



ORYZA OIL & FAT CHEMICAL CO., LTD.

MAQUI BERRY EXTRACT

Prevention of eye diseases
Anti-inflammatory · Anti-oxidant
Anti-diabetes
Prevention of atherosclerosis
Promotion of hair growth
Anti-photo aging of skin

■ **MAQUI BERRY EXTRACT-P35**
(WATER-SOLUBLE POWDER, FOOD)

■ **MAQUI BERRY EXTRACT-J**
(CONCENTRATED JUICE, FOOD)

■ **MAQUI BERRY EXTRACT-PC35**
(WATER-SOLUBLE POWDER, COSMETICS)

■ **MAQUI BERRY EXTRACT-LC**
(WATER-SOLUBLE LIQUID, COSMETICS)



ORYZA OIL&FAT CHEMICAL CO., LTD.

Ver. 2.0 YK

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1. Introduction

Maqui Berry (*Aristotelia chilensis*) or commonly known as Chilean Wineberry is a deep purple berry belongs to the Elaeocarpaceae family (Fig. 1). It grows in the temperate rain forests of Chile known as the Patagonia Region. The Maqui berries are consumed by the Mapuche, the indigenous of southern Chile (Fig. 2) as fermented beverage for stamina and energy. Historically, the nutritious value of Maqui Berry contributed to the ability of the Mapuche in fighting the Incas and Spaniards. Maqui Berry has been regarded as a symbol of health by the Mapuche due to its strong healing power. Traditionally, it is believed to heal wounds, relieve sore throats and as analgesic.

Today, Maqui Berry is regarded as “super fruit” due to its superior antioxidant properties. Its deep purple colour signifies the naturally rich content of anthocyanidin. In addition, Maqui Berry has been reported to have the highest ORAC value, thus confirming its superior antioxidant activities.



Fig. 1 Maqui berry



Fig.2 Mapuche

Oryza Oil & Fat Chemical Co., Ltd. has prompted further research and development studies on the extract of Maqui Berry. Results showed that Maqui Berry Extract is preventive from several degenerative conditions of the eyes, promotes hair growth and prevent photo-ageing. In addition, Maqui Berry Extract demonstrated superior protective effect on eye optic nerves compared with blueberry and blackcurrant.

The active component of Maqui Berry Extract is anthocyanins, delphinidin is the strongest among the anthocyanins. The rich content of delphinidin of Maqui Berry Extract compared with Bilberry and Blackcurrant, Maqui Berry Extract is excellent in preventing degenerative conditions of eyes and other health functions.

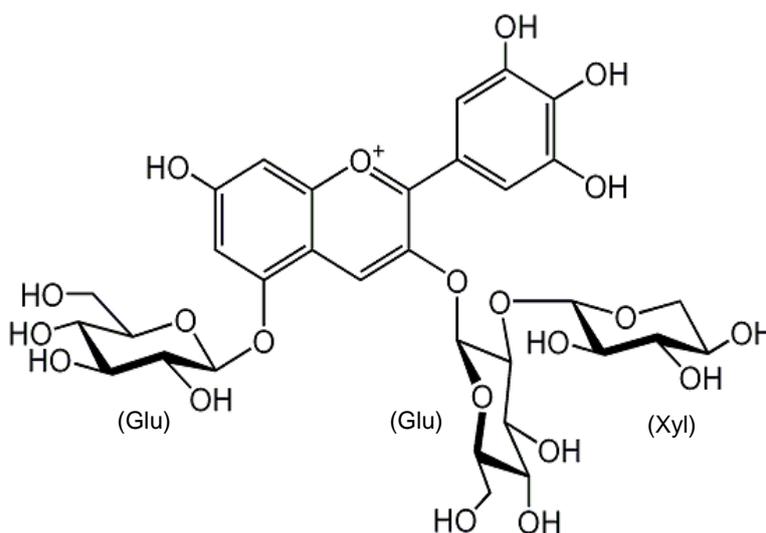


Fig. 3: Structure of Delphinidin, one of the specific anthocyanin of Maqui Berry Extract (Delphinidin-3-sambubioside-5-glucoside)

After bilberry and black currant, Maqui Berry Extract is a new generation natural ingredient for eye care, anti-aging and hair growth.

2. Functional Components of Maqui Berry Extract

The antioxidant effect of Maqui Berry Extract is based on the rich content and variety of anthocyanins. Among the anthocyanins, delphinidin content is the highest in Maqui Berry (more than blackcurrant and bilberry) and it is the most potent antioxidant of Maqui Berry Extract (Fig. 4).

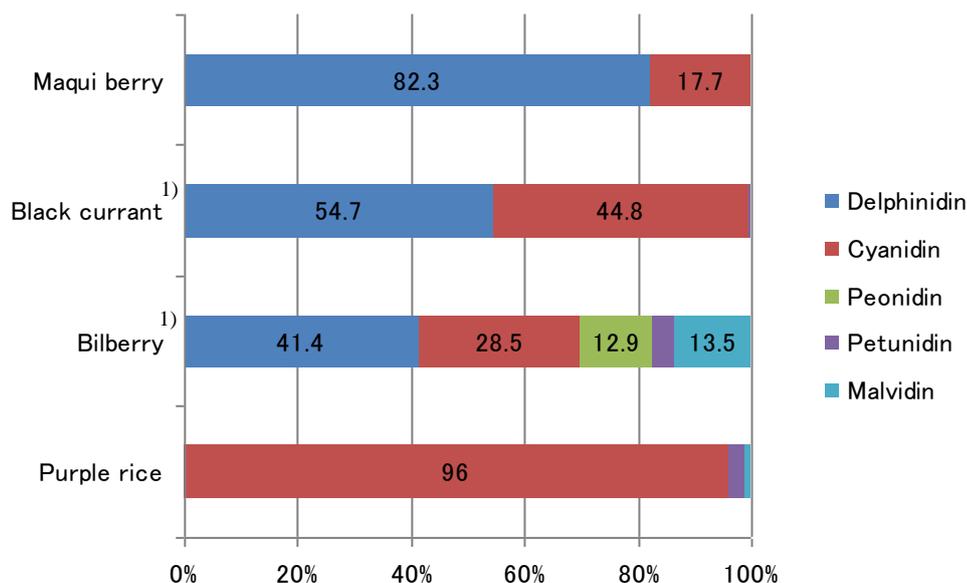
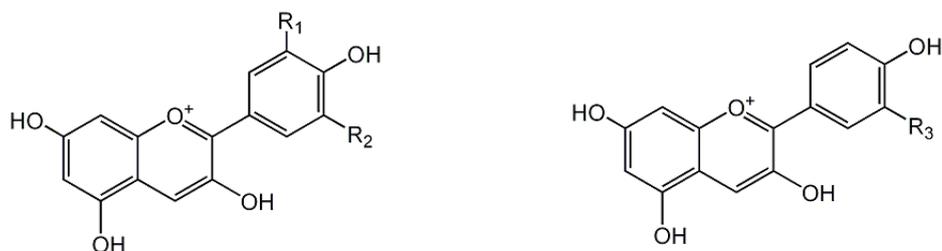


Fig. 4 Composition of anthocyanins among different type of berries



Name	R ₁	R ₂
Delphinidin	OH	OH
Petunidin	OMe	OH
Malvidin	OMe	OMe

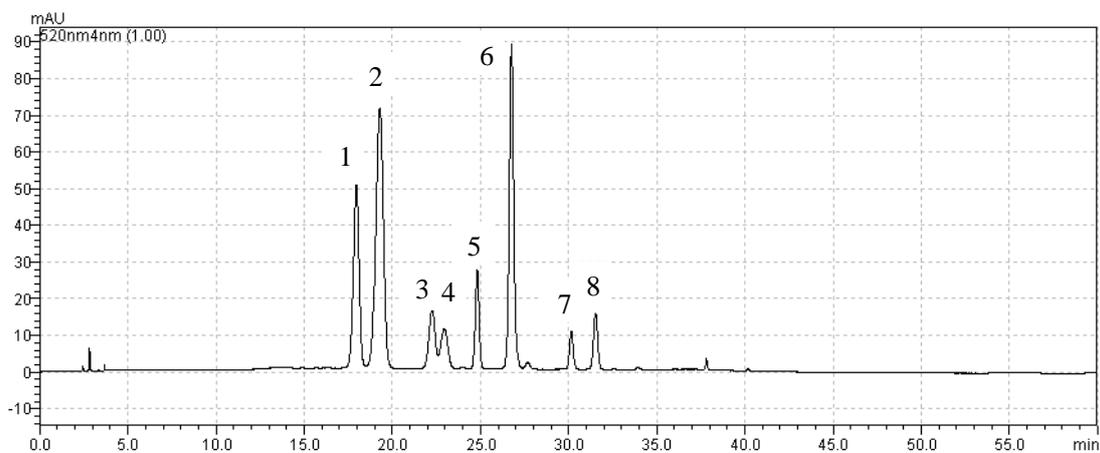
Name	R ₃
Cyanidin	OH
Peonidin	OMe

Fig. 5 Selected anthocyanins and their substitutions

Delphinidin has the most phenolic hydroxyl group (-OH) substitution among the anthocyanins which contributed to the powerful antioxidant activity with health promoting effect.

1) Marja P K *et al*, *J Sci Food Agric*, 83, 1403-11 (2003)

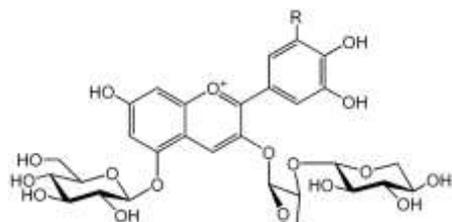
Analysis on the anthocyanins of Maqui Berry Extract, 8 distinctive types of anthocyanins have been identified (Fig. 6).



