, and **, $p < 0.01$ versus vehicle.

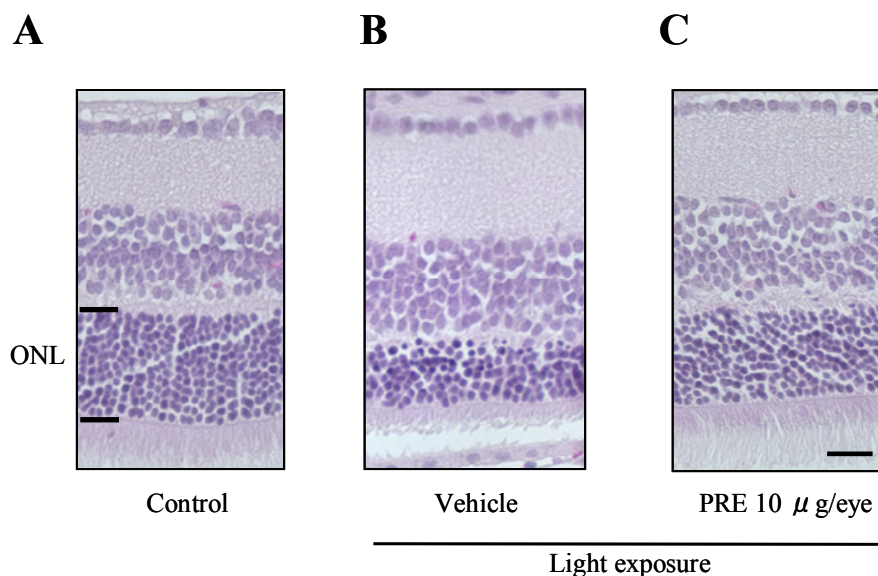


Fig. 22. Effects of PRE on retinal damage induced by exposure to light in mice. (A) Nontreated, (B) Light exposure (8,000 lux) plus vehicle-treated, and (C) Light exposure plus PRE-treated (10 µg/eye) retinal cross sections at 5 days after light exposure in mice. Outer nuclear layer (ONL). The scale bar (C) represents 25 µm.

2) Anti-angiogenic effects of anthocyanidins consist of anthocyanins in purple rice

Vascular endothelial growth factor (VEGF) is a key regulator of pathogenic angiogenesis in the pathology of diabetic retinopathy and cancer. We evaluated the protective effects of purple rice bran extract (PRE) and its constituents such as cyanidin and peonidin against VEGF-induced angiogenesis. Tube formation assay *in vitro*, human umbilical vein endothelial cells (HUVECs) and fibroblasts were co-cultured for 14 days with VEGF and PRE. To clarify the anti-angiogenic mechanism of PRE, VEGF-induced proliferations and migrations of HUVECs and/or human retinal microvascular endothelial cells (HRMECs) were examined. Moreover, the effect of PRE on VEGF-induced phosphorylations of extracellular signal-regulated kinase (ERK) and p38 were evaluated to investigate cell proliferation and migration of HRMECs. As a result, PRE cyanidin and peonidin significantly suppressed VEGF-induced tube formation (Fig.23), proliferation (Fig. 24), and migration (Fig. 25) in HUVECs and HRMECs. Furthermore, PRE (30 µg/ml) inhibited the VEGF-induced phosphorylations of ERK and p38 (Fig. 26). These findings indicate that PRE and anthocyanidins suppress VEGF-induced angiogenesis by inhibiting HUVECs and/or HRMECs proliferation and migration and the inhibition of phosphorylated-ERK and -p38 may be

involved in the mechanism. Therefore, PRE may be expected to prevent some diseases caused by angiogenesis.

