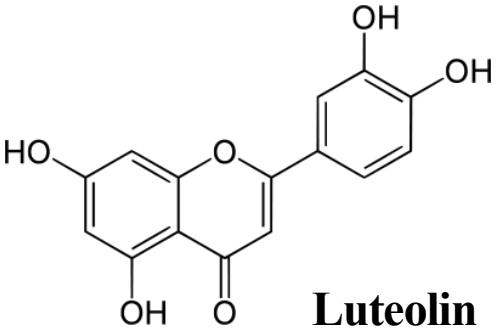
ORYZA OIL & FAT CHEMICAL CO., LTD.

KIKU FLOWER EXTRACT

Prevention of Gout・Inhibition of Uric Acid Production
Improvement of Sleep・Deep Care of Skin ・Anti-ageing
Skin Beautifying Food・Cosmetic Ingredient

- **KIKU FLOWER EXTRACT-P**
  (Powder, Food Grade)
- **KIKU FLOWER EXTRACT-WSP**
  (Water-soluble Powder, Food Grade)
- **KIKU FLOWER EXTRACT-PC**
  (Powder, Cosmetic Grade)
- **KIKU FLOWER EXTRACT-WSPC**
  (Water-soluble Powder, Cosmetic Grade)
- **KIKU FLOWER EXTRACT-LC**
  (Water-soluble Liquid, Cosmetic Grade)



Luteolin

ORYZA OIL & FAT CHEMICAL CO., LTD.

ver. 2.1 YF

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1. Introduction

【What is KIKU FLOWER】

KIKU Flower is Chrysanthemum flower in Japanese. It belongs to compositae family. And Chrysanthemum flower is the common name for *chrysanthemum morifolium*, one of the most well-known flowers in Japan. In floriography, the chrysanthemum signifies "nobleness." Its elegant beauty was believed to resemble a "person of virtue", and it was included as one of the "Four Noble Plants" along with plum tree, bamboo and orchid. In China, chrysanthemums have been cultivated for over 3,000 years and were traditionally used for both medicinal and food purposes. In the ancient Chinese herbal medicine book, "The Divine Farmer's Materia Medica" (Shen Nong Ben Cao Jing), the chrysanthemum was believed to prolong life and was described as being able to "enhance vitality, soothe the body and lengthen one's lifespan when taken for a prolonged period." In herbal medicine, the chrysanthemum is used to treat a variety of symptoms including eye disorders (decreased vision, blurred vision, bloodshot and stinging eyes, eyelid swelling or pain) and symptoms of the common cold (fever, headache, cough, sore throat).

Fig.1. Field of planted *Chrysanthemum morifolium* and harvested ones.

【Japan and the Chrysanthemum】

The chrysanthemum was brought to Japan from China at the end of the Nara Period (8th century). From the Heian Period (8th-12th century), the chrysanthemum came to be used for medicinal purposes, primarily in court society by the Emperor and aristocrats. By the late Heian Period, it was also widely cultivated by commoners for ornamental purposes. It is said that, after a historical event in China, the Chrysanthemum Festival began to be held at court each year on

the 9th of September in the Lunar calendar, and the Emperor presented chrysanthemum liquor to his vassals, wishing them good health and long life. Since the Kamakura Period (12th-14th century), the chrysanthemum has been used as the crest of the Imperial family, and is now printed on the cover of Japanese passports.

Although Japan does not have a national flower designated by law, the chrysanthemum and the cherry blossom are often considered symbols of Japan. Traditionally, the cherry blossom has been regarded as representing spring, while the chrysanthemum represents fall. Along with the mountain cherry blossom, the chrysanthemum is practically Japan's national flower as well as the flower of the Imperial family.

In 1868, Japan's "Dajokan Fukoku" law no. 195 (issued by the Grand Council of State) stipulated that the Emperor had sole use of the chrysanthemum as a symbol of his supremacy, and that only the Emperor could use the chrysanthemum as a crest. If any civilian tried to use the chrysanthemum crest in an unauthorized manner, they were severely punished for dishonoring the Emperor. Even after World War II, while the symbol of the chrysanthemum was no longer exclusively used by the Imperial family, the custom of respecting the chrysanthemum remained, and can still be seen across Japan even today. The chrysanthemum is not only used in the Imperial family's crest, but in the Metropolitan Police Department's emblem, National Diet members' badges and the design of the cover of Japanese passports.

【History of the Chrysanthemum as a Food Ingredient】

In China, it is said that the cultivation of chrysanthemums for medicinal and food purposes dates back more than 3,000 years. In particular, chrysanthemum tea is well-known, and is regarded as a typical remedy for eye strain, decreased vision, and blurred vision. The tea is used widely across the country as a remedy, not just in one specific region. Furthermore, the people of southern China use chrysanthemums in cuisine for remedial and preventative purposes.

In Japan, it is considered that people started eating chrysanthemums during the Edo Period (17th-19th century). At that time, the custom was limited to people living in Niigata, Tohoku, and a part of Hokuriku. In Niigata, chrysanthemums were eaten boiled or pickled, and have now become an essential delicacy for autumn. Nowadays, chrysanthemums are used in a variety of dishes across Japan. Famous dishes include pickled chrysanthemum flowers, sashimi, chrysanthemums dressed with mustard, flower-shaped sushi, cod and chrysanthemums wrapped in vegetables, citrus-flavored chrysanthemum sushi, and tempura.

Currently in Japan, the annual production of edible chrysanthemums is about 1000 tons. The prefectures that shipped the largest volumes of edible chrysanthemums in 2010 were: 1) Aichi Prefecture (612 tons); 2) Yamagata Prefecture (212 tons); 3) Niigata Prefecture (82 tons); and 4) Aomori Prefecture (80 tons). However, most chrysanthemum producers are elderly, and the volume produced is decreasing every year.

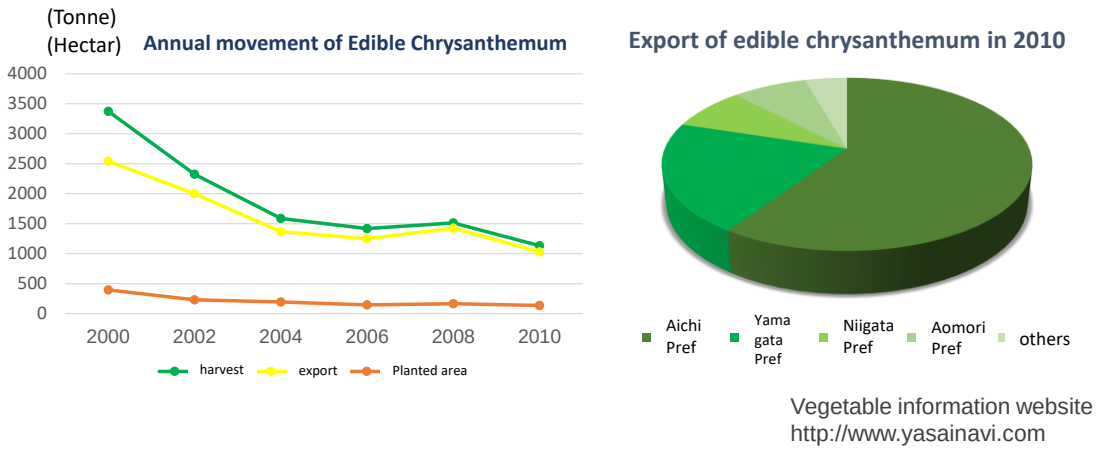
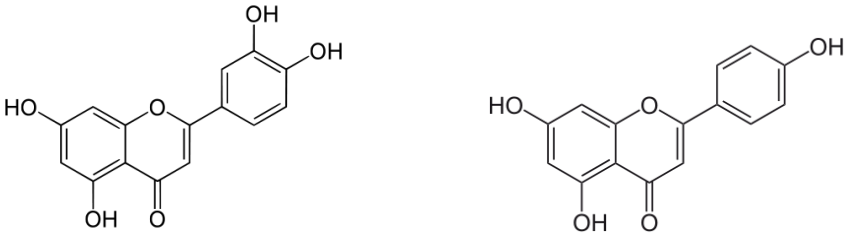


Fig.2. The amounts of production and shipment of edible Chrysanthemum flower in Japan

2. Active ingredients in Kiku (Chrysanthemum) Flower Extract

Chrysanthemum flowers contain several dozen kinds of flavonoids as active ingredients, with a high proportion of luteolin and apigenin in particular. In the product "Kiku Flower Extract (KFE)", luteolin and apigenin are identified, and the amount of luteolin in "Kiku Flower Extract-P" is standardized at over 10 %.



Luteolin　　Apigenin

Fig.3. Flavonoids compounds identified in Kiku Flower Extract

Since chrysanthemums have historically been used as a herbal remedy, a wealth of research exists regarding their functionality. Chrysanthemum flavonoids are reported to improve conditions of fatty liver[1)], have an antioxidant effect[2)], suppress melanin production[3)], enhance learning and memory[4)], improve sleep behavior[5)], protect against cardiovascular disease[6, 7)], have an anti-arrhythmic effect[8)], protect the brain [9)] and nerves[10)], and have anti-inflammatory [11)], anti-tumor [12-14)], antidotal [15)], anti-HIV[16)], anti-tuberculosis[17)] and anti-mutagenic effects[18)].

Oryza Oil & Fat Chemical Co., LTD., after undertaking extensive research over a ten year period, has discovered the secret of this edible flower's ability to promote long life. It lies in the flavonoids (e.g. luteolin) contained in the chrysanthemum's buds, and the company has succeeded in commoditizing this as an anti-gout product as well as a skincare product that acts from deep within the skin.

This catalog mainly introduces kiku flower extract's actions to prevent gout and relieve its symptoms as well as actions to prevent wrinkles and improve skin conditions by protecting and repairing the skin's basal membrane.

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3. Functionality of Kiku Flower Extract

(1) Anti-gout Action

"Gout" is a common word for Japanese people in modern times. Gout is caused by elevated levels of uric acid in the blood. Uric acid forms in needlelike crystals and they deposit in the inner surfaces of joints, causing pain.

Uric acid is a type of waste produced when cells are destroyed by metabolism or produced from purine contained in food. In our body, a certain amount of uric acid is generated every day and a certain amount of it is discharged from the kidneys. Therefore, the amount of uric acid in the blood and body is kept at a certain level.

Uric acid is "slightly soluble" in blood and urine. Therefore, when the uric acid level in the body elevates too much because of an imbalance between its production and discharge, it cannot be dissolved in blood or urine. In this condition, uric acid crystallizes and deposits in various parts of the body.

