ORYZA OIL & FAT CHEMICAL CO., LTD.

SEABERRY EXTRACT
SEABERRY FRUIT OIL

Prevention and improving actions of prostatic hyperplasia and overactive bladder, Metabolic syndrome improvement, Reducing effect of skin irritation (air pollutants and drying), Anti-inflammatory effect, Moisturizing

- **SEABERRY EXTRACT-P**
  (Powder, Food Grade)
- **SEABERRY EXTRACT-WSP**
  (Water-soluble Powder, Food Grade)
- **SEABERRY EXTRACT-J**
  (Concentrated juice, Food Grade)
- **SEABERRY FRUIT OIL**
  (Oil, Food Grade)
- **SEABERRY EXTRACT-PC**
  (Powder, Cosmetic Grade)
- **SEABERRY EXTRACT-WSPC**
  (Water-soluble Powder, Cosmetic Grade)

オリザ油化株式会社

Ver. 2.0 YF

SEABERRY EXTRACT
SEABERRY FRUIT OIL

Prevention and improving actions of
prostatic hyperplasia and overactive bladder
Metabolic syndrome improvement
Reducing effect of skin irritation (air pollutants and drying)
Anti-inflammatory effect
Moisturizing

1. Introduction

Seaberry (*Hippophae Rhamnoides*) is a fruit of deciduous shrub from the Elaeagnaceae family. It is eaten by people in temperate to subarctic zones including Northern Europe, the central areas of the Eurasian Continent, and Canada. It is a vigorous plant that can grow in harsh environments with extreme temperature variation, dry weather, sandstorms, denudation of soil, or even in barren areas. The plant already existed approximately 70 million years ago and has survived for this long time because of its strong vital power.

Seaberry is called various names, for example sea-buckthorn (English), 沙棘 (shājí, Chinese), Чацаргана (chatsargan, Mongolian), облепиха (oblepikha, Russian), and sanddorn (German).

Its fruit is approximately 5 to 10 mm in diameter and its color is yellow to orange. Flesh fruit contains a large amount of oil. According to the Encyclopedia of Chinese Drugs[1)], 100 g of the fruit contains at least 300 mg of vitamin C, 3 to 4 mg of carotene, 10 to 15 mg of vitamin E, 0.2 to 0.4 mg of vitamin B1, 0.4 to 0.5 mg of vitamin B2, and 0.5 to 0.8 mg of folic acid. It has also been confirmed to contain over 200 components including flavonoids, polyphenols, carotenoids, lipids, phytosterols, organic acids, amino acids, and minerals. In addition, it is known that its fatty acids contain a large amount of palmitoleic acid (ω-7), which is rare in natural plants.

Fig.1　Seaberry fruits（left）and trees（right）

Seaberry has been a precious nutrient source for wild animals and birds because it contains many components as described. Its scientific name *Hippophae rhamnoides* means “berry that makes horse hair shine.” According to Greek legend, it was a favorite food of the mystical white winged-horse Pegasus. In the historical story of Genghis Khan who established the Mongolian Empire used seaberry as a nutrient source for his soldiers and horses in battles. Seaberry is currently used in juices and health foods. Since it has high contents of vitamins C and E and polyphenols and anti-oxidant action, it supports our health from within and protects us from oxidative stress.

Seaberry is also used to protect the environment, because it can basically grow in any environment. It is cultivated to green deserts, prevent soil erosion, protect water sources, block wind, and strengthen soils structure.

ORYZA OIL & FAT CHEMICAL studied the functions of seaberry extract and seaberry fruit oil on urinary disorders caused by an enlarged prostate or overactive bladder. As a result, we discovered their actions to suppress an enlarged prostate and hyper-contraction of bladder smooth muscle, a world first.

Seaberry fruit oil rich in palmitoleic acid (ω-7) and seaberry extract containing triterpenic acids can be used in foods to reduce urinary problems caused by an enlarged prostate or urination disorders caused by hyper-contraction of bladder smooth muscle such as frequent urination.

1) Encyclopedia of Chinese Drugs edited by Shanghai Science and Technology Press and published by Shogakkan (1985)

2. Seaberry's Components and Newly-Found Component "Hippophaelic acid"

It has been reported that seaberry fruit contains at least 200 different components. ORYZA OIL & FAT CHEMICAL isolated the components and determined the structures in a joint research with Kyoto Pharmaceutical University. As a result, the structure of the components shown in Fig. 2 was determined.

Among these components, we discovered the new component "Hippophaelic acid" and named it after seaberry (*Hippophae rhamnoides*).

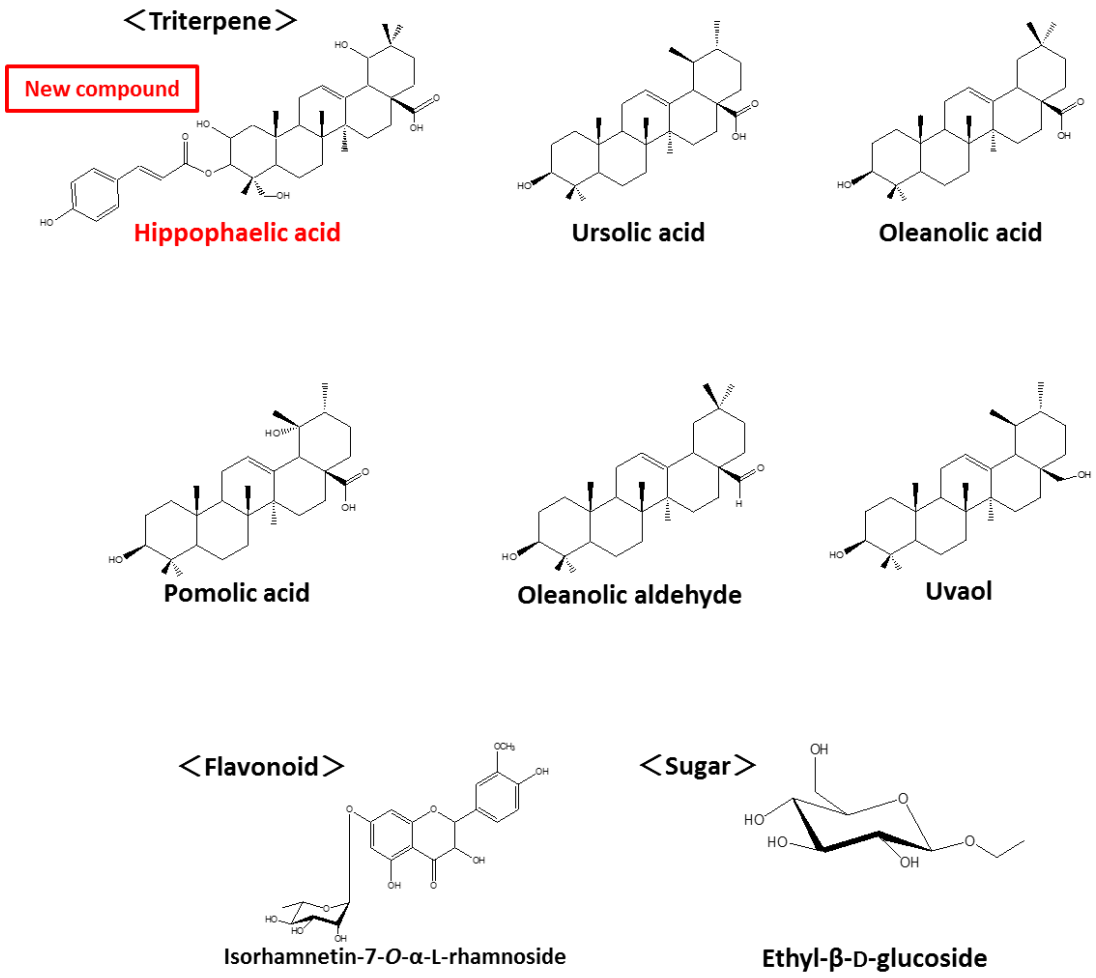


Fig.2 Components of seaberry

3. Action of Seaberry Extract and Seaberry Fruit Oil on Enlarged Prostate

（1）Enlarged prostate

Only men have a prostate under the bladder as shown in Fig. 3 and it is known that the prostate enlarges with aging. The rate of men with an enlarged prostate increases after they reach their fifties and 90 % of men aged eighties have an enlarged prostate histologically. Since the urethra passes through the center of the prostate, enlarged prostate often leads to the following problems: “difficulty in urination” which is urinary disorder where momentum or force is necessary for urination, urinary storage problems such as “frequent urination” and “impending incontinence,” and “dribbling after urination” where sensation of residual urine is felt. Although an enlarged prostate is not a life-threatening disease, it induces the lower urinary tract symptoms described above, negatively influencing on QOL. Patients may hesitate to go out or travel, because they are conscious about frequent urination or shortage of sleep because of frequent urination at night.

Recently, it is pointed out that there is a relationship between enlarged prostate and obesity, high blood pressure, high blood sugar level, and dyslipidemia. In addition, the relationship with metabolic syndrome has been studying.