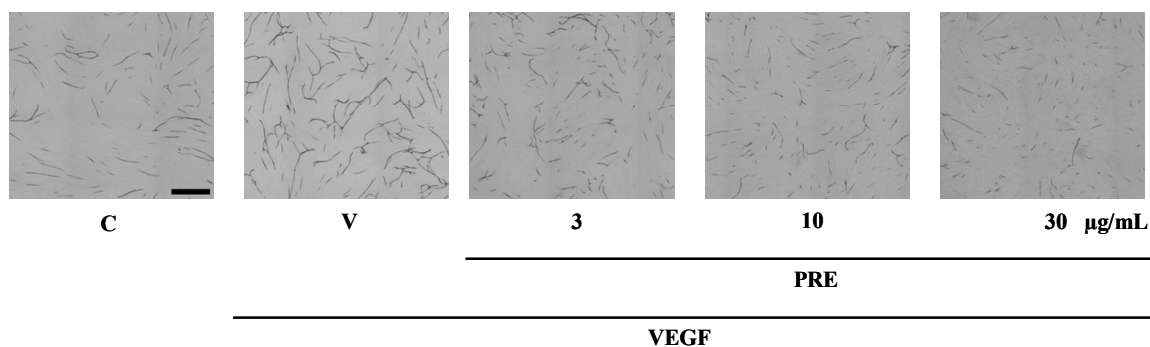


Fig. 23. Effects of PRE on tube formation induced by VEGF. Representative photographs of tube formation. The scale bar indicates 1 mm length. C, control; V, vehicle.