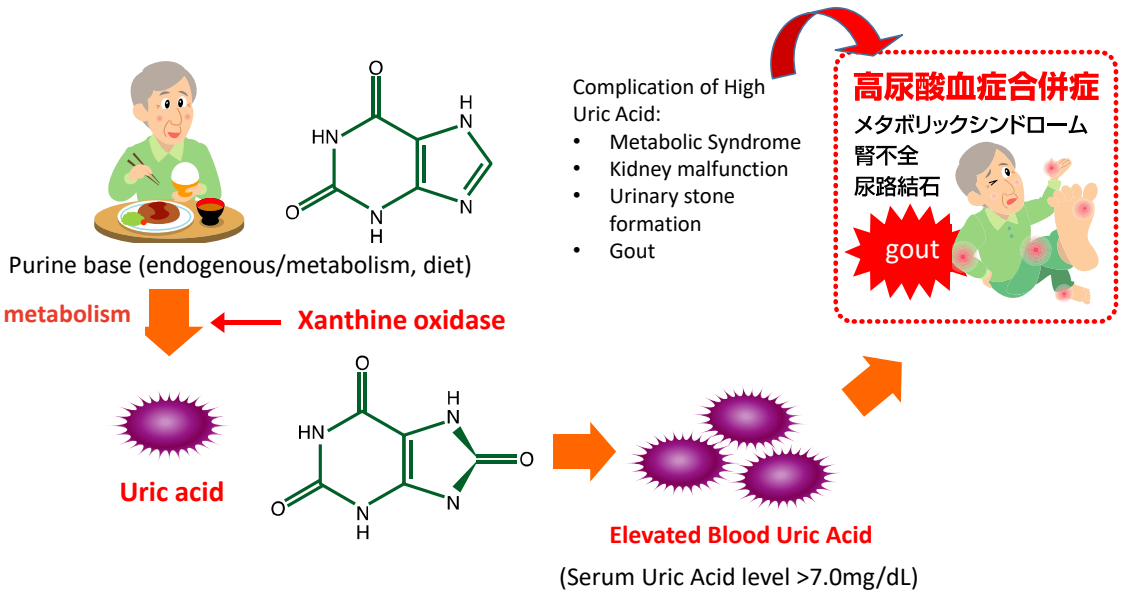
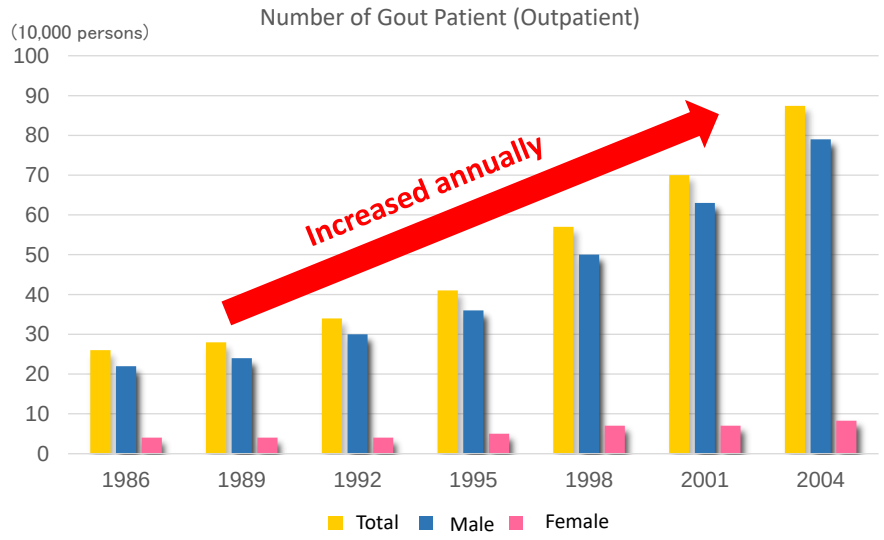


Fig.4. Mechanisms of hyperuricemia and gout

The state where the uric acid level in the blood (serum uric acid level) has increased exceeding 7.0 mg/dL is called "hyperuricemia." When deposited in the body for a long period of time, most uric acid crystals cause a gout attack and kidney impairment or induces urinary calculus. Lately, results of various types of studies suggest a strong relationship between hyperuricemia and metabolic syndrome. Although hyperuricemia does not develop any symptoms, appropriate treatment is necessary from the aspect of the prevention of other lifestyle-related diseases and hardened arteries.

Currently, there are 500,000 to 600,000 gout patients in Japan and it is estimated that there are 3 to 5 million hyperuricemia patients (whose serum uric acid level is 7.0 mg/dL or higher)

who are considered as potential gout patients. According to the Survey of National Living Standards conducted by the Ministry of Health, Labour and Welfare, there are 5 million potential gout patients in Japan and the number is increasing every year.



「Comprehensive Survey of Living Conditions」1986, 1989, 1992, 1995, 1998, 2001, 2004, compiled by Secretariat Statistical Information Department, Ministry of Health, Labor and Welfare
Cited from Hakoda Masayuki「**Epidemiology of hyperuricemia and gout in Japan**」「Nippon Rinsho」2008-4

Fig.5. Changes of gout patient (outpatient) number from year 1986 to year 2004

Uric acid that causes gout is made of purines produced by metabolism in the body and purines taken from meals. As a part of gout preventative measures, meals with reduced purine content are recommended. Fig. 6 shows the purine content of common foods and alcohols.

Variety		mg
Meat	Chicken liver	312
	Beef liver	219
	Chicken drumstick	122
Marine products	Dried sardine	746
	Bonito	211
	Prawn	195
Vegetables, grains	Maitake	98.5
	Soy bean	172
	Natto	113
Fish roe	Cod roe	120
	Salmon roe	15

Modified from Purine Base, Wikipedia

Alcoholic Beverages			Purine Base (mg/100mL)	Alc %
Distilled liquor	Distilled spirit		0	20-35
	Whiskey		0.1	40
	Brandy		0.4	40
Brewed alcohol	Japanese sake		1.2	15
	Wine		0.4	15
	beer	General (3 types)	4.4 – 6.9	5
		Local beer (11 types)	6.8 – 16.6	5
		Malt Beer (4 types)	2.8 – 3.8	5
		Purine cut	0.1	5
		Low alcohol (4 types)	2.8 – 13.0	< 1
	Shaoxing rice wine		11.6	10

Modified from Fujimori Shin「Living guidance for hyperuricemia and gout patients」「The Journal of Therapy」Vol.88, No.11 2006

Fig.6. Purine contents in principal foodstuffs and alcoholic beverages

1) Uric acid production inhibitory action (*in vitro*)

Since gout is caused by uric acid, it is considered that lowering uric acid levels in the blood can lower the risk of gout attacks. A test was conducted to study KFE's effect to prevent gout. In this test, the extract's action to suppress xanthine oxidase, which is an enzyme to produce uric acid, was evaluated. As a result, as shown in Fig. 7, KFE inhibited the action of xanthine oxidase, which is uric acid synthetase found in the body, concentration-dependently at concentrations 3 to 100 μg/ml. In this test, Allopurinol which is a drug to treat hyperuricemia was used as a positive control and it inhibited the action of xanthine oxidase approximately 95 % at the concentration of 10 μg/ml. The results above suggest that KFE and its main component luteolin inhibit uric acid synthesis in the body by their action to inhibit xanthine oxidase and in turn, they are expected to improve uric acid levels in the blood.

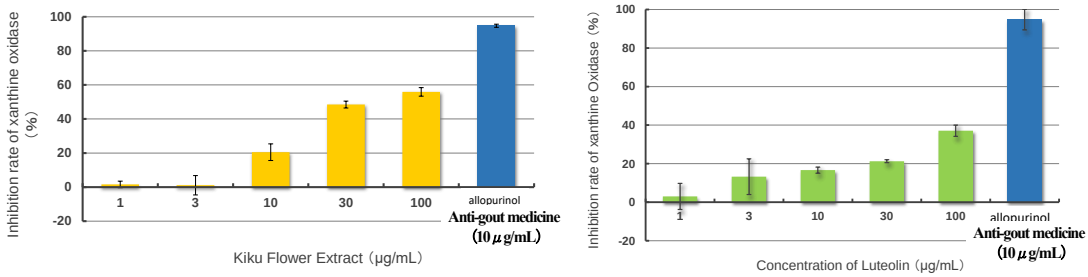


Fig.7. Inhibition of Kiku Flower Extract and its active compound, luteolin on xanthine oxidase

【Method】

The sample solution and xanthine oxidase solution (0.01 unit/ml) were placed in 96-well micro plates and processed at 25 °C for 15 minutes. Then, xanthine solution (150 μm) was added and the material was allowed to react at 25 °C for 30 minutes. Hydrochloric acid (1 N) was added to stop reaction and the absorbance was measured at 290 nm using a micro plate reader.

Uric acid content was calculated by deducting the absorbance in the absence of xanthine oxidase from the absorbance in the presence of xanthine oxidase.

The xanthine oxidase inhibitory action was calculated by the following expression.

Inhibition rate of xanthine oxidase （%）

＝｛1－（Absorbance of sample／Absorbance of solvent control） x 100

2) Inhibition of paw edema in rat gout model induced by monosodium urate crystal (*in vivo*)

Anti-inflammatory action of KFE and its main component luteolin was evaluated using a rat model with gout and paw edema induced by uric acid crystals. As shown in Fig. 8, paw edema on the rats in the control group peaked 24 hours after the administration of uric acid crystal. Luteolin (40 mg/kg) inhibited paw edema 24 hours later significantly ($P<0.05$). KFE (50 mg/ml) showed a tendency to inhibit paw edema in the second hour and after. The positive control indomethacin (5 mg/ml), an anti-inflammatory drug, significantly inhibited paw edema 24 and 48 hours later. Fig. 9 shows photos of paw edema on the rat model with gout and uric acid crystals.

The results above suggest that KFE has an anti-inflammatory effect on rat model with gout caused by uric acid crystals. Thus, the extract is expected to have effects to prevent gout and release gout symptoms by improving the uric acid level in the blood.

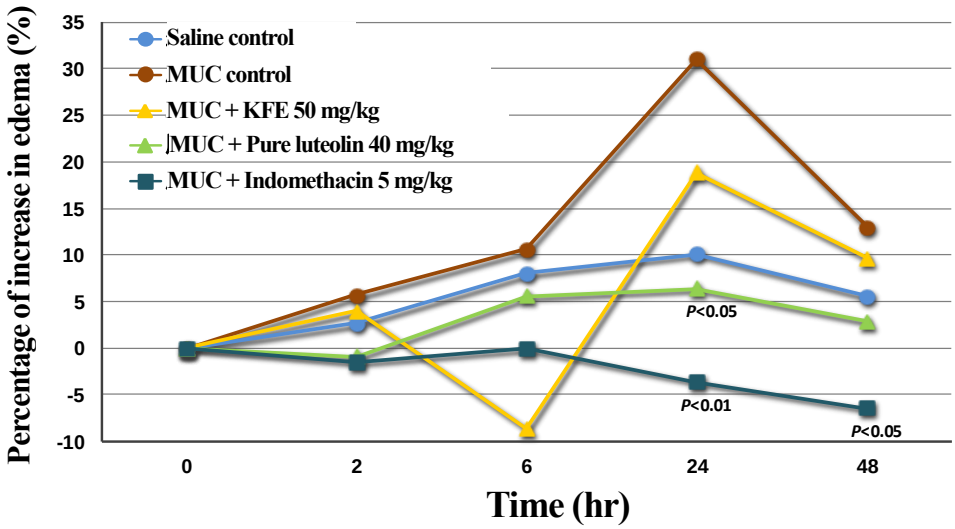


Fig.8. Inhibition of paw edema in rat gout model induced by monosodium urate crystal

【Method】

KFE or luteolin was administered to 5-week old male rats for one week. Then uric acid crystals were injected into the rats' back paw to create a gout model. The cubic volume of their paws was measured over time, the percentage of edema to the cubic volume was calculated, and the percentage was compared between positive groups and uric acid crystal control group.