Peak No.	Composition	MBE content (%)	Peak No.	Composition	MBE content (%)
1	Delphinidin-3-samb-5-glu	22.0	5	Delphinidin-3-samb	6.3
2	Delphinidin-3,5-glu	34.8	6	Delphinidin-3-glu	19.2
3	Cyanidin-3-samb-5-glu	7.6	7	Cyanidin-3-samb	2.3
4	Cyanidin-3,5-glu	4.8	8	Cyanidin-3-glu	3.0

samb: sambubioside, glu: glucoside

Fig. 6 HPLC chromatogram of Maqui Berry Extract

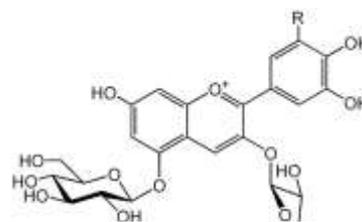


Peak No.1: R=OH:

Delphinidin-3-sambubioside-5-glucoside

Peak No. 3: R=H:

Cyanidin-3- sambubioside-5-glucoside

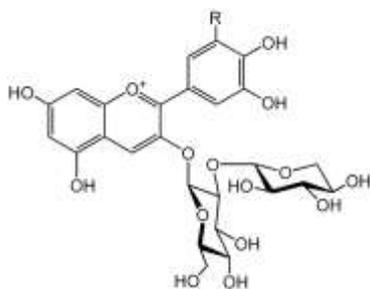


Peak No.2: R=OH:

Delphinidin-3,5-glucoside

Peak No. 4: R=H:

Cyanidin-3,5-glucoside

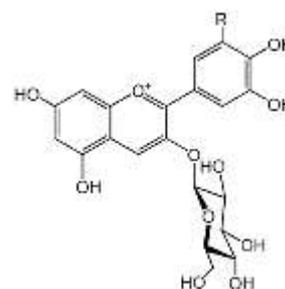


Peak No. 5: R=OH:

Delphinidin-3-sambubioside

Peak No. 7: R=H:

Cyanidin-3- sambubioside



Peak No. 6: R=OH:

Delphinidin-3-glucoside

Peak No. 8: R=H:

Cyanidin-3-glucoside

Fig. 7 Chemical Structure of anthocyanins of Maqui Berry Extract

3. Prevention of Eye Diseases

(A jointed research with Prof Hara of Gifu Pharmaceutical University)

Age-related macular degeneration (AMD) is a medical condition of the macula of the eye retina leading to vision impairment or loss of vision. AMD is the major cause of blindness affecting the aging population (age > 50) in Europe and the USA. AMD is also the 4th leading disease in Japan with increasing incidents being reported in recent years.

On the other hand, retinitis pigmentosa (RP) is a hereditary degenerative eye disorder where photoreceptor cells of the eye retina progressively deteriorate and eventually blindness. Patients may experience night blindness, tunnel vision, photophobia, ocular pain and discomfort. Retinitis pigmentosa has emerged as the 3rd leading cause of blindness in Japan.

Nevertheless, effective treatment for above mentioned eye disorders is yet to be available. Alternatively, preventive measure is important in suppressing the progression of disorders, e.g. preventing light damage to the photoreceptor cells of the eye retina.

Inhibition of visible light-induced damage of photoreceptor cells

An experiment was conducted to evaluate the effect of Maqui Berry Extract (MBE) on photoreceptor cells (isolated from mouse retina; 661W) upon irradiation of visible light. Results showed that Maqui Berry Extract at concentration as low as 1µg/mL significantly inhibited light-induced damage on photoreceptor cells of retina (Fig. 8). Besides, light-induced apoptosis of photoreceptor cells was observed (Fig. 9 & Fig. 10).

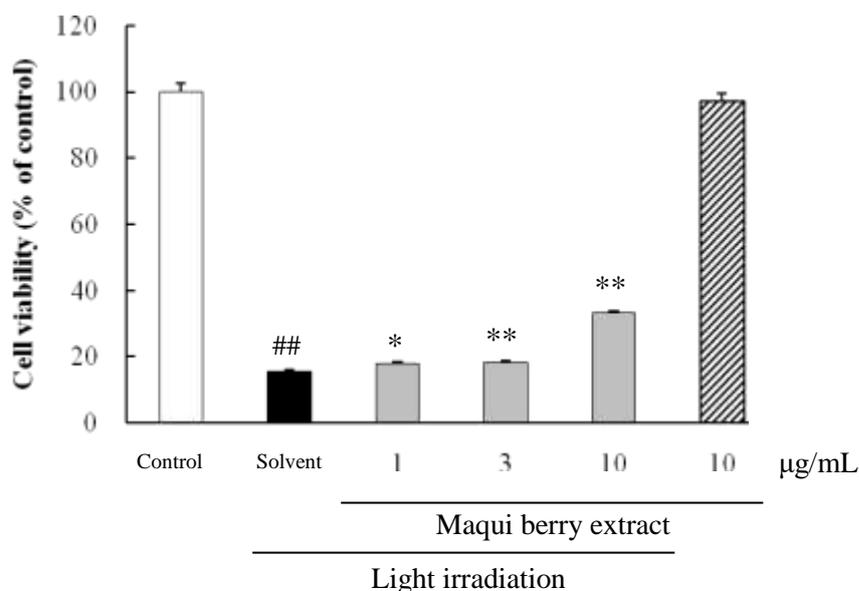


Fig. 8 The Effect of Maqui Berry Extract on light-induced damage on photoreceptor cells of eye retina
 Solvent: Cell culture medium
 Mean ± SE, n=6, ## $p < 0.01$ vs control,
 * $p < 0.05$, ** $p < 0.01$ versus vehicle.

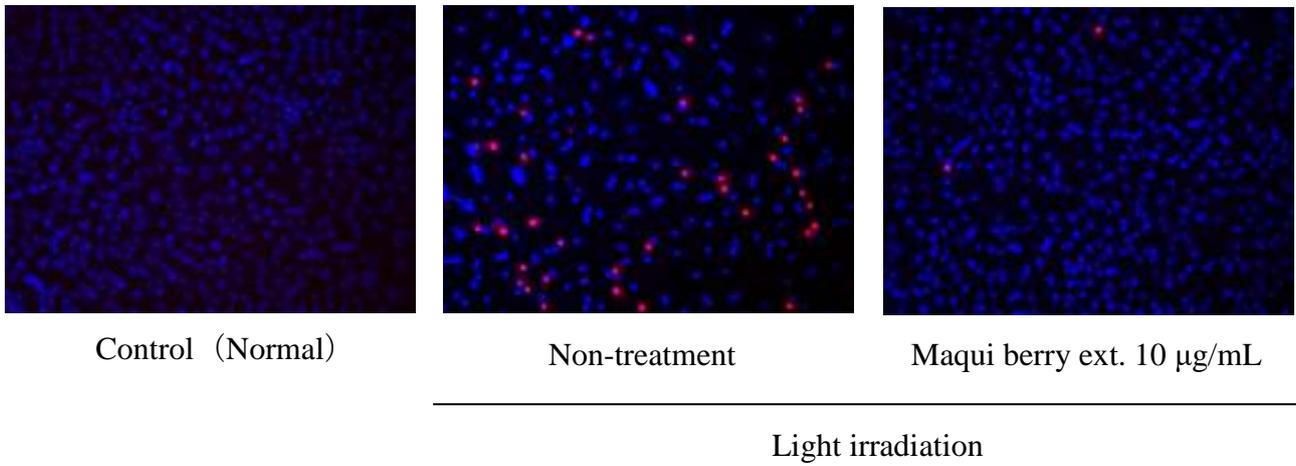


Fig 9 The Effect of Maqui Berry Extract on light-induced apoptosis of photoreceptor cells of eye retina
 Red dots indicate light-induced apoptosis. light-induced apoptosis is significantly observed in sample containing Maui berry extract.

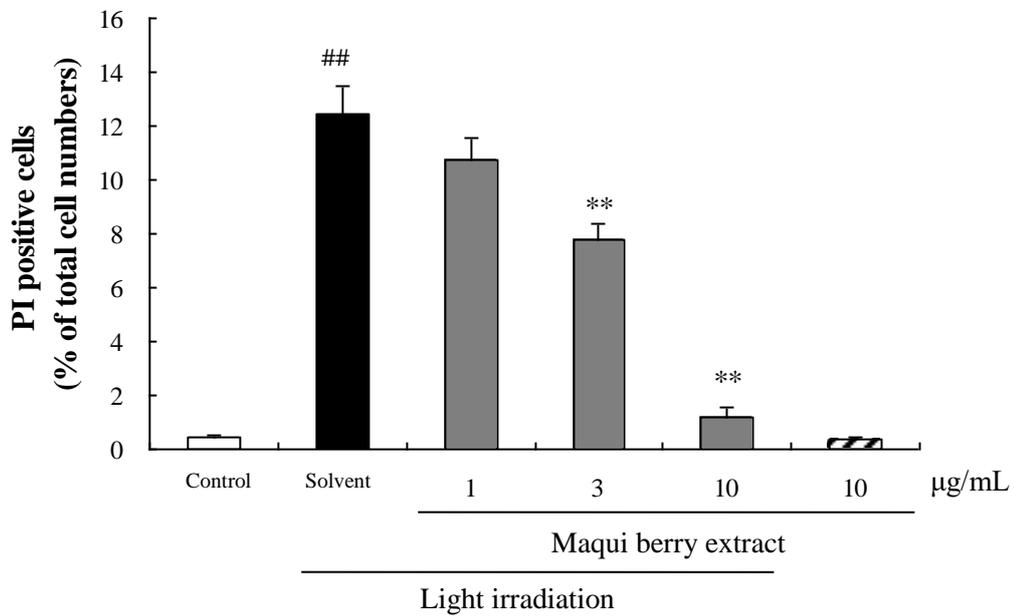


Fig. 10 The Effect of Maqui Berry Extract on light-induced apoptosis of photoreceptor cells of eye retina
 Solvent: Cell culture medium
 Mean \pm SE, n=6, ## $p < 0.01$ vs control, ** $p < 0.01$ versus vehicle.

Previously, we mentioned that Maqui Berry Extract is rich in anthocyanins, particularly, delphinidin-3,5-glucoside and delphinidin-3-sambubioside which are the characteristic functional compounds of Maqui Berry. The effect of delphinidin-3,5-glucoside and delphinidin-3-sambubioside on light-induced damage of photoreceptor cells of eye retina was studied. As showed in Fig. 11, both functional compounds of Maqui Berry Extract significantly inhibited light-induced apoptosis of photoreceptor cells. Therefore, delphinidin-3,5-glucoside and delphinidin-3-sambubioside are the functional compounds of Maqui Berry Extract.