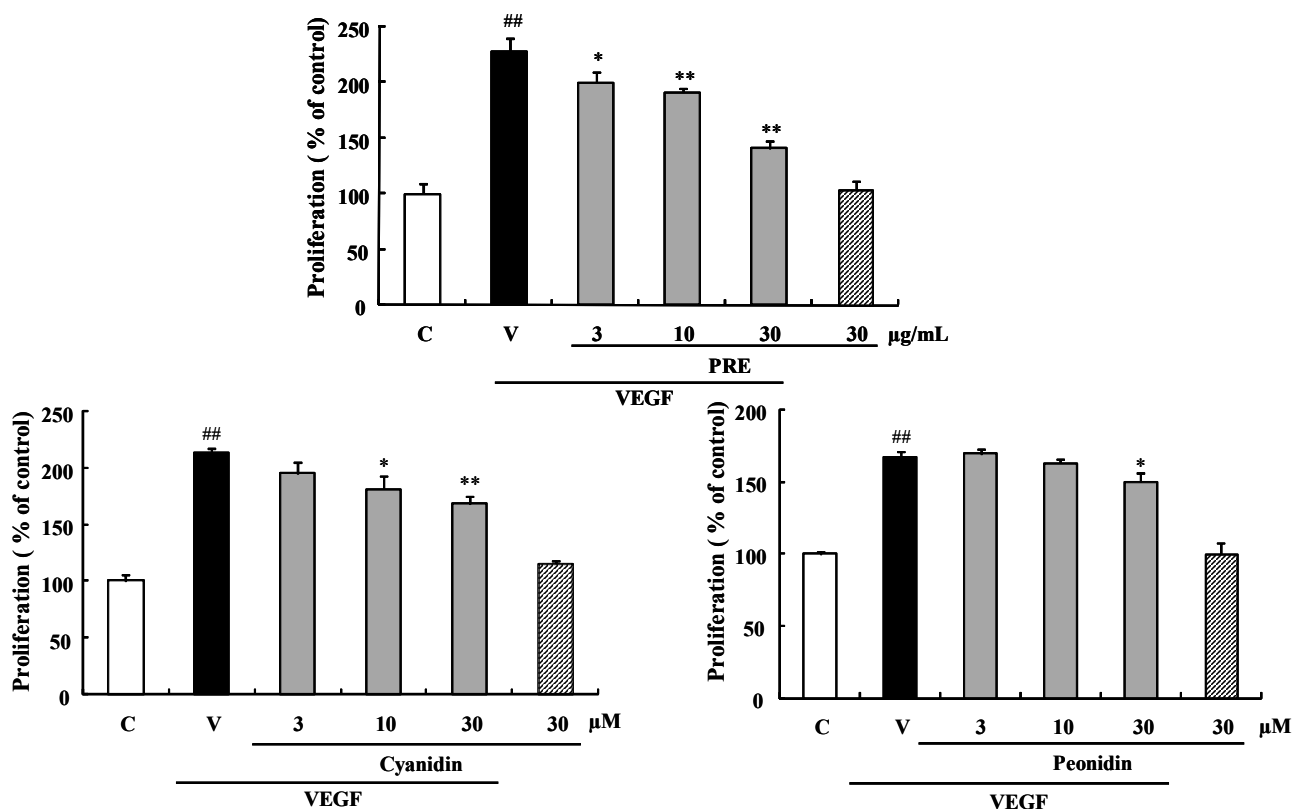


Fig. 24. Effects of PRE and anthocyanidins on VEGF-induced proliferation in HRMECs. Data are shown as mean \pm SEM ($n = 6$). C, control; V, vehicle. ^{##}, $P < 0.01$ versus control, and ^{*}, $P < 0.05$, ^{**}, $P < 0.01$ versus vehicle.

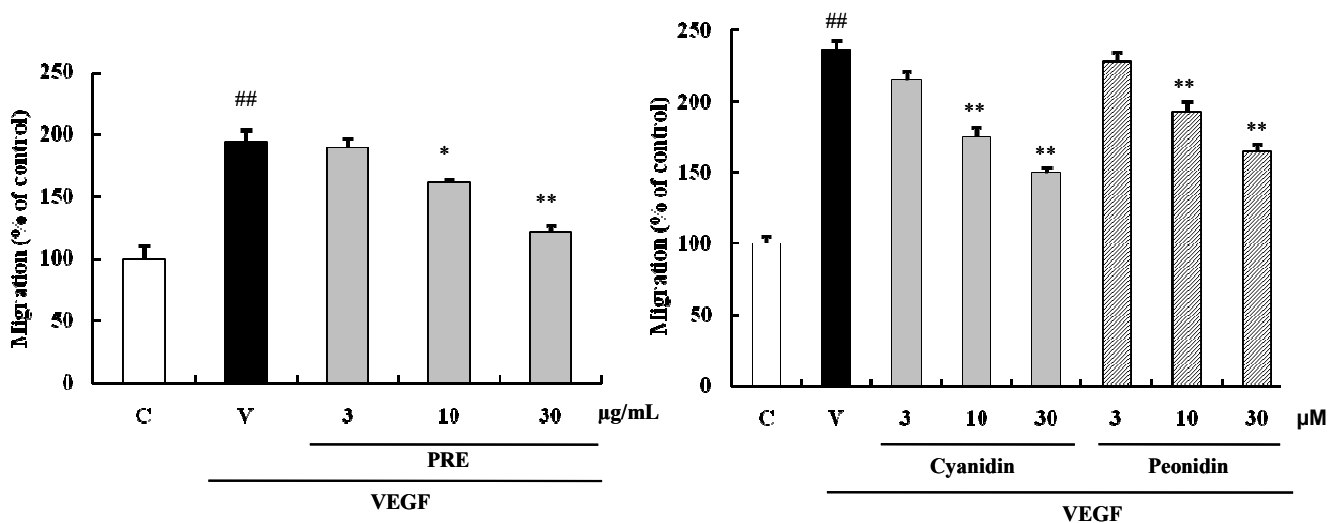


Fig. 25. Effects of PRE and anthocyanidins on VEGF-induced migration and *in vitro* wound healing. Migration was estimated by measuring the cell numbers within the wounded region after treatment with VEGF plus PRE, or cyanidin and peonidin. Data are shown as mean \pm SEM ($n = 3$ or 4). C, control; V, vehicle. $^{##}$, $P < 0.01$ versus control, and * , $P < 0.05$, ** , $P < 0.01$ versus vehicle.