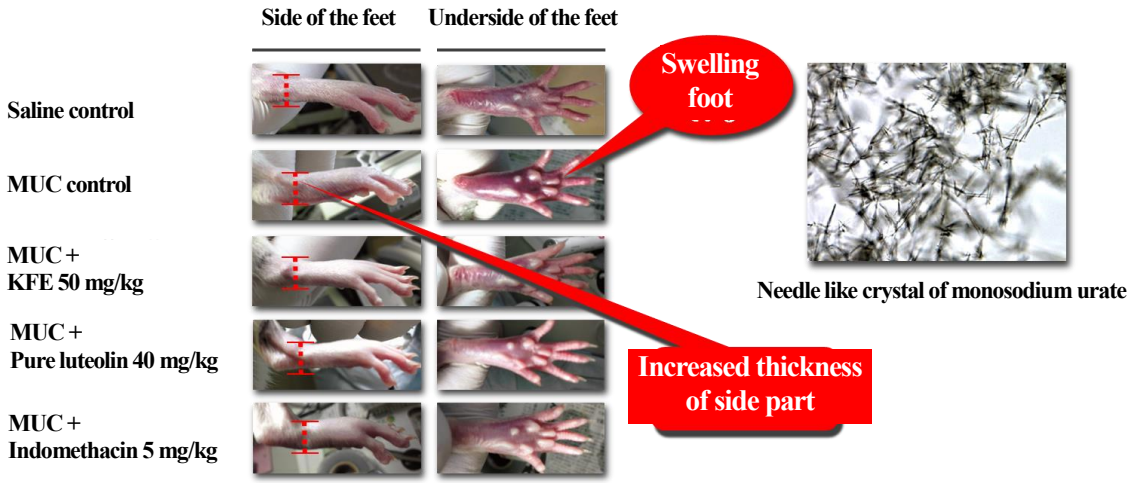


Fig.9. Pictures of the swelling feet of rat gout model and monosodium urate crystal

3) Human clinical test

3-1) Open test

Effects of continuous ingestion of KFE (luteolin content 20 % min.) on uric acid levels in the blood and safety-related blood parameters were studied. Seven male volunteer test subjects from Oryza Oil & Fat Chemical (uric acid level 6.8 to 7.9 mg / dL) freely ingested KFE (50 mg / capsule / day, luteolin content 10 mg) for 28 days. Then, uric acid levels in the blood and other parameters were compared before and after ingestion.

Target evaluation item: Uric acid level

Safety evaluation items:

Total bilirubin, total serum protein, albumin, A / G ratio, ALP, AST (GOT), ALT (GPT), LD (LDH), γ-GTP, neutral fat, phospholipid, free fatty acid, LDL cholesterol, total cholesterol, HDL cholesterol, urea nitrogen, creatinine, uric acid, sodium, potassium, chrome, blood sugar, white blood cell count, red blood cell count, hemoglobin, hematocrit level, MCV, MCH, MCHC, platelet

Blood inspection institute: FALCO Biosystems Ltd.

The average uric acid level in the blood of the seven subjects was 7.3 mg / dL (abnormal value) before ingestion. The average value lowered to 7.0 mg / dL (upper limit of normal value) after ingestion as shown in Fig. 10. The uric acid level of five of the seven subjects lowered. Moreover, the uric acid level of four of them lowered to a normal range. There was no change in uric acid level (6.8 mg / dL) of one subject which was in the normal range from the beginning.

Among other blood parameters, red blood cell count significantly lowered. However, the fluctuation was within the normal range and was not determined to be a harmful influence of the extract. During the capsule ingestion period, no adverse events or side effects deemed to be caused by the ingestion of kiku flower extract was observed. The test results indicate that ingestion of KFE (one month) lowers uric acid level in the blood without causing any adverse event.

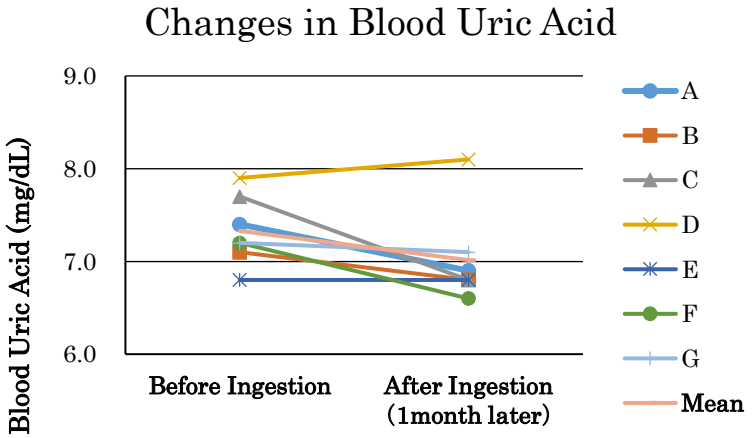


Fig.10. Changes in blood uric acid before and after ingestion of Kiku flower extract

Table 1. Effect of Kiku flower extract on safety related blood parameters (Mean ± SD)

Items	Before ingestion	After 1 month	Normal range	Unit
Total bilirubin	0.5±0.2	0.6±0.2	0.2～1.2	mg/dL
Total protein	7.5±0.4	7.5±0.3	6.5～8.3	g/dL
Albumin	4.6±0.3	4.7±0.2	3.8～5.3	g/dL
A/G ratio	1.7±0.2	1.7±0.2	1.1～2.3	
AST(GOT)	23.1±5.5	20.4±4.5	8～38	U/L
ALT(GPT)	28.7±12.0	26.0±8.6	4～43	U/L
ALP	208.7±51.6	211.3±48.1	110～354	U/L
LD(LDH)	183.1±13.8	179.7±17.5	121～245	U/L
γ-GTP	47.7±36.1	51.9±42.0	86以下	U/L
LDL-ｺﾚｽﾃﾛｰﾙ	121.1±32.9	124.1±34.1	70～139	mg/dL
Total cholesterol	200.7±30.8	208.6±30.1	130～219	mg/dL
Triglyceride(TG)	106.7±59.2	129.4±83.6	30～149	mg/dL
Phospholipid	221.0±22.3	229.7±17.5	150～260	mg/dL
Free fatty acid	0.56±0.23	0.59±0.16	0.13～0.77	mEq/L
HDL-cholesterol	63.3±16.4	64.0±16.7	40～77	mg/dL
Sodium	143.9±0.7	145.1±1.2	135～150	mEq/L
Chloride	102.9±1.8	103.1±2.0	98～110	mEq/L
Potassium	4.1±0.3	4.0±0.3	3.5～5.3	mEq/L
Urea nitrogen	13.7±2.9	13.5±3.1	8.0～22.0	mg/dL
Creatinine	0.9±0.2	0.9±0.2	0.61～1.04	mg/dL
Uric acid	7.3±0.4	7.0±0.5	3.6～7.0	mg/dL
Blood Glucose	89.6±10.3	93.3±6.0	60～109	mg/dL
HbA1c NGSP	5.4±0.4	5.2±0.2	4.6～6.2	%
Ketone body	28.3±24.1	24.6±14.8	74以下	μmol/L
White blood cell count	54.1±15.0	58.0±9.9	39～98	$\times 10^2/\mu L$
Red blood cell count	519.4±28.9	502.4±28.0	427～570	$\times 10^4/\mu L$
Hemoglobin	15.7±0.5	15.2±0.6	13.5～17.6	g/dL
Hematocrit	47.5±1.8	46.6±1.4	39.8～51.8	%
MCV	91.6±4.2	92.9±4.1	82.7～101.6	fL
MCH	30.2±1.8	30.4±1.6	28.0～34.6	pg
MCHC	33.0±0.8	32.7±0.5	31.6～36.6	%
Platelet	25.2±3.4	24.5±3.3	13.1～36.2	$\times 10^4/\mu L$

3-2) Placebo controlled double blind cross over trials

We conducted a clinical trial of supplementation with KIKU FLOWER EXTRACT (KFE) to assess the effect on serum uric acid levels. Thirty male employees of Oryza Oils & Fat Chemical CO.Ltd aged 22 to 71 years were recruited.

The subjects ingested continuously KFE (100 mg / capsule / day, luteolin content 10 mg) or the placebo capsules contained 100mg of dextrin for 4 weeks. Through the experiment period, 2 subjects dropped out from each of the placebo and KFE groups during the study period for personal reasons.

As a result of 4 weeks continuous intake, KFE group caused a uric acid level to decrease significantly (6.00→5.82 mg / dL) (Table 2).

Statistical analysis was also performed in subjects with a uric acid level of more than 5.5 and less than 7.0 mg / dL. As a result, significant reduction of the uric acid level was observed in KFE group compared to the placebo group (Table 3).

Thus, ingestion of KFE for 4 weeks suppressed the fasting serum uric acid level in Japanese men with mild hyperuricemia.

Table 2. Serum uric acid level before and after ingestion of placebo or KFE for 4 weeks.

		Before	After	Net change (Δ)
Uric acid levels (mg / dL)	Placebo	5.96 ± 0.22	5.85 ± 0.19	-0.11 ± 0.11
	CFE	6.00 ± 0.20	5.82 ± 0.19 †	-0.18 ± 0.07

Values represent the mean and SD. A two-tailed paired *t*-test was used for comparison between before and after ingestion of capsules. The unpaired *t*-test was used to compare the placebo group with the KFE groups. Significant differences between before and after ingestion †: $p < 0.05$ (n=26).

Table 3. Serum uric acid level before and after ingestion of placebo or KFE for 4 weeks. (Subjects with a uric acid level of more than 5.5 and less than 7.0 mg / dL.)

		Before	After	Net change (Δ)
Uric acid levels (mg / dL)	Placebo	5.91 ± 0.19	6.09 ± 0.16	0.18 ± 0.15
	CFE	6.18 ± 0.21	5.98 ± 0.17	-0.20 ± 0.12 *

Values represent the mean and SD. A two-tailed paired *t*-test was used for comparison between before and after ingestion of capsules. The unpaired *t*-test was used to compare the placebo group with the KFE groups. Significant differences between placebo and KFE *: $p < 0.05$ (n=13).

Published paper:

Hirano M., Takeda S., Hitoe S., Shimoda H. Luteolin-rich chrysanthemum flower extract suppresses baseline serum uric acid in Japanese subjects with mild hyperuricemia. *Integr Mol Med*, 4(2): 1-5 (2017).

(2) Sleep Improvement Action

It is said that one in five (20 %) Japanese people suffer from sleep disorders. Modern society is most certainly a stressful one and sleep disorders caused by stress are quite common. Chrysanthemums have been used to treat sleep disorders as a folk medicine for centuries. In China, people put chrysanthemum flowers in their pillows to improve their sleep. They also take chrysanthemum flowers with honey.

