Oryza

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)

オリザ油化株式会社

Ver. 2.0NS

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-Isolated Component

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.

In addition, it was the first to isolate the 3-*O*-coumaroyl 2,23-dihydroxy oleanolic acid from Seaberry.

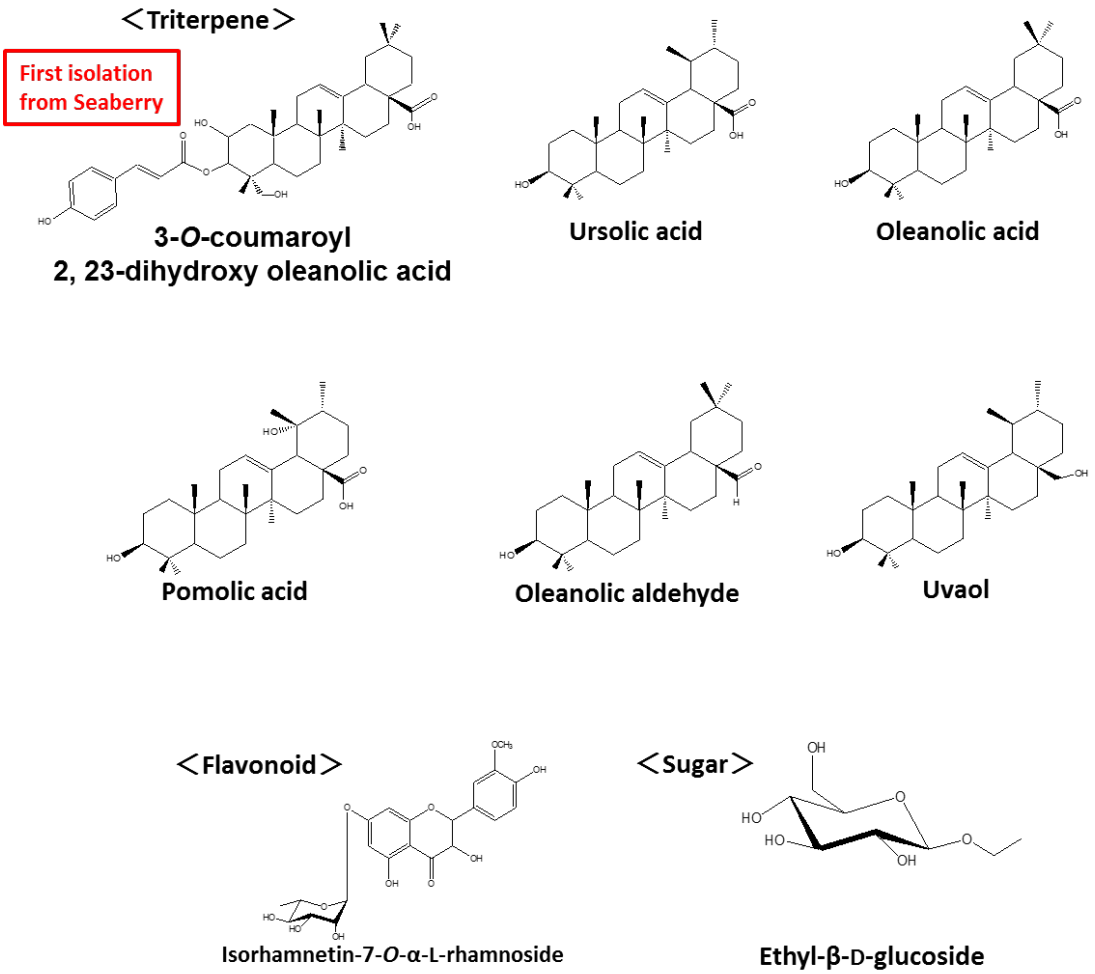


Fig.2 Components of seaberry

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.

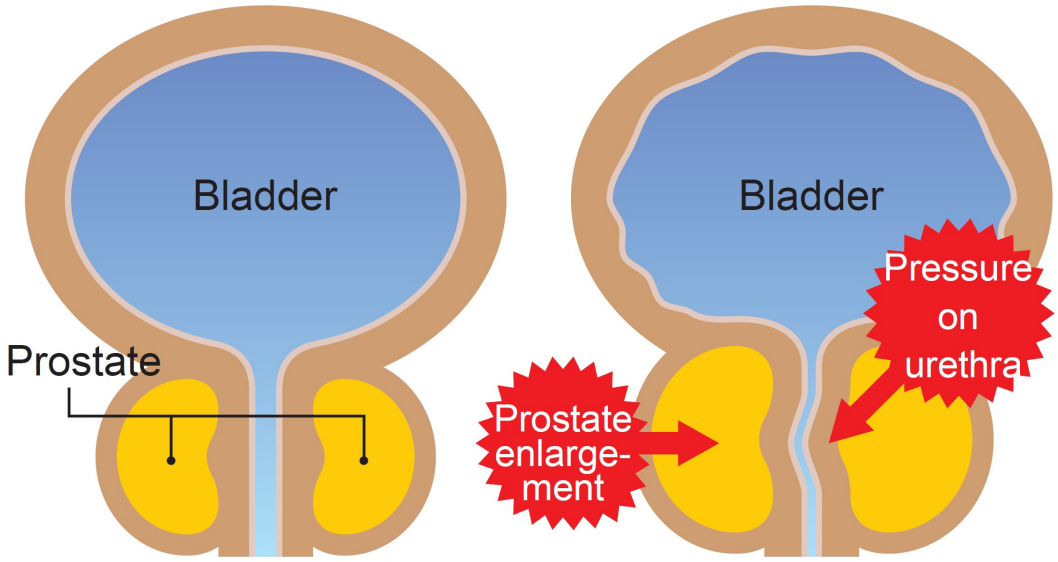


Fig.3 Image of the prostate and prostatic hyperplasia

（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).

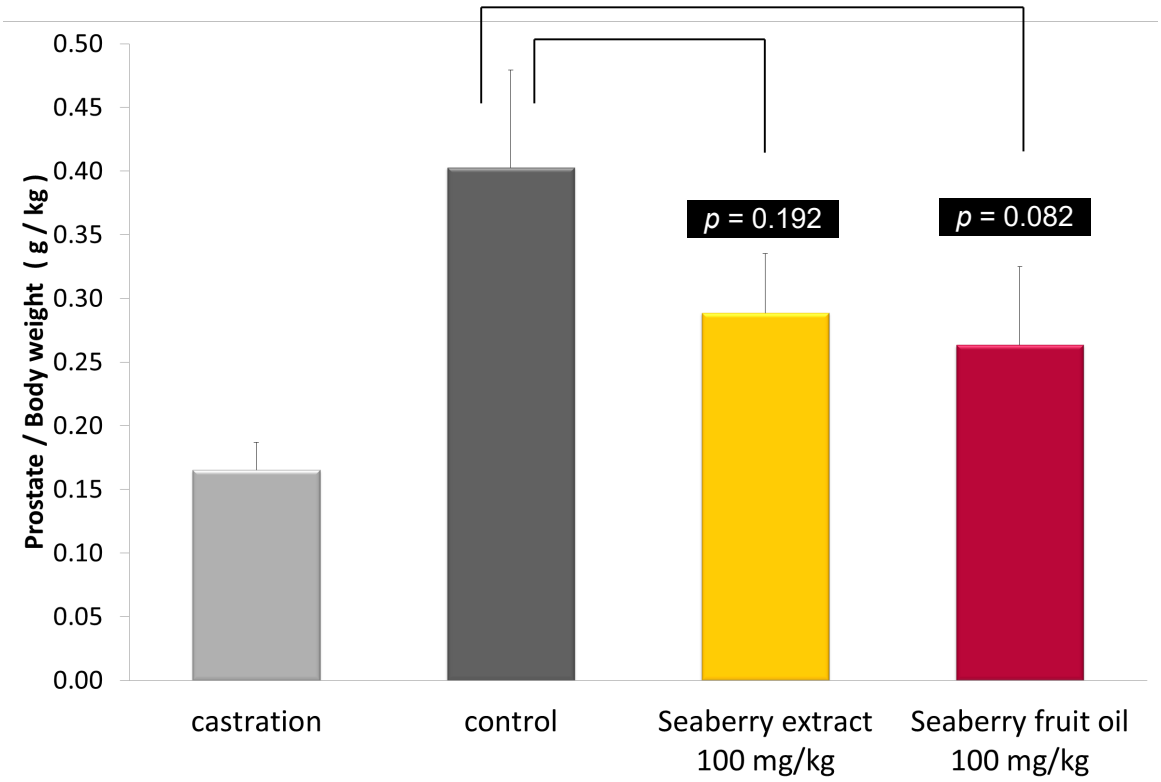


Fig.4 Effect of the seaberry extract and Seaberry fruit oil on prostate hypertrophy model mice (Mean ± S.D.; n=4-6)

(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).