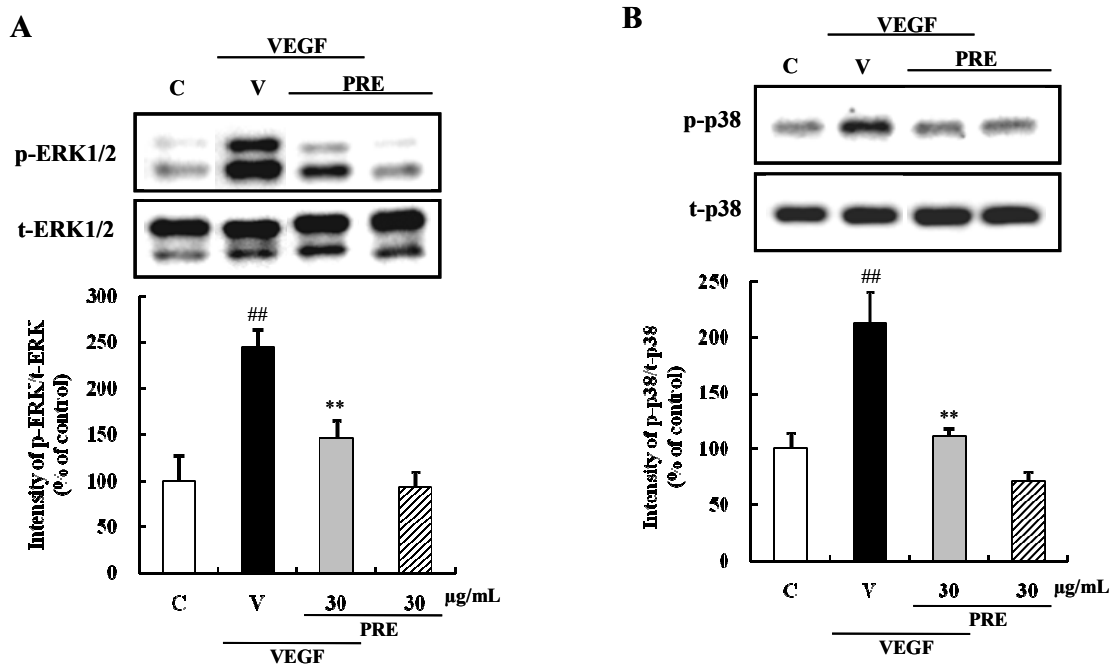


Fig. 26. Effects of PRE on phosphorylation of ERK 1/2 and p38 induced by VEGF. Phosphorylation of (A) ERK 1/2 and (B) p38 determined by immunoblotting assay. Blots were scanned and quantified by densitometric analysis, with the phosphorylated -ERK and -p38 blots (p-ERK1/2 and p-p38) expressed relative to the total -ERK and -p38 (t-ERK1/2 and t-p38). Data are shown as mean \pm SEM ($n = 5$). C, control; V, vehicle. $^{##}$, $P < 0.01$ versus control, and ** , $P < 0.01$ versus vehicle.

6. Stability of PURPLE RICE EXTRACT

(1) Thermal resistance

The pyrolysis of PURPLE RICE EXTRACT does not occur at a normal food processing temperature for 60 minutes.

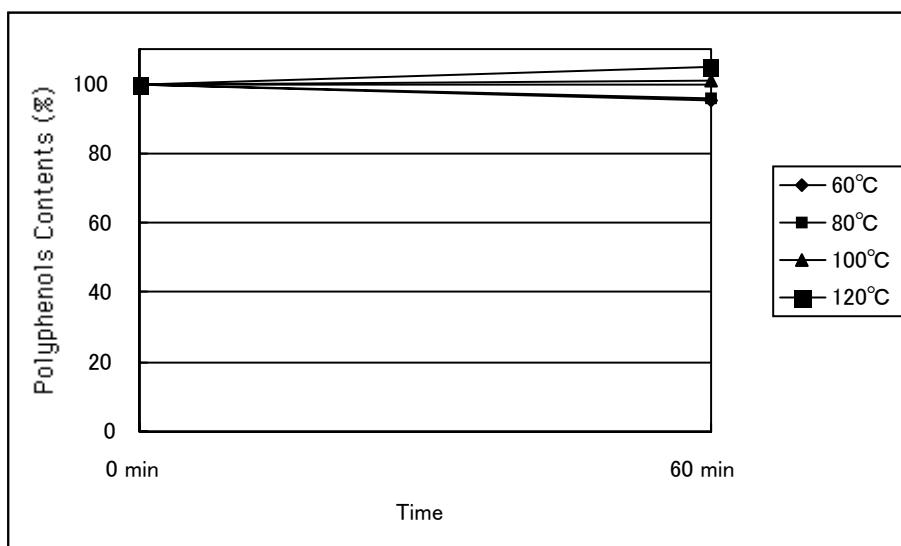


Fig. 27 Heat resistance of PURPLE RICE EXTRACT

(2) pH Stability

Polyphenols in PURPLE RICE EXTRACT remains stable especially from neutral to acid field of pH.