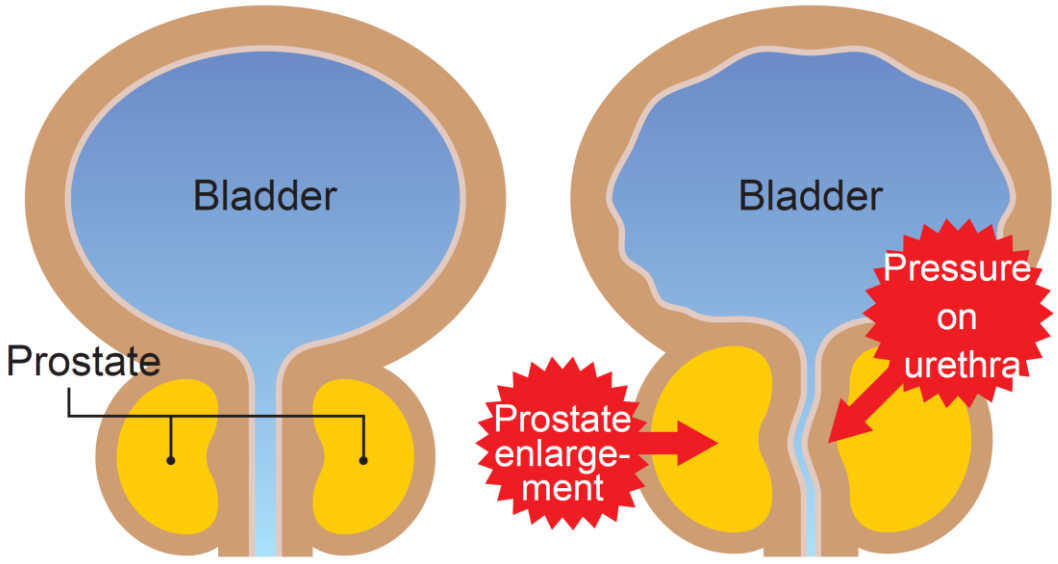


Fig.3　Image of the prostate and prostatic hyperplasia

（2）Action of seaberry extract and seaberry fruit oil on enlarged prostate model in mice

Enlarged prostate model were created by subcutaneous-injection of testosterone to castrated mice. After recovery period, seaberry extract (without binder) or seaberry fruit oil (without additive) was orally administrated to the mice for 14 days at 100 mg/kg/day and then the wet weight of their prostate was measured. As a result, both the extract and the oil showed a tendency to suppress the enlargement of the prostate (Fig. 4).

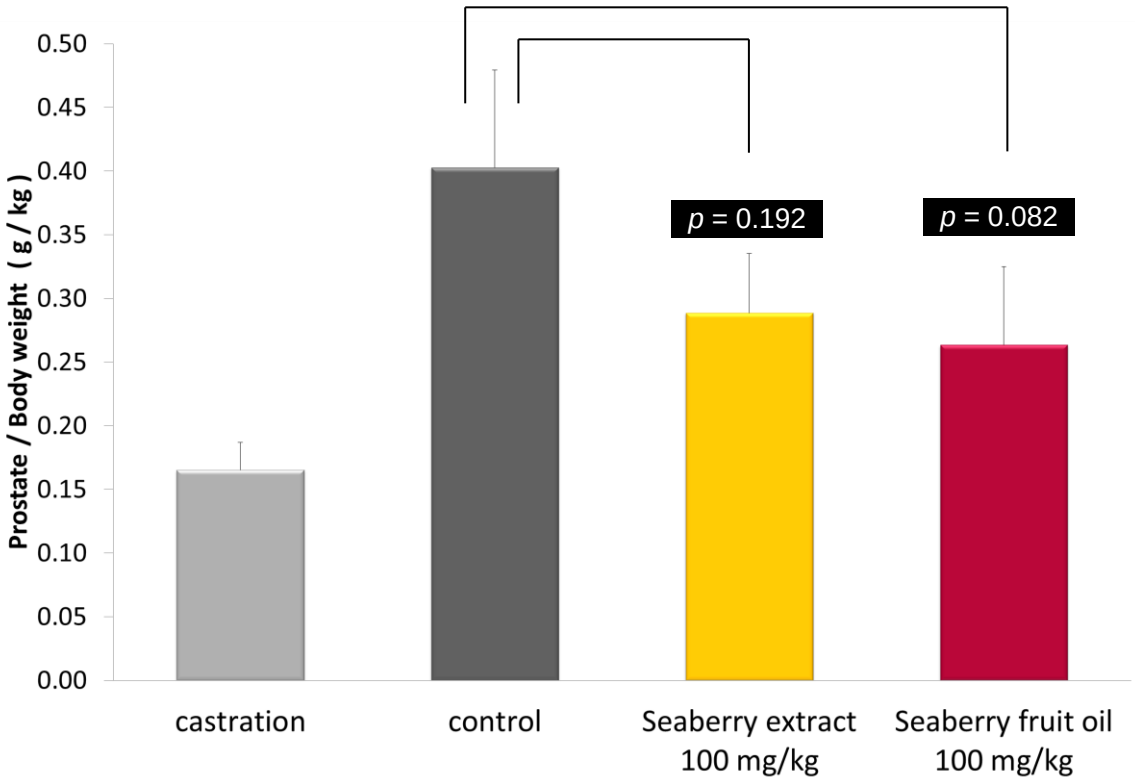


Fig.4 Effect of the seaberry extract and Seaberry fruit oil on prostate hypertrophy model mice (Mean ± S.D.; n=4-6)

(3) Human trial using seaberry extract and seaberry fruit oil

Ten male volunteers with high international prostate symptom score (IPSS) ingested seaberry extract (equivalent to 200 mg of Seaberry Extract-P, see page 24 for specifications of P) or seaberry fruit oil (equivalent to 450 mg of fruit oil product, see page 27 for specifications of fruit oil) for 4 weeks. A questionnaire was conducted about the subjects' IPSS and QOL before and after the ingestion. As a result, both IPSS and QOL score decreased (Fig. 5, 6).

Comparing a blood parameters before and after the ingestion, they did not show a significant difference in the values. The result indicates the safety of seaberry extract and seaberry fruit oil (Table 1, 2).

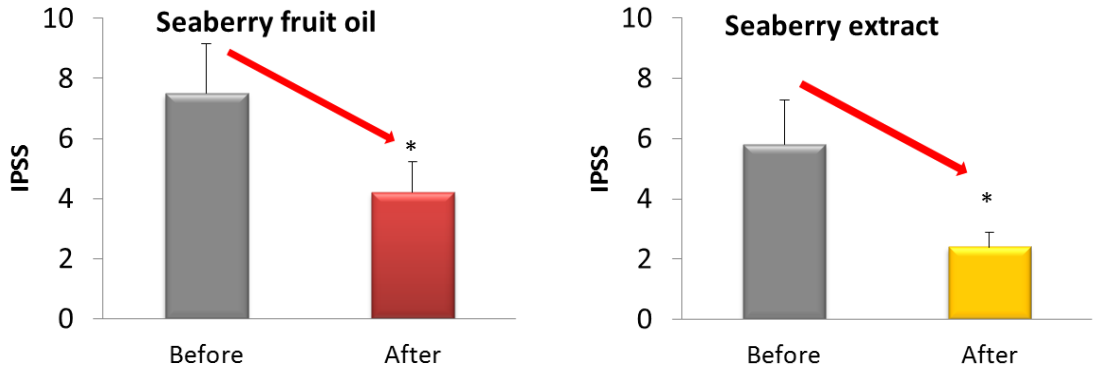


Fig.5 Change in international prostate symptom score (IPSS) before and after oral ingestion of the seaberry fruit oil and seaberry extract (Mean±SE, n=10, * : $p < 0.05$)

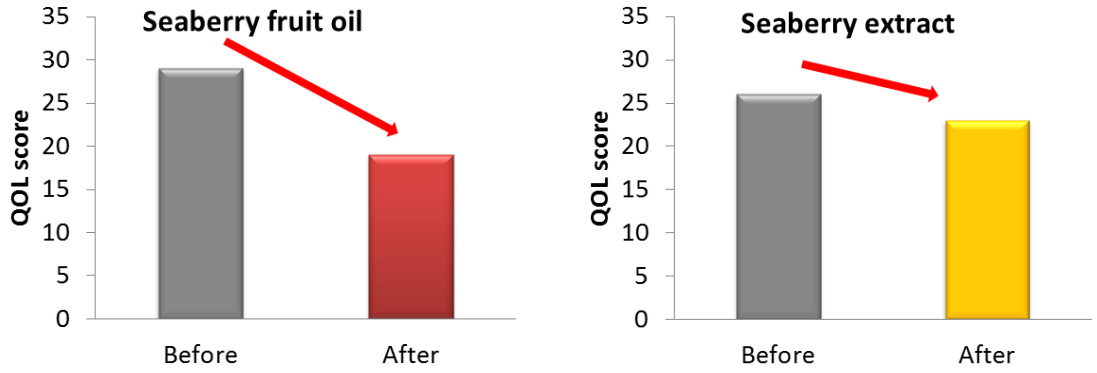


Fig.6 Change in QOL score before and after oral ingestion of the seaberry fruit oil and seaberry extract (n=10)

Table 1. Blood parameters of test subjects before and after Seaberry fruit oil ingestion

