



ORYZA OIL & FAT CHEMICAL CO., LTD.

## *Evening Primrose Extract*

For Anti-Diabetes & Anti-Obesity

- **Evening Primrose Extract—P**  
(Food, Powder)
- **Evening Primrose Extract—WSPS**  
(Food, Water-Soluble Powder)
- **Evening Primrose Extract—PC**  
(Cosmetics, Powder, No binder)
- **Evening Primrose Extract—LC**  
(Cosmetics, Liquid)

ORYZA OIL & FAT CHEMICAL CO., LTD.

Ver. 6.1 JT

# **EVENING PRIMROSE EXTRACT**

## **Foodstuff for Diabetes Mellitus and Health**

### **1. Introduction**

Evening Primrose is a dicotyledonous plant of genus *Oenothera*. The seeds have been used for edible oil and also known to be a medicine in Europe. Evening Primrose oil has contains much  $\gamma$ -linolenic acid, which has well known to relieve obesity, diabetes mellitus, hypercholesterolemia, premenstrual syndrome (PMS), and so on.

Recently, polyphenols in plant seeds have been paid attention. Polyphenols prevent oxidation of lipids and scavenge active oxygen as a trigger of cause of various diseases. ORYZA OIL & FAT CHEMICAL CO., LTD. has been studying for the physiological function of polyphenols of the Evening Primrose seeds. We have recently developed an EVENING PRIMROSE EXTRACT (EPE), in which polyphenols are highly concentrated. The product contains more polyphenols than any other products, showing a strong antioxidative activity. Further, we found that EVENING PRIMROSE EXTRACT has an anti-diabetic activity.



**Evening Primrose**

## **2. What is “EVENING PRIMROSE EXTRACT”?**

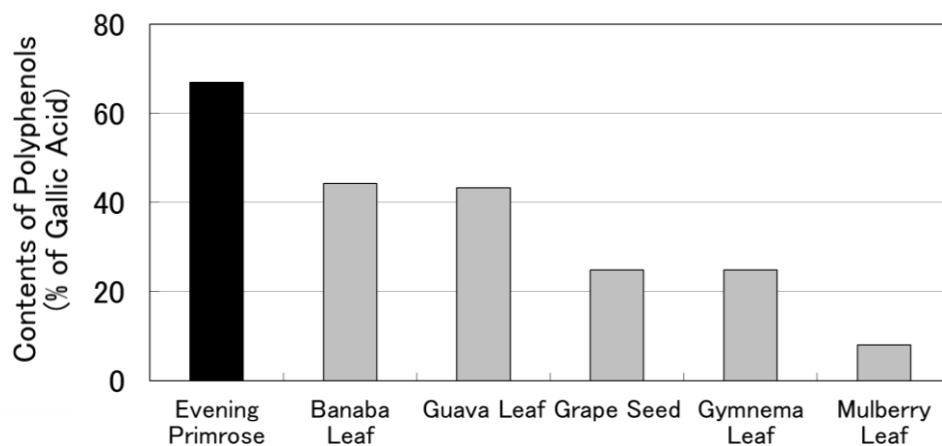
### **2-1. EVENING PRIMROSE**

EVENING PRIMROSE EXTRACT has been cultivated in North America or China to obtain oils from the seeds. Evening primrose is introduced to Japan as decorative plants which is also seen on the riverside or seashore. The following 4 species are known to be evening primrose: *Oenothera laciniata*, *Oenothera striata*, *Oenothera biennis* and *Oenothera erythrosepaa*.

### **2-2. Contents of Total Polyphenols**

The contents of total polyphenols in EVENING PRIMROSE EXTRACT were superior high comparison to other plant extracts.

**Fig. 1 Contents of total polyphenols of EVENING PRIMROSE EXTRACT**



### 2-3. Components of EVENING PRIMROSE EXTRACT

EVENING PRIMROSE EXTRACT contains polyphenols such as gallic acid, ellagic acid, pentagalloylglucose, catechin, *etc.* Our research has shown that EVENING PRIMROSE EXTRACT would be effective for prevention of diabetes and obesity by test of carbohydrase inhibition *in vitro* and by sugar tolerance *in vivo*.