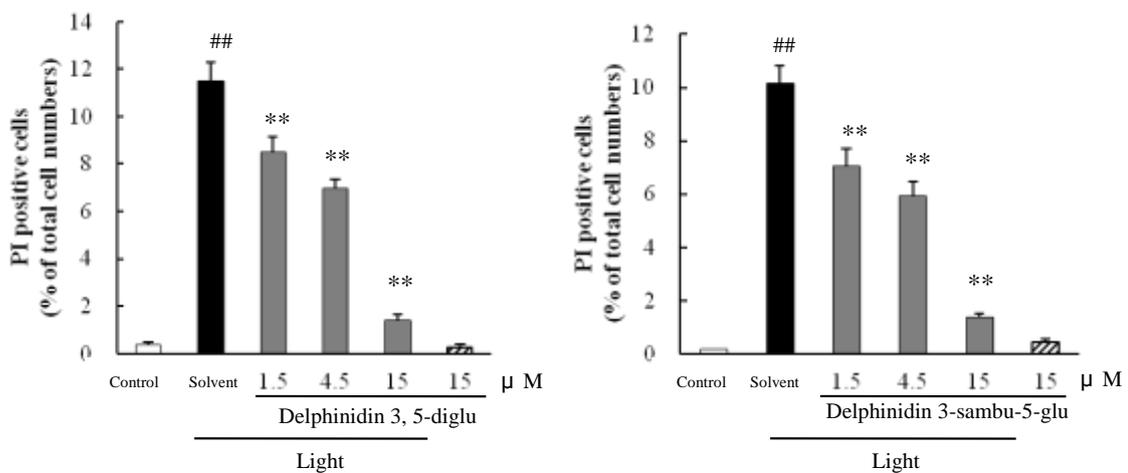


Fig. 11 The effect of delphinidins on light-induced apoptosis of photoreceptor cells
Solvent: Cell culture medium
Mean ± SE, n=6, ## $p < 0.01$ vs control, ** $p < 0.01$ versus vehicle.

Among various types of berries, Maqui Berry Extract demonstrate the most potent protective effect on photoreceptor cells of the eye retina (Fig. 12).

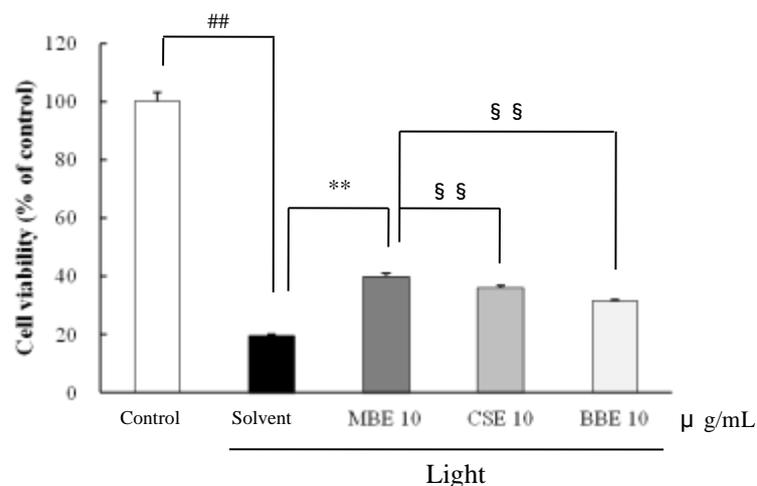


Fig. 12 The protective effect of various berries on photoreceptor cells
Solvent: Cell culture medium, MBE: Maqui berry extract,
CSE: cassis

Upon light irradiation on photoreceptor cells, reactive oxygen species (ROS) is increased due to oxidative stress. Meanwhile, the genetic expression of p38 – mitogen-activated protein kinase is up-regulated in response to stress stimuli brought by ultraviolet irradiation resulting in apoptosis (Fig. 13). Maqui Berry Extract and its functional compound, delphinidin inhibit the production of ROS in photoreceptor cells upon light-irradiation (Fig. 14 & 15) while down-regulating the genetic expression of p38 preventing apoptosis of photoreceptor cells (Fig. 16).

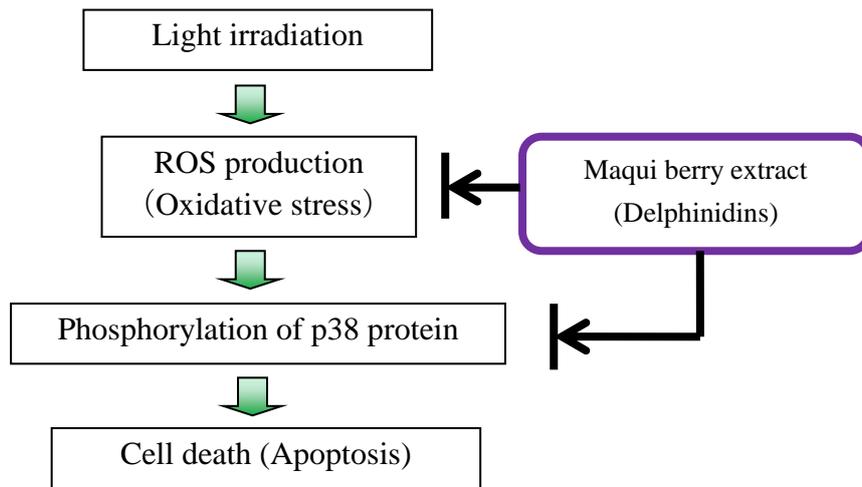


Fig. 13 The Mechanism of Maqui Berry Extract & Delphinidin in the prevention of UV-induced apoptosis

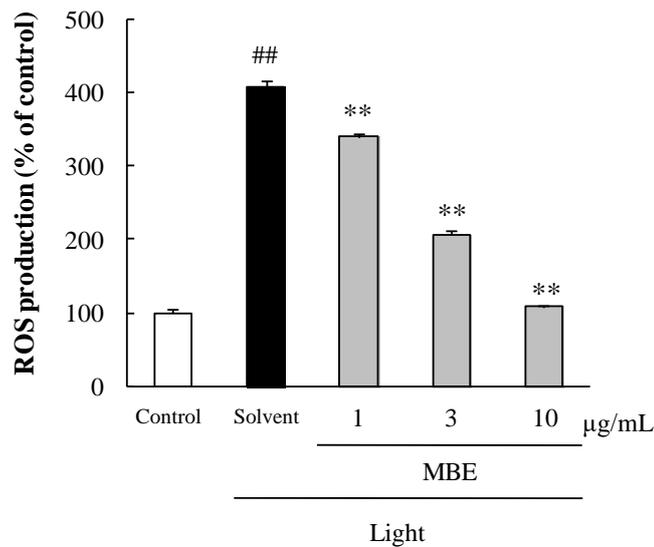


Fig. 14 The Effect of Maqui Berry Extract on the production of ROS upon light-irradiation on photoreceptor cells.

Mean ± SE, n=6, ## $p < 0.01$ vs control, ** $p < 0.01$ versus vehicle.

Solvent: Cell Culture medium

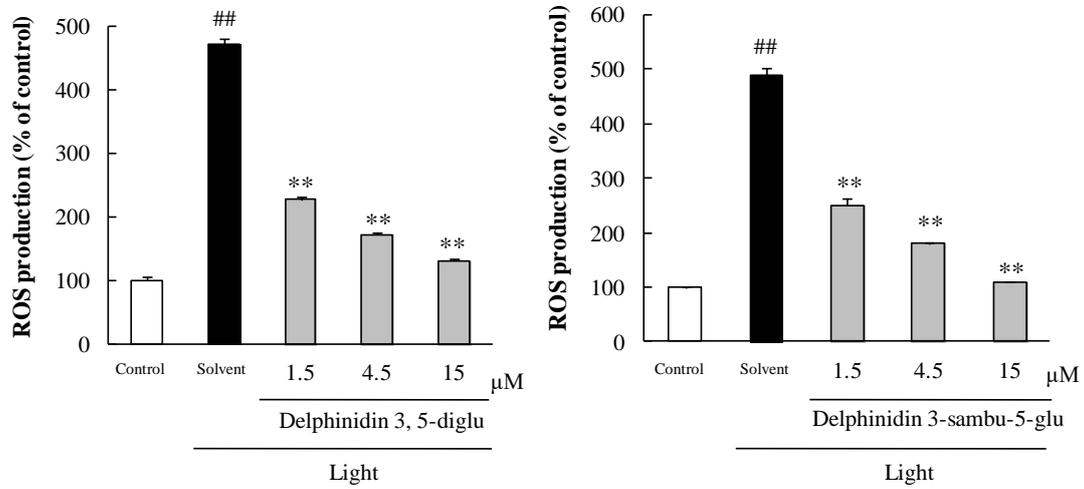


Fig. 15 The Effect of delphinidin on the production of ROS upon light-irradiation on photoreceptor cells.

Mean ± SE, n=6, ## $p < 0.01$ vs control, ** $p < 0.01$ versus vehicle.)

Solvent: Cell culture medium

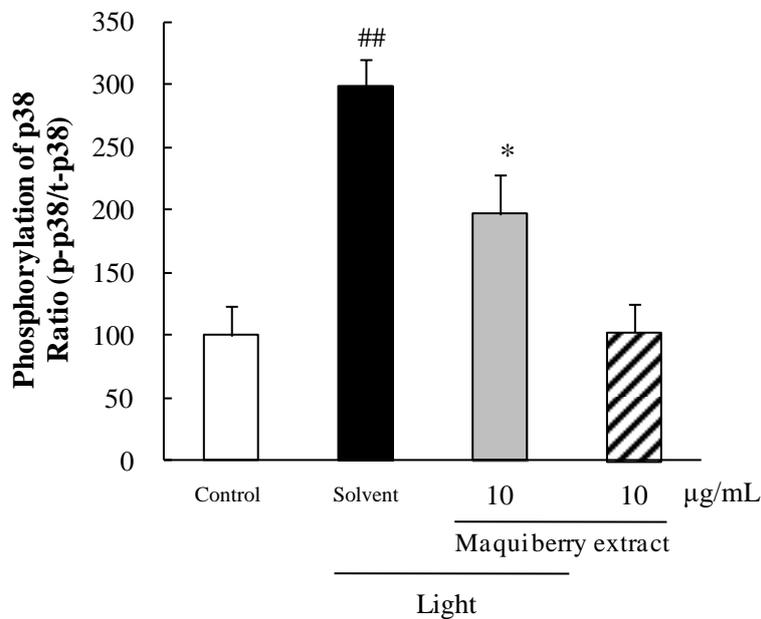


Fig. 16 The Effect of Maqui Berry Extract on the genetic expression of p38 upon light-irradiation on photoreceptor cells

Solvent: Cell culture medium

Mean ± SE, n=6, ## $p < 0.01$ vs control, ** $p < 0.01$ versus vehicle.