Items	Before ingestion	After 1 month	Normal range	Unit
Total bilirubin	0.6±0.2	0.6±0.1	0.2～1.2	mg/dL
Total protein	7.7±0.3	7.5±0.3 $^{<0.05}$	6.5～8.3	g/dL
Albumin	4.7±0.3	4.7±0.3	3.8～5.3	g/dL
A/G ratio	1.6±0.3	1.7±0.3	1.1～2.3	
AST(GOT)	24.6±4.7	23.4±2.5	8～38	U/L
ALT(GPT)	30.4±10.8	29.3±10.6	4～43	U/L
ALP	240.9±76.5	236.5±78.8	110～354	U/L
LD（LDH）	199.6±44.5	196.0±43.4	121～245	U/L
γ−GTP	44.6±24.5	44.6±35.5	< 86	U/L
LDL-cholesterol	144.7±24.2	141.0±30.8	70～139	mg/dL
Total cholesterol	222.7±26.1	220.2±26.8	130～219	mg/dL
Triglyceride（TG）	96.3±52.1	108.6±62.8	30～149	mg/dL
Phospholipid	236.6±26.4	230.5±25.2	150～260	mg/dL
Free fatty acid	0.6±0.3	0.7±0.2	0.13～0.77	mEq/L
HDL-cholesterol	65.1±18.4	63.2±18.1	40～77	mg/dL
Sodium	146.3±2.1	145.2±3.4	135～150	mEq/L
Chloride	104.2±1.6	104.0±1.7	98～110	mEq/L
Potassium	4.2±0.3	4.1±0.3	3.5～5.3	mEq/L
Urea nitrogen	14.8±4.4	15.0±2.4	8.0～22.0	mg/dL
Creatinine	0.8±0.1	0.8±0.1	0.61～1.04	mg/dL
Uric acid	5.7±1.0	5.6±1.1	3.6～7.0	mg/dL
Blood Glucose	96.6±11.0	94.1±6.7	60～109	mg/dL
HbA1c NGSP	5.6±0.6	5.7±0.5	4.6～6.2	%
Ketone body	31.7±27.4	31.5±19.8	<74	mmol/L
White blood cell count	63.0±13.9	58.0±9.9	39～98	$\times 10^2$/mL
Red blood cell count	502.1±22.3	495.1±27.6 $^{<0.05}$	427～570	$\times 10^4$/mL
Hemoglobin	15.1±0.7	15.0±0.8	13.5～17.6	g/dL
Hematocrit	47.0±2.5	46.0±2.8 $^{<0.05}$	39.8～51.8	%
MCV	93.6±2.7	92.9±3.2	82.7～101.6	fL
MCH	30.1±0.9	30.3±0.9	28.0～34.6	pg
MCHC	32.2±0.4	32.6±0.7	31.6～36.6	%
Platelet	27.1±2.4	26.1±3.1	13.1～36.2	$\times 10^4$/mL

Table 2. Blood parameters of test subjects before and after Seaberry extract ingestion

Items	Before ingestion	After 1 month	Normal range	Unit
Total bilirubin	0.6±0.2	0.5±0.1	0.2～1.2	mg/dL
Total protein	7.5±0.4	7.4±0.4	6.5～8.3	g/dL
Albumin	4.7±0.3	4.6±0.3	3.8～5.3	g/dL
A/G ratio	1.7±0.3	1.7±0.3	1.1～2.3	
AST(GOT)	24.5±6.6	23.3±6.1	8～38	U/L
ALT(GPT)	27.2±14.3	24.2±9.2	4～43	U/L
ALP	206.6±49.0	203.6±45.8	110～354	U/L
LD(LDH)	205.6±38.9	199.1±37.1	121～245	U/L
γ-GTP	35.0±16.9	31.8±13.3	<86	U/L
LDL-cholesterol	126.7±20.3	125.6±15.8	70～139	mg/dL
Total cholesterol	204.3±21.4	199.9±19.8	130～219	mg/dL
Triglyceride(TG)	110.5±61.1	104.3±46.0	30～149	mg/dL
Phospholipid	225.9±36.1	196.8±63.3	150～260	mg/dL
Free fatty acid	0.6±0.2	0.5±0.2	0.13～0.77	mEq/L
HDL-cholesterol	58.0±13.0	57.7±12.8	40～77	mg/dL
Sodium	145.2±2.5	145.4±3.0	135～150	mEq/L
Chloride	103.8±2.8	103.8±3.2	98～110	mEq/L
Potassium	4.1±0.3	4.2±0.3	3.5～5.3	mEq/L
Urea nitrogen	15.3±3.2	16.9±4.9	8.0～22.0	mg/dL
Creatinine	0.9±0.1	0.9±0.1	0.61～1.04	mg/dL
Uric acid	5.6±1.4	5.8±1.6$^{<0.05}$	3.6～7.0	mg/dL
Blood Glucose	99.6±10.1	102.5±16.9	60～109	mg/dL
HbA1c NGSP	5.8±0.5	5.8±0.5	4.6～6.2	%
Ketone body	21.7±13.6	19.4±9.1	<74	mmol/L
White blood cell count	60.9±13.3	55.6±9.0	39～98	$\times10^2$/mL
Red blood cell count	495.5±34.1	488.2±28.4	427～570	$\times10^4$/mL
Hemoglobin	15.0±0.6	14.8±0.6	13.5～17.6	g/dL
Hematocrit	47.0±1.9	45.6±1.7$^{<0.05}$	39.8～51.8	%
MCV	95.0±3.5	93.5±3.4$^{<0.05}$	82.7～101.6	fL
MCH	30.3±1.1	30.4±0.9	28.0～34.6	pg
MCHC	31.8±0.5	32.5±0.7$^{<0.01}$	31.6～36.6	%
Platelet	23.6±3.3	22.4±2.4$^{<0.05}$	13.1～36.2	$\times10^4$/mL

4. Action of Seaberry Extracts and Its Components on Overactive Bladder

(1) Overactive bladder

The bladder muscle (bladder smooth muscle) repeatedly contracts and relaxes mainly under control of parasympathetic nerve to control urination (Fig. 7). When the bladder becomes overactive, bladder smooth muscle contracts excessively inducing “urinary urgency”. Urgent urination “impending incontinence” occurs suddenly, and you cannot hold urination until you get to a bathroom. “Frequent urination” means the symptom that required to go to the bathroom eight times a day or at least once in your sleep.

According to a recent report about the mechanism of overactive bladder, the following conditions were observed in chronic hyper-contraction: increase of the concentration of the proliferation factor (TGF-β1) that induces the fibrosis within bladder cells, denaturation of muscle fibers (actin filaments) that induce over contraction, and the expression of stress fibers[2)].

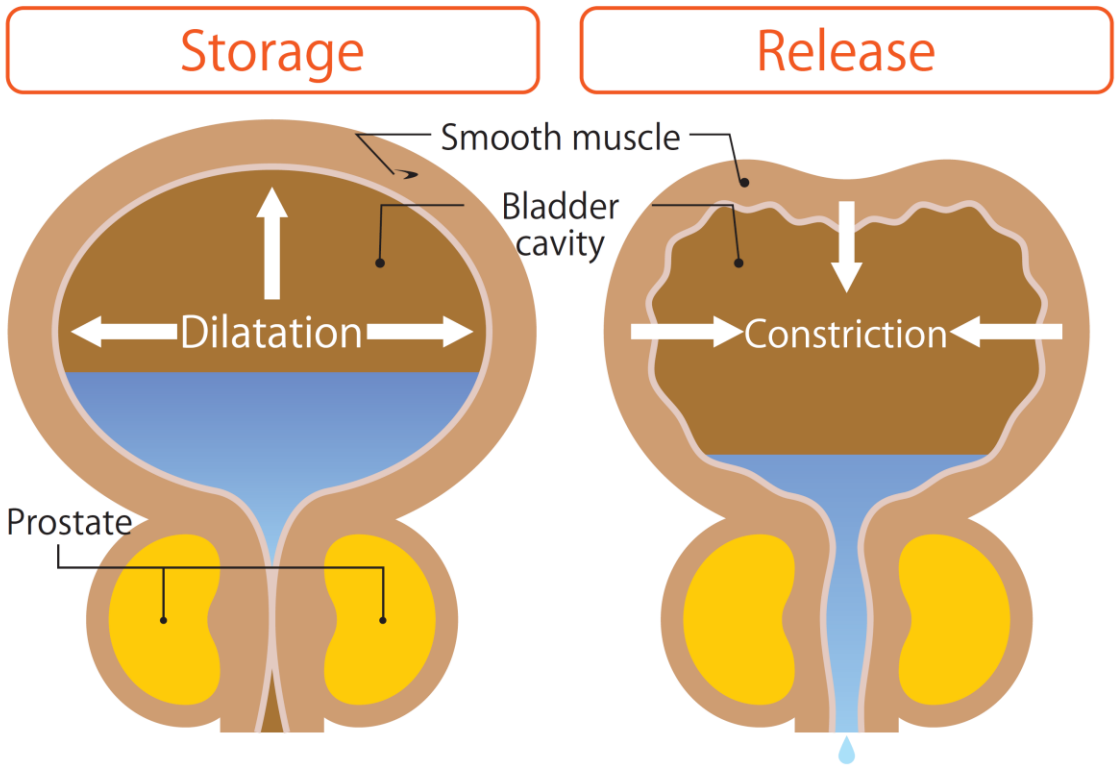


Fig.7 Images of the bladder and urination mechanism

2) Ramachandran, Aruna, *et al*. "JunB Mediates Basal-and TGF-β1-Induced Smooth Muscle Cell Contractility." *PloS one* 8.1 (2013): e53430.

(2) Action of seaberry extract and its components on collagen gel hyper-contraction model containing bladder smooth muscle cells

One of the cause of chronic hyper-contraction of bladder smooth muscle is an increase in the TGF-β1 of cells as described previously. Therefore, we stimulated collagen gel embedded with human bladder smooth muscle cells by TGF-β1 to study the action of seaberry on the contraction of the gel. As a result, seaberry extract and its components suppressed the contraction. As shown in Fig. 8, the gel area treated with the extract became at least similar to that of the normal compared to the control. An action to suppress contraction was confirmed in seaberry extract (without binder) and its contents, ursolic acid, hypophaeric acid, pomolic acid, oleanolic aldehyde, isorhamnetin rhamnoside, and uvaol (Figs. 9-1, 2). The results confirmed that seaberry extract has an action to suppress chronic hyper-contraction of bladder caused by TGF-β1 stress.