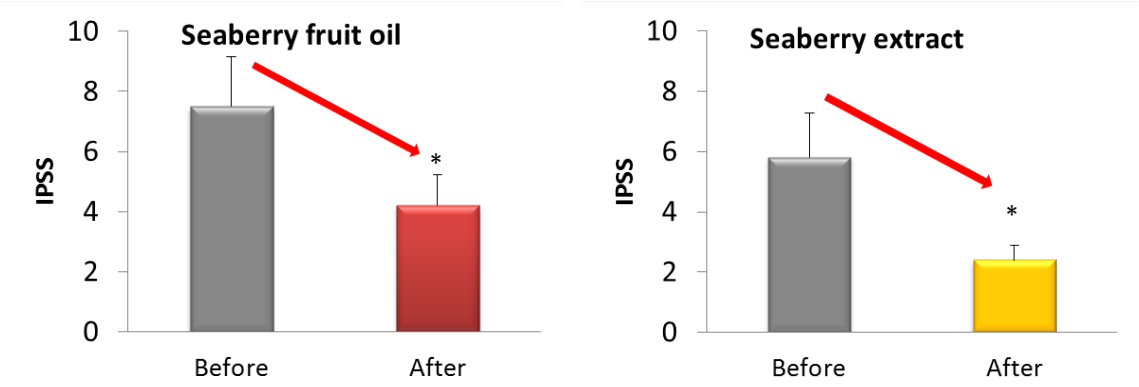


Fig.5 Change in international prostate symptom score (IPSS) before and after oral ingestion of the seaberry fruit oil and seaberry extract (Mean±SE, n=10, * : $p < 0.05$)

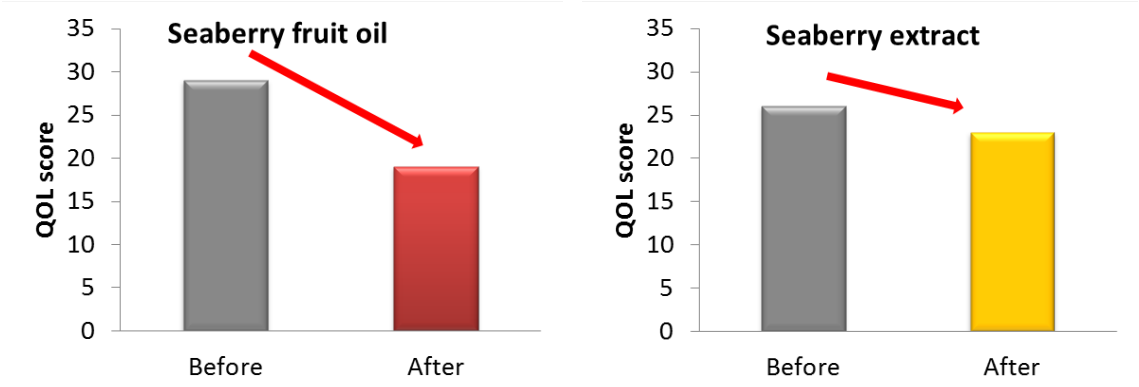


Fig.6 Change in QOL score before and after oral ingestion of the seaberry fruit oil and seaberry extract (n=10)

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	$\times10^{2}$/mL
Red blood cell count	502.1±22.3	495.1±27.6$^{<0.05}$	427～570	$\times10^{4}$/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	$\times10^{4}$/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	$5.8\pm1.6^{<0.05}$	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	$45.6\pm1.7^{<0.05}$	39.8～51.8	%
MCV	95.0±3.5	$93.5\pm3.4^{<0.05}$	82.7～101.6	fL
MCH	30.3±1.1	30.4±0.9	28.0～34.6	pg
MCHC	31.8±0.5	$32.5\pm0.7^{<0.01}$	31.6～36.6	%
Platelet	23.6±3.3	$22.4\pm2.4^{<0.05}$	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].

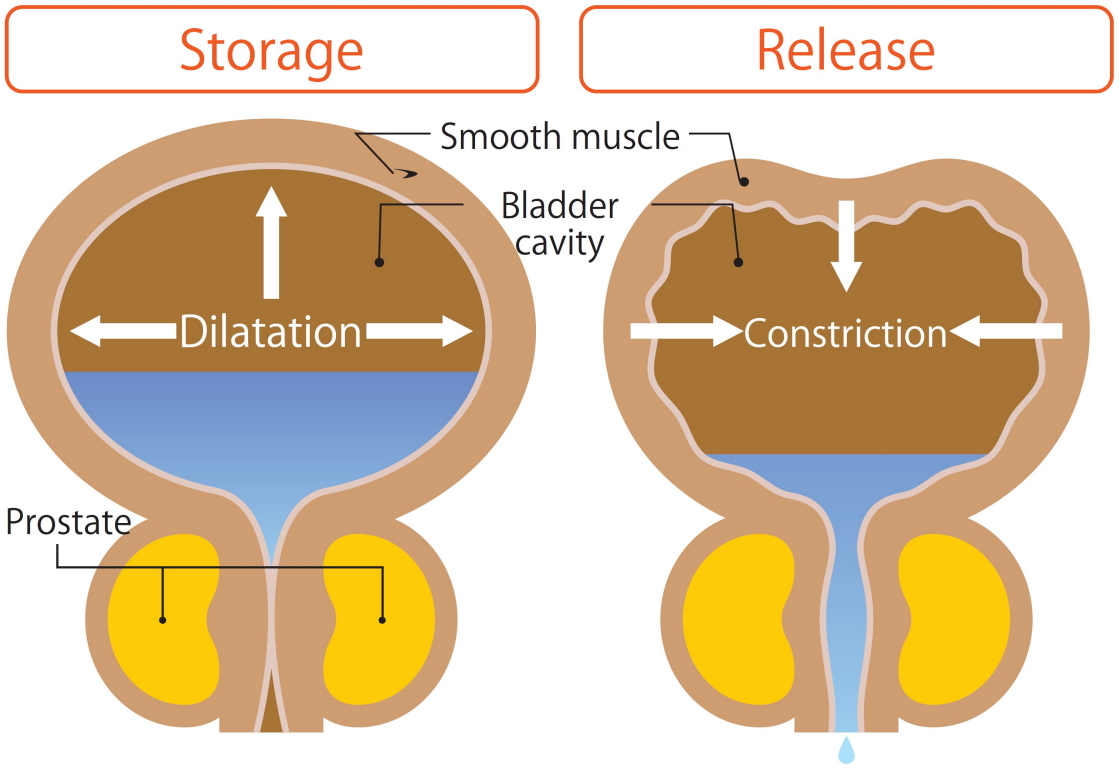


Fig.7 Images of the bladder and urination mechanism

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, 3-O-coumaroyl 2,23-dihydroxy oleanolic 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.

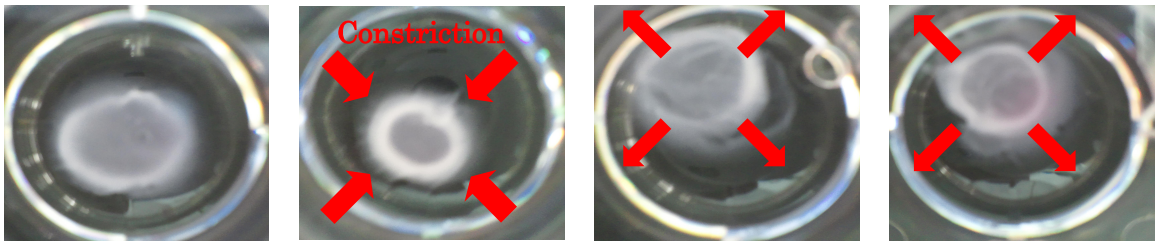


Fig.8 The relaxation effect of Seaberry extract on the constriction of collagen gel containing bladder smooth muscle cells