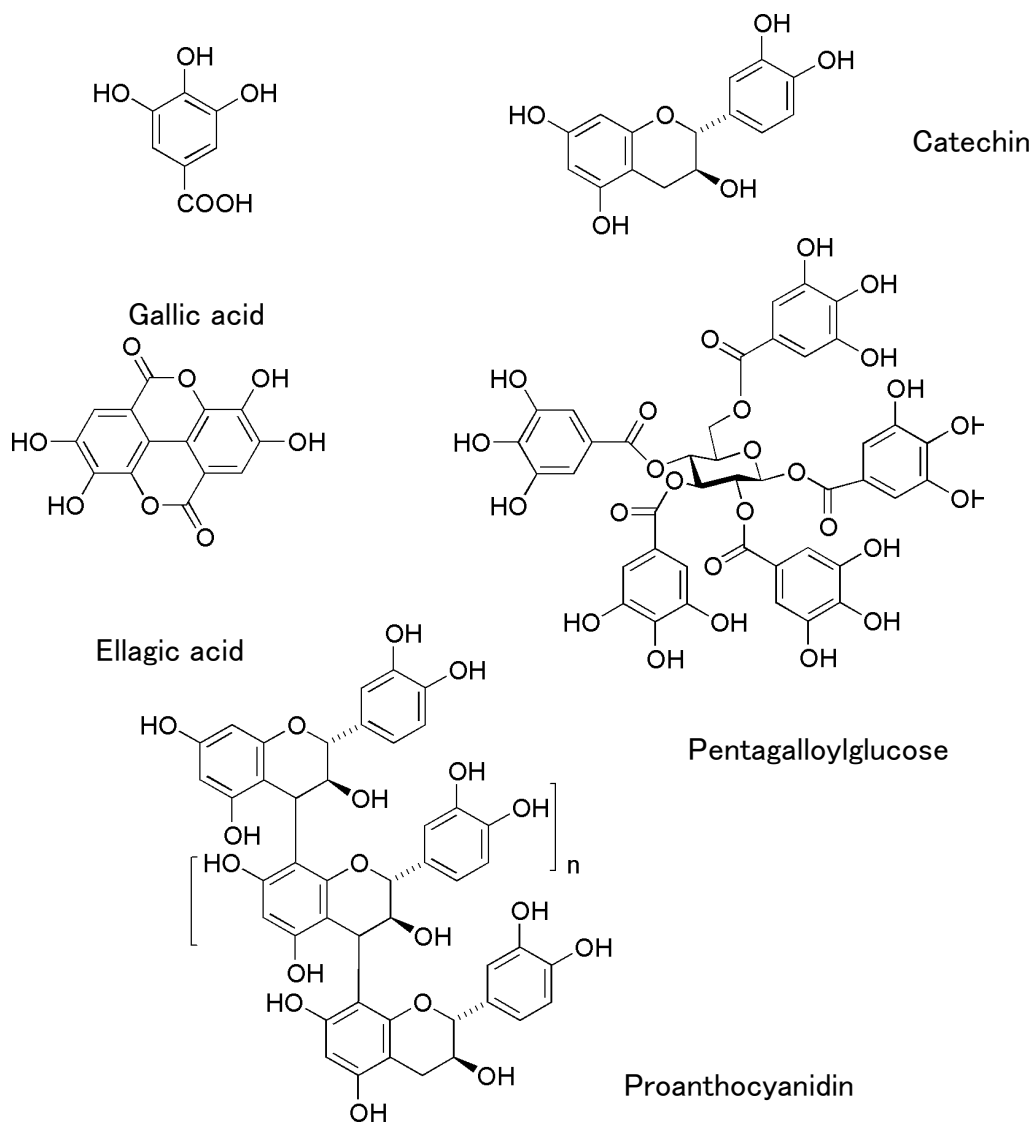


Fig. 2 Major polyphenols of EVENING PRIMROSE EXTRACT

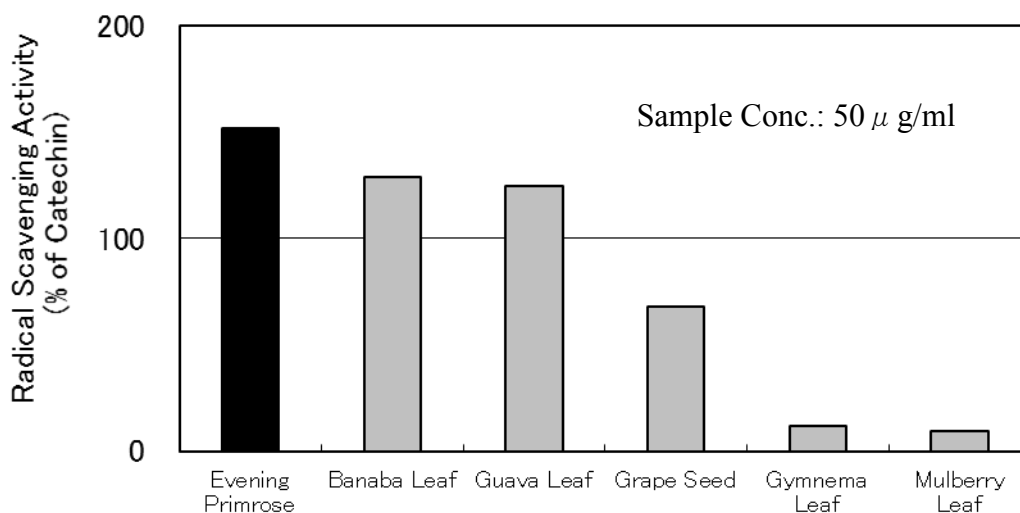
### 2-4. Antioxidant Activity

Assayed Items	Result	Assaying Method
Superoxide dismutase activity	$3.5 \times 10^5$ units/g	ESR method

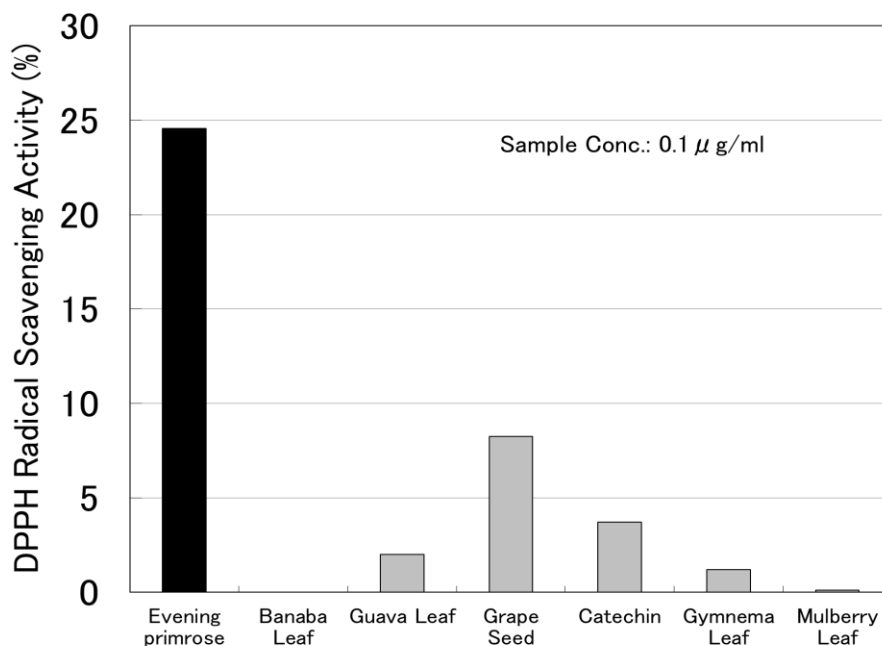
Tested by: Japan Food Research Center Foundation

Research results issue number: 302010023-001

EVENING PRIMROSE EXTRACT has antioxidant activity stronger than other plant extracts. It is said that taking foods which have such a strong SOD-like activity or radical scavenging activity is good for prevention of several disease from habit of life style.



**Fig. 3 SOD-like activity of several plant extracts**



**Fig. 4 DPPH radical scavenging activity of several plant extracts**

## 2-5. Mechanism of Diabetes Mellitus

Diabetes mellitus is caused by the lack of insulin secretion and manifests chronic hyperglycemia. The disease is ascribed to the genetic background and environmental factor (a habit of life). The non-insulin dependent diabetes mellitus (NIDDM), which account for more than 90% of the incidence of diabetes mellitus, is caused by over-eating, ingestion of high-fat diet, lack of exercise, obesity, or aging. These factors decrease in responsiveness and sensitiveness of insulin. In particular, obesity is one of the highest risk factors of the diabetes mellitus among them. Therefore, it is important to control diet or to take exercise for prevention of the incidence of diabetes mellitus.