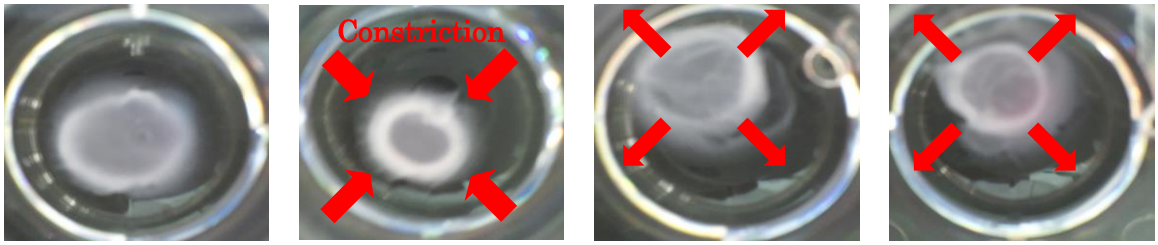


Fig.8 The relaxation effect of Seaberry extract on the constriction of collagen gel containing bladder smooth muscle cells

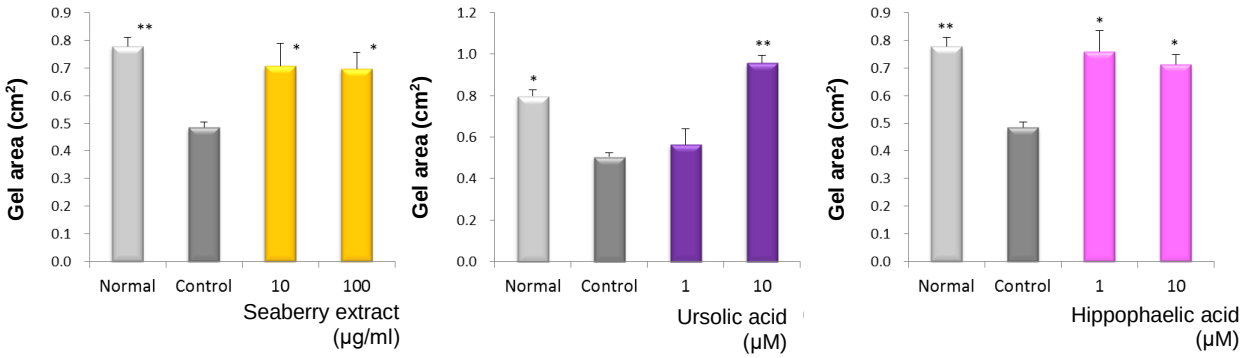


Fig.9-1 The relaxation effect of Seaberry extract and its components on the constriction of collagen gel containing bladder smooth muscle cells (Mean±SE, n=10, ** : $p < 0.01$, * : $p < 0.05$)

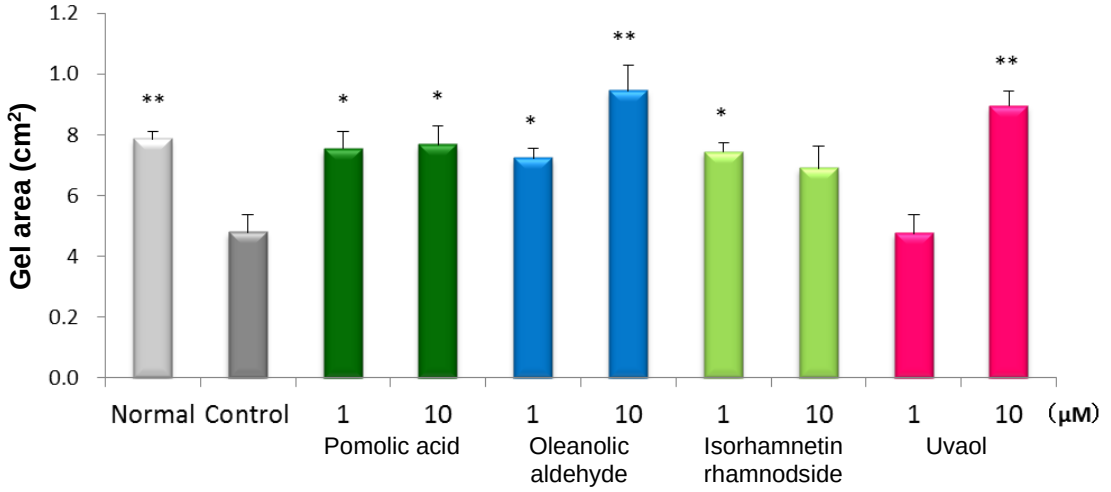


Fig.9-2 The relaxation effect of Seaberry extract and its components on the constriction of collagen gel containing bladder smooth muscle cells (Mean±SE, n=10, ** : $p < 0.01$, * : $p < 0.05$)

(3) Effect of seaberry extract and its components on the contraction of bladder smooth muscle

As described in the previous section, seaberry extract and its components were confirmed to suppress hyper-contraction on the model of bladder smooth muscle hyper-contraction. A test was conducted to study the effect on bladder contraction induced by a smooth muscle neurotransmitter by using Magnus method (Fig. 10). Bladder was removed from a rat and the specimen was fixed. In this method, the upper fitting is pulled and its force is converted into an electric signal when the smooth muscle contracts so that the contraction level can be measured. The fixed smooth muscle was immersed in nutrient buffer and the sample was added 30 minutes later. The sample was not added to the control. The sample and control were left for ten minutes for stabilization. Then the smooth muscle was contracted by carbachol (CCh: a stimulator of parasympathetic nerve).

As shown in gray lines in Fig. 11, the contraction of the control increased as the concentration of carbachol. Seaberry extract (10 μg/ml) suppressed the contraction. One of the major components in seaberry, ursolic acid (1 to 100 μM) clearly suppressed the contraction. Isorhamnetin rhamnoside (10 μM) significantly suppressed the contraction caused by CCh at 3×10^{-7} M. Pomolic acid, uvaol, and oleanolic aldehyde also showed a tendency to suppress the contraction. These results confirmed that seaberry extract suppresses bladder contraction.

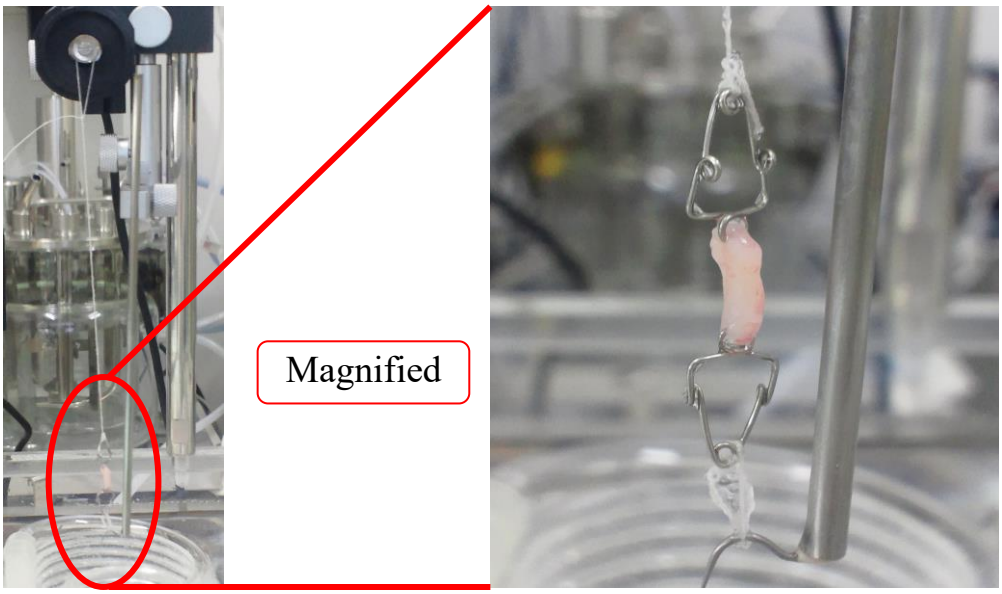


Fig.10 Magnus apparatus and a tissue

A pharmacological tester that can convert tension caused by contraction and relaxation to electric signals. Smooth muscle of organs such as the bladder is hung and soaked in nutrient buffer.