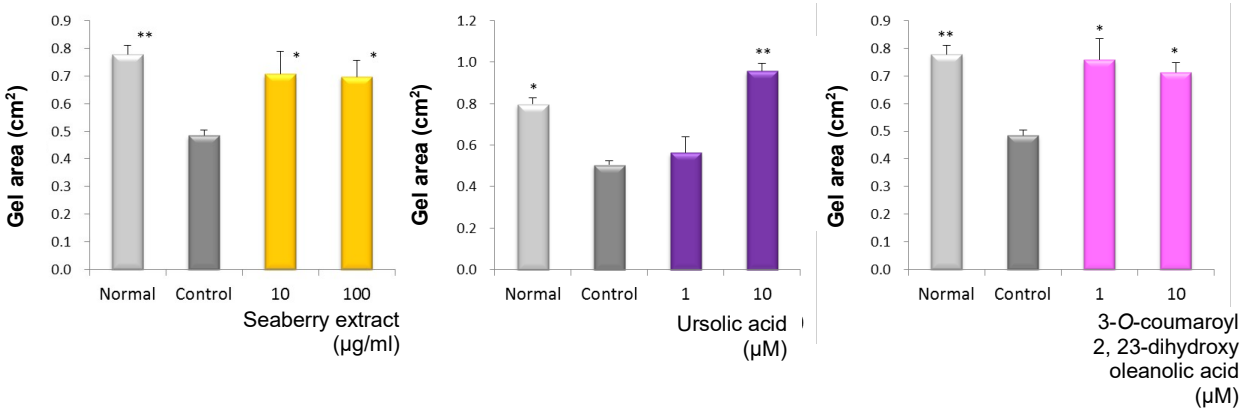


Fig.9-1 The relaxation effect of Seaberry extract and its components on the constriction of collagen gel containing bladder smooth muscle cells (Mean±SE, n=10, ** : $p < 0.01$, * : $p < 0.05$)

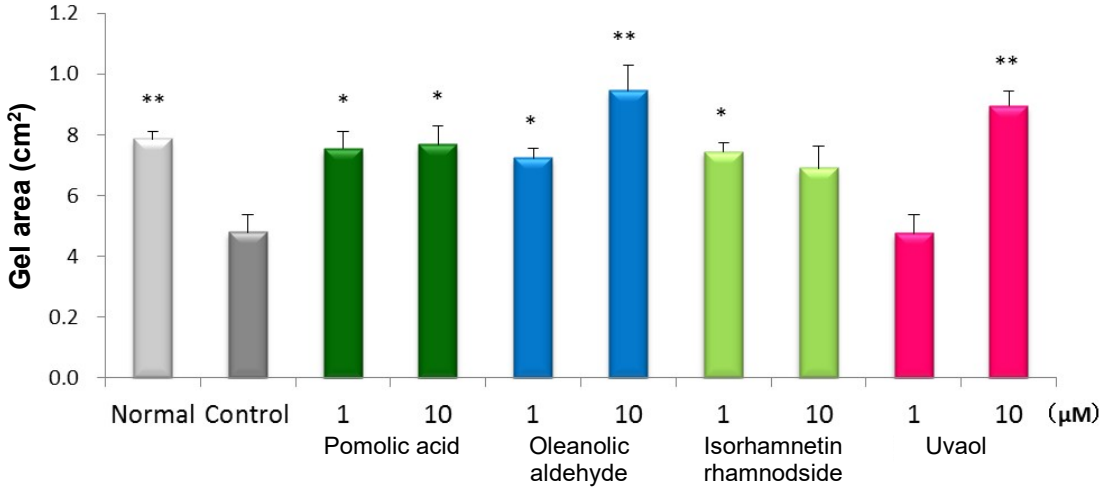


Fig.9-2 The relaxation effect of Seaberry extract and its components on the constriction of collagen gel containing bladder smooth muscle cells (Mean±SE, n=10, ** : $p < 0.01$, * : $p < 0.05$)

(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.

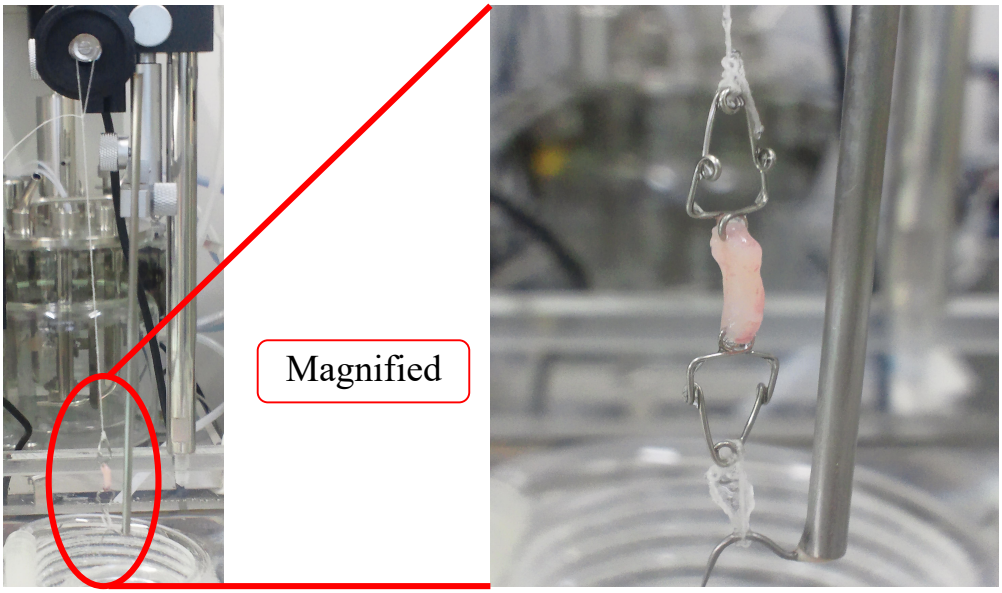


Fig.10 Magnus apparatus and a tissue

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.

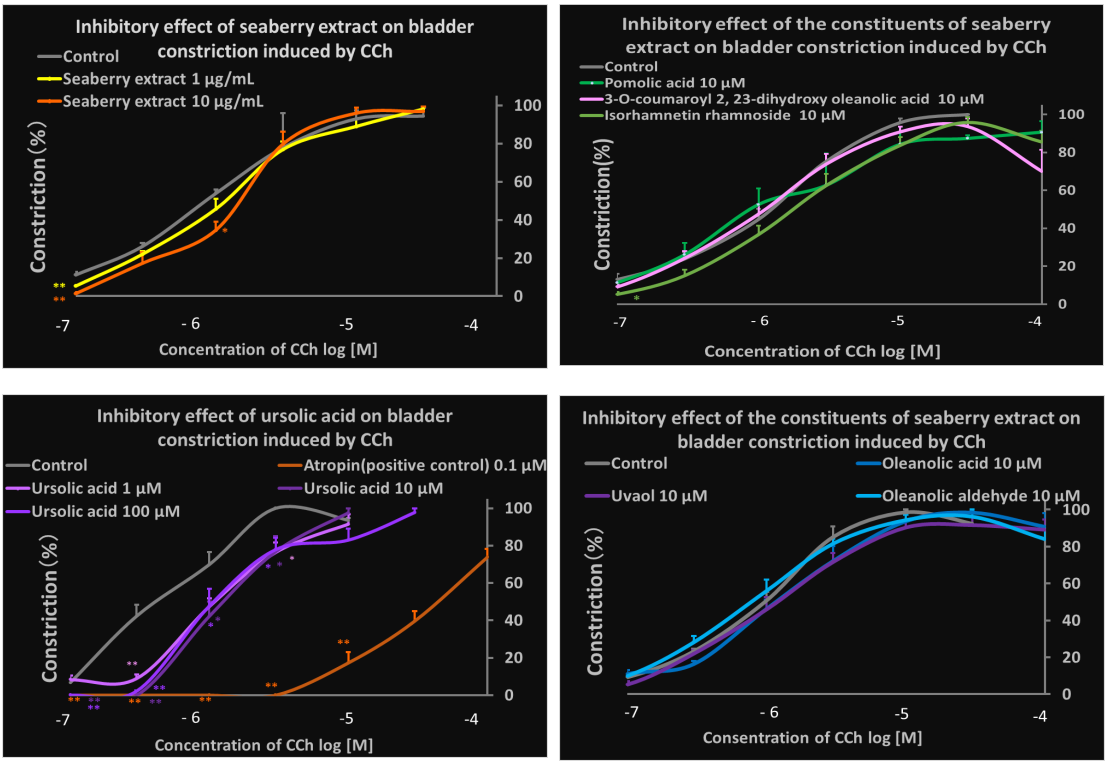
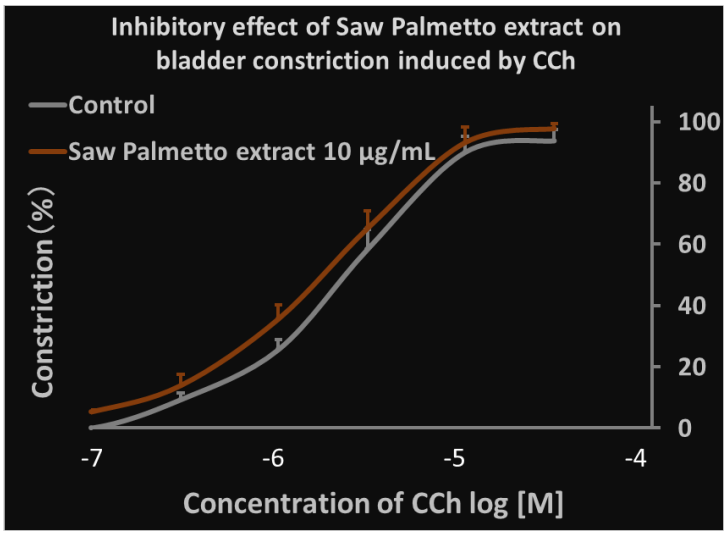


Fig.11 Inhibitory effects of Seaberry extract and its components on contraction of rat bladder smooth muscle (Mean±SE, n=4, ** : $p < 0.01$, * : $p < 0.05$)

In addition, we evaluated the effect of three commercially extracts including saw palmetto extract, pumpkin seed oil and banana peel extract which are said to be effective on dysuria. As a result of Magnus method, all extracts did not suppress smooth muscle contraction at a concentration 10 µg/ml (Fig. 12). One of the mechanisms for Seaberry extract to improve dysuria is considered to be inhibition of hyper smooth muscle constriction, but saw palmetto extract, pumpkin seed oil and banana peel extract have no effect. Therefore, combination of Seaberry extract with saw palmetto extract, pumpkin seed oil and banana peel extract can be expected a better solution against dysuria.