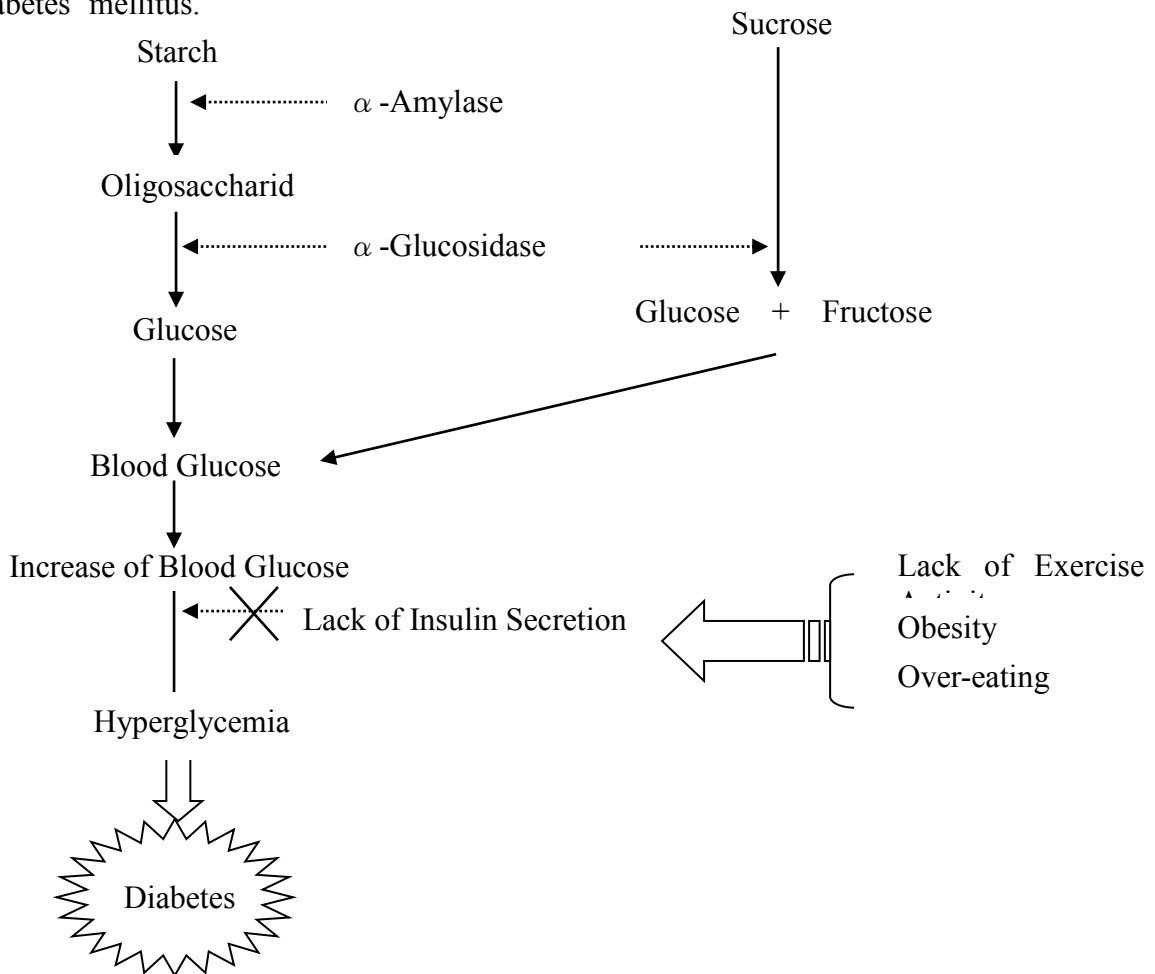
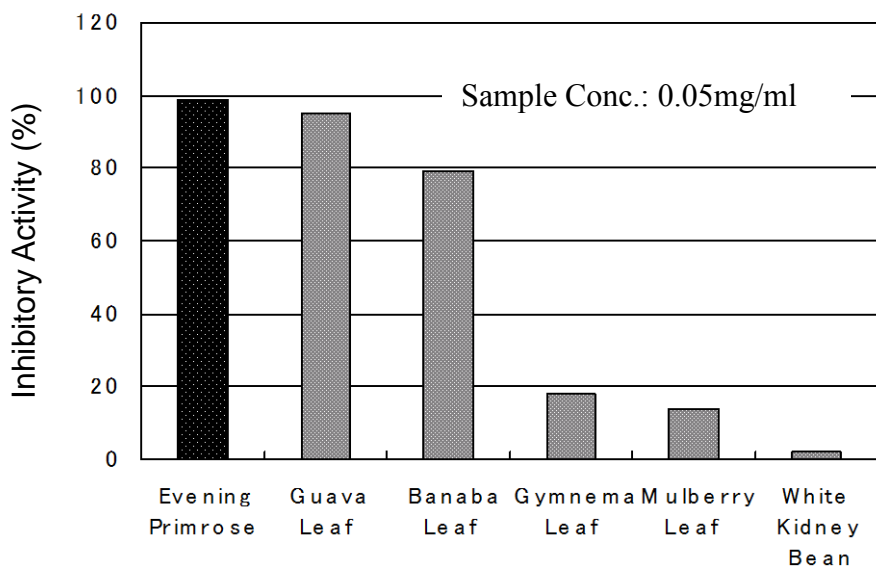


Fig. 5 Relation between digestion and absorption of sugar and diabetes

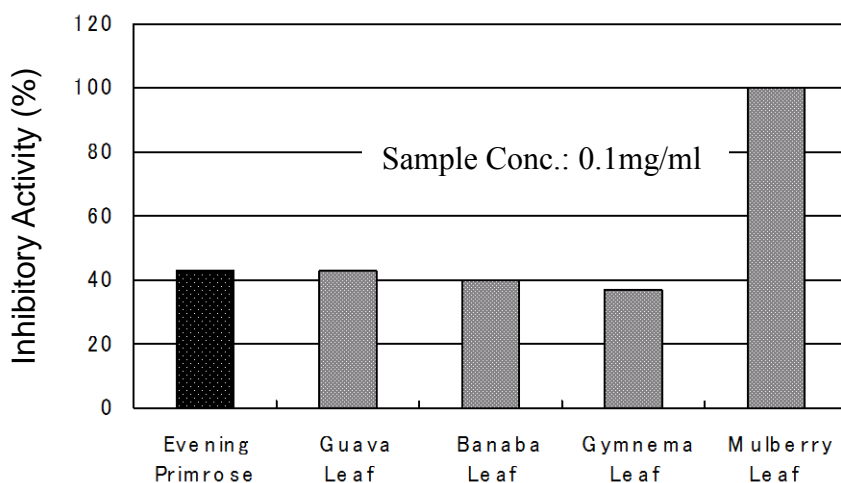
### 3. Function of “EVENING PRIMROSE EXTRACT”

#### 3-1. Inhibitory Effect on Sugar Degradation Enzyme

EVENING PRIMROSE EXTRACT has inhibitory efficacy on  $\alpha$ -amylase and  $\alpha$ -glucosidase. Especially the inhibitory activity to  $\alpha$ -amylase showed stronger than other plant extracts.



**Fig. 6** Inhibitory activity of EVENING PRIMROSE EXTRACT and several plant extracts on  $\alpha$ -amylase



**Fig. 7** Inhibitory activity of EVENING PRIMROSE EXTRACT and several plant extracts on  $\alpha$ -glucosidase

The inhibitory activity of polyphenols contained in EVENING PRIMROSE EXTRACT on sugar degradation enzyme were investigated. Pentagalloylglucose showed the highest inhibitory contribution in monomeric polyphenols.

**Table 1. Inhibitory activity of evening primrose polyphenols on sugar degradation enzyme**

Polyphenol	Contents (%)	$\alpha$ -Glucosidase		$\alpha$ -Amylase	
		IC <sub>50</sub> (mg/ml)	Contribution (%)	IC <sub>50</sub> (mg/ml)	Contribution (%)
Evening primros	100.0	0.34	100.0	0.026	100
PGG	2.7	0.10	9.3	0.05	1.4
Gallic acid	3.1	0.19	5.5	N.D.	—
(+)-Catechin	3.4	6.50	0.18	0.10	0.9
PB1+PB3	1.5	>2.0	<0.26	0.041	1.0
Total PAC	41.4	0.27	52.4	0.016	68