J. W. Kim *et al*. studied the action of ethanol extract of chrysanthemum flowers to improve sleeping on a pentobarbital-induced sleep mouse model. Pentobarbital was injected into the abdominal cavity of mice at the amount of 40 mg/kg 30 minutes after kiku flower extract was orally administered and 15 minutes after muscimol (GABA receptor agonist, sleeping drug) was administered. Then, mice's sleeping time was measured. The sleeping time of mice that kiku flower extract (50, 100 mg/kg) was administered to significantly increased (Fig. 11). Sleeping time of mice in the positive control muscimol group also increased significantly.

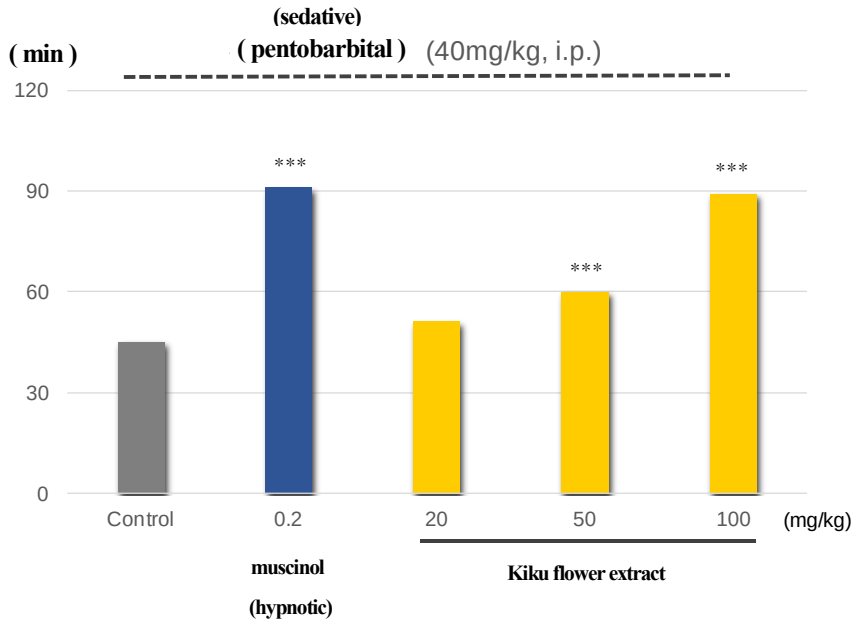


Fig.11. Effect of Kiku flower extract on Pentobarbital-Induced Sleep Behaviors
*** : P<0.005. Modified from J. W. Kim *et al*., *Evid Based Complement Alternat Med.* **2011**:109164 (2011).

KFE (100 mg/kg) and pentobarbital (40 mg/kg) were administered to mice for three days and the expression of glutamate decarboxylase (GAD_{65}) protein in mice's brain hippocampus was analyzed using Western blot technique. As a result, KFE significantly increased the expression of GAD_{65} (Fig. 12). Pentobarbital did not cause any influence on the expression of GAD_{65} in mice's brain hippocampus. The test results suggest that KFE may increase sleeping time by promoting the action of GAD_{65} in the brain and promoting the synthesis of GABA which is an inhibitory neuro-transmitter from glutamic acid.

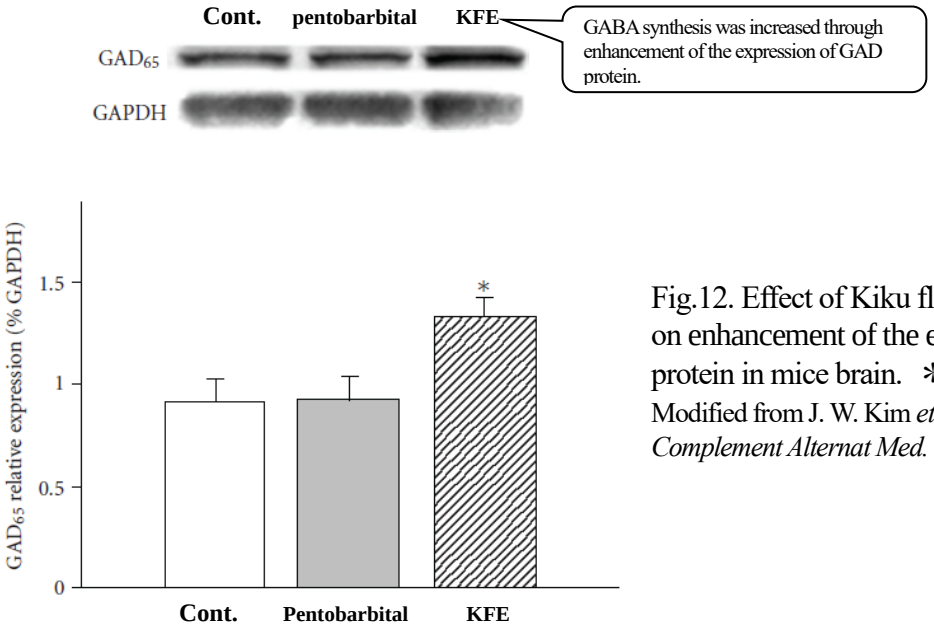
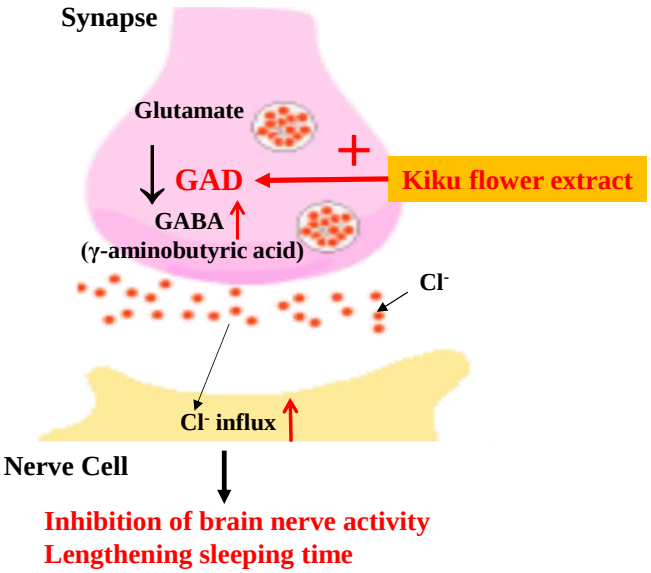


Fig.12. Effect of Kiku flower extract (KFE) on enhancement of the expression of GAD_{65} protein in mice brain. ＊ : $P<0.05$.
Modified from J. W. Kim *et al.*, *Evid Based Complement Alternat Med.* **2011**:109164 (2011).

Moreover, KFE was confirmed to increase the amount of Cl^- ions flowing from the outside of the cells into the cells on a test using neuronal cell culture. When Cl^- ions flow in, the electric potential inside the cells tends to become negative even more (hyperpolarization), suppressing nervous activities.

The results above indicate that kiku flower extract performs its action to increase sleeping time by the mechanism shown in Fig. 13. KFE is considered to increase the action of GAD_{65} in the brain and thus promote the synthesis of GABA. The increase of the amount of Cl^- ions flowing into cells suppress nervous activities.



GAD: glutamate decarboxylase

Fig.13. Proposed mechanism of kiku flower extract in augmenting pentobarbital-induced sleep.

(3) Anti-wrinkle and Skin Improvement Actions by Protecting Skin's Basal Membrane

In the skin's basal membrane, the epidermis and dermis make contact with each other and are connected by the basal membrane. Type IV collagen is the basic skeleton of the basal membrane. By bonding to extracellular matrix components such as fibronectin, type IV collagen helps cells to adhere to each other, connect the epidermis and dermis firmly so that they can link smoothly, and keep the movement of each layer healthy (Fig. 14). When MMP-9, extracellular matrix degrading enzyme is produced because of irritation from UV rays and oxidation stress, it destroys the basal membrane, causing skin's aging and wrinkles. Table 2 shows extracellular matrix components around the basal membrane.

Table 2 Extracellular Matrix Components Around the Basal Membrane

Component	Definition and Functions in the Skin
Basal Membrane	A thin membrane structure where the epidermis and dermis contact. It controls the growth of the epidermis and maintains skin's normal functions.
Type IV Collagen	A type of protein. It is an extracellular matrix component and the basic skeleton of the basal membrane structure.
Type I Collagen	A type of protein. It is an extracellular matrix component found in the dermis in a high concentration.
Fibronectine	A glycoprotein and adhesion molecule. Promotes adhesion, growth, migration, and differentiation of cells.
MMP-9	An enzyme that degrades extracellular matrix protein components. It mainly degrades type IV collagen.
MMP-1	An enzyme that degrades extracellular matrix protein components. It mainly degrades type I collagen.

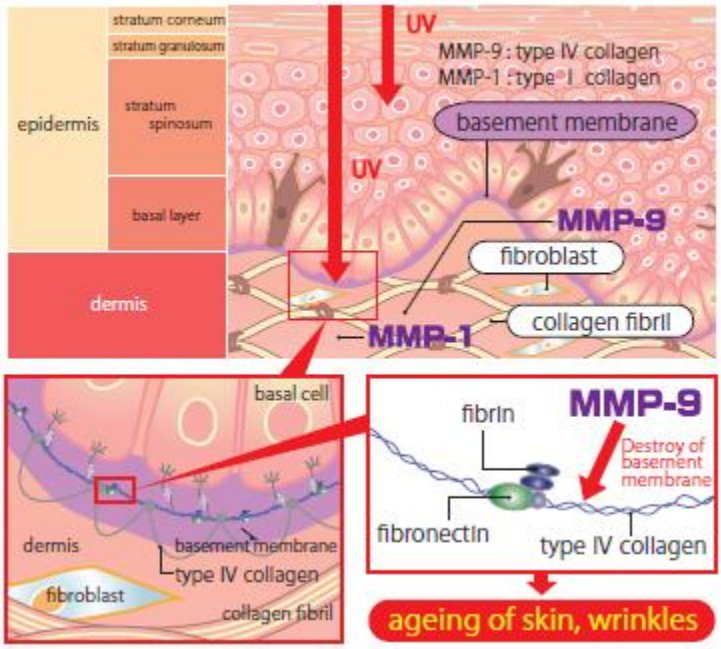
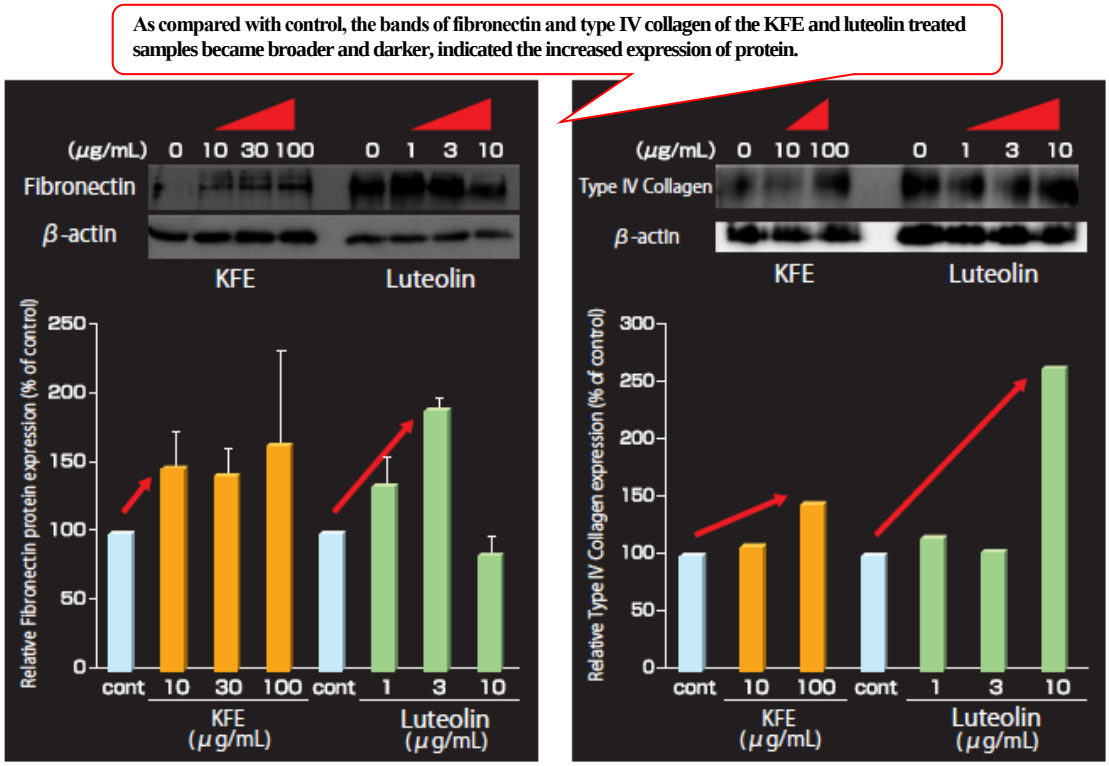