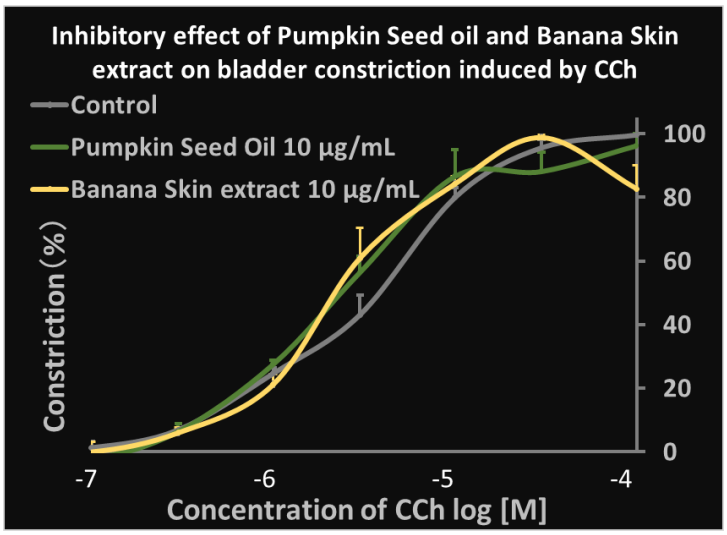


Fig.12 Inhibitory effects of Saw Palmet extract, Pumpkin Seed oil and Banana Skin extract on contraction of rat bladder smooth muscle (Mean±SE, n=4)

(4) Effect of ursolic acid on the rat overactive bladder model

This research was supported by Aichi Prefecture Research and Development Subsidy (2016) from Aichi prefecture.

1) Evaluation of bladder pressure and urination in rats with overactive bladder

Overactive bladder model was induced by intraperitoneal injection of cyclophosphamide to rats. Isosamidin, the compound of *Peucedanum japonicum* are known as the active compound for the smooth muscle relaxant. To compare the effect of ursolic acid and isosamidin on overactive bladder model, we performed cystometric study, a method for measurement of the pressure/volume relationship of the bladder (Figure 13).

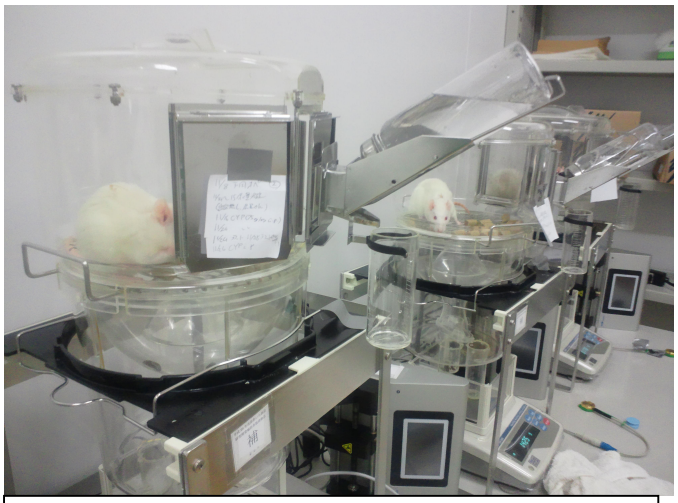


Fig 13. The system for measurement of the bladder pressure and the amount of urine in rats

Experimental results are shown in Table 3. Intravesical administration of ursolic acid (100uM) extended voiding interval and increased the volume of each voiding and the pressure slope between basal and voiding threshold pressure (Fig.14; f→e). Moreover, its treatment decreased the voiding frequency and the number of non-voiding contraction in the bladder. These results indicated that ursolic acid improves frequent urination and increase the amount of urine in the bladder. Especially, it is worthy of attention that ursolic acid increased the voiding interval and voided volume more than isosamidin. Furthermore, its treatment decreased the number of non-voiding contraction. Conversely, isosamidin have no effect on this parameter. Our results suggested that ursolic acid may have the unique effect on overactive bladder.

Table 3 Effect of ursolic acid and isosamidin on cyclophosphamide-induced overactive bladder in rats.

	Amplitude of overactive contraction (mmHg) a	Voiding Interval (s) b	Number of NVCs※ c	Bladder pressure during NVCs (mmHg) c	Maximum voiding pressure (mmHg) d
Control	0.63±0.26	260±55	3.75±0.63	21.9±6.0	55.8±12.3
		(89.2±18.0)			(87.6±10.1)
Ursolic acid (100 μM)	0.67±0.26	463±93	2.00±0.55	11.8±3.9	43.1±5.2
		(121.8±23.0)			(80.2±1.9)
Isosamidin (100 μM)	2.57±1.12	407±127	2.67±0.67	20.3±6.9	57.6±14.0
		(138.5±18.3)			(86.0±13.3)
Normal	1.35±0.65	253±39	2.25±0.85	20.9±9.1	40.4±6.7
		(200.4±82.6)			(89.7±15.3)

※NVCs; non-voiding contractions

	basal pressure (mmHg) e	Maximum pressure-basal (mmHg) d-e	Voided volume (mL each)	Pressure slope between basal and threshold (mmHg/s)	Threshold -basal pressure (mmHg) f-e
Control	14.2±4.2	41.5±9.1	0.71±0.14	0.014±0.005	8.6±2.6
	(103.8±9.7)	(83.5±11.2)	(100.0±23.2)		
Ursolic acid (100 μM)	7.4±2.9	35.7±4.0	1.38±0.27	0.025±0.008	9.3±1.3
	(139.6±43.2)	(165.8±24.6)	(120.8±17.0)		
Isosamidin (100 μM)	12.1±4.2	45.4±12.6	1.17±0.37	0.034±0.016	14.0±4.5
	(100.9±12.1)	(86.9±15.2)	(140.1±17.7)		
Normal	9.4±5.5	31.0±3.5	0.76±0.1	0.020±0.007	8.6±0.6
	(64.0±9.1)	(91.8±14.9)	(124.7±42.0)		

Data are represented as the mean ± SE (n=4-5), The values in the parentheses is the percentage of changes in each parameter before and after treatment