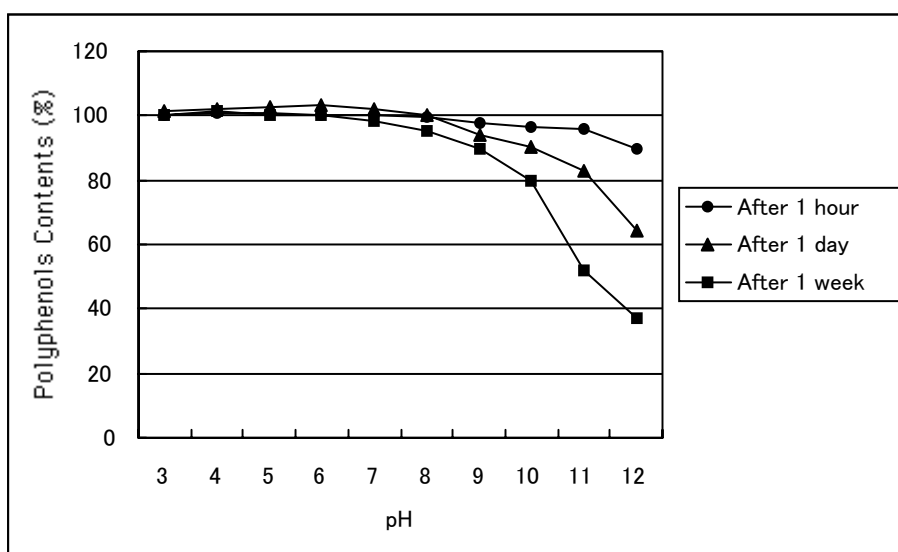


Fig. 28 Influence of pH on Polyphenols Contents

7. Daily dosage of PURPLE RICE EXTRACT

It is recommended to take more than 200~400mg/day of PURPLE RICE EXTRACT-P.
More marked effects are expected when PURPLE RICE EXTRACT is used in combination with vitamin C, collagen, hyaluronic acid, and ceramide.

8. Nutrition facts of PURPLE RICE EXTRACT

Items Analyzed	Result
Water	2.5g/100g
Protein* ¹	6.9g/100g
Fat	0.5g/100g
Ash	6.6g/100g
Available carbohydrate* ²	83.5g/100g
Energy* ³	366kcal/100g
Sodium	64.9mg/100g
Iron	4.25mg/100g
Calcium	21.5mg/100g

*1 N×5.95

*2 100-(Moisture + Protein + Fat + Ash)

*3 Factors for calculating the energy value: Protein - 4, Fat - 9,
Available carbohydrate - 4

Tested by : Japan Food Research Center Foundation

Research result issue number : 302090006-001

9. Acute toxicity and safety

(1) Residual agricultural chemicals

The PURPLE RICE EXTRACT (with no diluents added) was again examined for 475 residual agricultural chemical compounds following the provisions of the Food Hygiene Law and pesticide legislation. As a result, contents of all compounds were confirmed to be below the standard values (measurable limits).

Test trustee: Kyusai Analytical Research Laboratory Co. Ltd.

Date of issue of the test result report: July 19, 2006

Research result issue number: No. 2006061304-01

(2) Acute toxicity

Five weeks old mice were orally given PURPLE RICE EXTRACT (5000mg/kg) and then fed a laboratory chow for two weeks. No toxic effect were observed, thus the LD50 (mouse) is more than 5000mg/kg

(3) Acute Eye Irritation Study

The solution of PURPLE RICE EXTRACT-PC (0.1 g) was applied into the conjunctival sac of the left eye of 3 rabbits. The conjunctival of iris and corneal lesions were observed approximately 24, 48 and 72 hours after instillation. Under the experimental condition, PURPLE RICE EXTRACT-PC was found to be non-irritant for eyes of the rabbits.