Fig.14. Diagram of the basement membrane

1) Action to increase the expression of fibronectine and type IV collagen

In order to study KFE's actions on skin's basal membrane, KFE or luteolin was applied to fibroblast (TIG-108) and the expression level of fibronectin and type IV collagen was measured. As a result, it was confirmed that KFE promoted the expression of fibronectin in the concentration range 10 to 100 μg/ml and luteolin promoted the expression in the concentration range of 1 and 3 μg/ml (Fig. 15). It was also confirmed that KFE promoted the expression of type IV collagen in the concentration range 10 to 100 μg/ml and luteolin promoted the expression in the concentration range of 1 to 100 μg/ml.



A: Enhancement of fibronectin protein expression

B: Enhancement of type IV collagen protein expression

Fig.15. Enhancing effect of Kiku flower extract on protein expression of fibronectin and type IV collagen in fibroblast.

When type IV collagen is in its normal condition, skin's epidermal cells grow, thickening the epidermis. Maintaining type IV collagen in its normal condition may prevent decrease of firmness and elasticity caused by thinning of the skin due to aging.

KFE and luteolin are expected to restore the basal membrane, prevent wrinkles, and improve skin conditions by increasing the expression of fibronectin and type IV collagen that are extracellular matrix components of the skin.

【Method】

Fibroblasts (TIG-108, 3×10^4 cells/ml) were inseminated on 6-well plates by 2 ml, cultivated for three days, and then the medium was replaced with a medium containing 0.5 % FCS. Samples were added 24 hours later and the cells were cultivated for another 24 hours. The cells were collected and the expression of fibronectin and type IV collagen was checked using the Western blotting technique.

2) MMP-9 inhibitory action of luteolin and apigenin

E. Tahanian et al. induced accelerated production of MMP-9 (type IV collagen degrading enzyme) by adding cell stimulation agent (PMA) on human vascular endothelial cell culture. They also added flavonoids such as luteolin and apigenin and reported their action to suppress accelerated production of MMP-9.

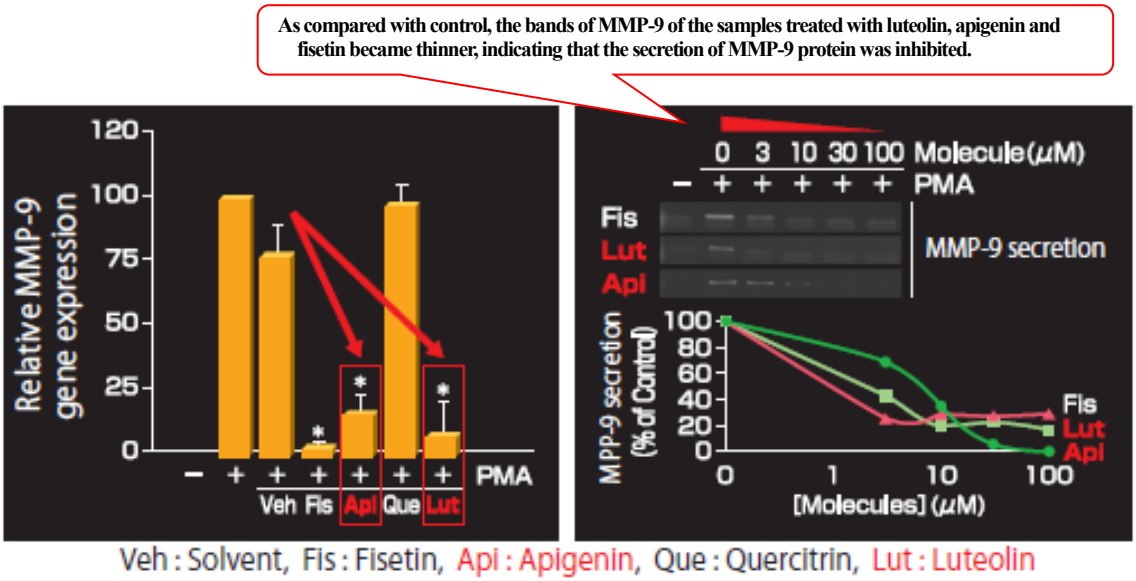


Fig.16. Inhibitory effect of luteolin and apigenin on MMP-9
Modified from E. Tahanian et al. *Drug Design, Development and Therapy*, 5: 299-309, 2011.

As shown in Fig. 16, luteolin, apigenin, and fisetin suppressed the secretion of MMP-9, extracellular matrix degrading enzyme, in the concentration range of 3 to100 μM concentration-dependently. Luteolin, apigenin, and fisetin suppressed the expression of mRNA of MMP-9 at the concentration of 30 μM. A type of flavonoid quercitrin did not suppress the expression. By suppressing MMP-9, extracellular matrix fibronectin and type IV collagen can be protected. Since they are involved in protecting and maintaining the basal membrane, they are expected to prevent wrinkles and improve skin conditions. Since luteolin and apigenin are main components of KFE, KFE is considered to have the same functions.

3) MMP-1 suppressive action of luteolin and apigenin

Y.P. Hwang et al. induced accelerated production of MMP-1 (type I collagen degrading enzyme) by UVA radiation using human keratinocyte culture. They also added luteolin and apigenin and reported their action to suppress accelerated production of MMP-1.

On human keratinocytes, UVA radiation increased the expression of MMP-1, extracellular matrix degrading enzyme. Luteolin and apigenin significantly suppressed the increase of the expression of MMP-1 when UVA was irradiated at the concentration of 1 and 5 mM (Fig. 17). When UVA was not irradiated, luteolin and apigenin suppressed the expression of MMP-1 at the concentration of 5 mM. MMP-1 mainly hydrolyzes type I collagen, namely it decomposes collagen. When MMP-1 is suppressed, type I collagen that exists in the dermis in high concentration can be protected, skin's strength and elasticity can be maintained, and skin conditions can be improved as a result.

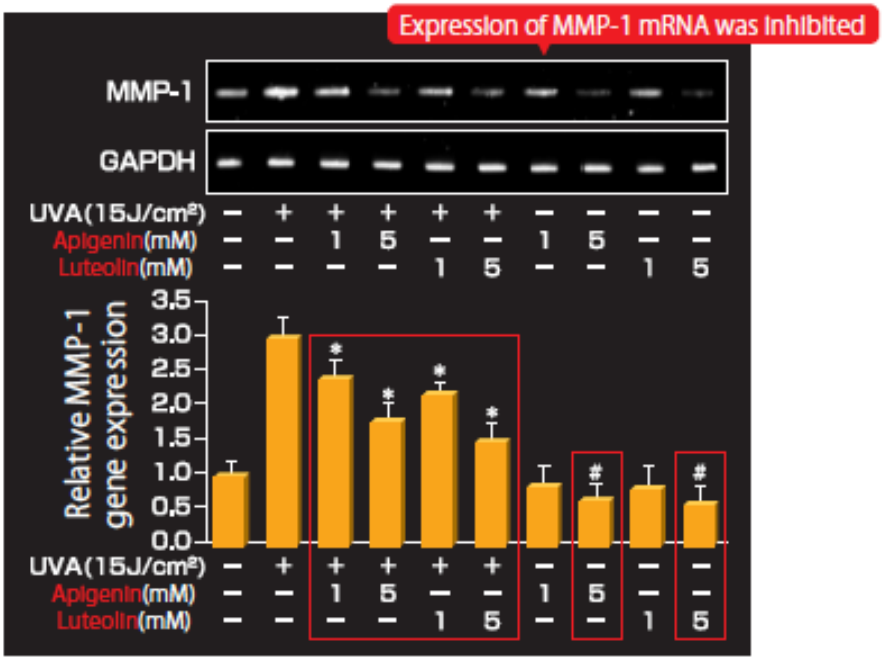


Fig.17. Inhibitory effect of luteolin and apigenin on MMP-1.
Modified from Y. P. Hwang et al. *J. Dermatol. Sci.*, 61(1): 23-31, 2011.

4) Female hormone-like action

Female hormone-like action of kiku flower extract and luteolin was studied using cells (MCF-7) that grow according to the amount of female hormones (estrogens). In the test, kiku flower extract (10 to100 μg/ml) and luteolin (1 to10 μg/ml) demonstrated a proliferative action just like isoflavones daidzein and genistein known as phytoestrogens (Fig. 18). The results clearly indicate that kiku flower extract and luteolin have a female hormone-like action.

Lately, mutagenicity of flavonoides has raised discussions. However, K. Shimoi et al. from University of Shizuoka has reported that luteolin has the strongest anti-oxidation property and

inhibits chromosome defects the most of all flavonoids [Shimoi K. et al., Carcinogenesis, 15(11):2669-2672(1994)].