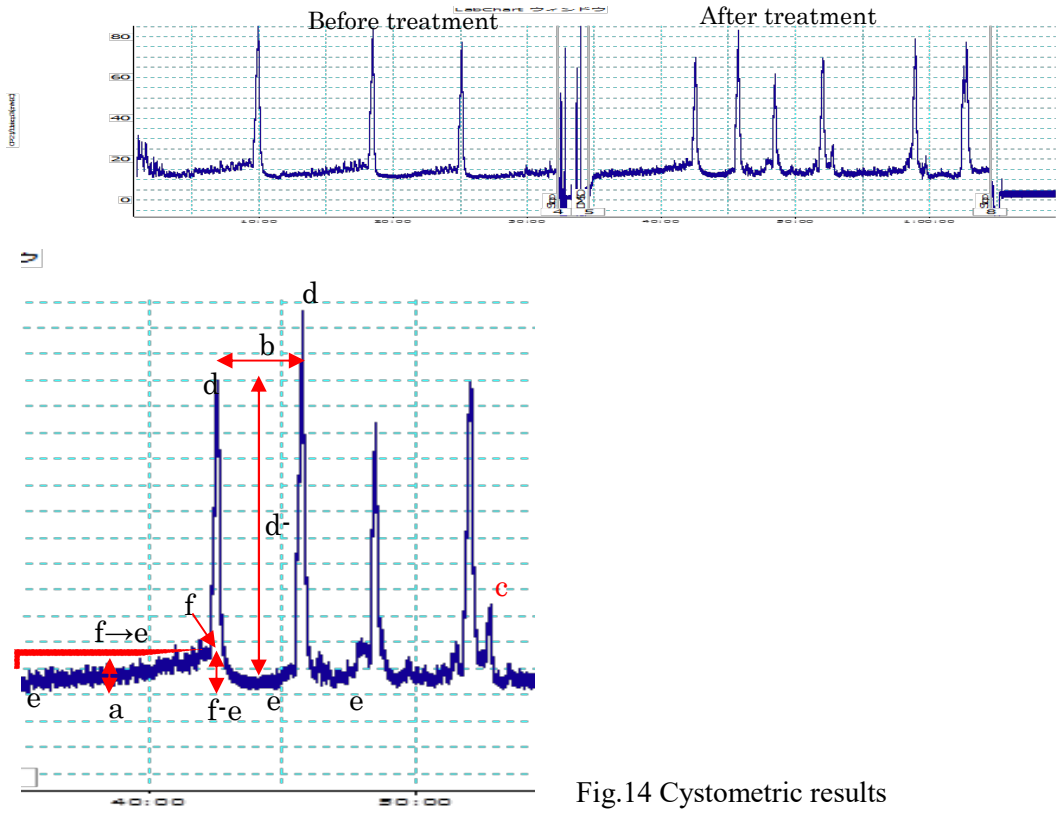


Fig.14 Cystometric results

Next, the rats were orally administered twice, either ursolic acid or isosamidin at 25mg/kg. We evaluated the changes in voiding behavior of mice in each group during night time (12 hr) by measurement of the total amount and number of voiding. Although there was no significant difference between the group in the total amount of urine, ursolic acid significantly decreased voiding frequency. On the other hand, isosamidin increased voiding frequency and decreased the total amount of urine. These results confirmed that ursolic acid has the ability to ameliorate frequent urination during the activity period.

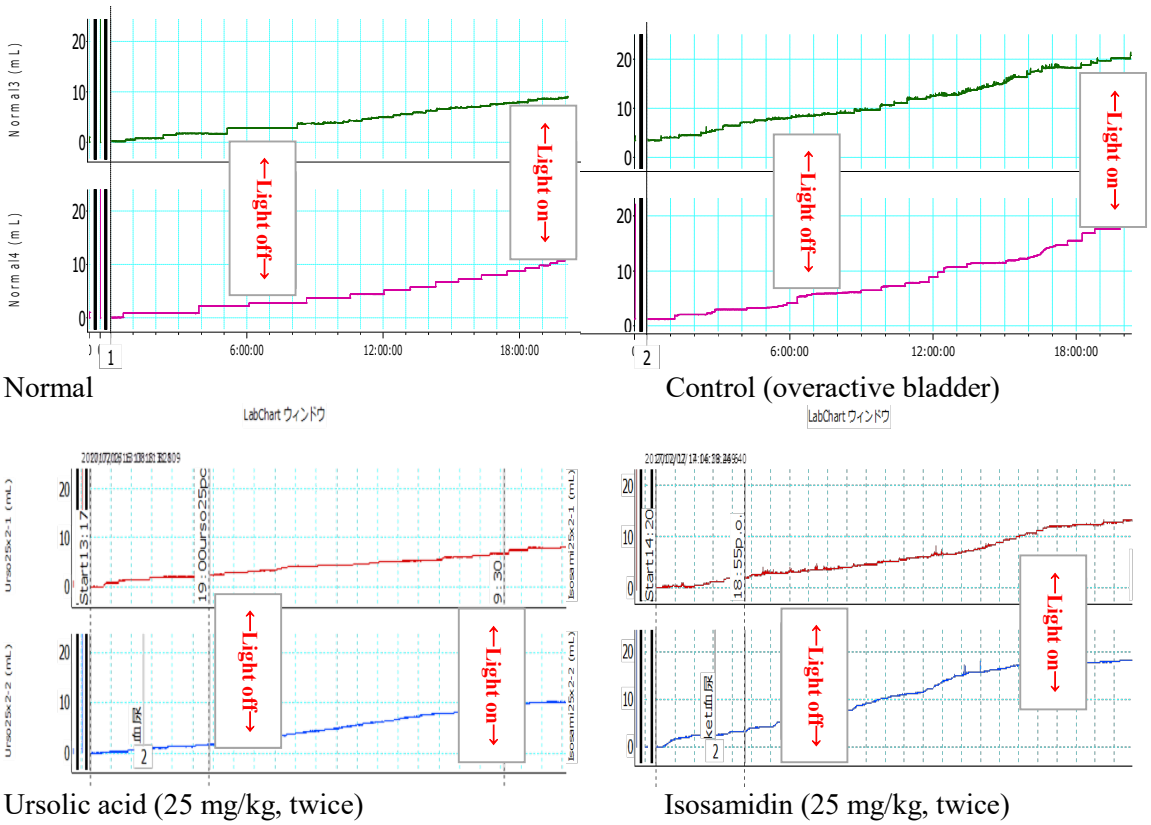


Fig 15. The pattern of voiding at night time after oral administration of ursolic acid or isosamidin

(vertical axis: amount of urine (ml); horizontal axis: time)

Table 4. Effect of ursolic acid and isosamidin on cyclophosphamide-induced overactive bladder in rats (Mean±SE (n=3-4)).

	Voided volume (mL/at night)	Number of voiding	Volume of each voiding (ml each)
Control	9.97±0.94	50.5±8.6	0.23±0.06
Ursolic acid (25 mg/kg, twice)	11.67±5.79	30.3±6.3	0.20±0.03
Isosamidin (25 mg/kg, twice)	11.36±3.18	102.0±24.8	0.11±0.02
Normal	8.13±1.05	28.3±6.5	0.34±0.08

(5) Human clinical trials -Quality of life improvements (Randomized double-blind placebo-controlled crossover study)

Overactive bladder is one of the urinary dysfunction with frequent urination and urinary incontinence which occur both day and night. The overactive bladder is a condition based on the excessive contraction of bladder smooth muscle. In Japan, although 8 million people suffer from this disease and related symptoms, many people go without treatment because they feel uncomfortable discussing incontinence with their doctor. In order to examine the effect of seaberry extract on humans, we conducted clinical trial in healthy subjects. The subjects were divided into three groups (placebo; 16 subjects, Seaberry extract-P 200 mg/day; 16 subjects or Seaberry extract-P 400 mg/day; 16 subjects). After 8 weeks of seaberry extract treatment, urinary symptoms were evaluated by using the King's Health Questionnaire and the overactive bladder syndrome score.

King's Health Questionnaire

Question	Condition	Score
1. How would you describe your health at the present?	Very good	1
	Good	2
	Fair	3
	Poor	4
	Very poor	5
2. How much do you thinkyour bladder problem affects your life?		
3. Role limitations		
a. Does your bladder problemaffect your household tasks?		
b. Does your bladder problem affect your job,or your normal daily activities outside the home?	Not at all	1
	A little	2
4. Physical / Social limitations	Moderately	3
a. Does your bladder problem affect your physical activities?	A lot	4
b. Does your bladder problem affect your ability to travel?		
c. Does your bladder problem limit your social life?		
d. Does your bladder problem limit your ability to see and visit friends?		

Question	Condition	Score
1. Personal relationships		
a. Does your bladder problem affect your relationship with your partner?	Not applicapable	1
b. Does your bladder problem affect your sex life?	Not at all	2
	Slightly	3
c. Does your bladder problem affect your family life?	Moderately	4
	A lot	5
2. Emotions		
a. Does your bladder problem make you feel depressed?		
b. Does your bladder problem make you feel anxious or nervous?	Not at all	1
c. Does your bladder problem make you feel bad about yourself?	Slightly	2
	Moderately	3
3. Sleep / Energy	Veru much	4
a. Does your bladder problem affect your sleep?		
b. Does your bladder problem make you feel worn out and tired?		
4. Do you do any of the following? If so, how much?		
a. Wear pads to keep dry?	Never	1
b. Be careful how much fluid you drink?	Sometimes	2
c. Change your underclothes because they get wet?	Often	3
d. Worry in case you smell?	All the time	4

Question	Calculation
General health perception	(Score for Q1-1)/4×100
Incontinence impact	(Score for Q2-1)/3 ×100
Role limitations	[{(Sum of scores for Q3a and Q3b)-2}/6]×100
Physical limitations	[{(Sum of scores for Q4a and Q4b)-2}/6]×100
Social limitations	If score for Q5c ≧ 1: [{(Sum of scores for Q4c, Q4d and Q5c)-3}/9] ×100
	If score for Q5c is 0: [{(Sum of scores for Q4c and Q4d)-2}/6]×100
Personal relationships	If sum of scores for Q5a and 5b≥2: [{(Sum of score s for Q5a and Q5b)-2}/6]×100
	If sum of scores for Q5a and Q5b=1: [{(Sum of scores for Q5a and Q5b)-1}/3]×100
	If sum of scores for Q5a and Q5b=0: treat as a missing value
Emotions	[{(Sum of scores for Q6a, Q6b and Q6c)-3}/9]×100
Sleep/Energy	[{(Sums of scores for Q7a and Q7b)-2}/6]×100
Severity/coping	[{(Sum of scores for Q8a, Q8b, Q8c and Q8d)-4}/12]×100