(4) Acute Skin Irritation Study

The solution of PURPLE RICE EXTRACT-PC (0.5 g) was applied on the skin of 3 rabbits for 1 hour. The treated lesions were observed approximately 24, 48 and 72 hours after removal of the dressing. Under the experimental conditions, PURPLE RICE EXTRACT-PC was found to be non-irritant for skin of rabbits.

(5) Skin Sensitisation Study

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 PURPLE RICE EXTRACT-PC is free of any sensitising capacity in the guinea-pig.

(6) Patch Test

0.025 g of PURPLE RICE EXTRACT 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 irritating or sensitizing effect on the skin of human was found.

(7) Mutagenicity Test

Ames test was performed under the conditions with/without the presence of S9mix using *Salmonella* strains of TA98 and TA100. PURPLE RICE EXTRACT showed no mutagenicity at concentrations from 19.5 to 5000 µg/plate.

10. Practical applications of PURPLE RICE EXTRACT

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

11. Packing

PURPLE RICE EXTRACT-P (powder type)

3kg Interior packaging : alminum-coated plastic bag
Exterior packaging : 18 L thin and cardboard box

PURPLE RICE EXTRACT-PC (powder type for cosmetics)

3kg Interior packaging : alminum-coated plastic bag
Exterior packaging : 18 L thin and cardboard box

12. Storing method

Store in cool, dry place. Avoid humidity.

13. Expression of PURPLE RICE EXTRACT

PURPLE RICE EXTRACT-P (food)

PURPLE RICE EXTRACT

PURPLE RICE EXTRACT-PC (cosmetics)

INCI Name : Oryza Sativa (Rice) Extract, Dextrin

※Please refer to your nation's standards.

Test Methods

Fig. 3 Inhibition of α -Amylase by PURPLE RICE EXTRACT

α -Amylase from pig pancreatic juice was purchased from Wako Pure Chemical Ind., Ltd., Japan. α -Amylase activity was assayed by using Amylase-Test Wako. In advance, sample were diluted with ethanol and 10 μ l of the sample solution was added to the assay system. The inhibitory activity (%) was calculated by using the following equation: 100-(% of Control).

Fig. 4 Inhibition of α -Glucosidase by PURPLE RICE EXTRACT

Acetone powder of rat intestine was homogenized in phosphate buffer and centrifuged. The supernatant was used as a crude enzyme solution containing α -glucosidase.

α -Glucosidase activity was determined by the fluorescent assay with 4-methylumbelliferyl- α -D-glucopyranoside as substrate. The inhibitory activity (%) was calculated as described above.

Fig. 5 Inhibition of α -Glucosidase by PURPLE RICE EXTRACT and Several Plant Extracts

Acetone powder of rat intestine was homogenized in phosphate buffer and centrifuged. The supernatant was used as a crude enzyme solution containing α -glucosidase.

α -Glucosidase activity was determined by the fluorescent assay with 4-methylumbelliferyl- α -D-glucopyranoside as substrate. The inhibitory activity (%) was calculated as described above.

Fig. 6 Sugar Tolerance Test (Starch)

After 24h without foods, six weeks old rats were loaded 2g/kg of soluble starch and 3000mg/kg of PURPLE RICE EXTRACT. Every 30 minutes, blood samples were bled from the tail vein into heparinized capillary tubes, and the plasma glucose level were measured by Glucose B-Test Wako.

Fig. 7 Sugar Tolerance Test (Sucrose)

After 24h without foods, six weeks old rats were loaded 2g/kg of soluble sucrose and 1000mg/kg of PURPLE RICE EXTRACT. Every 30 minutes, blood samples were bled from the tail vein into heparinized capillary tubes, and the plasma glucose level were measured by Glucose B-Test Wako.

Fig. 8 Sugar Tolerance Test in Normal Human Subjects (Rice)

The blood glucose levels of the subjects after fasting for 11 hours were in the range of 70-100mg/dl. Subjects took meal (200g boiled rice) with or without PURPLE RICE EXTRACT-P (400mg) at 9:00 am. Blood was withdrawn from finger at 0, 30, 60, and 90 min and measured blood glucose using a portable analyzer equipped glucose sensor (MediSense Precision Q.I.D, Dainabot Co., Ltd.).