4. Anti-dry eye Effect

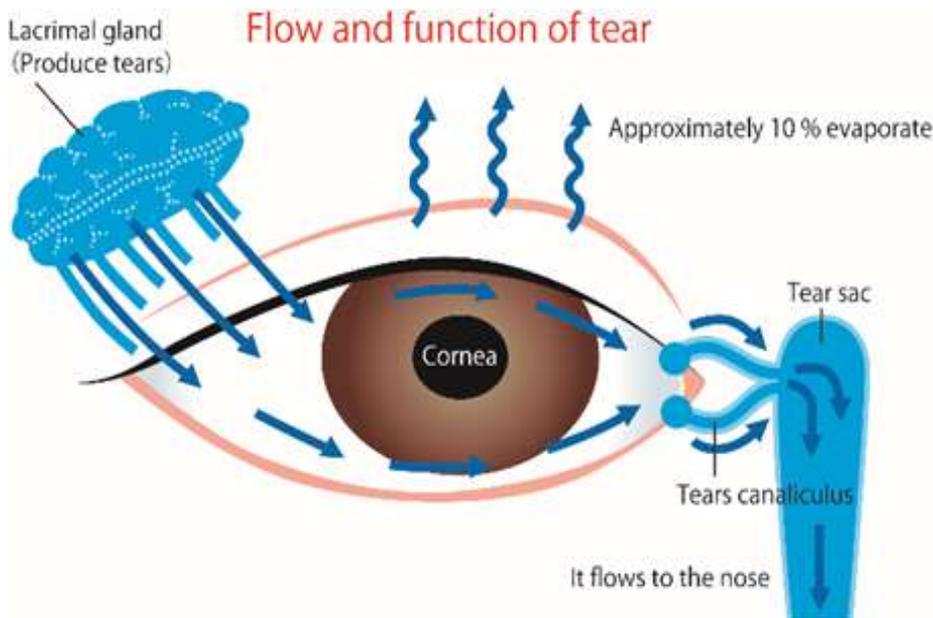
ORYZA OIL & FAT CHEMICAL CO., LTD found that maqui berry extract ameliorates dry eye related symptoms by joint study department of ophthalmology, Keio University of Medicine.

Composition of tear

- ①Water : Moisturizing eyes
- ②Protein : It works for retaining moisture on the surface of cornea
- ③Lipids: Prevents from evaporation of moisture

Function of tear

- ①It carries oxygen and nutrition to the surface of the eyes
- ②It protects eyes against bacteria
- ③It eliminates dust



※Blink produces tear. In general, we blink 20 times a minute. But it decreases in four times when we get absorbed in playing games, and the surface of eyes dries.

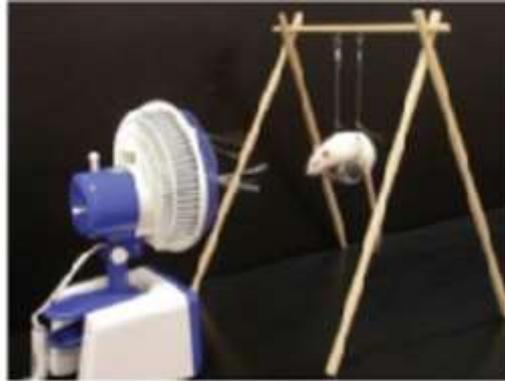
What's dry-eye

- The condition that the health of surface of eyes, cornea and conjunctival health are spoiled by decrease, or changing of tears
- Asthenia accompanied with visual impairments
- The numbers of patients are approximately 24 million in Japan
- It occurs by complex causes such as aging, stress, and excessive use of information computer

Animal dry eye modes

①Rat model

The rat was placed on a swing 7.5 hours/day for 10 days. This experiment was repeated under the dry condition and a constant air flow (temperature $23 \pm 2^\circ \text{C}$, humidity $25 \pm 5\%$, 2 – 4 m/sec air flow).



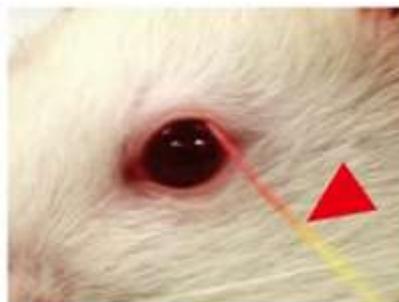
②Mouse model

The mouse was constrained in a centrifuge tube and send air to mouse face (0.5-1.0m/sec air flow) for 4 hours/day.



Measurement of tear secretion

Cotton yarn was placed on the mouse external canthus for 15 seconds, and discolored in the length of red part change from yellow by permeation of tear fluid was measured. The length was gauged with the precision of the 0.5 mm.

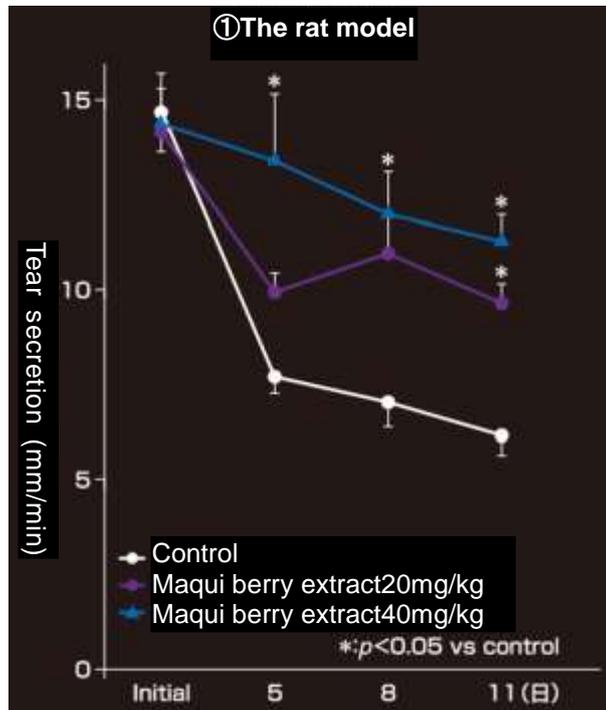


Features of the model

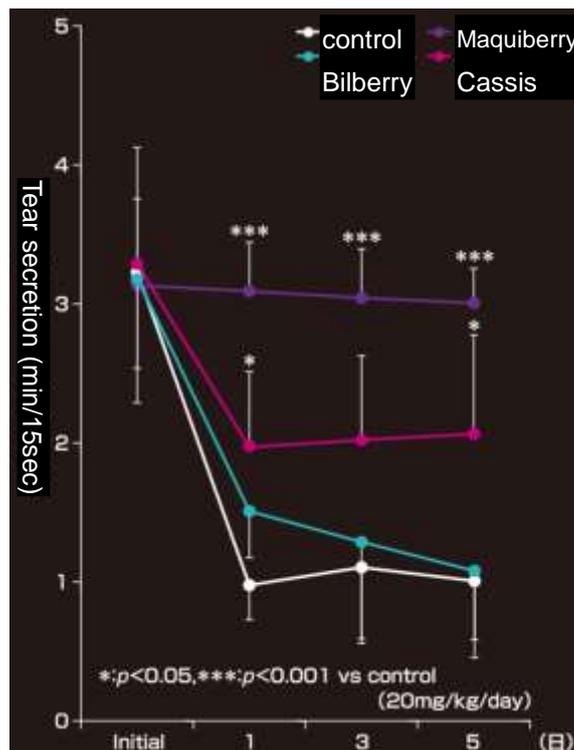
- Corneal epithelium disorder is induced by a decrease of tear secretion and aggravation of tear circulation as well as human dry-eye.
- Number of blink decreases to one third when a rat is a swing as well as human is working VDT

Dry eye improving action

The oral administration of maqui berry extract dose dependently suppressed the reduction in tear secretion in a rat dry eye model. Thus, this findings suggest that the maqui berry extract has anti dry eye effect.

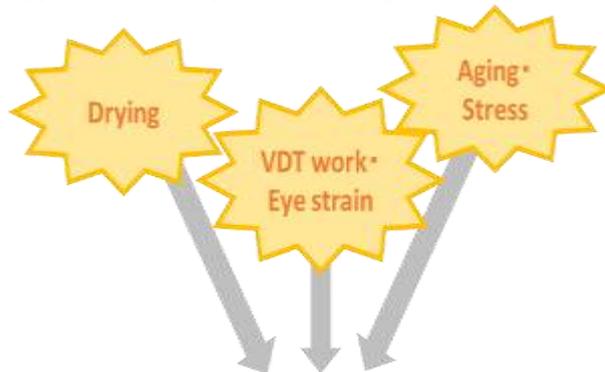


In addition, Maqui berry extract showed the strongest effect compared with the other potential eye care materials including bill berry and cassis.



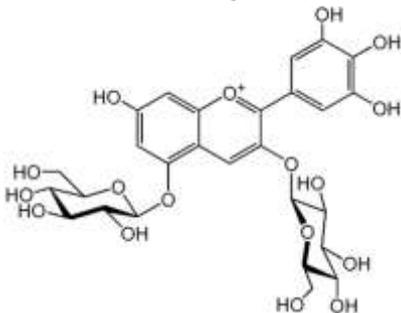
Dry-eye improving action (mechanism)

Increase of reactive oxygen species (ROS) in lacrimal gland cells is reported to be responsible factor of dry-eye. So we assessed the effective due of maqui berry extract and components on oxidative stress in lacrimal gland cells. As a result, maqui berry extract and only delphinine 3, 5-O-diglucoside (D3S5G) among its constituents suppressed ROS production significantly with strongest inhibitory effect.



Increase of reactive oxygen species (ROS) in lacrimal gland cell (Oxidative stress)

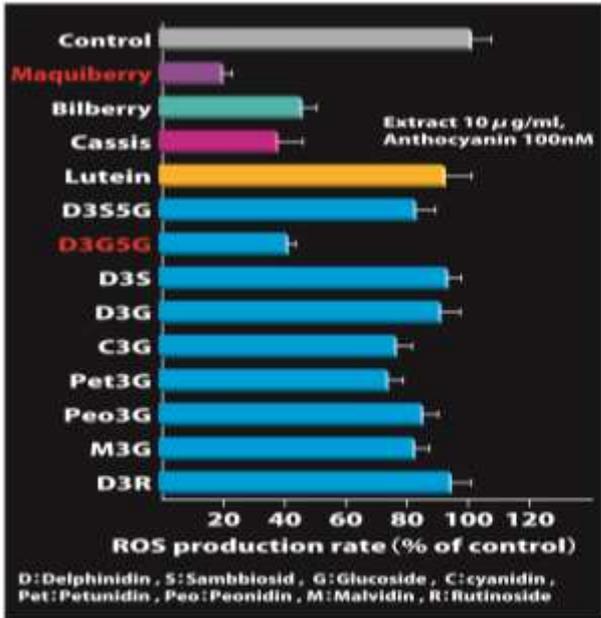
A decrease of tear secretion
Developed dry eye



Delphinine 3-O-glucoside (D3G5G) is special active ingredient in maqui berry extract.