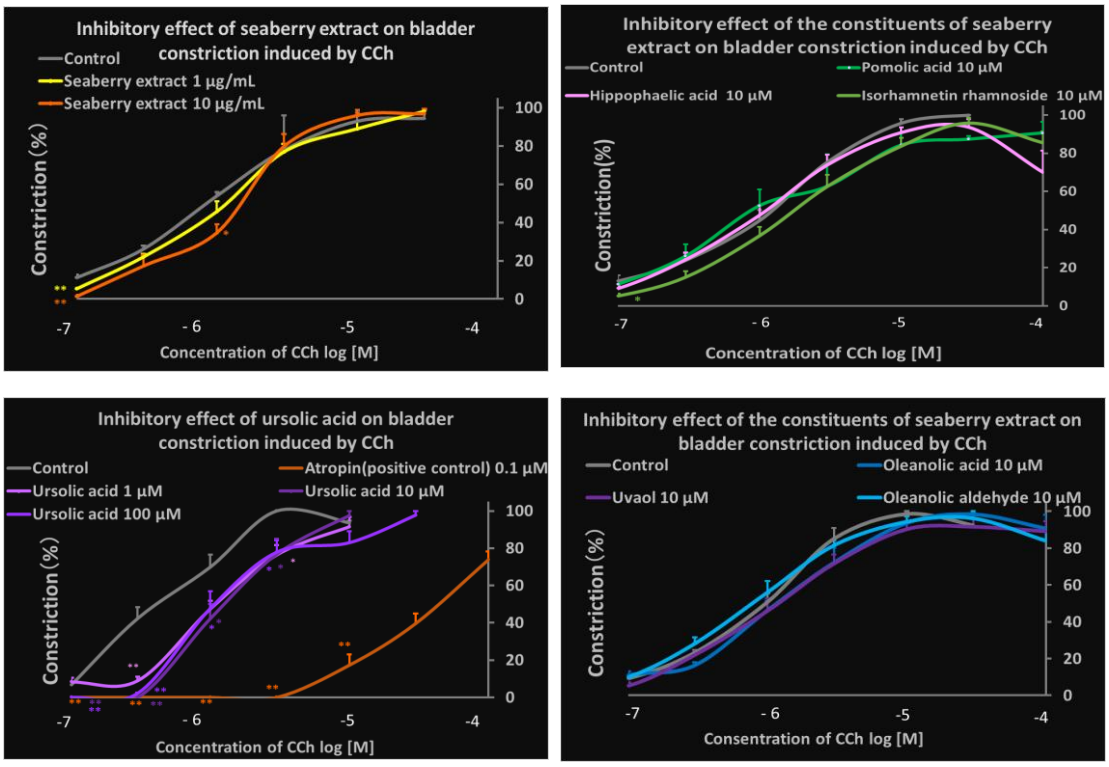
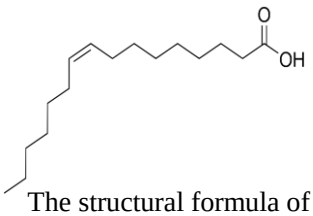


Fig.11 The inhibitory effects on the contraction of rat bladder smooth muscle by Seaberry extract and its components (Mean±SE, n=10, ** : $p < 0.01$, * : $p < 0.05$)

5. Anti-Metabolic Syndrome Action of Palmitoleic Acid (ω-7)

（1） ω－7

Palmitoleic acid contained in seaberry fruit oil is called ω-7 and is a monounsaturated fatty acid. It has a dual structure on the seventh carbon from the methyl group as shown in the figure to the right. It is contained in human liver and skin in a larger amount than other tissues. Among substances in the natural world, seaberry is known to have the highest percentage of ω-7 in fatty acid composition[3].



The structural formula of palmitoleic acid(ω-7)

（2） Effects and efficacy of ω-7

It is considered that ω-7 lowers the blood sugar level by increasing insulin sensitivity and in turn reduces symptoms of type II diabetes. Stefan *et al.* found that people with high blood ω-7 concentration have a high insulin sensitivity in human clinical trial such as an oral glucose tolerance test (Fig. 12, A) that is insulin resistance evaluation method and euglycemic hyperinsulinemic clamp test (Fig. 12, B)[4]. In other words, active ingestion of ω-7 may accelerate the activity of insulin and reduce symptoms of type II diabetes in turn. After a placebo-controlled double blind comparative study, Bernstein *et al.* reported that ingestion of ω-7 lowered C-reactive protein (inflammation marker), blood triglyceride, and LDL-cholesterol (bad cholesterol) and increased HDL-cholesterol (good cholesterol)[5]. An effect to reduce body weight has also been reported[6]. Various types of drugs are used for metabolic syndrome. The reports above indicate that ω-7 may be used as a safe anti-metabolic syndrome component (Table 3).

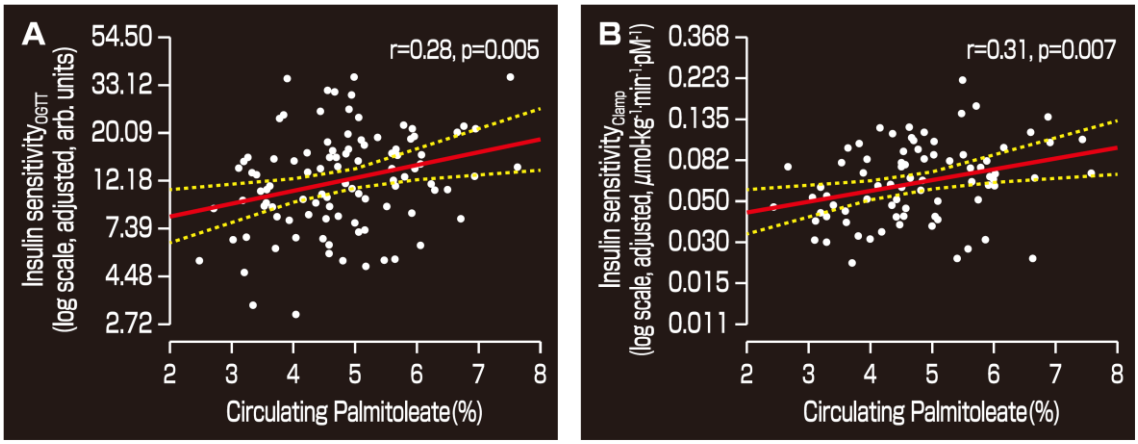


Fig.12 Cross-sectional relationships of circulating palmitoleate with insulin sensitivity estimated from the OGTT (A) and measured by the clamp (B)

Table 3. Comparison of ω-7 and medicines on the metabolic syndrome

	ω7 (Palmitoleic acid)	Statins (Anti-cholesterol)	Fibrates (Lipids lowing)	Glitazone (Blood suger lowing)	Sulfonylurea (Blood suger lowing)
Decrease in LDL-cholesterol	○	○	○	×	—
Increase in HDL-cholesterol	○	—	○	○	×
Decrease in blood suger	○	×	—	○	○
Improving insulin resistance	○	—	—	○	△
Body weight/composition	Reduce appetite	Increase weight Decrease fat-free mass	May increase weight and fat mass	Decrease fat	Increase
Anti-inflammatory	○	○	○	○	—
Adverse effect	No report	Muscle pain, risk of diabetes	Gallstones, Muscle pain	May increase risk of cardiovascular death	increase risk of cardiovascular death

（3）Skin-lightening action of ω-7

Cloudy or darkened skin and pigmentation are caused by melanin. Melanin is generated by tyrosine, a type of amino acid. It is known that tyrosinase, tyrosinase related proteins (TRP1 and TRP2), and microphthalmia-associated transcription factor (MITF) that is necessary for the generation of these enzymes are involved in the generation of melanin. Yoon, *et al.*[7] evaluated actions of ω-7 on melanin generation using melanocytes (B16F10) by a stimulation of melanocyte-stimulating hormone (α-MSH). As a result, melanin generation was suppressed concentration dependently (Fig. 13). The mechanism of the performance is related to the inhibition of the protein expression of tyrosinase, TRP1, TRP2, and MITF (Fig. 14).

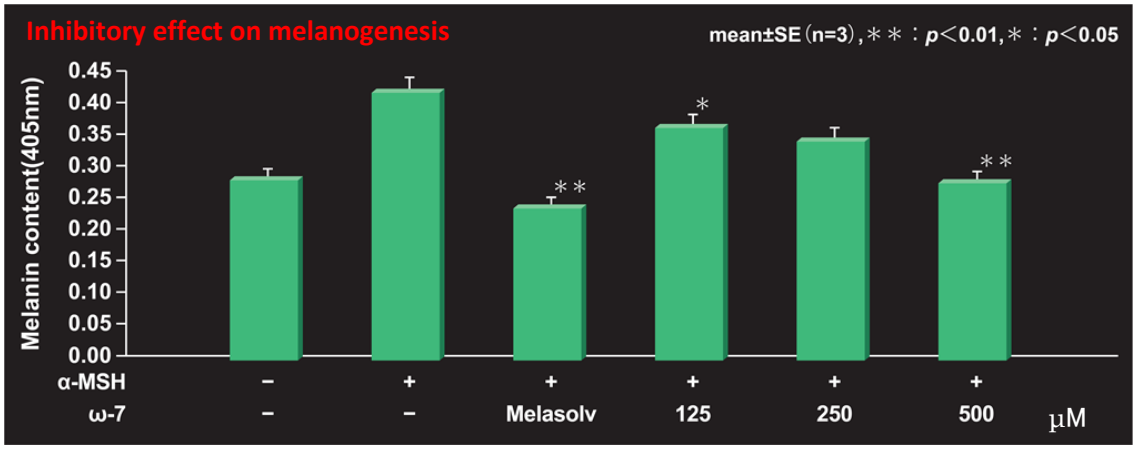


Fig.13 Inhibitory effect on melanogenesis of palmitoleic acid in B16F10 cells
α-MSH：melanocyte-stimulating hormones, melasolv：positive control

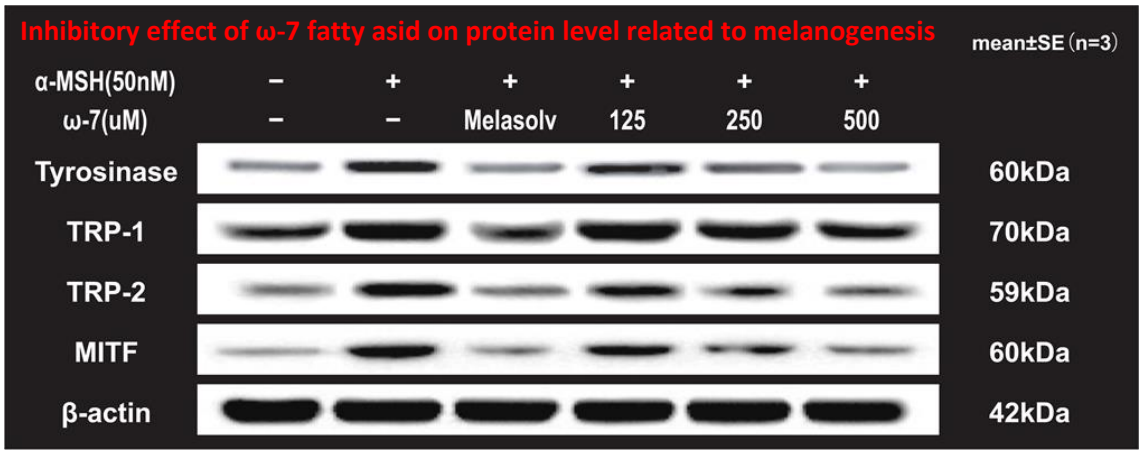


Fig.14 Inhibitory effect of palmitoleic acid on protein level related melanogenesis in B16F10 cells

α-MSH : melanocyte-stimulating hormones, melasolv : positive control

3) Fatima, Tahira, *et al.* "Fatty acid composition of developing sea buckthorn (*Hippophae rhamnoides* L.) berry and the transcriptome of the mature seed." *PloS one* 7.4 (2012) e34099.