PGG: Pentagalloylglucose

PB1: ProcyanidinB1

PB3: ProcyanidinB3

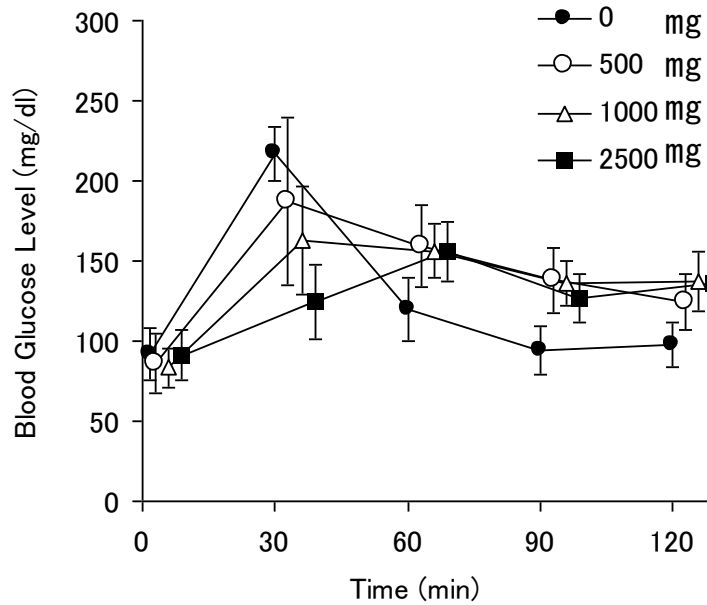
PAC: Proanthocyanidin

N.D.: not detected

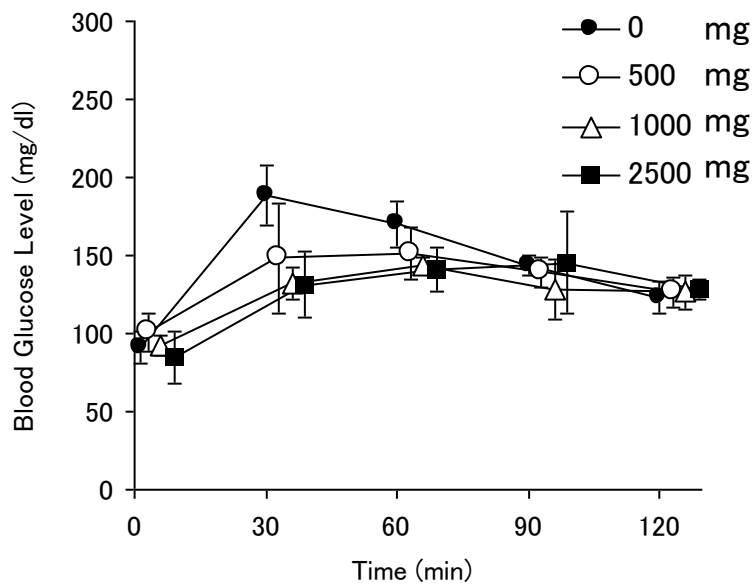


**3-2. Suppressive Effect on Blood Plasma Glucose Level (*in vivo*)**

EVENING PRIMROSE EXTRACT significantly suppressed the elevation of blood glucose level by sugar tolerance test in rats.



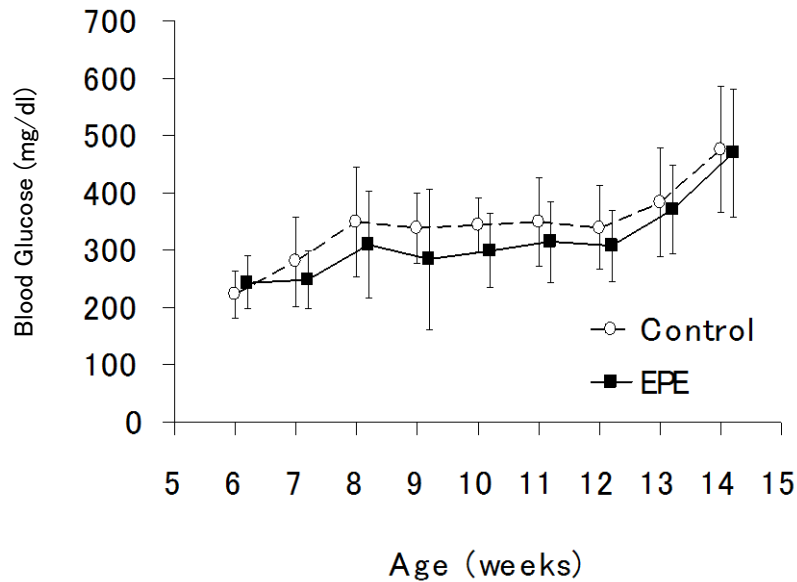
**Fig. 8 Sugar tolerance test (starch)**



**Fig. 9 Sugar tolerance test (sucrose)**

**3-3. Effect of Anti-Diabetic Activity (*in vivo*)**

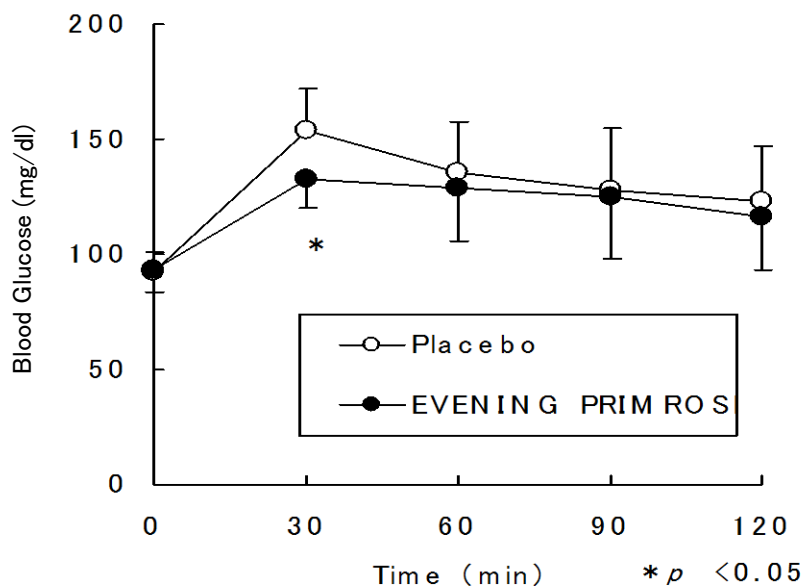
Hereditary diabetic mice (Type II, KK-Ay/Ta Jcl) were fed a test diet containing 1% of EVENING PRIMROSE EXTRACT for 6 weeks. As shown in Fig. 10, the elevation of blood plasma glucose level of the test group were almost entirely suppressed compared with control group.



**Fig. 10 Effect of feeding on diabetes in KK-Ay mice**

**3-4. Suppressive Effect on Blood Glucose Level in Normal Subjects**

The post-prandial increases in blood glucose levels were significantly suppressed by EVENING PRIMROSE EXTRACT.



**Fig. 11 Sugar tolerance test in normal human subjects**

### 3-5. Suppressive Effect on Blood Glucose Level in Diabetic Subjects

We examined effects of single intake of an extract of seeds of EVENING PRIMROSE EXTRACT on postprandial blood glucose levels on 18 subjects suffering mild diabetes and borderline diabetes. In the all subjects, the rises of postprandial blood glucose levels were reduced by EVENING PRIMROSE EXTRACT ingestion, compared to those when placebo taking. These results suggest that EVENING PRIMROSE EXTRACT may be a useful food ingredient for prevention of diabetes mellitus.

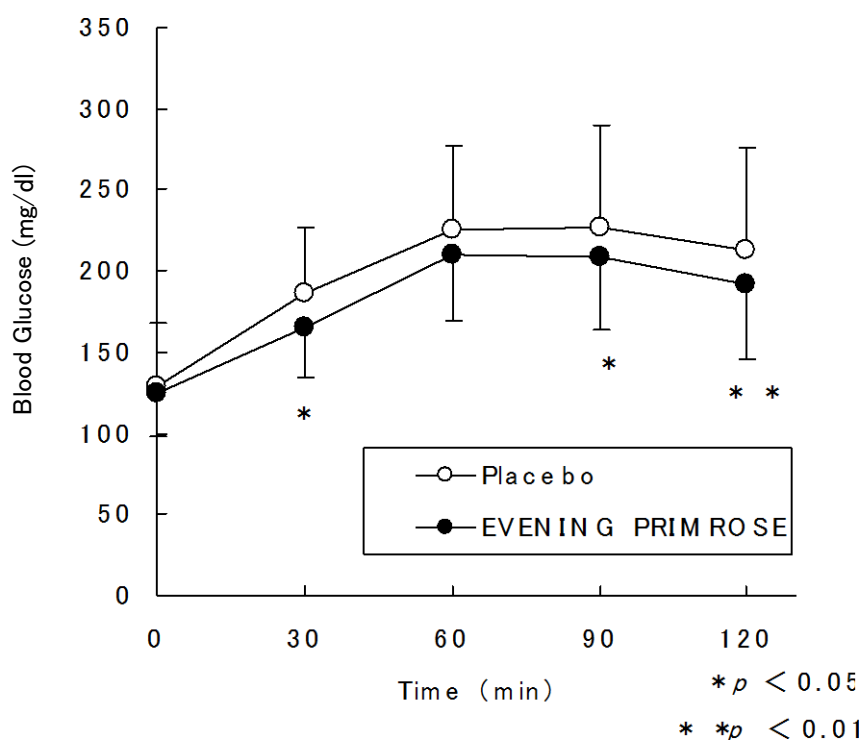


Fig. 12 Sugar tolerance test in diabetic patients

Table 2 Postprandial insulin levels in diabetic patients

	Insulin level ( $\mu$ U/dl) after diet loading					AUC (mg·hr/dl)
	0min	30min	60min	90min	120min	
Active	12.7 ± 9.8	22.4 ± 14.5	35.8 ± 33.1	43.7 ± 35.9	42.7 ± 31.4	39.4 ± 34.5
Placebo	13.3 ± 15.0	28.4 ± 23.9	40.0 ± 31.1	43.6 ± 28.1	50.1 ± 30.0	46.8 ± 31.2
Significance of effects	n.s	p<0.1	n.s	n.s	n.s	n.s