Method

- The excited lacrimal gland tissue of SD Rat was homogenized using mil mixer
- Each material and DCFH-DA were incubated for 1hour at 37°
- The intensity of fluorescence measurement (ex 485nm, em528nm)



Published papers ; Nakamura S., Tanaka J., Imad Foods 10, 346-354 (2014).

Clinical trials. (Dry-eye improving action)

【Test Method】

(1) Test materials

The subjects took Maqui berry extract-P35 (30 mg or 60 mg) once a day for two months.

(2) Study participants

The volunteers were screened by “Dry Eye-related Quality of life Score” and nominals subjects with this value of 20 or higher . We investigated 13 healthy subjects with moderately dry eyes using schemer test. The subjects were randomly assigned to one of two dosage groups.

1) Maqui berry extract-P35 (30 mg/day)

The group consisting of 7 subjects (2 women, 5 men, aged range from 28 to 60 years) received a tablet of maqui berry extract-P35 (30mg) a day in the morning.

2) Maqui berry extract-P35 (60 mg/day)

The second group consisting of 6 subjects (2 women, 4 men, aged from 25 to 60 years) received a tablet of maqui berry extract-P35 (60mg) a day in the morning.

(3) Test Method

Before and after one and two months of treatment, subjects were refrained to eat except water from 9pm on the before test day. Subjects were gathered in an examination room under controlled temperature and humidity to measure blood pressure. Then they were required to fill out the DEQS question and measured tear fluid secretion.

Tear fluid secretion was measured in both eyes simultaneously with Schirmer test strip, “Zone-Quick” phenol red thread manufactured by Showa Yakunin Kako Co Ltd, (Tokyo, Japan) for fifteen seconds without anesthesia.

(4) Statistical analysis

Statistical comparison was carried using a one way analysis of variance (ANOVA), followed by a Student’s *t*-test. A value of $p < 0.05$ was considered indicative for statistical significance. Data are presented as mean±standard error.

(5) Ethical codes

The study was performed according to the Helsinki Declaration.

【Results and Discussion】

Daily dosing of maqui berry extract-P35 (30 mg) increased tear fluid secretion significantly after 30 day - treatment. Furthermore, after a further 30 day - treatment of 30 mg extract daily, the amount of tear fluid still remained significantly higher than initial value. (Figure 17)

Improvement effect was bound on score of DEQS after a month treatment of 30 mg extract daily. After a further 30 day - treatment of 30 mg extract daily, significantly higher than initial value. (Figure 18)

Daily dosing of maqui berry extract-P35 (60 mg) increased tear fluid secretion significantly. (Figure 19)

Improvement effect was confirmed on the score of DEQS after a month treatment of 60 mg extract daily. After further 30 day - treatment of 60 mg extract daily, the score was still significantly higher than initial value. (Figure 20)

From the above results, maqui berry extract-P35 is expected to improve dry eye.

Published papers ; Hitoe, S., J. Tanaka, and H. Shimoda. "MaquiBright™ extract significantly increases tear fluid production and ameliorates dry eye-related symptoms in a clinical pilot trial." *Panminerva medica* 56.3 Suppl 1 (2014): 1-6.

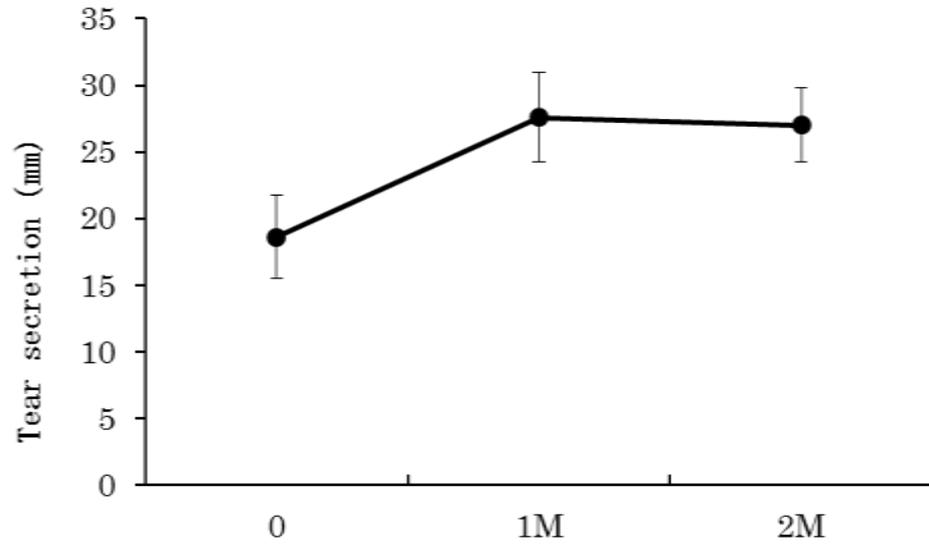


Figure 17. Effect of maqui berry extract-P35 (30 mg/day) a tear secretion (mean \pm standard error , n=7 , *: $p < 0.05$)

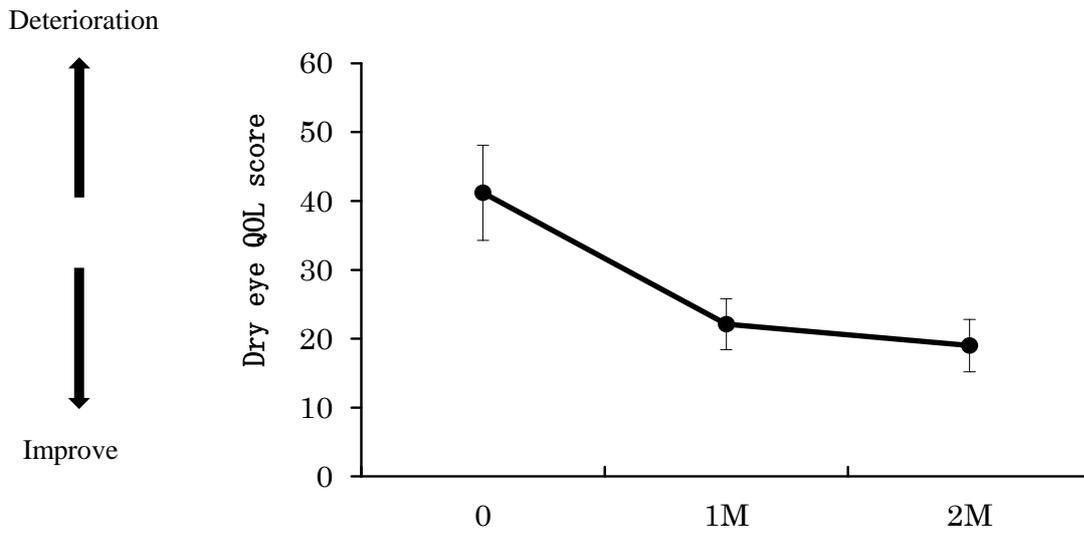


Figure 18. Effect of maqui berry extract-P35 (30 mg/day) on dry eye QOL score (mean \pm standard error , n=7 , **: $p < 0.01$)

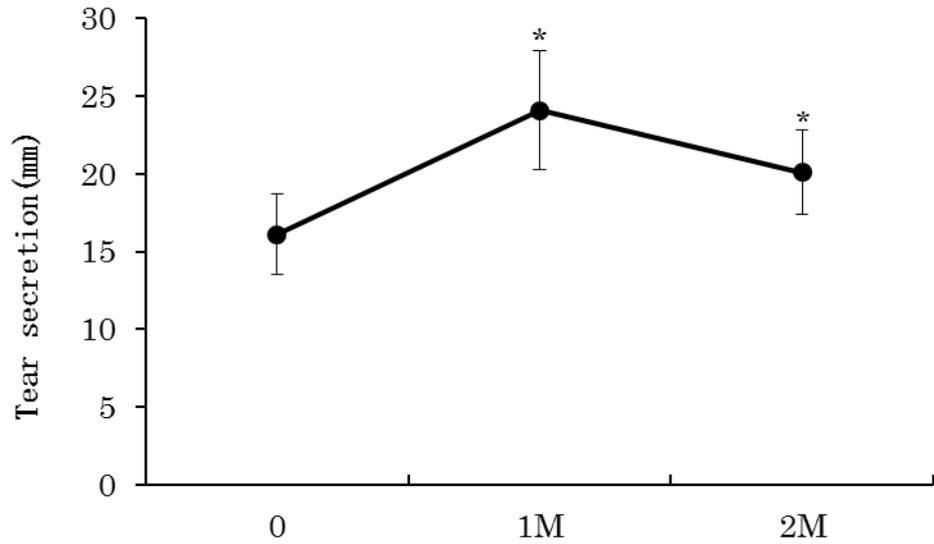


Figure 19. Effect of maqui berry extract-P35 (60 mg/day) a tear secretion (mean±standard error , n=6 , *: $p < 0.05$)

Deterioration

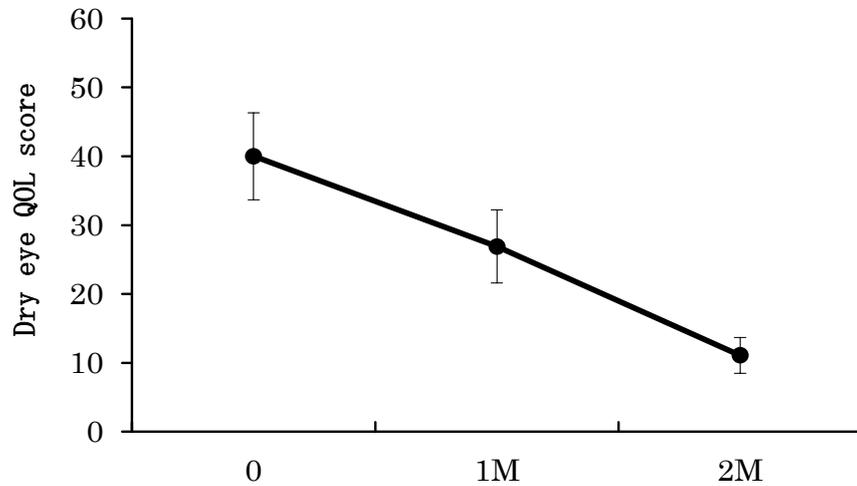


Figure 20. Effect of maqui berry extract-P35 (60 mg/day) on dry eye QOL score (mean±standard error , n=6 , *: $p < 0.05$)

【Bibliography】

- 1) DRY EYE SOCEITY HP (http://www.dryeye.ne.jp/qol_monshin/index.html)
- 2) Sakane Y., et al JAMA Ophthalmol. 131(10), 1331-1338, 2013.