Overactive bladder Syndrome score

Question	Frequency	Score
1. How many times do you typically urinate from waking in the morning until sleeping at night?	≤7	0
	8-14	1
	≥15	2
2. How many times do you typically wake up to urinate from sleeping at night until waking in the morning?	0	0
	1	1
	2	2
	≥3	3
3. How often do you have a sudden desire to urinate, which is difficult to defer?	Not at all	0
	Less than once a week	1
	Once a week or more	2
	About once a day	3
	2-4 times a day	4
	5 times a day or more	5
4. How often do you leak urine because you cannot defer the sudden desire to urinate?	Not at all	0
	Less than once a week	1
	Once a week or more	2
	About once a day	3
	2-4 times a day	4
	5 times a day or more	5

As figure 16 shows, by ingestion of Seaberry extract-P (400 mg/day) for 8 weeks, significant improvement was observed in three items of King's Health Questionnaire and one item of the overactive bladder syndrome score. From these results, seaberry extract improved the anxieties in terms of urinary system in healthy subjects. In figure 16, the intake of seaberry extract-P (400 mg/day) improved not just the anxiety related to urination, but the problem about frequent urination and urinary urgency. The results

obtained in this section suggest that the intake of seaberry extract is useful for improving quality of life of urinary problems. Additionally, side effects were not observed under these conditions.

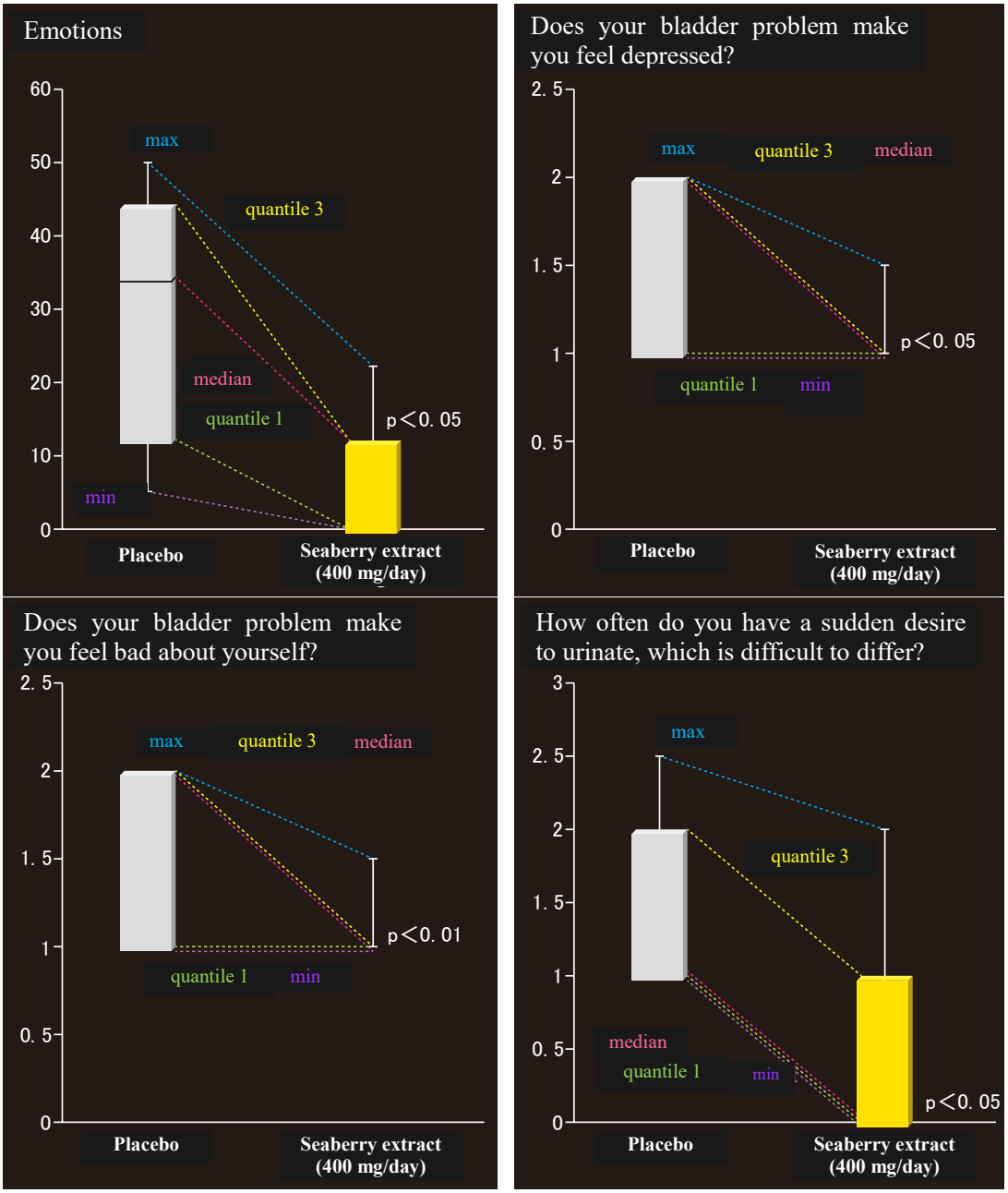


Fig 16. Seaberry extract -P alleviated discomfort caused by overactive bladder

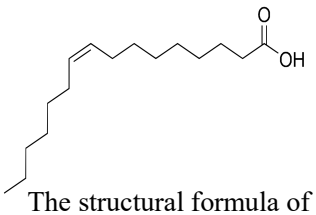
Median: the median of all values; quantile 1: the median of the values below the median; quantile 3: the median of the values above the median

This research was supported by Aichi Prefecture Research and Development Subsidy (2016) from Aichi prefecture.

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. 13, A) that is insulin resistance evaluation method and euglycemic hyperinsulinemic clamp test (Fig. 13, 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).

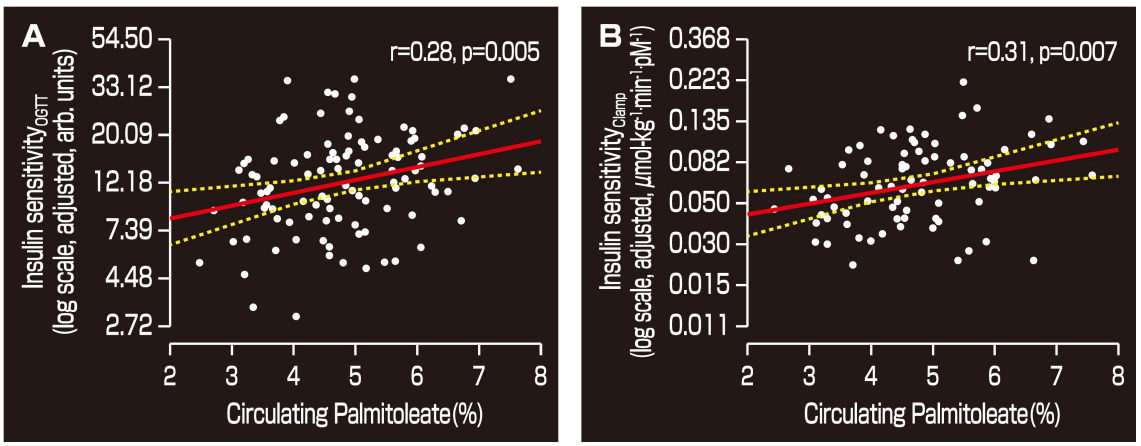


Fig.13 Cross-sectional relationships of circulating palmitoleate with insulin sensitivity estimated from the OGTT (A) and measured by the clamp (B)

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. 14). The mechanism of the performance is related to the inhibition of the protein expression of tyrosinase, TRP1, TRP2, and MITF (Fig. 15).