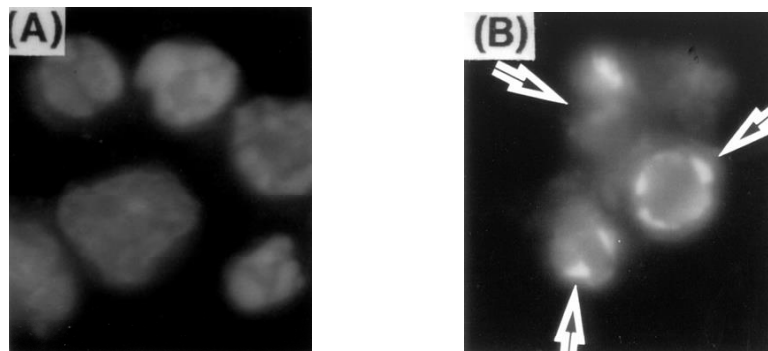
n.s : not significant

paired t test

AUC : Area under the Insulin elevation curve

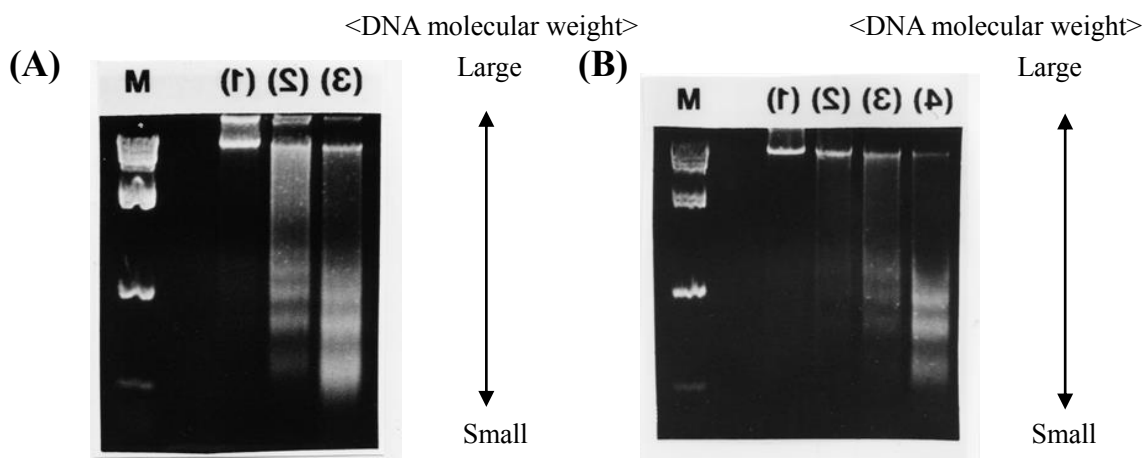
### 3-6. Induction Effects of Apoptosis in Human Stomach Cancer Cells by EVENING PRIMROSE EXTRACT

For the stomach cancer cells any a change was not found in controls. DNA fragmentation effect in cells was found by EVENING PRIMROSE EXTRACT addition.



**Fig. 13 Morphological changes of human stomach cancer cells**

(A) Non-treated cells (B) Cells treated with 2mg/ml EVENING PRIMROSE EXTRACT



**Fig. 14 DNA fragmentation effect in human stomach cancer cells**

Dose-dependency (A) and time-dependency (B) of induction of apoptosis by EVENING PRIMROSE EXTRACT in human stomach cancer cells.

(A) M: DNA marker

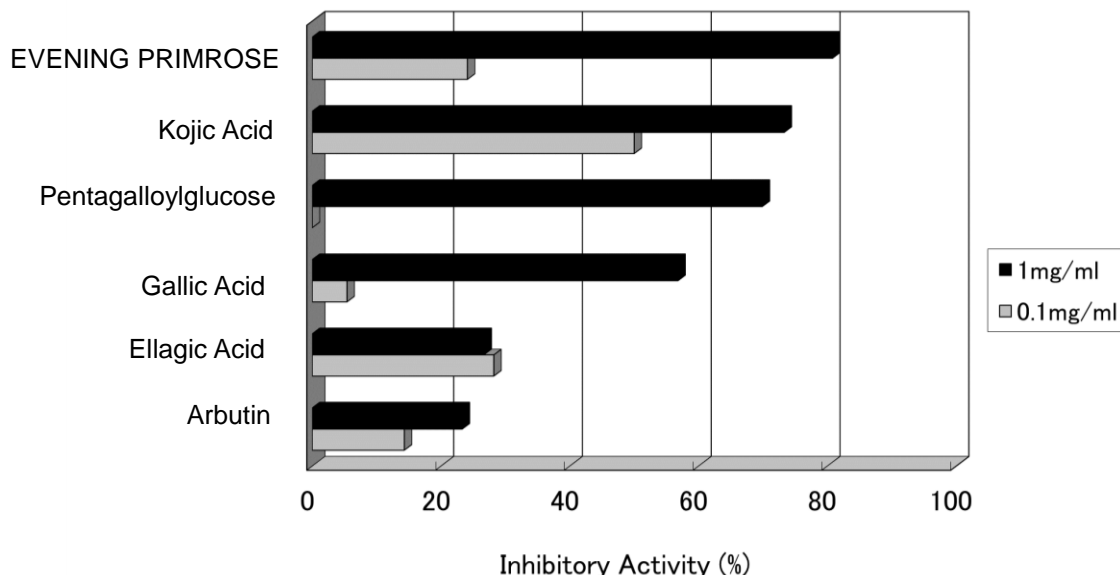
- (1) EVENING PRIMROSE (0mg/ml)
- (2) EVENING PRIMROSE (1mg/ml)
- (3) EVENING PRIMROSE (2mg/ml)

(B) M: DNA marker

- (1) EVENING PRIMROSE (0mg/ml)
- (2) EVENING PRIMROSE (2mg/ml) 1days
- (3) EVENING PRIMROSE (2mg/ml) 2days
- (4) EVENING PRIMROSE (2mg/ml) 3days

**3-7. Inhibition of Tyrosinase**

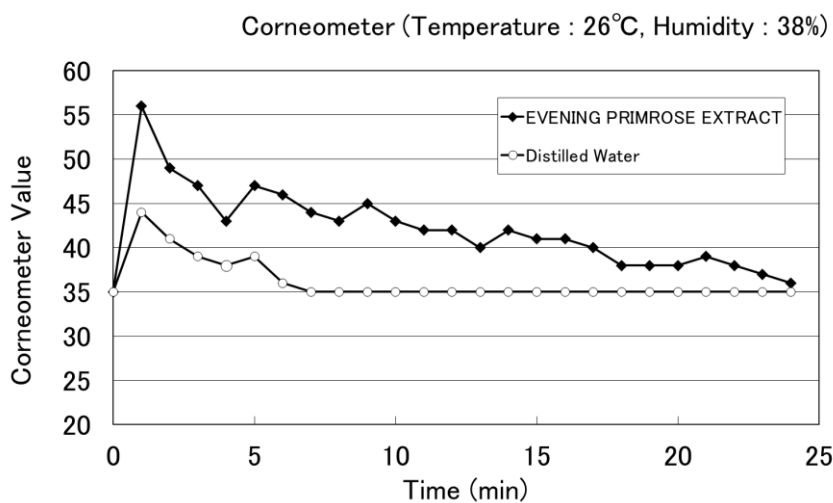
EVENING PRIMROSE EXTRACT inhibits tyrosinase activity and appears to be applicable to foods for whitening.