Fig. 9 Sugar Tolerance Test in Normal Human Subjects (Sucrose)

The blood glucose levels of the subjects after fasting for 11 hours were in the range of 70-115mg/dl. Subjects took meal (50g boiled sucrose) with or without PURPLE RICE EXTRACT-P (400mg) at 9:00 am. Blood was withdrawn from finger at 0, 30, 60, and 90 min

and measured blood glucose using a portable analyzer equipped glucose sensor (MediSense Precision Q.I.D, Dainabot Co., Ltd.).

Fig. 10 Inhibition of elastase by PURPLE RICE EXTRACT

PURPLE RICE EXTRACT was dissolved in distilled water, and the amount of DQ elastin degraded by elastase was measured. Fluorescence was measured after 60 minutes at an excitation wavelength of 485 nm and a measurement wavelength of 530 nm.

Fig. 11 Inhibition of collagenase by PURPLE RICE EXTRACT

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

Fig. 12 Inhibition of hyaluronidase by PURPLE RICE EXTRACT

PURPLE RICE EXTRACT was dissolved in distilled water, and hyaluronic acid was reacted with hyaluronidase. After reaction with p-dimethylaminobenzaldehyde, absorbance was measured.

Fig. 13 Tyrosinase inhibition action reinforcement effect of PURPLE RICE EXTRACT

PURPLE RICE EXTRACT was dissolved in distilled water, and the test was performed. After addition of the extract to mushroom-derived tyrosinase solution, the reaction from L-tyrosine to dopa quinone was measured in terms of the absorbance of dopa quinone.

Fig. 14 Inhibition of melanin formation by PURPLE RICE EXTRACT

Mouse B16 melanoma 4A5 cells were cultured in medium not containing sodium lactate but containing PURPLE RICE EXTRACT (containing sodium lactate and PURPLE RICE EXTRACT?) The medium was removed, and 1N-sodium hydroxide was added. After heating at 100°C for 30 minutes, absorption was measured to determine the amount of melanin.

Fig. 15 SOD-like Activity of PURPLE RICE EXTRACT

PURPLE RICE EXTRACT was dissolved in distilled water, and the test was performed. SOD-like activity was measured using an SOD test Wako kit.

Fig. 16 DPPH Radical Scavenging Activity of PURPLE RICE EXTRACT

PURPLE RICE EXTRACT was dissolved in 70% ethanol, and the test was performed. PURPLE RICE 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.17 Inhibitory effect on decrease in reduced vitaminC of PURPLE RICE EXTRACT

Initial concentration of reduced vitamin C was assumed to be 100%. Solution of samples keeping at 40°C in shade were taken on 1, 2, and 11 days, for reduced vitamin C determination.

Figs. 18, 19 Polyphenol content

Samples diluted with distilled water were measured by the Folin-Denis method described in the Food Function Study Method. Gallic acid was used as a standard reference material.

PRODUCT STANDARD

PRODUCT NAME

PURPLE RICE EXTRACT-P

(FOOD)

This product is extracted with aqueous ethanol from purple rice, the seeds of *Oryza sativa* Linne (Gramineae). It includes more than 15.0% of polyphenols and more than 5.0% of anthocyanins. This powder is water-soluble.