4) Stefan, Norbert, *et al.* "Circulating palmitoleate strongly and independently predicts insulin sensitivity in humans." *Diabetes Care* 33.2 (2010) 405-407.

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5. Beautifying actions of Seaberry Extract

（1）Action to Reduce/Suppress Irritation by Air Pollutants

We are constantly exposed to air pollutants. Examples of common air pollutants are cigarette smoke and exhaust gas from gas and diesel engine vehicles. These air pollutants irritate the skin and cause inflammation. When air pollutants (standard environmental substances, vehicle exhaust particulate, cigarette butts, particles in diesel car muffler) are added to human epidermal keratinocytes (horny cells), we found the production of the inflammatory factor, prostaglandin E_2 (PGE_2) increases. In a test, addition of seaberry extract suppressed the increase of PGE_2 (Fig. 15). This result suggests that seaberry extract is expected to have an action to reduce skin irritation caused by air pollutants.

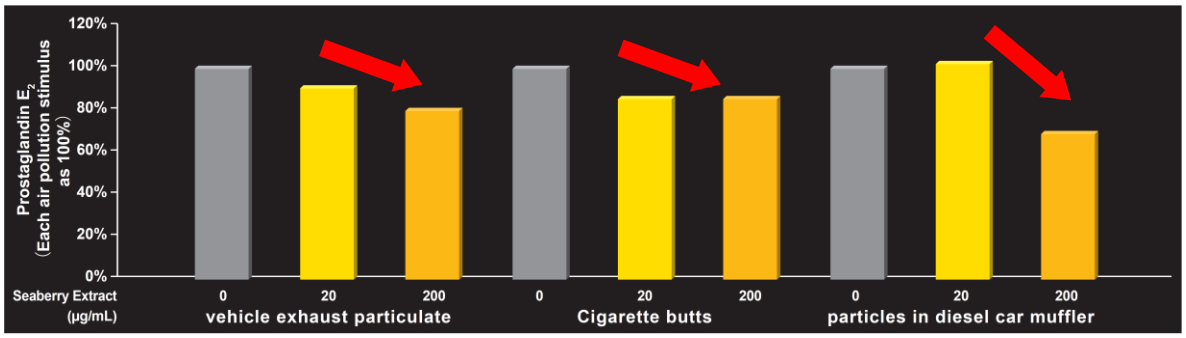

Fig.15　Effect of inhibit against PGE_2 production exposure air pollutants

（2）Action to Reduce/Suppress Irritation from Dryness

Dryness is a well-known factor that damages the skin. Even in summer, we are exposed to dryness when we are in an air-conditioned room. When the skin is irritated by dryness, inflammatory factors such as interleukins (IL) are produced. A test was conducted regarding this background. When 3D-cultured epidermis were dried, the concentration of IL-1α increased in the medium. When seaberry extract was added in this condition, it suppressed the increase in IL-1α concentration (Fig. 16). According to the result, seaberry extract is expected to have an action to reduce or suppress irritation caused dryness or moisturize the skin.

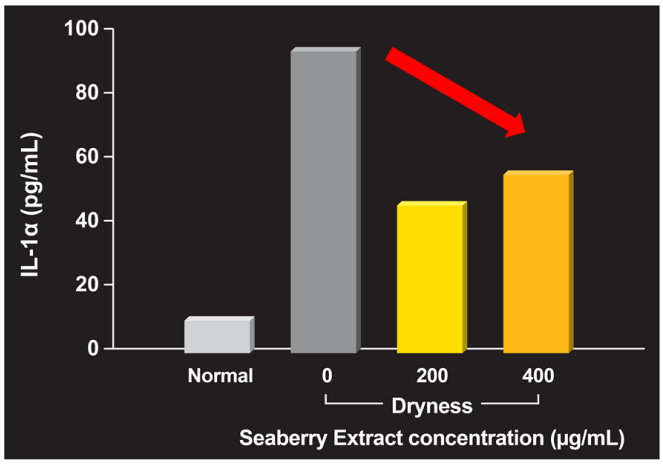

Fig.16 Inhibitory effect of seaberry extract against IL-1α production by exposure to dryness

（3） Anti-Oxidative Action (Ability to Scavenge DPPH Radicals)

Reactive oxygen species protect our body from the attack of bacteria and viruses. However, since it attacks normal cells as well as bad cells, excessive reactive oxygen species negatively influence the body. When the skin is exposed to UV rays, dryness, bacteria, and chemical substance, reactive oxygen species are produced. In order to evaluate the ability of seaberry extract on exclusion of reactive oxygen species, DPPH radical scavenging ability was measured. As a result, the extract showed concentration-dependent anti-oxidative activity (Fig. 17). This indicates that seaberry extract has an action to protect bodies against attacks of reactive oxygen species caused by external irritation.

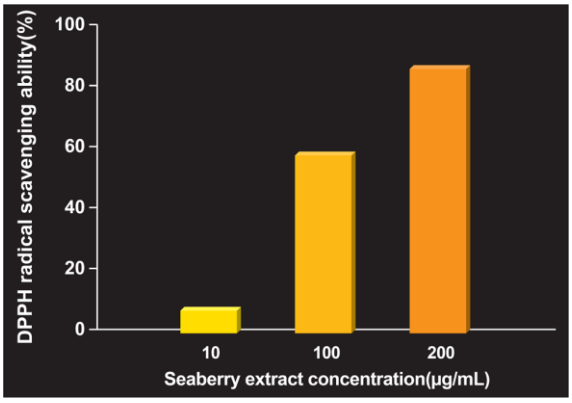

Fig.17 DPPH radical scavenging ability of Sea berry extract

（4） Wound Healing Effect

Wounds mean damages on a wide range of body surface tissues including cuts, stabs, and burns. Wounds start to heal during the inflammation period and then progress to the proliferation stage and maturity stage. In these healing stages, fibroblasts proliferate, while collagen fibers are produced to heal damaged parts. Seven *et al.*[8] applied seaberry extract on a burn on the femur of rats and measured the change of blood flow using the ^{133}Xe (radioisotope of xenon) clearance method. They applied seaberry extract on the right thigh with burned damage in mice and their left thigh untreated. Then ^{133}Xe was injected intradermally. The count rate shown in the vertical line in Fig. 18 indicates the residual volume of ^{133}Xe, which decreases when blood flow is enhanced and a large amount is discharged. In the group that seaberry extract was applied, the count rate significantly lowered. However, there was no significant difference in the group dexpanthenol (panthenol: used in cosmetics often) was applied. In other words, seaberry extract is expected to have an effect to promote the recovery of skin tissue by enhancing blood flow.