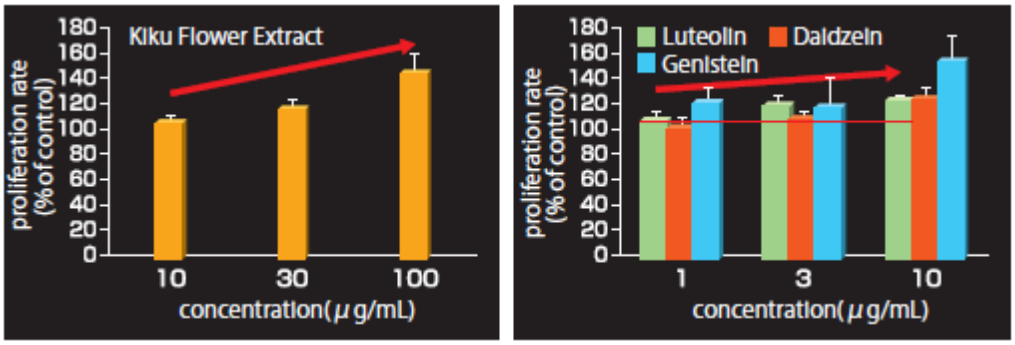


Fig.18. Female hormone-like actions of Kiku flower extract and luteolin

Estrogen is known to act on fibroblasts and promote the production of fibronectin. It is considered that kiku flower extract and luteolin involve in the production of fibronectin because their female hormone-like actions have been confirmed.

For female hormone-like actions, kiku flower extract is expected to have actions similar to that of isoflavones such as daidzein and genistein.

4) Human Clinical Trial (topical use)

The improvement effect of Kiku Flower Extract-LC on wrinkles was evaluated by using a lotion (referring to table 3) prescribed with 3% Kiku Flower Extract-LC when topically using on the tails of the eyes. In-house volunteers were requested to topically use the sample lotion and control lotion on the right and left tails of the eyes, respectively, twice a day in the morning and at night for 5 weeks. Wrinkle replicas before and after use of lotions were prepared and evaluated using the parameters listed in table 4. Comparison was made between the right and left in individual subject. Furthermore, the feeling of using the lotion was investigated by a questioair.

Table 3. Compositions of sample and control lotion

Composition	Sample (%)	Control (%)
Kiku Flower Extract-LC	3	-
30% propanediol	-	3
Propanediol	48.5	48.5
Water	48.5	48.5
Total	100	100

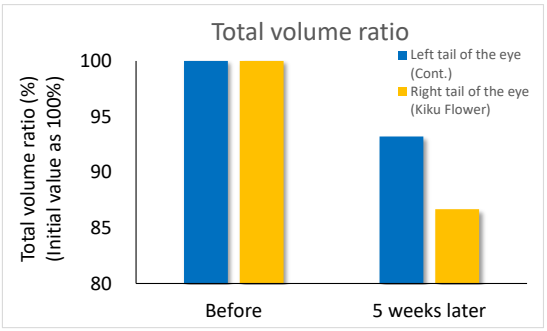
As shown in Fig. 19A and 19B, after 5 weeks, the use of the sample lotion contained Kiku Flower Extract-LC decreased the wrinkles as compared with that of the control lotion. The results of replica analyses showed the improvement of deep and fine wrinkles on the right tails of the eyes. Therefore, it is expected that continuously topical use of Kiku Flower Extract-LC will decrease the wrinkles.

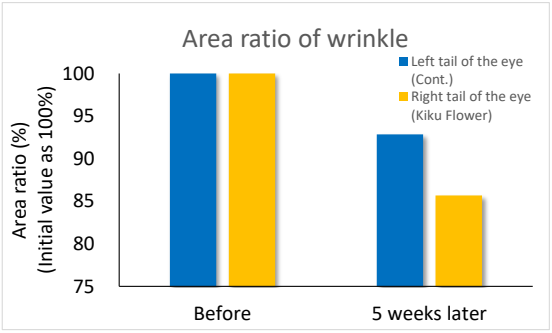
Table 4. Parameters related to improvement of wrinkles

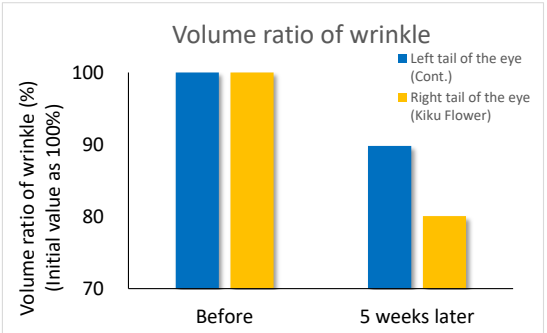
Items for wrinkle analysis	Contents of wrinkle analysis
Total volume ratio: $\mu m^3/mm^2 \times 1/100$	Total volume ratio of wrinkles within the designated range (10㎜×10㎜), expressed as %
Area ratio: %	Identification value: area ratio of wrinkles with the width within 250 μm
Volume ratio: $\mu m^3/mm^2 \times 1/100$	Identification value: volume ratio of wrinkles with the width within 250 μm, expressed as %
Widest wrinkle: μm	The widest wrinkle among the lines which are recognized as wrinkles
Average deepth: μm	The quotient obtained by dividing the sum of deepth of the wrinkles by number of them

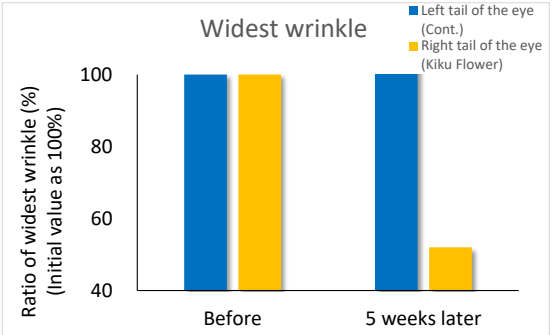
Note: Replica analysis was performed by A-KIT CORPORATION using an instrument of ASA-03RXD made by ASAHIBIOMED CORPORATION.

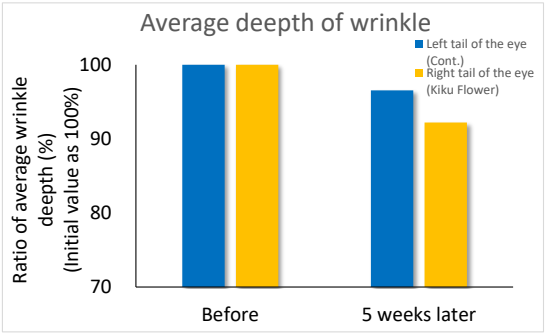
【Volunteer 1】











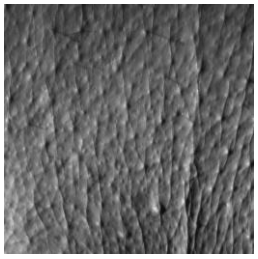
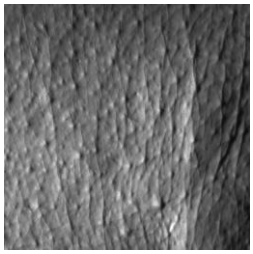
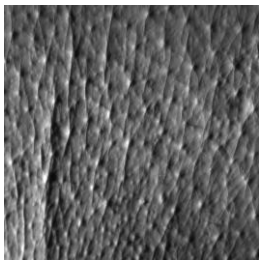
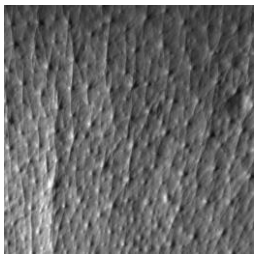
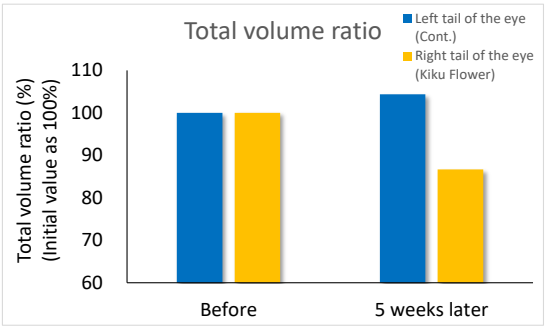
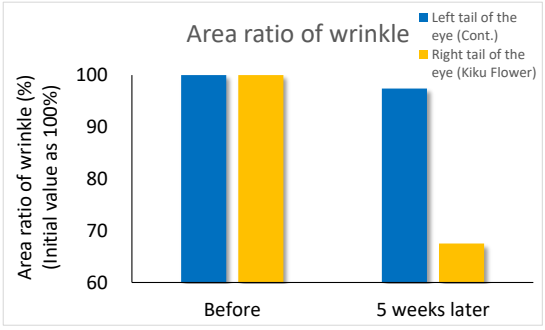
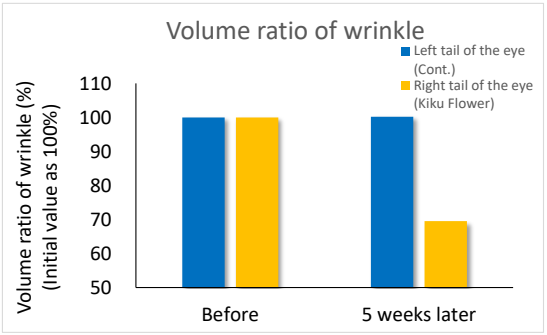
	Before	5 weeks later
Left tail of the eye: Lotion prescribed with propanediol		
Right tail of the eye: Lotion prescribed with Kiku Flower Extract-LC		

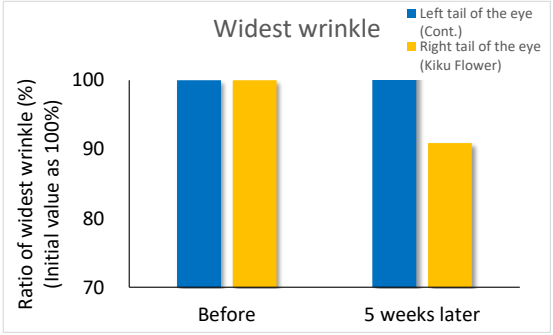
Fig. 19A Improvement effect of Kiku Flower Extract-LC on wrinkles

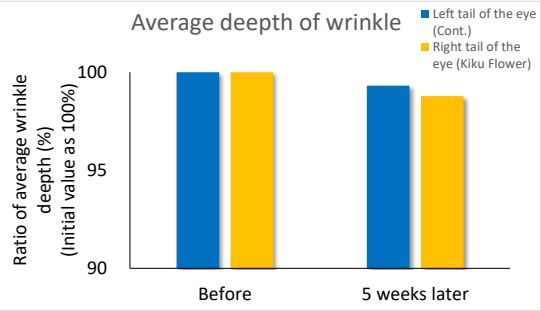
【Volunteer 2】











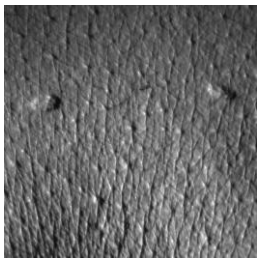
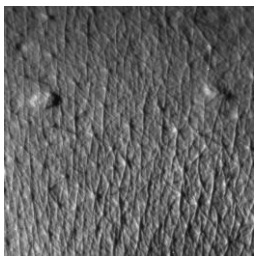
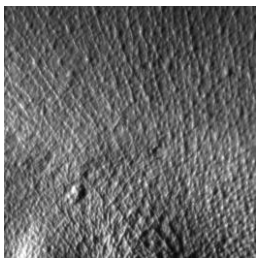
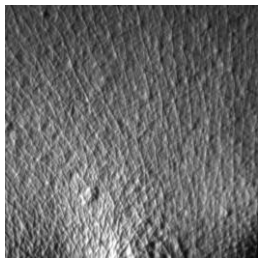
	Before	5 weeks later
Left tail of the eye: Lotion prescribed with propanediol		
Right tail of the eye: Lotion prescribed with Kiku Flower Extract-LC		

Fig. 19B. Improvement effect of Kiku Flower Extract-LC on wrinkles

Fig. 20 showed the result of questionair on the use of Kiku Flower Extract-LC containing lotion. Better comments were obtained in each individual question as compared with that of the use of control lotion. Especially, with regard to moisturization, much more good comments were obtained. No side-effects such as rash or itching were observed.