<u>Appearance</u>	It is purple-black color powder with slightly unique smell.									
<u>Content of Polyphenols</u>	Min. 15.0 %	(Folin-Denis method)								
<u>Content of Anthocyanin</u>	Min. 5.0 %	(Absorbance method)								
<u>Loss on Drying</u>	Max. 8.0 %	(Analysis for Hygienic Chemists,1g, 105°C, 2h)								
<u>Purity Test</u>										
(1) Heavy Metals	Max. 20 ppm	(The Japanese Standards for Food Additives)								
(2) Arsenic	Max. 2 ppm	(Standard Methods of Analysis in Food Safety Regulation)								
<u>Standard Plate Counts</u>	Max. 1×10^3 cfu/g	(Analysis for Hygienic Chemists)								
<u>Moulds and Yeasts</u>	Max. 1×10^2 cfu/g	(Analysis for Hygienic Chemists)								
<u>Coliforms</u>	Negative	(Analysis for Hygienic Chemists)								
<u>Composition</u>	<table><tr><td><u>Ingredients</u></td><td><u>Contents</u></td></tr><tr><td>PURPLE RICE EXTRACT</td><td>50 %</td></tr><tr><td>Dextrin</td><td>50 %</td></tr><tr><td>Total</td><td>100 %</td></tr></table>		<u>Ingredients</u>	<u>Contents</u>	PURPLE RICE EXTRACT	50 %	Dextrin	50 %	Total	100 %
<u>Ingredients</u>	<u>Contents</u>									
PURPLE RICE EXTRACT	50 %									
Dextrin	50 %									
Total	100 %									

PRODUCT STANDARD

PRODUCT NAME

PURPLE RICE EXTRACT-PC

(COSMETIC)

This product is extracted with aqueous ethanol from purple rice, the seeds of *Oryza sativa* Linne (Gramineae). It includes more than 15.0% of polyphenols and more than 5.0% of anthocyanins. This powder is water-soluble.

<u>Appearance</u>	It is purple-black color powder with slighaly unique smell.	
<u>Content of Polyphenols</u>	Min. 15.0 %	(Folin-Denis method)
<u>Content of Anthocyanin</u>	Min. 5.0 %	(Absorbance method)
<u>Loss on Drying</u>	Max. 8.0 %	(Analysis for Hygienic Chemists,1g, 105□, 2h)
<u>Purity Test</u>		
(1) Heavy Metals (as Pb)	Max. 20 ppm	(The Second Method of The Japanese Standards of Quasi-Drug Ingredients)
(2) Arsenic (as As ₂ O ₃)	Max. 2 ppm	(The Second Method of The Japanese Standards of Quasi-Drug Ingredients)
<u>Standard Plate Counts</u>	Max. 1×10 ² cfu/g	(Analysis for Hygienic Chemists)
<u>Moulds and Yeasts</u>	Max. 1×10 ² cfu/g	(Analysis for Hygienic Chemists)
<u>Coliforms</u>	Negative	(Analysis for Hygienic Chemists)

<u>Composition</u>	<u>Ingredients</u>	<u>Contents</u>
	Oryza Sativa (Rice) Extract	50 %
	Dextrin	50 %
	Total	100 %

Ref: The Japanese Standards of Quasi-drug Ingredients.

PRODUCT STANDARD

PRODUCT NAME

PURPLE RICE EXTRACT-LC0.1

(COSMETIC)

This product is extracted from purple rice, the rice bran of *Oryza sativa* Linne (*Gramineae*) with aqueous 1,3-butylene glycol.

Appearance Purplish-black color liquid. Scentless or light unique smell.

Certification Test

- (1) Anthocyanin Add the solution of 0.1 ml in 5 ml of methanol and add 0.2 ml of hydrochloric acid, heat to boiling, the color of the solution changes to red.
- (2) Polyphenols Add 200μl of the water solution of this product (1→10) in 3.2 ml of water. Then add 0.2 ml of phenol reagent and 0.4 ml of sodium carbonate solution. The color of the solution changes to blue.
- (3) Sugar To 2 ml of this product, add 5 ml of fehling solution, heat to boiling. Red precipitate is formed.

Purity Test

- (1) Heavy Metals (as Pb) Max. 10 ppm (The Second Method of The Japanese Standards of Quasi-Drug Ingredients)
- (2) Arsenic (as As₂O₃) Max. 1 ppm (The Second Method of The Japanese Standards of Quasi-Drug Ingredients)

Standard Plate Counts Max. 1×10² cfu/g (Analysis for Hygienic Chemists)

Moulds and Yeasts Max. 1×10² cfu/g (Analysis for Hygienic Chemists)

Coliforms Negative (Analysis for Hygienic Chemists)

Composition

Ingredients	Contents
Water	50.0 %
Butylene Glycol	49.9 %
<u>Oryza Sativa (Rice) Extract</u>	0.1 %
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.

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