5. Anti-oxidant Effect

(1) ORAC Value

Based on the measuring standard of antioxidant capacity of ORAC (Oxygen Radical Absorbance Capacity), Maqui Berry was identified to have the highest ORAC value among different fruits, e.g. the antioxidant capacity of Maqui Berry is 20 times stronger than lemon, 3.5 times stronger than blackcurrant and 2.9 times stronger than wild blueberry (Fig. 21).

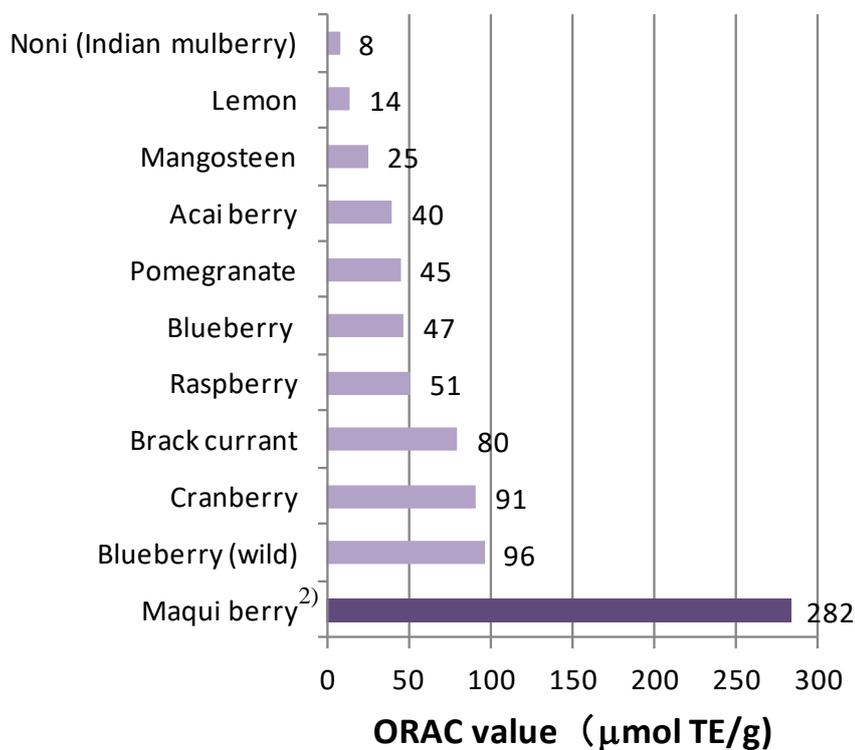


Fig. 21 ORAC value of different variety of raw fruits
quoted reference 3)

2) Brunswick Laboratories (USA)

3)“USDA Database for the Oxygen Radical Absorbance Capacity (ORAC) of Selected Foods, Release 2”

The antioxidant capacity (ORAC) of Maqui Berry Extract-P35 was analyzed by Brunswick Laboratories (USA). Table 1 below showed the total ORAC of Maqui Berry Extract-P35 is greater than 26,000 $\mu\text{mol TE/g}$.

Table 1 Total ORAC of Maqui Berry Extract-P35 ⁴⁾

Reactive Oxygen Species	Activity Value ($\mu\text{mol TE/g}$)
Peroxide radical	4,611
Hydroxyl radical	14,372
Peroxyl nitrite	835
Superoxide anion	5,699
Singlet oxygen	1,245
Total ORAC	26,762

4) Brunswick Laboratories (USA)

(2) Antioxidant Activity - in vitro

(a) Superoxide anion radical scavenging activity

The effect of Maqui Berry Extract and its anthocyanins (e.g. delphinidins) on superoxide anion radical scavenging activity was studied. As illustrated in Fig. 22, the superoxide anion radical scavenging activity of delphinidin is the strongest among the anthocyanins in which the IC_{50} required is the lowest.

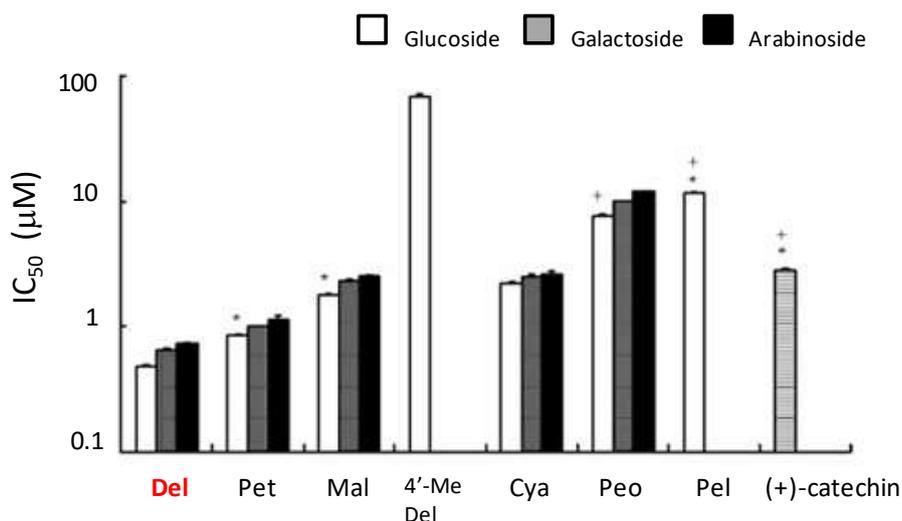


Fig. 22 The radical scavenging activity of various anthocyanin on superoxide anion ⁵⁾

Del: Delphinidin, Pet: Petunidin, Mal: Malvidin,
 Cya: Cyanidin, Peo: Peonidin, Pel: Pelargonidin,
 4'-Me Del: 4'-Methyldelphinidin

(b) Peroxyl nitrite scavenging activity

Similarly, delphinidin demonstrated the most potent radical scavenging activity on peroxy nitrite radical with highest inhibition rate on nitration (Fig. 23).

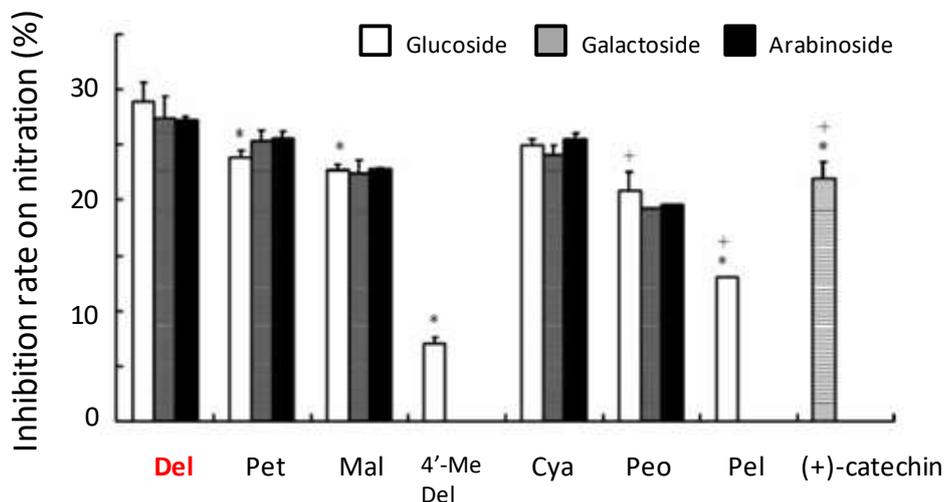


Fig. 23 The radical scavenging activity of various anthocyanin on peroxy nitrite⁵⁾
 Del: Delphinidin, Pet: Petunidin, Mal: Malvidin,
 Cya: Cyanidin, Peo: Peonidin, Pel: Pelargonidin,
 4'-Me Del: 4'-Methyl delphinidin

(c) Inhibition on lipid peroxidation

The effect of anthocyanins on lipid peroxidation was examined in-vitro (using artificial membrane lipid bilayer model). Results showed that anthocyanins strongly inhibited lipid peroxidation by Fe²⁺ ion, particularly, delphinidin demonstrated powerful inhibitory effect (Fig. 24).

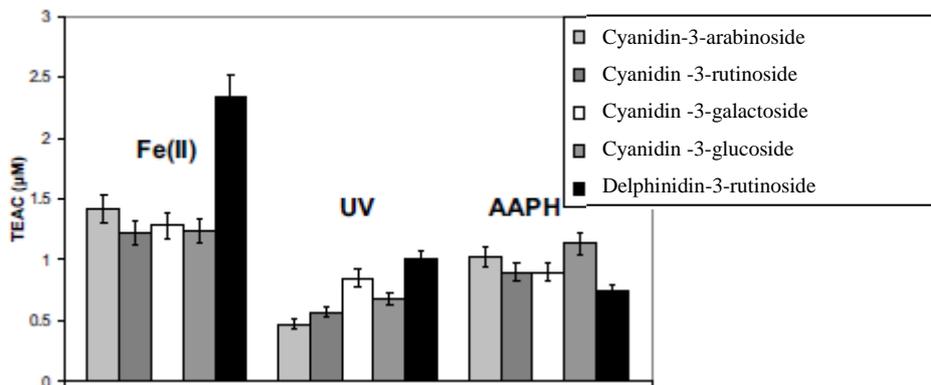


Fig. 24 The effect of anthocyanins on lipid peroxidation of liposomes⁶⁾
 Fe(II): Fe²⁺ lipid peroxidation (Fe²⁺ NADPH)
 UV: UV-induced lipid peroxidation, AAPH: radical inducer

(d) Inhibition on hydrogen peroxide of membrane lipids

Hydrogen peroxide (H₂O₂) is the simplest peroxide with powerful oxidizing capacity, hence a highly reactive oxygen species. The effect of anthocyanins on hydrogen peroxide was examined on membrane lipids (using rat brain homogenate). As showed in Fig. 21, delphinidin exhibited strongest inhibitory effect on hydrogen peroxidation of membrane lipids with lowest ID₅₀.