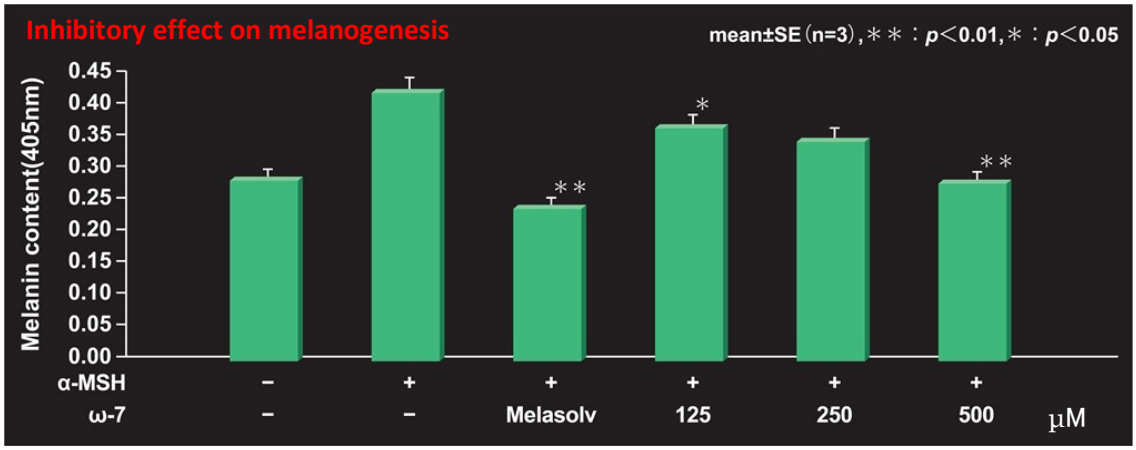


Fig.14 Inhibitory effect on melanogenesis of palmitoleic acid in B16F10 cells
α-MSH : melanocyte-stimulating hormones, melasolv : positive control

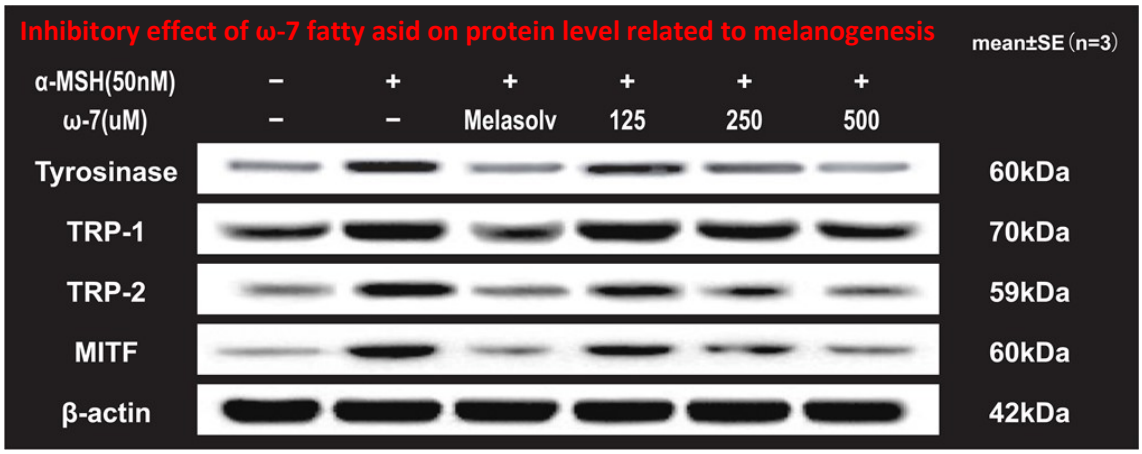


Fig.15 Inhibitory effect of palmitoleic acid on protein level related melanogenesis in B16F10 cells

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.

5) Bernstein, Adam M., Michael F. Roizen, and Luis Martinez. "Purified palmitoleic acid for the reduction of high-sensitivity C-reactive protein and serum lipids: A double-blinded, randomized, placebo controlled study." *Journal of clinical lipidology* 8.6 (2014) 612-617.

6) Yang, Zhi-Hong, Hiroko Miyahara, and Akimasa Hatanaka. "Chronic administration of palmitoleic acid reduces insulin resistance and hepatic lipid accumulation in KK-A (y) mice with genetic type 2 diabetes." *Lipids Health Dis* 10.8 (2011) 120.

7) Yoon, Weon-Jong, *et al.* "Effect of palmitoleic acid on melanogenic protein expression in murine B16 melanoma." *Journal of oleo science* 59.6 (2009) 315-319.

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. 16). This result suggests that seaberry extract is expected to have an action to reduce skin irritation caused by air pollutants.

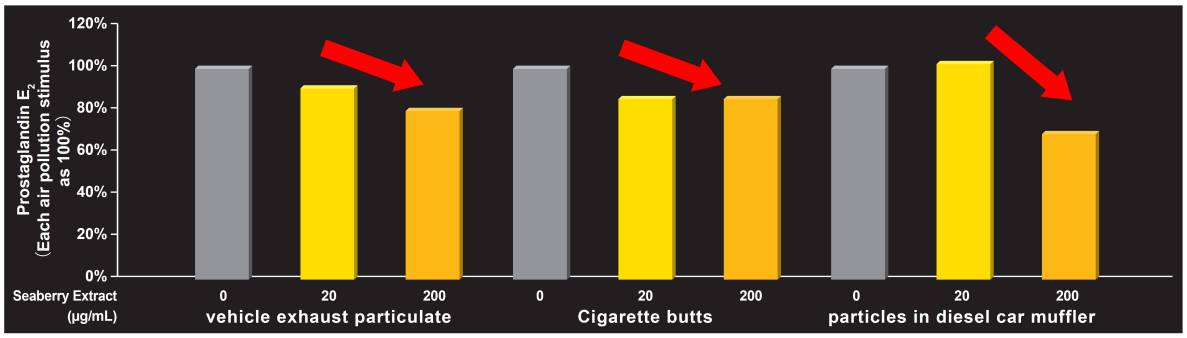


Fig.16 Effect of inhibit against PGE_2 production exposure air pollutants

（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. 17). According to the result, seaberry extract is expected to have an action to reduce or suppress irritation caused dryness or moisturize the skin.

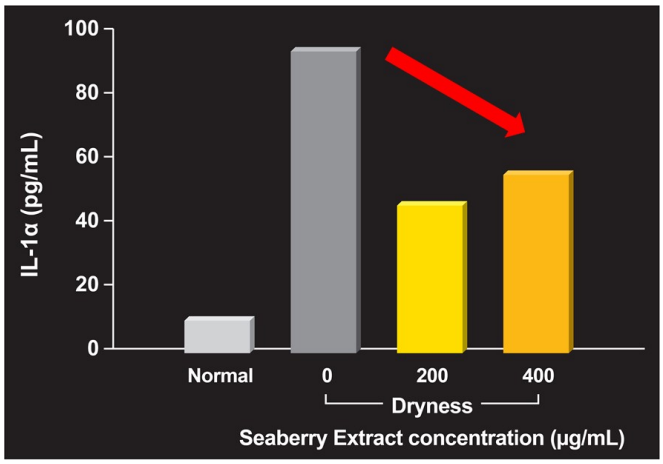


Fig.17 Inhibitory effect of seaberry extract against IL-1α production by exposure to dryness

（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. 18). This indicates that seaberry extract has an action to protect bodies against attacks of reactive oxygen species caused by external irritation.

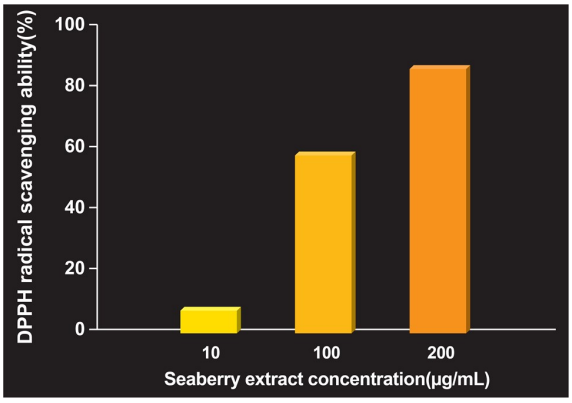


Fig.18 DPPH radical scavenging ability of Sea berry extract

（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. 19 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.