**Fig. 15. Tyrosinase Inhibitory Activity**

**3-8. Moisture Retention Test**

After direct topical application of samples to human skin, moisture retention ability was measured. When only distilled water was applied, the water content returned to the pre-application level after about 5 minutes. However, moisture was retained for more than 20 minutes after application of EVENING PRIMROSE EXTRACT.

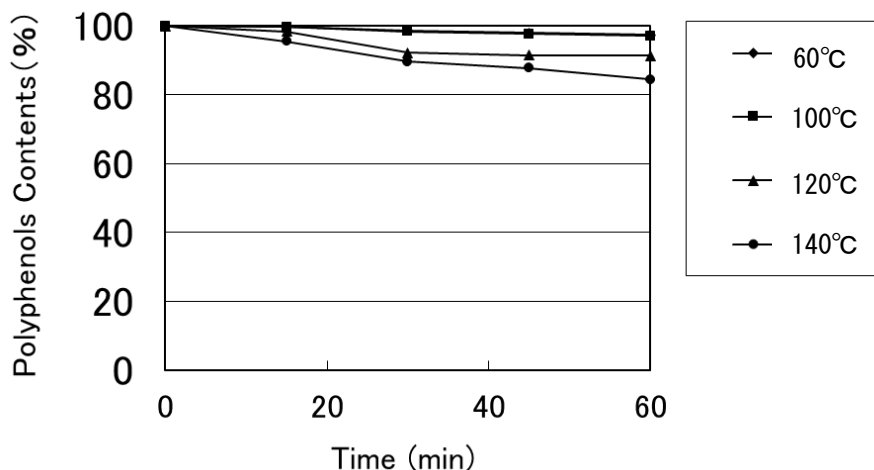


**Fig. 16 Moisture retention test of EVENING PRIMROSE EXTRACT**

## 4. Stability of EVENING PRIMROSE EXTRACT

### 4-1. Thermal Resistance

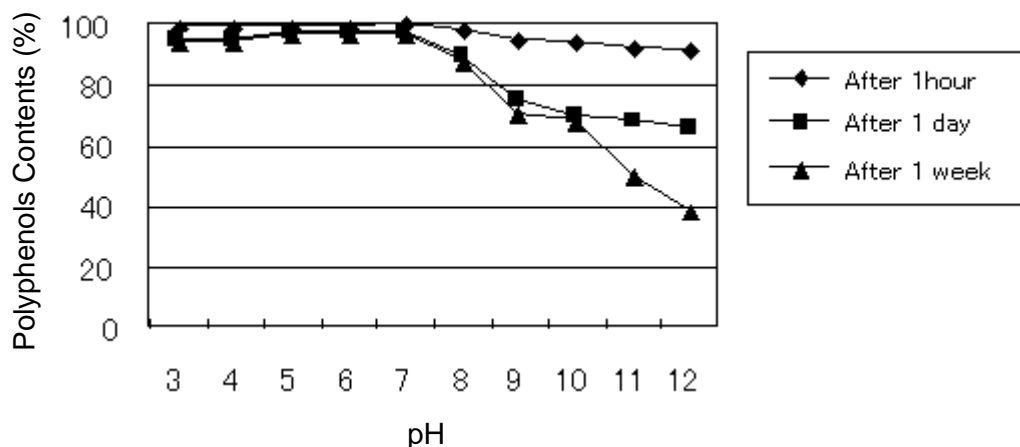
The pyrolysis of EVENING PRIMROSE EXTRACT dose not occurs at a normal food processing temperature for 60 minutes.



**Fig. 17 Heat-resistance of EVENING PRIMROSE EXTRACT**

### 4-2. pH Stability

Polyphenols in EVENING PRIMROSE EXTRACT remains stable specially at neutral to acid field of pH.



% of polyphenols in 0.05% solution (80% aqueous methanol)

**Fig. 18 Influence of pH on the polyphenols contents**

## 5. Daily Dosage of EVENING PRIMROSE EXTRACT

It is recommended to take more than 50mg/time or 100~500mg of total EVENING PRIMROSE EXTRACT-P per day.

## 6. Nutrition Information

Description	Evening primrose extract-P	Evening primrose extract-WSPS
Moisture	1.5g/100g	7.0g/100g
Protein	6.6g/100g	3.2g/100g
Fat	4.9g/100g	0.7g/100g
Ash	3.7g/100g	8.4g/100g
Carbohydrate	82.3g/100g	79.0g/100g
Energy	400kcal/100g	339kcal/100g
Dietary fiber	1.0g/100g	1.7g/100g
Sodium	14.4mg/100g	53mg/100g

Test trustee: Japan Food Research Center Foundation, Hokuriku Environmental Science Laboratory (WSPS)

Date of analysis: August 25, 2000, December 20, 20013 (WSPS)

Test No.: 300080303-002, 13D-0105701 (WSPS)

## 7. Acute Toxicity and Safety

### 7-1. Residual Agricultural Chemicals

Assayed Items	Results	Detection Limits	Assay Method
BHC	Not Detected	0.02ppm	Gas Chromatography
DDT	Not Detected	0.02ppm	Gas Chromatography
Aldrin	Not Detected	0.01ppm	Gas Chromatography
Dieldrin	Not Detected	0.01ppm	Gas Chromatography
Endrin	Not Detected	0.01ppm	Gas Chromatography
Parathion	Not Detected	0.05ppm	Gas Chromatography
Malathon	Not Detected	0.05ppm	Gas Chromatography

Tested by: Japan Food Research Center Foundation  
 Research results issue number: 300080303-001

### 7-2. Acute Toxicity

5 weeks old rats had been bred for two weeks after administering 5000mg/kg. No toxic effects were observed, thus the LD<sub>50</sub> (rat) is more than 5000mg/kg.

### 7-3. Chromosome Aberration Test

The chromosome aberration test was examined using cultured mammalian cells (CHL/IU).

EVENING PRIMROSE EXTRACT was considered to be non-induce for chromosome aberration.

#### **7-4. Reverse Mutation Assay “AMES TEST”**

Salmonella typhimurium strains and Escherichia coli strain were treated with suspensions of the material using the Ames plate incorporation method at five dose levels both with and without the addition of a rat liver homogenate metabolising system. No significant increases in the frequency of revertant colonies were recorded for any of the bacterial strains, with any dose of the test material (EVENING PRIMROSE EXTRACT), either with or without metabolic activation.

EVENING PRIMROSE EXTRACT was considered to be non-mutagenic under the conditions of this test.

#### **7-5. Micronucleus Test in Mice**

The micronucleus study was conducted using the intraperitoneal route in groups of seven mice (males) at the maximum tolerated dose (MTD) 200 mg/kg with 100 and 50 mg/kg as the two lower dose levels. Animals were killed 24 or 48 hours later, the bone marrow extracted and smear preparations made and stained. Polychromatic (PCE) and normochromatic (NCE) erythrocytes were scored for the presence of micronuclei.

There was no evidence of a significant increase in the incidence of micronucleated polychromatic erythrocytes in animals dosed with the test material (EVENING PRIMROSE EXTRACT) when compared to the concurrent vehicle control groups.

EVENING PRIMROSE EXTRACT was considered to be non-genotoxic under the conditions of the test.