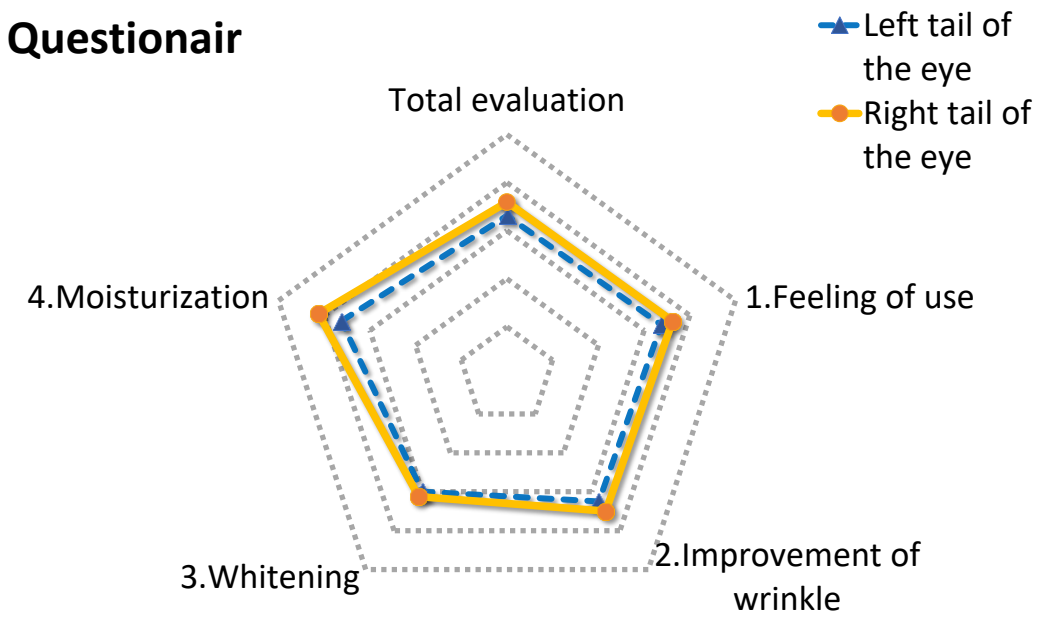


Fig. 20. Questionair on the use of Kiku Flower Extract-LC containing lotion

4. Stability of Kiku Flower Extract

(1) Thermal Stability

The thermostability of Kiku Flower Extract-P (-PC) was examined by heating at 100°C and 120°C continuously for 1 hour. As shown in Fig. 21, content of luteolin, the principal component of Kiku Flower Extract-P (-PC) were not reduced after heating for 1 hour. Therefore, Black Ginger Extract-P is highly stable upon heating at normal food processing temperature.

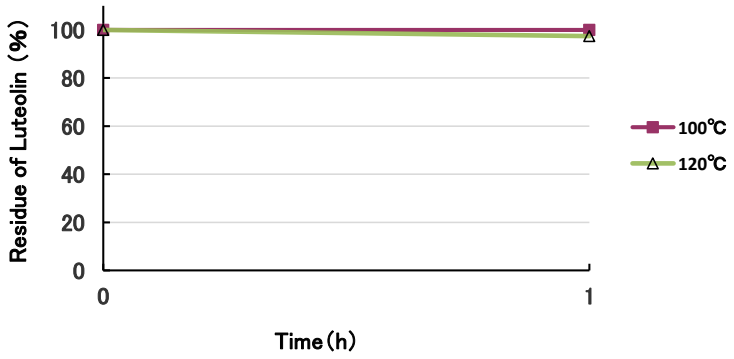


Fig. 21. Thermal Stability of Kiku Flower Extract

(2) Thermal Stability of Kiku Flower Extract-LC

Kiku Flower Extract-LC was diluted ten-fold and heated at 90 °C for seven hours. To check the change in the color, absorbance values at 450 nm was measured every hour and the results were compared against the initial value. As a result, no color change was observed even seven hours after and Kiku Flower Extract-LC was confirmed to be stable when heated at 90 °C.

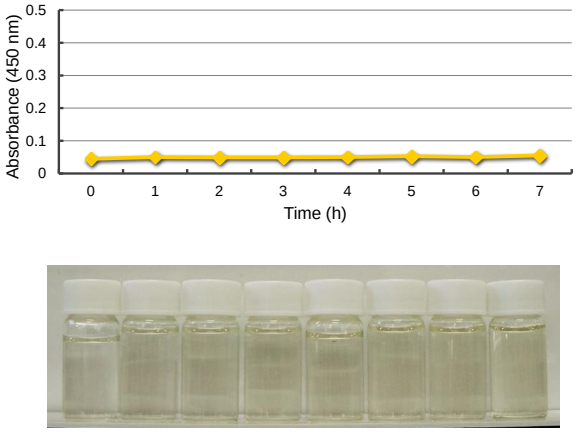


Fig.22. Thermal stability of Kiku Flower Extract-LC

(3) pH Stability of Kiku Flower Extract-LC

The pH of Kiku Flower Extract-LC was adjusted to 2 to 12 and absorbance at 450 nm was compared to check color change. No significant color change was observed in pH 2 to 5. However, the color noticeably changed to yellowish brown in pH 6 and higher and the absorbance increased. Be careful with color change because of high pH during use.

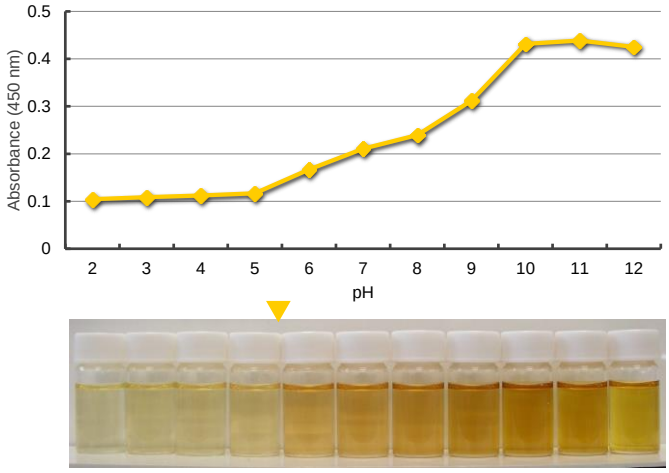


Fig.23. pH stability of Kiku Flower Extract-LC

5. Water Solubility of Kiku Flower Extract-WSP (Comparison with Other Companies' Products)

Luteolin and apigenin, active ingredients of kiku flower extract, are very poorly soluble in water. Through dedicated studies, Oryza Oil & Fat Chemical successfully developed highly water-soluble Kiku Flower Extract-WSP (-WSPC) containing at least 0.1 % luteolin.

Water solubility of Kiku Flower Extract-WSP was compared with that of commercially-available products manufactured by companies A, B, and C. 2 % solutions of each product's samples were prepared and their characteristics were compared. Company A's product did not have lacked clarity. Deposition occurred when it was left two to three days. Company B's product was a suspended solution. Company C's product started to look like an emulsified liquid and material similar to oil settled on the bottom of the sample bottle. When it was left to sit for a while, drops of oil started to float. Oryza Oil & Fat Chemical's Kiku Flower Extract-WSP (-WSPC) was a highly transparent solution and its appearance did not change even when it was left for a week, indicating excellent water solubility.

Oryza A B C **Oryza A B C**

Fig. 24. Comparison of the water solubility of Kiku Flower Extract-WSP (-WSPC) with products manufactured by other campanies

6. Recommended Dosage（Food）

Product	Claims	Recommended Dosage
Kiku Flower Extract-P	1) Prevention of Gout 2) Inhibition of Uric Acid Production 3) Improvement of Sleep 4) Deep Care of Skin /Anti-ageing 5) Antioxidant	100 mg/day

7. Recommended Dosage（Cosmetics）

Products	Claims	Recommended Dosage
Kiku Flower Extract-PC	1) Protection of basement membrane 2) Deep Care of Skin /Anti-ageing 3) Antioxidant	0.1～0.5%
Kiku Flower Extract -WSPC		1.0～5.0%
Kiku Flower Extract -LC		

8. Nutritional Composition

Items	Kiku Flower Extract-P (100 g)	Analytical Method
Energy	373 kcal	Atwater Method (Revised)*1
Protein	0.9 g	Kjeldahl Method *2
Fat	1.0 g	Acid degradation
Carbohydrate	89.4 g	*3
Sodium	21.5 mg	Atomic absorption spectrophotometory
Water	5.9 g	Air Oven Method
Ash	1.2 g	Direct Incineration
Dietary fiber	1.6 g	Prosky Method

*1 Conversion factor: Protein 4, fat 9, sugar 4, dietary fiber 2
*2 Nitrogen, protein conversion factor: 6.25
*3 Carbohydrate expression standard (Ministry of Health and Welfare's announcement No. 176)
Calculation: 100 – (water + protein + fat + ash)

Test trustee: Hokuriku Environmental Science Institute Co., Ltd.
Date of analysis: April 1, 2014
Test No.: Kanken No. 13D-0162401

9. Safety Profiles of Kiku Flower Extract

(1) Residual Agricultural Chemicals

Kiku Flower Extract (without binder) was examined for 532 residual agricultural chemical compounds following the provisions of the Food Hygiene Law and Pesticides Control Act. As a result, contents of all compounds were confirmed to be below the standard values (measurable limits).

Test Trustee: Masis Co., Ltd.; Center for Food Safety Evaluation and Analysis
Date of test report issued: May 8, 2014
Report No.: 68893

(2) Acute Toxicity (LD_{50})

Acute Toxicity test was conducted according to the Guidelines for Single-Dose Toxicity Tests for Pharmaceutical Products where Kiku Flower Extract (without binder) 2000mg/kg was orally given to mice (male & female ICR, 6 weeks old, weight 20.9-27.2g) for 14 days. The mice were housed at 23 ± 2°C and at 50 ± 10% humidity with free access to feed and drinking water for 14 days. No abnormal change was found in their weight as compared to the control group. No abnormalities were found in their organs upon autopsy after the test either. LD_{50} of Kiku Flower Extract is deduced to be more than 2,000 mg/kg.

(3) Subacute toxicity (28 days)

We examined the safety profile of the KFE standardized with 10% luteolin by consecutive (28 days) oral administration (250, 500 mg / kg) in rats. No remarkable change was observed in rats given the extract as results of performance status, body weight, organs and blood analysis. The extract was found to exhibits no toxicity in rats by 4-week oral administration.