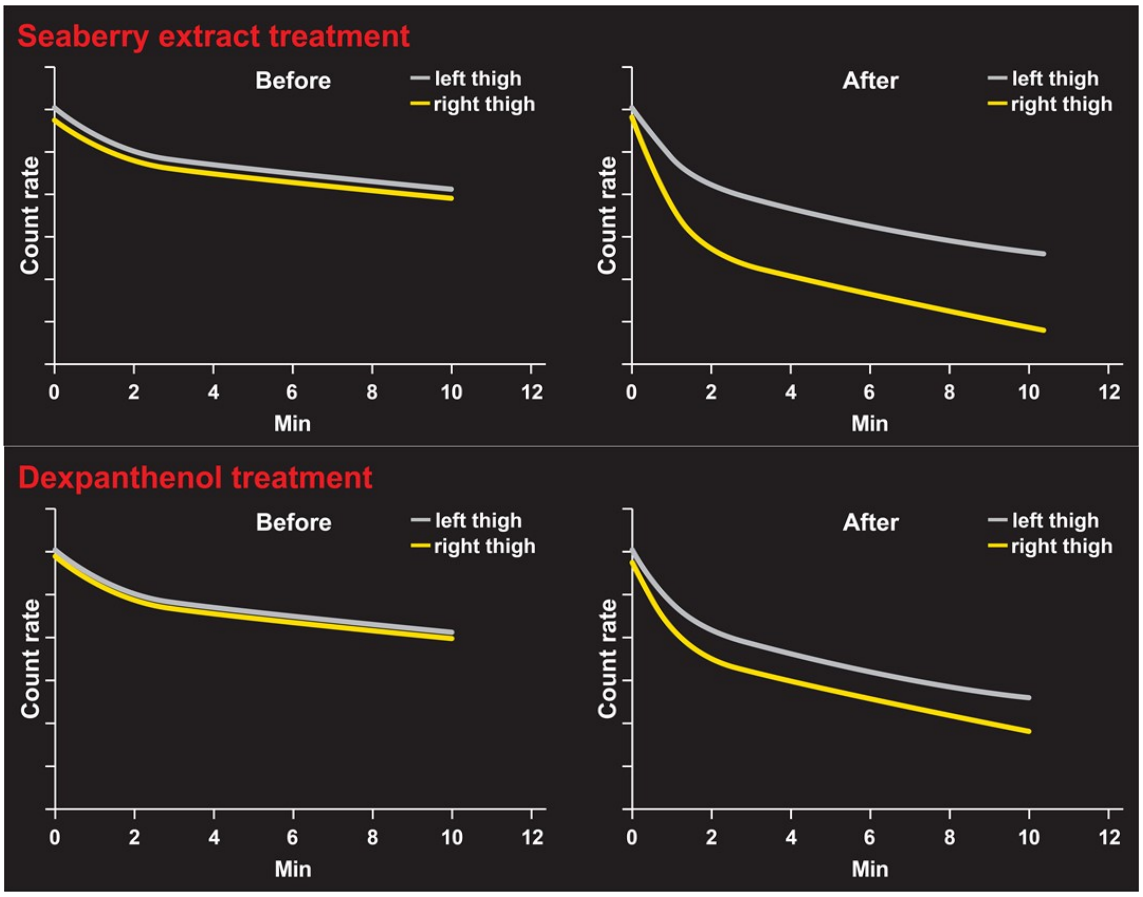


Fig.19 Wound healing effect of Seaberry extract

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.

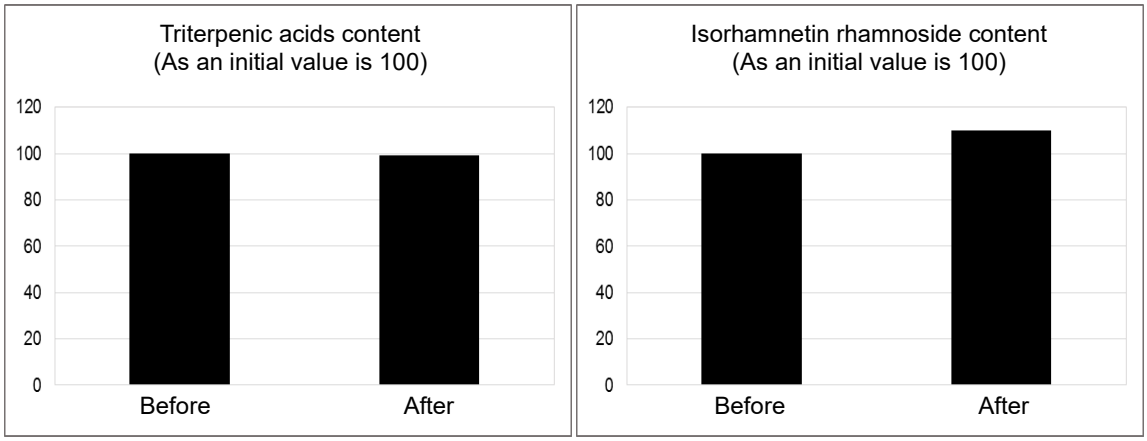


Fig.20 The heat stability of Seaberry extract-P

(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. 21), isorhamnetin rhamnoside content of Seaberry Extract-WSP is stable between pH 3 – 8 (Fig. 22). Isorhamnetin rhamnoside is not stable under alkaline environment.

pH3 pH4 pH5 pH6 pH7 pH8 pH9

Fig.21 The color change by pH of Sea berry extract-WSP in aqueous solution

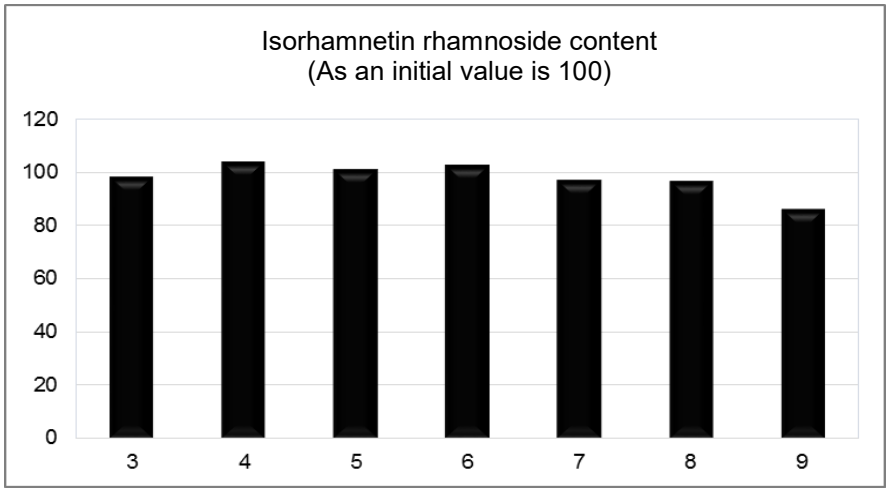


Fig.22 pH Stability of Seaberry Extract-WSP in aqueous solution

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. 23, 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.

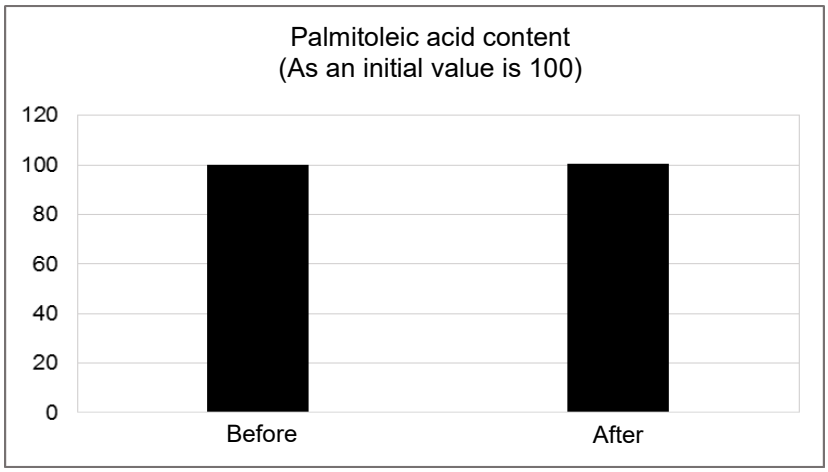


Fig.23 The heat stability of Seaberry Fruit Oil

8. Nutrition Profiles

Analyzed item (/100g)	P	WSP	Oil note4	Method
Water (g)	2.5	4.3	0	Heating drying method under normal pressure
Protein (g)	2.2	1.8	0	Kjeldahl method, nitrogen protein conversion factor: 6.25
Fat (g)	1.1	0.3	100	Acid decomposition method
Ash (g)	1.5	1.4	0	Direct incineration method
Carbohydrate (g)	92.7	92.2	0	Refer note 1
Energy (kcal)	388	376	900	Refer note 2
Fiber (g)	0.9	1.4	0	Prosky's method
Sodium (mg)	129	97.7	0	Atomic absorption spectrophotometory
Sodium chloride equivalent (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

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.	
Triterpenic acids*	Min. 0.2 %	(HPLC)
Isorhamnetin rhamnoside	Min. 0.2 %	(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.

*Total amounts of ursolic acid, oleanolic acid and pomolic acid.

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	A peak is detectable (as β-carotene)	(HPLC)
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.	

ORYZA OIL & FAT CHEMICAL CO., LTD. striving for the development of the new functional food materials to promote health and general well-being.

From product planning to OEM - For any additional information or assistance, please contact：

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“The catalog was created based on academic data. For expressions of consumer products containing this product, follow the Health Promotion Law, Pharmaceutical Low, and other related laws and regulations.”

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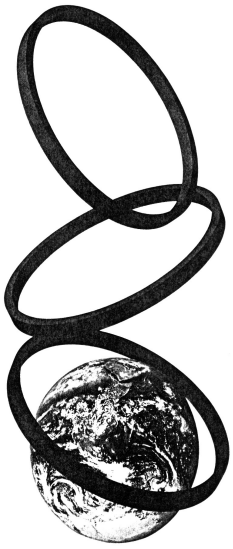

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