#### **7-6. Cutaneous Sensitization (Clinical Research)**

EVENING PRIMROSE EXTRACT (5%) was always applied to the same area, either on the right or left side of the back, duly protected. Applications were conducted 3 times a week for 3 consecutive weeks, every other day, totaling 9 applications. The patch was removed by the researchers (50 people) 24 hours after it was applied. After 9 consecutive applications, a 10 day rest period followed, when no patches were applied. After this rest period, a single sample patch was applied either on the right or left side of the back (fresh area) where no patches had been applied before. The patch was removed by the researchers after 48 hours of contact with the skin, and reactions were noted 30 minutes after removal. As a result, EVENING PRIMROSE EXTRACT did not induce cutaneous sensitization and is considered as approved for topical use.



## 8. Practical Applications of EVENING PRIMROSE EXTRACT

Applications	Examples
Drinks	Tea, Blended tea, Protein shakes, and Nutritional drinks.
Dried Foods	Soup, Dried noodles, Seasoning, Pasta, Cereal, Oatmeal, and Topping for pizza.
Confectionery	Candies, Gum, Cookies, Pudding, Jelly, Yogurt, Chocolate
Snacks	Rice crackers, Cookies, and Wafers.
Fermentative Foods	Bread and Yogurt
Others	Health foods, Nutraceutical foods, and Functional foods
Cosmetics	Base cosmetics (Lotion, Milk, Cream, and so on) Body cosmetics (Body lotion, Body cream, and so on) Cleansing cosmetics (Soap, and so on) Makeup cosmetics (Lipstick, Foundation, and so on), <i>etc.</i>

## 9. Packaging

○EVENING PRIMROSE EXTRACT-P

○EVENING PRIMROSE EXTRACT-WSPS

5kg Interior packaging: aluminium-coated plastic bag  
Exterior packaging: 18L thin and cardboard box

○EVENING PRIMROSE EXTRACT-PC

4kg Interior packaging: aluminium-coated plastic bag  
Exterior packaging: 18L thin and cardboard box

○EVENING PRIMROSE EXTRACT-LC

5kg Interior packaging: cubic polyethylene container  
Exterior packaging: cardboard box

## 10. Storing Method

Store in cool, dry place. Avoid humidity.

## **11. Indication of Evening Primrose Extract**

○ EVENING PRIMROSE EXTRACT-P  
Evening Primrose Seed Extract

○EVENING PRIMROSE EXTRACT-WSPS  
Evening Primrose Seed Extract


○EVENING PRIMROSE EXTRACT-PC  
INCI name: Oenothera Biennis (Evening Primrose) Seed Extract

○EVENING PRIMROSE EXTRACT-LC  
INCI name: Butylene Glycol (and) Water (and)  
Oenothera Biennis (Evening Primrose) Seed Extract

※Please consult your nation's regulations.

## 12. International patent

### United States Patent



US006932990B2

(12) **United States Patent**  
Konishi et al.

(10) **Patent No.:** US 6,932,990 B2  
(45) **Date of Patent:** Aug. 23, 2005

### European Patent

(19)  europäisches Patentamt  
European Patent Office  
Office européen des brevets



(11) **EP 1 312 374 B1**

(12) **EUROPEAN PATENT SPECIFICATION**

(45) Date of publication and mention of the grant of the patent:  
10.09.2008 Bulletin 2008/37

(51) Int Cl.:  
A61K 36/185 (2006.01) A61K 31/05 (2006.01)  
A61P 3/10 (2006.01)

(21) Application number: 01954345.3


(86) International application number:  
PCT/JP2001/006436

(22) Date of filing: 26.07.2001

(87) International publication number:  
WO 2002/009734 (07.02.2002 Gazette 2002/06)

### China Patent

[19] 中华人民共和国国家知识产权局



[12] 发明专利说明书  
专利号 ZL 01816661. X

[51] Int. Cl.  
A61K 36/185 (2006.01)  
A61K 131/00 (2006.01)  
A61K 31/192 (2006.01)  
A61K 31/352 (2006.01)  
A61K 31/7024 (2006.01)  
A61K 31/7048 (2006.01)

[45] 授权公告日 2006年8月9日

[11] 授权公告号 CN 1268351C

## Methods of Experiments

### Fig. 1 Contents of total polyphenols

Samples dissolved in methanol were assayed by using Folin-Denis Method.

### Fig. 3 SOD-like activity of several plant extracts

Samples dissolved in ethanol were assayed by using SOD-Test Wako.

### Fig. 4 DPPH radical scavenging activity of several plant extracts

DPPH radical scavenging activity was assayed by using DPPH method.

### Fig. 6 Inhibitory activity of EVENING PRIMROSE EXTRACT and several plant extracts on $\alpha$ -amylase

$\alpha$ -amylase from human saliva was purchased from Wako Pure Chemical Ind., Ltd., Japan.  $\alpha$ -Amylase activity was assayed by using Amylase-Test Wako. In advance, sample were diluted with ethanol and 10  $\mu$ l of the sample solution was added to the assay system. The inhibitory activity (%) was calculated by using the following equation: 100- (% of Control).

### Fig. 7 Inhibitory activity of EVENING PRIMROSE EXTRACT and several plant extracts on $\alpha$ -glucosidase

Acetone powder of rat intestine was homogenized in phosphate buffer and centrifuged. The supernatant was used as a crude enzyme solution containing  $\alpha$ -glucosidase.  $\alpha$ -Glucosidase activity was determined by the fluorescent assay with 4-methylumbelliferyl- $\alpha$ -D-glucopyranoside as substrate. The inhibitory activity (%) was calculated as described above.

### Fig. 8 Sugar tolerance test (Starch)

### Fig. 9 Sugar tolerance test (Sucrose)

After 24h without foods, six weeks old rats were loaded 2g/kg of soluble starch (or sucrose) and 0~2500mg/kg of EVENING PRIMROSE EXTRACT. Every 30 minutes, blood samples were bled from the tail vein into heparinized capillary tubes, and the plasma glucose level were measured by Glucose B-Test Wako.

### Fig. 10 Effect of feeding on diabetes in KK-Ay mice

Five weeks old male mice (Type II diabetes, KK-Ay/Ta Jcl, Clea Japan, Inc., Osaka) were fed freely giving water and basal diets (CE-2) for one week to accustom them to the surroundings. The mice were divided into two groups. One group (control group) was fed on basal diets, another group (experimental group) was fed on basal diets containing 1% of EVENING PRIMROSE EXTRACT for 8 weeks. The plasma glucose levels were assayed every week.

**Fig. 11 Sugar tolerance test in normal human subjects**

The blood glucose levels of the subjects after fasting for 11 hours were in the range of 70-110mg/dl. Subjects took meal (200g boiled rice) with or without EVENING PRIMROSE EXTRACT-P (200mg) at 9:00 am. Blood was withdrawn from finger at 0, 30, 60, and 90 min and measured blood glucose using a portable analyzer equipped glucose sensor.

**Fig. 12 Sugar Tolerance Tests in Type II Diabetic Patients**

Eighteen adult type II diabetic patients whose fasting blood glucose levels were ranged 110 to 180 mg/dl were obliged to the tests. A crossover diet loading study using a placebo as a control drug was conducted. Subjects were fasted after 9:00 pm the day before this study. The subjects took meal (200g boiled rice) with placebo capsules or capsules containing EVENING PRIMROSE EXTRACT at 8:50 am. Blood was withdrawn from finger at 0, 30, 60, and 90 min and measured blood glucose and serum insulin levels.

**Fig. 13 Morphological Changes of Human Gastric Cancer Cells**

Human gastric cancer cells were grown in RPMI 1640 medium with 10% heat-inactivated fetal bovine serum at 37°C in 95% air-5% CO<sub>2</sub> atmosphere of saturated humidity. After 3-day cultivation in the presence of EVENING PRIMROSE EXTRACT (2 mg/ml), the cellular morphology was examined by a epifluorescence microscope equipped with a CCD camera digital imaging system.