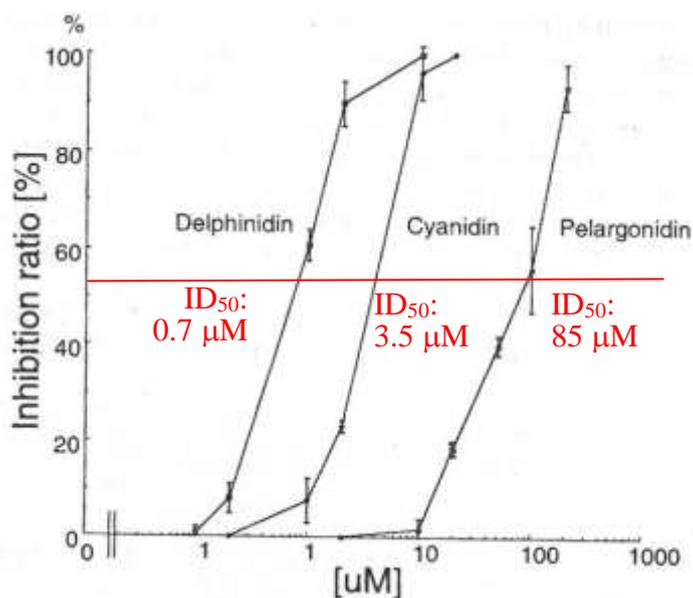


Fig. 25 The effect of anthocyanins on hydrogen peroxidation of membrane lipids⁷⁾

(e) Inhibition of peroxidation of LDL

Further *in-vitro* experiment was conducted to examine the effect of delphinidins and cyanidins on the peroxidation of LDL. Fig. 26 showed that delphinidins demonstrated highest inhibition rate on the formation of hexanol and its effect is stronger than that of cyanidins. LDL peroxidation has been suggested as the major risk factors of atherosclerosis, delphinidins with potent inhibitory effect on LDL peroxidation is preventive of cardiovascular diseases.

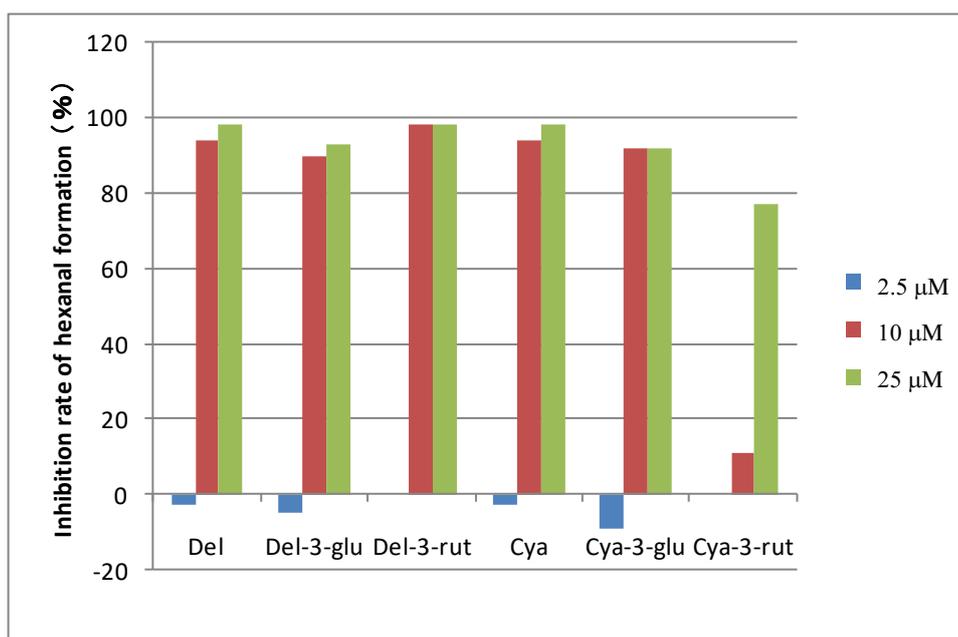


Fig. 26 Inhibition of peroxidation of LDL modified from reference ⁸⁾
 Del: Delphinidin, Cya: Cyanidin,
 glu: glucoside, rut: rutinoside

- 5) M. M. Rahman *et al.*, *Free Radic Res*, 40(9), 993-1002 (2006)
- 6) J. Gabrielska *et al.*, *Z Naturforsch*, 60C, 399-407 (2005)
- 7) Y. Noda *et al.*, *J Agric Food Chem*, 50, 166-171 (2002)
- 8) M. P. Kahkonen *et al.*, *J Agric Food Chem*, 51, 628-633 (2003)

(3) Antioxidant Effect on UV-irradiated Keratinocytes

UV light has intense effect on skin. Symptoms of premature skin aging such as erythema, inflammation, pigmentation, photoaging (wrinkles, blemishes, sagging) and skin cancer are consequences of excessive UV light exposure. Upon UV light irradiation, reactive oxygen is generated, triggering series of oxidation processes in cells resulting in oxidized DNA, lipid peroxidation and apoptosis (cell death).

The effect of delphinidin on UV-irradiated keratinocytes (HaCaT) was examined. Results showed that delphinidin exerts antioxidative effect on UV-irradiated keratinocytes with a concentration-dependent inhibition on cell apoptosis (Fig. 27).

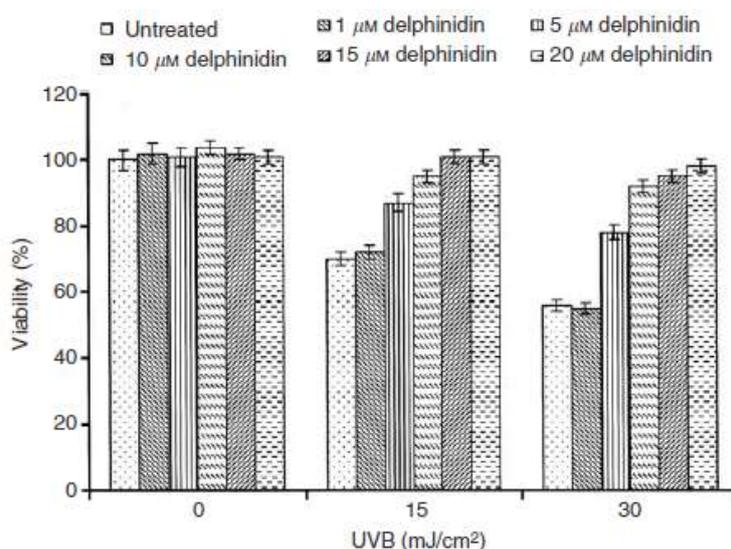


Fig. 27 The effect of delphinidin on UV-irradiated keratinocytes ⁹⁾

Upon UV-irradiation on keratinocytes, series of oxidation reactions is observed. Lipid peroxidation is suppressed in samples treated with delphinidin (Fig. 28).

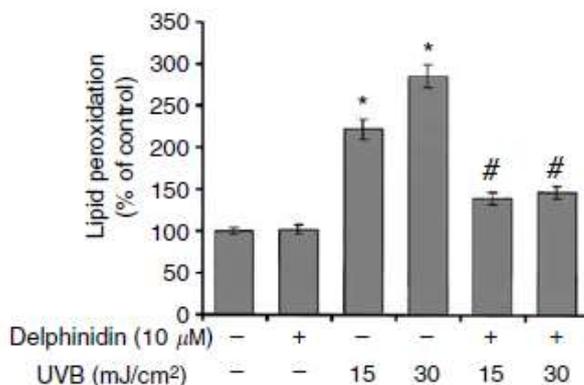


Fig. 28 The effect of delphinidin on UV-induced lipid peroxidation ⁹⁾

9) F. Afaq et al., *J Invest Dermatol*, 127, 222-232 (2006)

6. Anti-inflammatory Effect

The anti-inflammatory effect of anthocyanins was evaluated using mouse macrophage cells (RAW 264.7). Upon addition of LPS (lipopolysaccharides, inflammation inducer) to macrophage cells RAW264.7, the expression of cyclo-oxygenase-2 (COX-2) markedly up-regulated in response to activation of inflammatory cascades. However, in sample treated with delphinidin, up-regulation of COX-2 was inhibited (Fig. 29). Meanwhile, the expression of COX-1 was not affected indicating that delphinidin is a COX-2 selective anti-inflammatory agent. COX-1 is important in the healthy maintenance of physiological functions.

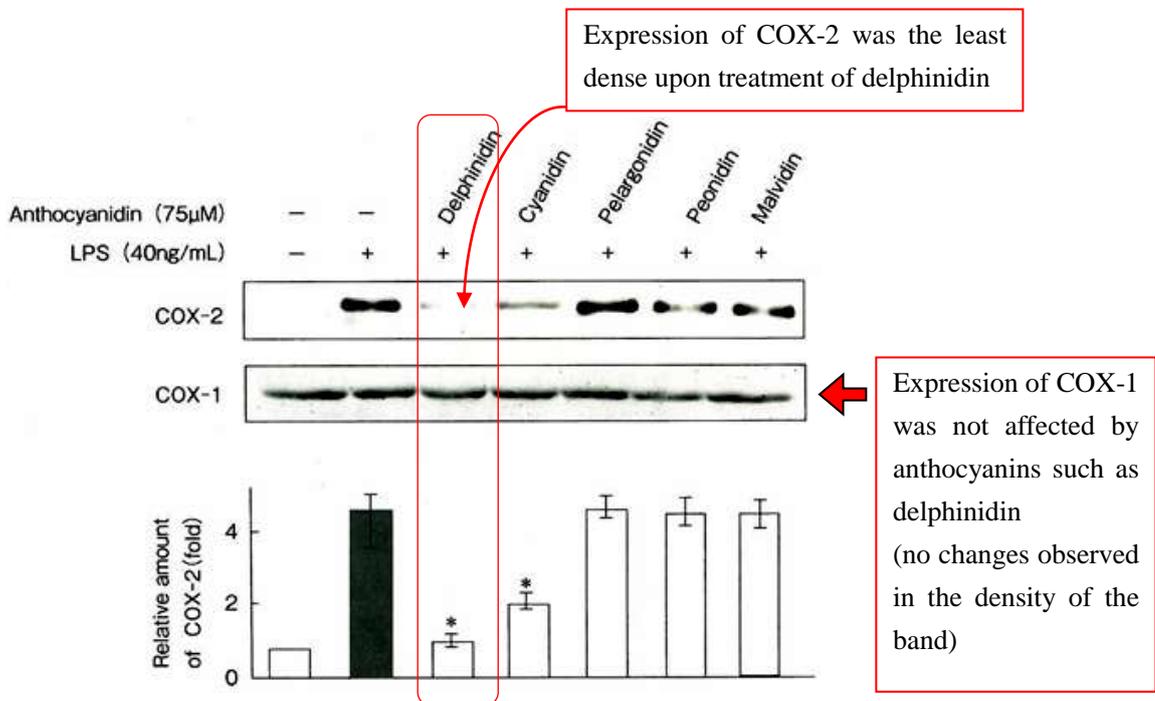


Fig.29. The effect of delphinidin on COX-2 expression ¹⁰⁾

Upon UVB-irradiation on the skin, inflammatory cascade is activated with up-regulation of COX-2 and release of pro-inflammatory prostaglandins E2 (PGE₂). Fig. 30a showed that expression of COX-2 protein and production of PGE₂ was inhibited in UVB-irradiated cultured mouse skin cells (JB6P+) treated with delphinidin. Similarly, Fig. 30b showed that expression of COX-2 was down-regulated upon UVB-irradiation by topical application of delphinidin on mouse skin.