(4) Chronic Toxicity

It is reported that chronic toxicity was performed by giving SD rats a methanol extract of Kiku Flower at dosages of 320, 640 and 1280 mg/kg for 26 weeks*. No abnormal change was found in their weight as compared to the control group. No abnormalities were found in their organs upon autopsy after the test either.

*: Li L et al., Toxicity study of ethanolic extract of *Chrysanthemum morifolium* in rats. *J Food Sci*. 75(6):T105-9 (2010).

(5) Phototoxicity (3T3-NR Method)

Phototoxicity on Kiku Flower Extract-LC was conducted by using mouse fibroblast (Balb/c 3T3 A31) according to OECD GUIDELINE FOR TESTING OF CHEMICALS 432 (Adopted: 13 April 2004) protocol. Cells in confluent condition were co-cultured with Kiku Flower Extract-LC (7.8−1000 μg/ml) for 1hr, and then irradiated by UVA/Vis (5 J/cm^2). After irradiation, culture medium was changed and the cells were further cultured overnight. Survival rate of the cells was measured and compared with control (without UVA/Vis irradiation). The result showed no reduction of the survival rate was observed. It is concluded that there is no phototoxicity for Kiku Flower Extract-LC.

(6) Eye Irritation (HCE Method)

Eye irritation test was performed by using the Human Corneal Epithelial Model (HCE) on Kiku Flower Extract-LC. The survival rate of the tissue cells exposed to 1% aqueous solution of Kiku Flower Extract-LC was evaluated below the COLIPA （The European Cosmetics Association） criteria. It is concluded that there is no eye irritation for Kiku Flower Extract-LC.

(7) Mutagenicity (Ames Test)

Ames test was conducted to evaluate the mutagenicity of Kiku Flower Extract (without binder) using Salmonella typhimurium strain TA98 and TA100. There was no increase in the number of colonies (19.5 ~ 5000 μg / plate) in both direct method and metabolism activation method. Kiku Flower Extract was considered as non-mutagenic.

(8) The safety in case of excessive consumption (5 times higher dosage)

We evaluated safety of excessive consumption of Kiku Flower Extract-P (500 mg / day). The study evaluated at double blind parallel test in healthy male and female aged from 23 to 46 years old. Questionnaire and measurements of blood pressure, pluse count, blood parameter, urinary parameter and subjective symptoms were performed. As a result of 4-week treatments any abnormal changes were not observed in all parameters. Furthermore there were no dropouts and subjects complaining health problem.

10. Applications

	Applications Claims Examples	Claims	Examples
Food	Food, Nutritional Supplement, Skin Beautifying Food	1. Prevention of Gout 2. Inhibition of Uric Acid Production 3. Improvement of Sleep 4. Deep Care of Skin 5. Anti-ageing	Beverages Hard & soft capsules, tablets Candies, chewing gums, chocolates, wafers, jellies Ham, sausage, etc.
Cosmetic	Skin Beautifying Cosmetic		Lotions, toner, serum, rinse, treatment care, pack, body gel etc.

11. Nutritional Composition

Kiku Flower Extract -P (powder, food grade)
Kiku Flower Extract -WSP (water soluble powder, food grade)
Kiku Flower Extract –PC (powder, cosmetics grade)
Kiku Flower Extract -WSPC (water soluble powder, cosmetics grade)
1kg, 5kg interior packing: Aluminium bag
Exterior packing: Cardboard box

Kiku Flower Extract -LC (liquid, cosmetics grade)
1kg, 5kg Interior packing: plastic container or cubic container
Exterior packing: Cardboard box

12. Storage

Store in a cool, dry, ventilated area with desiccant. Keep away from high temperature and store in a closed container.

13. Expressions

Food grade:

Kiku Flower Extract-P
Expression 1: Processed Kiku Flower Extract powder
Expression 2: Kiku Flower Extract and dextrin

Kiku Flower Extract-WSP
Expression 1: Processed Kiku Flower Extract powder
Expression 2: Kiku Flower Extract and cyclodextrin

It is suggested to reconfirm with the Regional Agricultural Administration Office for public health and food labeling.

Cosmetic grade:

Kiku Flower Extract-PC
INCI name: Chrysanthemum Morifolium Flower Extract (and) dextrin
Expression: Chrysanthemum Morifolium Flower Extract, dextrin

Kiku Flower Extract-WSPC
INCI name: Cyclodextrin (and) Maltosyl Cyclodextrin (and) Dimaltosyl Cyclodextrin (and) Maltose (and) Chrysanthemum Morifolium Flower Extract
Expression: Cyclodextrin, Maltosyl Cyclodextrin, Dimaltosyl Cyclodextrin, Maltose, Chrysanthemum Morifolium Flower Extract

Kiku Flower Extract-LC
INCI name: Propanediol (and) Chrysanthemum Morifolium Flower Extract
Expression: Propanediol, Chrysanthemum Morifolium Flower Extract

PRODUCT STANDARD

PRODUCT NAME : **KIKU FLOWER EXTRACT-P** (FOOD)

This product is extracted with aqueous ethanol from the bud and/or flower of *Chrysanthemum morifolium* (*Compositae*). It contains not less than 10 % of luteolin.

Appearance	Light grey brown to light brown powder with unique aroma	
Luteolin	Min. 10 %	(HPLC)
Loss on Drying	Max. 10.0 %	(Analysis for HygienicChemists, 1g, 105 ℃, 2 hr)
Purity Test		
(1)Heavy Metals (as Pb)	Max. 10 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
	Kiku Flower Extract	50 %
	Dextrin	50 %
	Total	100 %
Expiry date	2 years from date of manufacturing.	
Storage	Store in a cool, dry, ventilated area with desiccant. Keep away from high temperature and sunlight, and st ore in a closed container.	

PRODUCT STANDARD

PRODUCT NAME : **KIKU FLOWER EXTRACT-WSP** (FOOD)

This product is extracted with aqueous ethanol from the bud and/or flower of *Chrysanthemum morifolium* (*Compositae*). It contains not less than 0.1 % of luteolin. This product is water soluble.

Appearance	Light pale yellow to light yellowish powder with unique aroma	
Luteolin	Min. 0.1 %	(HPLC)
Loss on Drying	Max. 10.0 %	(Analysis for Hygienic Chemists, 1g, 105 ℃, 2 hr)
Purity Test		
(1)Heavy Metals (as Pb)	Max. 10 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	
	Kiku Flower Extract — 5 %	
	Cyclodextrin — 95 %	
	Total — 100 %	
Expiry date	2 years from date of manufacturing.	
Storage	Store in a cool, dry, ventilated area with desiccant. Keep away from high temperature and sunlight, and store in a closed container.	

PRODUCT STANDARD

PRODUCT NAME : **KIKU FLOWER EXTRACT-PC** (COSMETIC)

This product is extracted with aqueous ethanol from the bud and/or flower of *Chrysanthemum morifolium* (*Compositae*). It contains not less than 10 % of luteolin.

Appearance	Light grey brown to light brown powder with unique aroma.	
Luteolin	Min. 10 %	(HPLC)
Loss on Drying	Max. 10.0 %	(Analysis for HygienicChemists, 1g, 105 ℃, 2 hr)
Purity Test		
(1)Heavy Metals (as Pb)	Max. 10 ppm	(The Second Method of The Japanese Standards of Quasi-Drug Ingredients)
(2)Arsenic (as As_2O_3)	Max. 1 ppm	(The Third Method of The Japanese Standards of Quasi-Drug Ingredients)
Standard Plate Counts	Max. 1×10^2 cfu/g	(Analysis for Hygienic Chemists)
Moulds and Yeasts	Max. 1×10^2 cfu/g	(Analysis for Hygienic Chemists)
Coliforms	Negative	(Analysis for Hygienic Chemists)

Composition

Ingredient	Content
Chrysanthemum Morifolium Flower Extract	50 %
Dextrin	50 %
Total	100 %

Expiry date	2 years from date of manufacturing.
Storage	Store in a cool, dry, ventilated area with desiccant. Keep away from high temperature and sunlight, and store in a closed container.

PRODUCT STANDARD

PRODUCT NAME : **KIKU FLOWER EXTRACT-WSPC** (COSMETIC)

This product is extracted with aqueous ethanol from the bud and/or flower of *Chrysanthemum morifolium* (*Compositae*). It contains not less than 0.1 % of luteolin. This product is water soluble.

Appearance	Light pale yellow to light yellowish powder with unique aroma.	
Luteolin	Min. 0.1 %	(HPLC)
Loss on Drying	Max. 10.0 %	(Analysis for Hygienic Chemists, 1g, 105 ℃, 2 hr)
Purity Test		
(1)Heavy Metals (as Pb)	Max. 10 ppm	(The Second Method of The Japanese Standards of Quasi-Drug Ingredients)
(2)Arsenic (as As_2O_3)	Max. 1 ppm	(The Third Method of The Japanese Standards of Quasi-Drug Ingredients)
Standard Plate Counts	Max. 1×10^2 cfu/g	(Analysis for Hygienic Chemists)
Moulds and Yeasts	Max. 1×10^2 cfu/g	(Analysis for Hygienic Chemists)
Coliforms	Negative	(Analysis for Hygienic Chemists)

Composition

Ingredient	Content
Maltosyl Cyclodextrin Cyclodextrin Dimaltosyl Cyclodextrin Maltose	95 %
Chrysanthemum Morifolium Flower Extract	5 %
Total	100 %

Expiry date	2 years from date of manufacturing.
Storage	Store in a cool, dry, ventilated area with desiccant. Keep away from high temperature and sunlight, and store in a closed container.

PRODUCT STANDARD

PRODUCT NAME : **KIKU FLOWER EXTRACT-LC** (COSMETIC)

This product is an aqueous solution adding 1,3-propanediol into water-extract which obtained from extracting the flowers of *Chrysanthemum morifolium* Ramat. (*Compositae*).

Description

Yellowish brown to reddish tan color solution, having slight characteristic odor

Identification

(1) Phenol Compounds

Add 1 to 2 drops of Ferric chloride (Ⅲ) solution TS to this product; The color of the solution develops black to blackish green color.

(2) Sugar

Add α-Naphtol・ethanol solution (1→20) to 0.5mL of this solution and shake well, and add gently 1 to 2 drops of sulfuric acid; The contact zone of the solution develops reddish purple color.

Purity Test

(1)Heavy Metals (as Pb)	Max. 10 ppm	(Method 2, JSQI)
(2)Arsenic (as As_2O_3)	Max. 1 ppm	(Method 3, JSQI)

Microbiological Examinations

(1) Standard Plate Counts	Max. 1×10^2 cfu/g	(General Test, JP)
(2) Molds and Yeast	Max. 1×10^2 cfu/g	(General Test, JP)

Expiry Date

2 years after manufacturing date

Composition

INCI Name	Composition
Water	69.7%
Propanediol	30.0%
Chrysanthemum Morifolium Flower Extract	0.3%
Total	100.0 %

Storage

Store in a dry, ventilated area with desiccant. Keep away from high temperature and sunlight, and store in a closed container.

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: October 21, 2015
Revised Date: May 15, 2019

ORYZA OIL & FAT CHEMICAL CO., LTD.