**Fig. 14 DNA Fragmentation Effect in Human Gastric Cancer Cells**

Human gastric cancer cells were cultured as described in the Fig. 13 legend. Exponentially growing cells were placed at the initial density of  $5 \times 10^5$  cells/ml in culture flasks. After cultivation in the presence of vehicle, EVENING PRIMROSE EXTRACT for 1, 2, or 3 days, the cells were collected and rinsed. DNA was extracted from the cells as described previously. Equivalent amounts of DNA were put into the well of 2% agarose gel.

**Fig. 15 Tyrosinase Inhibitory Activity**

EVENING PRIMROSE EXTRACT was dissolved in distilled water to make solutions of a series of concentrations. After addition of the extract to mushroom-derived tyrosinase solution, the reaction from L-tyrosine to dopa quinone was measured in terms of the absorbance of dopa quinone.

**Fig. 16 Moisture Retention Tests of EVENING PRIMROSE EXTRACT**

EVENING PRIMROSE EXTRACT was dissolved in distilled water to make 1% solution. One drop of the solution was topically applied to the medial side of the left brachium. The drop was spread over a 2-cm square area and absorbed to the skin. One minute later, the solution on the surface was wiped out with soft paper. Another one minute later, the epidermal moisture content was measured using a corneometer CM825. The atmospheric condition was 26°C with relative humidity of 38%.



PRODUCT STANDARD

PRODUCT NAME

**EVENING PRIMROSE EXTRACT-P**

(FOOD)

The product is extracted with aqueous ethanol from the seeds of evening primrose (*Oenothera biennis*). It contains more than 60.0 % of polyphenol.

<u>Appearance</u>	Brown-red powder with slight unique smell	
<u>Polyphenols</u>	Min. 60.0 %	(Folin-Denis method)
<u>Loss on Drying</u>	Max. 5.0 %	(1 g, 105°C, 2 hours)
<u>Purity Tests</u>		
(1) Heavy Metals	Max. 10 ppm	(The Japanese Standards for Food Additives)
(2) Arsenic	Max. 1 ppm	(Standard Methods of Analysis in Food Safety Regulation)
<u>Standard Plate Counts</u>	Max. $1 \times 10^3$ cfu/g	(Analysis for Hygienic Chemists)
<u>Moulds and Yeasts</u>	Max. $1 \times 10^2$ cfu/g	(Analysis for Hygienic Chemists)
<u>Coliforms</u>	Negative	(Analysis for Hygienic Chemists)
<u>Composition</u>		
	<u>Ingredient</u>	<u>content</u>
	Evening Primrose Seed Extract	100%



PRODUCT STANDARD

PRODUCT NAME

**EVENING PRIMROSE EXTRACT-WSPS**

(FOOD)

The product is extracted with water from the seeds of evening primrose (*Oenothera biennis*). It contains more than 50.0 % of polyphenol. This product is water soluble.

<u>Appearance</u>	Brown-red powder with slight unique smell.	
<u>Content of Polyphenols</u>	Min. 50.0 %	(Folin-Denis Method)
<u>Loss on Drying</u>	Max. 5.0 %	(1 g, 105°C, 2 hours)
<u>Purity Tests</u>		
(1) Heavy Metals	Max. 10 ppm	(The Japanese Standards for Food Additives)
(2) Arsenic	Max. 1 ppm	(Standard Methods of Analysis in Food Safety Regulation)
<u>Standard Plate Counts</u>	Max. $1 \times 10^3$ cfu/g	(Analysis for Hygienic Chemists)
<u>Moulds and Yeasts</u>	Max. $1 \times 10^2$ cfu/g	(Analysis for Hygienic Chemists)
<u>Coliforms</u>	Negative	(Analysis for Hygienic Chemists)

Composition

<u>Ingredient</u>	<u>Content</u>
Evening Primrose Seed Extract	100%



**EVENING PRIMROSE EXTRACT Ver. 6.1 JT**

PRODUCT STANDARD

PRODUCT NAME

**EVENING PRIMROSE EXTRACT-PC**

(COSMETIC)

The product is extracted with aqueous ethanol from the seeds of evening primrose (*Oenothera biennis*). It contains more than 60.0% of polyphenols.

<u>Appearance</u>	Brown-red powder with slight unique smell	
<u>Polyphenols</u>	Min. 60.0 %	(Folin-Denis Method)
<u>Loss on Drying</u>	Max. 5.0 %	(1 g, 105°C, 2 hours)
<u>Purity Tests</u>		
(1) Heavy Metals	Max. 10 ppm	(The Second Method)
(2) Arsenic	Max. 1 ppm	(The Third Method)
<u>Standard Plate Counts</u>	Max. $1 \times 10^2$ cfu/g	(Analysis for Hygienic Chemists)
<u>Moulds and Yeasts</u>	Max. $1 \times 10^2$ cfu/g	(Analysis for Hygienic Chemists)
<u>Coliforms</u>	Negative	(Analysis for Hygienic Chemists)
<u>Composition</u>		
	<u>Ingredient</u>	<u>Content</u>
	Oenothra Biennis (Evening Primrose) Seed Extract	100%

Ref: *The Japanese Standard of Quasi-Drug Ingredients*.



PRODUCT STANDARD

PRODUCT NAME

**EVENING PRIMROSE EXTRACT-LC**

(COSMETIC)

This product is extracted with aqueous 1,3-butylene glycol from the seeds of evening primrose (*Oenothera biennis*).

<u>Appearance</u>	Brown-red liquid with slight unique smell	
<u>Certification Test</u>	(1) The solution (1→5) of this product turns dark blue to black when ferric chloride is added. (Tannins)	
	(2) The solution (1→5) of this product turns the color off when potassium permanganate is added. (Polyphenols)	
<u>Polyphenols</u>	Min. 0.5 %	(Folin-Denis Method)
<u>pH</u>	4.0 ~ 5.5	(10 % solution)
<u>Specific Gravity</u>	1.020~1.060	(25 °C)
<u>Purity Tests</u>		
(1) Heavy Metals	Max. 10 ppm	(The Second Method)
(2) Arsenic	Max. 1 ppm	(The Third Method)
<u>Standard Plate Counts</u>	Max. $1 \times 10^2$ cfu/g	(Analysis for Hygienic Chemists)
<u>Moulds and Yeasts</u>	Max. $1 \times 10^2$ cfu/g	(Analysis for Hygienic Chemists)
<u>Coliforms</u>	Negative	(Analysis for Hygienic Chemists)

Composition

<u>Ingredients</u>	<u>Contents</u>
Butylene Glycol	69 %
Water	30 %
Oenothera Biennis (Evening Primrose) Seed Extract	1 %
Total	100 %

Ref: *The Japanese Standards of Quasi-Drug Ingredients.*

**ORYZA OIL & FAT CHEMICAL CO., LTD.**, striving for the development of the new functional food materials to promote your health.



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

**ORYZA OIL & FAT CHEMICAL CO., LTD.**

**Numata 1, Kitagata, Ichinomiya, Aichi, 493-8001 JAPAN**

**Tel: +81-586-86-5141**

**Fax: +81-586-86-6191**

**URL/http: //www.oryza.co.jp/**

**E-mail: info@oryza.co.jp**

**Tokyo Office**

**5F in Daitokyo Building**

**1-24-10 Kanda-Suda, Chiyoda, Tokyo, 101-0041 JAPAN**

**Tel: +81-3-5209-9150**

**Fax: +81-3-5209-9151**

**E-mail: oryza-tokyo@okbnet.ne.jp**

**Change:** Revise to Nutrition Information (WSPS) (p14)

\* The contents of this catalogue may be changed without prior notice.

Established Date: November 1, 2001

Revised Date: January 6, 2014