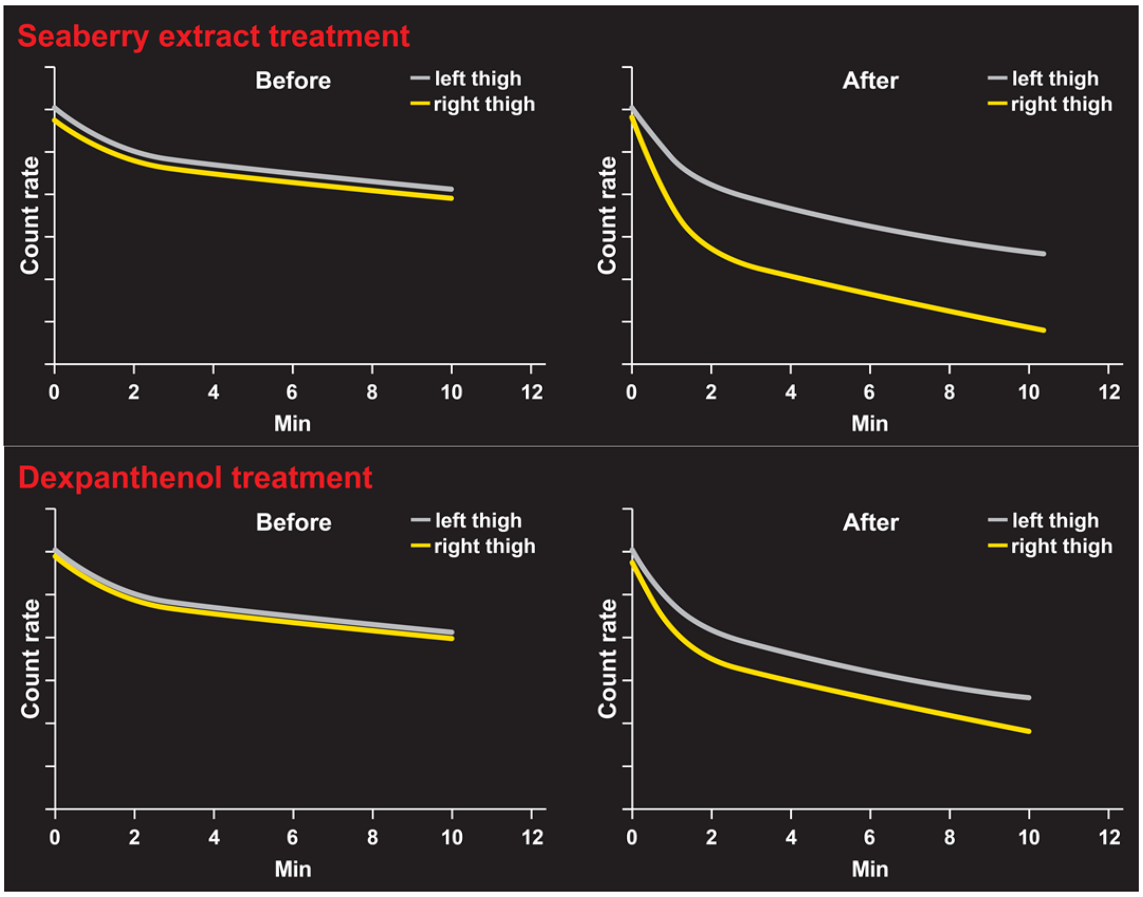

Fig.18 Wound healing effect of Seaberry extract

8) Seven *et al.* "*Hippophae rhamnoides* L. and dexpanthenol-bepanthene on blood flow after experimental skin burns in rats using ^{133}Xe clearance technique." *Hellenic J. Nuclear Med.* 12.1 (2008) 55-58.

6. Product Stability of Seaberry Extract

(1) Heat Stability

The heat stability of Seaberry Extract-P was examined by heating at 100°C continuously for 1 hour. As shown in Fig. 19, content of triterpenic acids and isorhamnetin rhamnoside were not reduced after heating for 1 hour. Therefore, Seaberry Extract-P is highly stable upon heating at normal food processing temperature.

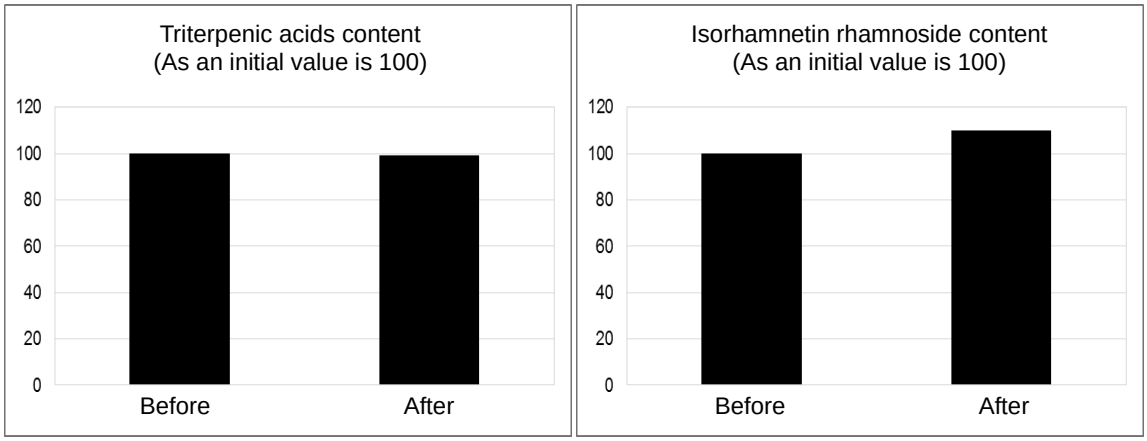

Fig.19 The heat stability of Seaberry extract-P

(2) pH stability

The pH stability of Seaberry extract was examined stored at different pH value at room temperature for a week. The isorhamnetin rhamnoside content of Seaberry Extract-WSP was measured. Results showed that yellow color disappeared when pH >5 (Fig. 20), isorhamnetin rhamnoside content of Seaberry Extract-WSP is stable between pH 3 – 8 (Fig. 21). Isorhamnetin rhamnoside is not stable under alkaline environment.

pH3 pH4 pH5 pH6 pH7 pH8 pH9

Fig.20 The color change by pH of Sea berry extract-WSP in aqueous solution

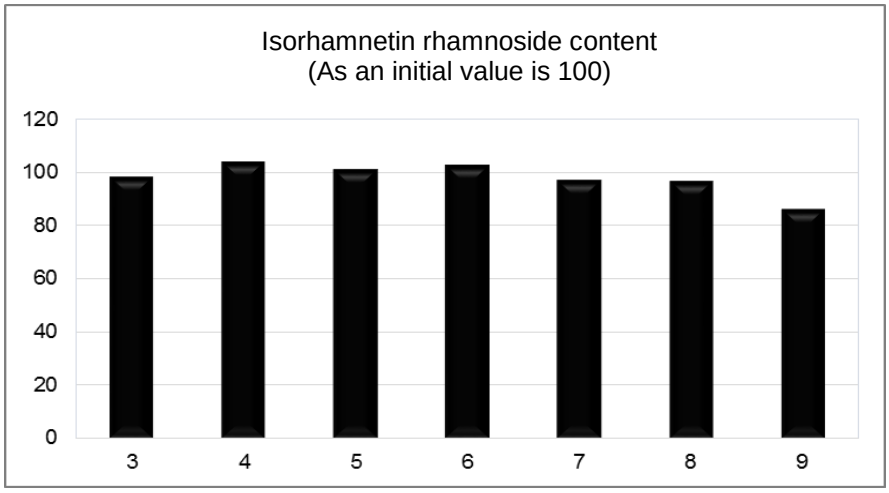

Fig.21 pH Stability of Seaberry Extract-WSP in aqueous solution

7. Product Stability of Seaberry Fruit Oil

Heat stability

The heat stability of Seaberry Fruit Oil was examined by heating at 120°C continuously for 1 hour. As shown in Fig. 22, content of palmitoleic acid was not reduced after heating for 1 hour. Therefore, Seaberry Fruit Oil is highly stable upon heating at normal food processing temperature.

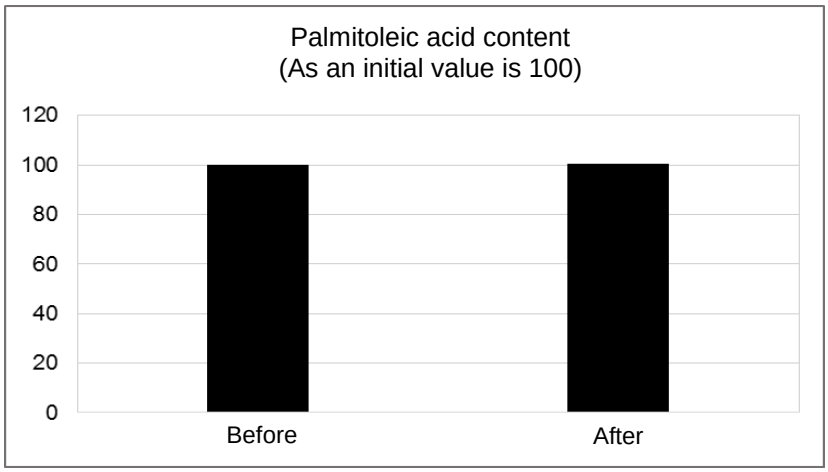

Fig.22 The heat stability of Seaberry Fruit Oil

8. Nutrition Profiles

Analyzed item	P	WSP	Oil note4	Method
Water (g/100 g)	2.5	4.3	0	Heating drying method under normal pressure
Protein (g/100 g)	2.2	1.8	0	Kjeldahl method, nitrogen protein conversion factor: 6.25
Fat (g/100 g)	1.1	0.3	100	Acid decomposition method
Ash (g/100 g)	1.5	1.4	0	Direct incineration method
Carbohydrate (g/100 g)	91.8	90.8	0	Refer note 1
Energy (kcal/100 g)	388	376	900	Refer note 2
Fiber (g/100 g)	0.9	1.4	0	Prosky's method
Sodium (mg/100 g)	129	97.7	0	Atomic absorption spectrophotometory
Sodium chloride equivalent (g/100 g)	0.3	0.2	0	Refer note 3

The nutritional information of Seaberry Extract and Oil was analyzed according to the standard in nutrition labeling (March 30, 2015; No 139 Eishin)
Note 1: Calculation: 100-(water + protein + fat + ash)
Note 2: Energy conversion factor: Protein 4, fat 9, sugar 4, dietary fiber 2
Note 3: In terms of sodium
Test trustee: SUNATECH / Date of analysis: May 18, 2015
Test No.: 150430251-001-01
Note 4: Since the fruit oil is soluble in ether, it is considered as 100% of lipid. Its energy was calculated using the conversion factor of 9.

9. Safety Profile

(1) Residual Agricultural Chemicals

Dried Seaberry Fruit was screened and analyzed for residual agricultural chemicals (308 items) stipulated under the Food Sanitation Act and Pesticides Control Act, presence of the test items was lower than the allowed limits.

Test Trustee: Masis Co., Ltd.; Center for Food Safety Evaluation and Analysis
Date: April 17, 2015
Report No.: 76323

（2）Acute Toxicity（LD_{50}）

Acute Toxicity test was conducted according to the Guidelines for Single-Dose Toxicity Tests for Pharmaceutical Products where Seaberry Extract and Seaberry Fruit Oil 2000 mg/kg was orally given to fasted ICR mice (male and female ddY, 6 weeks old, weight approx. 30 g) for 14 days. No abnormalities and fatal event observed at 2000 mg/kg. No abnormalities were observed under macroscopic examination upon autopsy. Thus, LD50 of Seaberry Extract and Seaberry Fruit Oil is deduced to be >2000 mg/kg.

10. Recommended Dosage

In accordance to the result of human clinical trials, the recommended dosage of Seaberry Extract-P is 200 mg/day and Seaberry Extract-WSP is 400 mg/day.

In accordance to the result of human clinical trials and enlarged prostate model mice test, the recommended dosage of Seaberry Fruit Oil is 150 – 450 mg/day.

11. Application

	Applications	Claims	Examples
Food	Nutritional Supplement, Beauty Food	Prevention and improving action of prostatic hyperplasia and overactive bladder, Metabolic syndrome improvement, Reducing effect of skin irritation (air pollutants and drying), Anti-inflammatory effect, Moisturizing	Be capsu Ham verages Hard & soft les, tablets Candies, chewing gums, chocolates, wafers, jellies , sausage, etc.

12. Packing

Seaberry Extract-P, -WSP, -PC, -WSPC

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

Seaberry Extract -J

1 kg	interior packing: : polyethylene bottle
	Exterior packing : Cardboard box
5 kg, 20 kg	interior packing: : Cubic polyethylene container
	Exterior packing : Cardboard box

Seaberry Fruit Oil

1 kg, 5 kg, 16 kg	interior packing: : Tin can
	Exterior packing : Cardboard box

13. Storage

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

14. Expression

<Food>

Seaberry Extract-P, -WSP
Maltodextrin, Seaberry Extract

Seaberry Extract-J
Seaberry juice

Seaverry Fruit Oil
Seaberry fruit oil, Triglyceride, Mix tocopherols, L-Ascorbic acid palmitate

PRODUCT STANDARD

SEABERRY EXTRACT-P (FOOD)

This product is extracted with aqueous ethanol from the dried fruits of seaberry fruits (*Hippophae rhamnoides* L.). It contains a minimum of 0.2% triterpenoic acids and 0.2% isorhamnetin rhamnoside.

Appearance	Pale brown to brown powder with slightly characteristic odor.	
Ursolic acid	Min. 0.1 %	(HPLC)
Loss on Drying	Max. 10.0 %	(Analysis for Hygienic Chemists, 1 g, 105ºC, 2 hr)
Purity Test		
(1) Heavy Metals (as Pb)	Max. 20 ppm	(Sodium Sulfide Colorimetric Method)
(2) Arsenic (as As_2O_3)	Max. 1 ppm	(Standard Methods of Analysis in Food Safety Regulation, The Third Method, Apparatus B)
Standard Plate Counts	Max. 1×10^3 cfu/g	(Analysis for Hygienic Chemists)
Moulds and Yeasts	Max. 1×10^2 cfu/g	(Analysis for Hygienic Chemists)
Coliforms	Negative	(Analysis for Hygienic Chemists)

Composition

Ingredient	Content
Maltodextrin	50%
Seaberry extract	50%
Total	100%

Expiry date 2 years from date of manufacturing.

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

PRODUCT STANDARD

SEABERRY EXTRACT-WSP (FOOD)

This product is extracted with water from the dried fruits of seaberry fruits (*Hippophae rhamnoides* L.). It is a water-soluble powder. It contains a minimum of 0.1% isorhamnetin rhamnoside.

Appearance	Pale yellow to pale brown powder with slightly characteristic odor.	
Isorhamnetin rhamnoside	Min. 0.1 %	(HPLC)
Loss on Drying	Max. 10.0 %	(Analysis for Hygienic Chemists, 1 g, 105ºC, 2 hr)
Purity Test		
(1) Heavy Metals (as Pb)	Max. 20 ppm	(Sodium Sulfide Colorimetric Method)
(2) Arsenic (as As_2O_3)	Max. 1 ppm	(Standard Methods of Analysis in Food Safety Regulation, The Third Method, Apparatus B)
Standard Plate Counts	Max. 1×10^3 cfu/g	(Analysis for Hygienic Chemists)
Moulds and Yeasts	Max. 1×10^2 cfu/g	(Analysis for Hygienic Chemists)
Coliforms	Negative	(Analysis for Hygienic Chemists)

Composition

Ingredient	Content
Maltodextrin	67%
Seaberry extract	33%
Total	100%

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

PRODUCT STANDARD

SEABERRY EXTRACT-J (FOOD)

This product is 6-fold concentrated juice from seaberry (*Hippophae rhamnoides* L.) fruits juice.

Appearance	Orange suspension with unique smell	
Purity Test		
(1) Heavy Metals (as Pb)	Max. 20 ppm	(Sodium Sulfide Colorimetric Method)
(2) Arsenic (as As_2O_3)	Max. 2 ppm	(Standard Methods of Analysis in Food Safety Regulation, The Third Method, Apparatus B)
Standard Plate Counts	Max. 3×10^3 cfu/g	(Analysis for Hygienic Chemists)
Moulds and Yeasts	Max. 1×10^2 cfu/g	(Analysis for Hygienic Chemists)
Coliforms	Negative	(Analysis for Hygienic Chemists)

Composition

Ingredient	Content
Seaberry concentrate juice	100%

Expiry date 2 years from date of manufacturing.

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

PRODUCT STANDARD

PRODUCT NAME : SEABERRY FRUIT OIL (FOOD)

This oil is extracted and refined from seaberry fruits (*Hippophae rhamnoides* L.).

Appearance	Reddish brown to brown liquid oil with slightly characteristic odor.	
Acid Value	Max. 5.0	
Palmitoleic Acid (ω-7)	Min. 30.0 %	(GC)
Certification Test		
Carotenoids	Dissolve the product in hexane (1→5) and prepare a standard β-carotene solution (50 μg/mL). Develop both solutions on a thin layer (silica gel) chromatography using hexane. As compared with the standard, an orange spot can be found in the sampleat the same point of β-carotene.	
Purity Test		
(1)Heavy Metals (as Pb)	Max. 20 ppm	(Sodium Sulfide Colorimetric Method)
(2)Arsenic (as As_2O_3)	Max. 1 ppm	(Standard Methods of Analysis in Food Safety Regulation, The Third Method, Apparatus B)
Standard Plate Counts	Max. 1×10^2 cfu/g	(Analysis for Hygienic Chemists)
Moulds and Yeasts	Negative	(Analysis for Hygienic Chemists)
Coliforms	Negative	(Analysis for Hygienic Chemists)

Composition

Ingredient	Content
Seaberry fruit oil	75.00 %
Triglyceride	24.90 %
Mix tocopherols	0.05 %
L-Ascorbic acid palmitate	0.05 %
Total	100.00 %

Expiry date 2 years from date of manufacturing.

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

PRODUCT STANDARD

SEABERRY EXTRACT-PC (COSMETIC)

This product is extracted with water from the dried fruits of seaberry (Hippophae rhamnoides L.). It is a water-soluble powder. It contains a minimum of 0.1% isorhamnetin rhamnoside.

Appearance	Pale brown to brown powder with slightly characteristic odor.	
Ursolic acid	Min. 0.1 %	(HPLC)
Loss on Drying	Max. 10.0 %	(Analysis for Hygienic Chemists, 1 g, 105ºC, 2 hr)
Purity Test		
(1) Heavy Metals (as Pb)	Max. 20 ppm	(Sodium Sulfide Colorimetric Method)
(2) Arsenic (as As_2O_3)	Max. 1 ppm	(Standard Methods of Analysis in Food Safety Regulation, The Third Method, Apparatus B)
Standard Plate Counts	Max. 1×10^2 cfu/g	(Analysis for Hygienic Chemists)
Moulds and Yeasts	Max. 1×10^2 cfu/g	(Analysis for Hygienic Chemists)
Coliforms	Negative	(Analysis for Hygienic Chemists)

Composition

Ingredient	Content
Maltodextrin	50%
Seaberry extract	50%
Total	100%

Expiry date 2 years from date of manufacturing.

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

PRODUCT STANDARD

SEABERRY EXTRACT-WSPC (COSMETIC)

This product is extracted with water from the dried fruits of seaberry (Hippophae rhamnoides L.). It is a water-soluble powder. It contains a minimum of 0.1% isorhamnetin rhamnoside.

Appearance	Pale yellow to pale brown powder with slightly characteristic odor.	
Isorhamnetin rhamnoside	Min. 0.1 %	(HPLC)
Loss on Drying	Max. 10.0 %	(Analysis for Hygienic Chemists, 1 g, 105ºC, 2 hr)
Purity Test		
(1) Heavy Metals (as Pb)	Max. 20 ppm	(Sodium Sulfide Colorimetric Method)
(2) Arsenic (as As_2O_3)	Max. 1 ppm	(Standard Methods of Analysis in Food Safety Regulation, The Third Method, Apparatus B)
Standard Plate Counts	Max. 1×10^2 cfu/g	(Analysis for Hygienic Chemists)
Moulds and Yeasts	Max. 1×10^2 cfu/g	(Analysis for Hygienic Chemists)
Coliforms	Negative	(Analysis for Hygienic Chemists)

Composition

Ingredient	Content
Maltodextrin	67%
Seaberry extract	33%
Total	100%

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

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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Established Date : August 8, 2015
Revised Date：May 28, 2019

ORYZA OIL & FAT CHEMICAL CO., LTD.