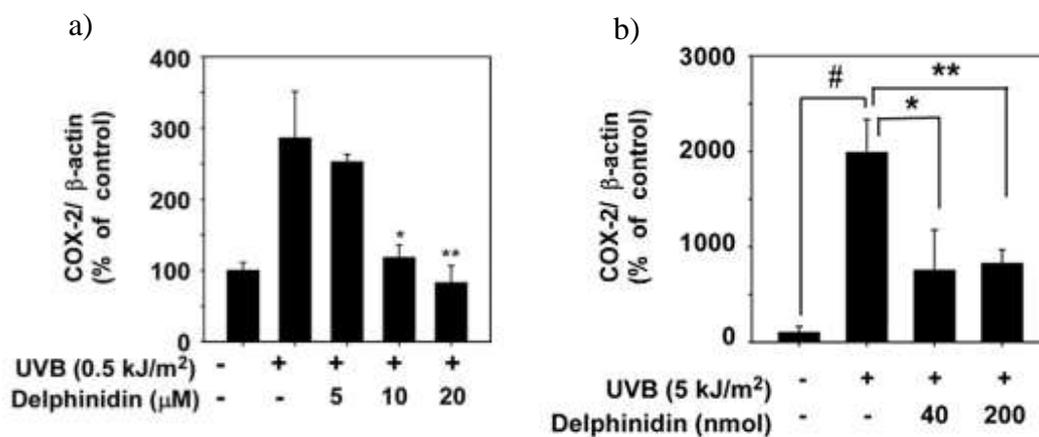


Fig.30 The effect of Delphinidin on the expression of COX-2 protein upon UVB-irradiation ¹¹⁾

a): COX-2 expression of UVB-irradiated culture mouse skin cell (JB6P+)

b): COX-2 expression of mouse skin upon UVB-irradiation (*in vivo*)

10) D. X. Hou *et. al.*, *Biochem Pharmacol*, 70, 417-425 (2005)

11) J. Y. Kwon *et. al.*, *Carcinogenesis*, 30, 1932-1940 (2009)

7. Anti-diabetes Effect

The effect of Maqui Berry Extract on blood sugar level was examined using hereditary Type II diabetes mouse model (C57BL/6J). First, high blood sugar level was stimulated in mouse by introducing high calorie / high fat diet, Maqui Berry Extract with rich content of anthocyanins was orally given to Type II diabetes mouse. Blood sugar level was measured at 4-hour and 6-hour after oral administration of Maqui Berry Extract. As shown in Fig. 27, blood sugar level decreases with increasing concentration of Maqui Berry Extract. Sambubioside-5-delphinidin-3-glucoside, the active component of Maqui Berry Extract is strongly suggested to contribute to the blood sugar lowering effect.

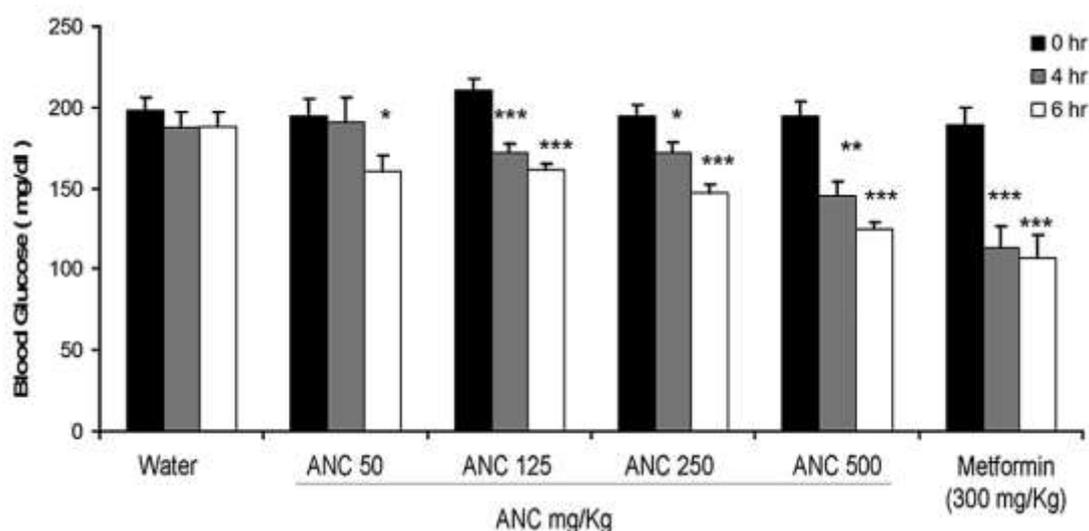


Fig.31 The effect of Maqui Berry Extract on mouse blood sugar level ¹²⁾

ANC: Maqui berry extract (anthocyanin 60%)

Metformin: Prescribed medication for lowering blood sugar level

In an experiment conducted using rat liver cells (H4IIE) found that Maqui Berry Extract inhibited the synthesis of sugar by enhancing insulin uptake to the liver cells (suppression of glucose-6-phosphatase) ¹²⁾. Further experiment conducted on L6 muscle cells confirmed that Maqui Berry Extract enhances the uptake of sugar into muscle cells and thus energy production.

Maqui Berry Extract inhibit glucose synthesis in Type II diabetic mouse by enhancing the uptake of sugar for energy production. It is recommended as a natural anti-diabetes agent.

12) L. E. Rojo *et al.* *Food Chemistry*, 131, 387-396 (2012)

8. Prevention of atherosclerosis

Atherosclerosis has been reported as the consequences of oxidative stress on LDL cholesterol in the vascular wall. Oxidized LDL support foam cells formation and is a potent inducer of inflammatory molecules which leads to apoptosis of vascular endothelial cells thus progression of atherosclerosis.

In an experiment using bovine aortic endothelial cells (BAECs), endothelial cells apoptosis was induced with the addition of actinomycin D and 7 β -hydroxycholesterol (oxidized LDL with apoptotic effect). The effect of delphinidin on endothelial cells apoptosis above was examined. Results showed that sample treated with delphinidin, apoptosis of endothelial cells was inhibited (Fig. 32).

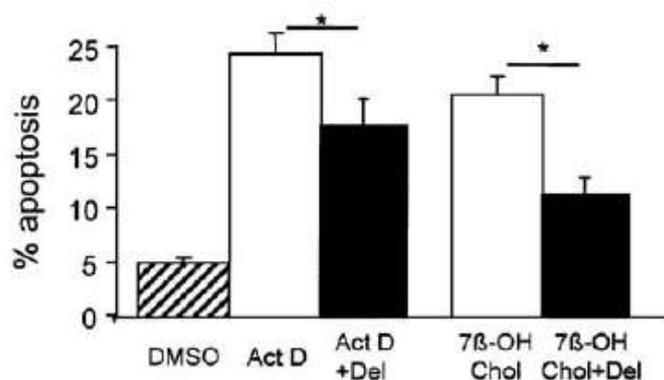


Fig.32 The Effect of delphinidin on vascular endothelial cells apoptosis ¹³⁾

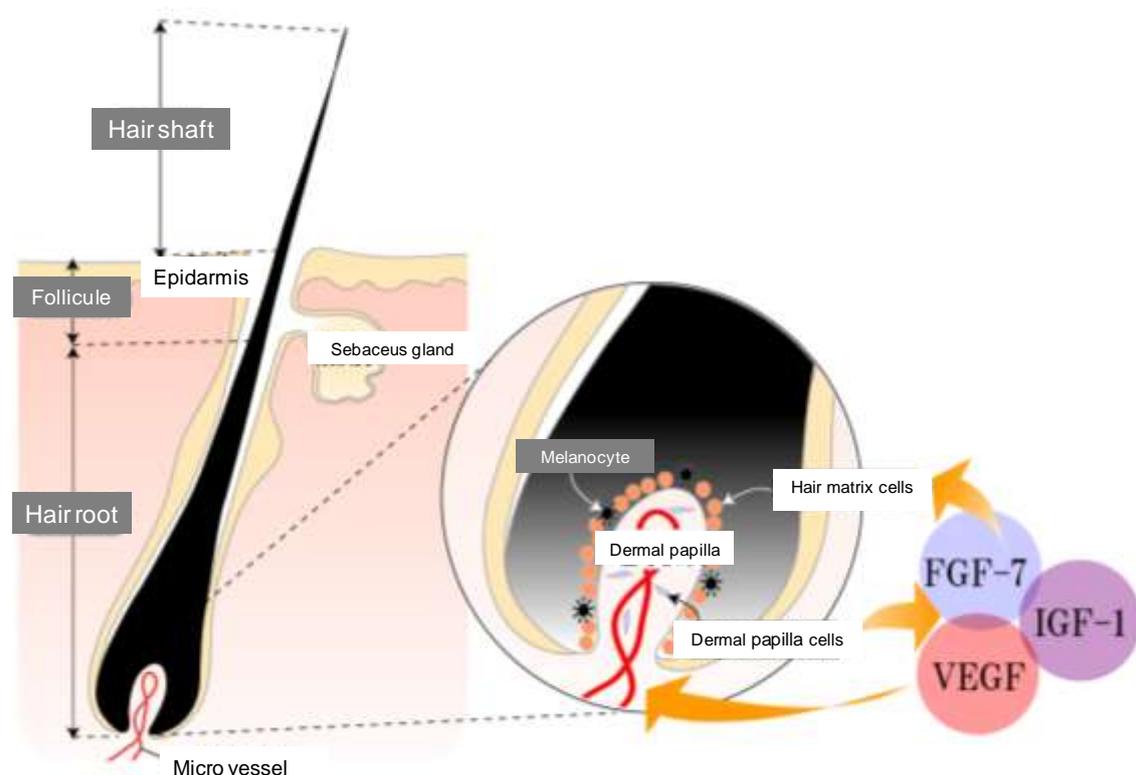
DMSO: Dimethylsulfoxide (control) , Act D: Actinomycin D,

7 β -OH Chol: 7 β -Hydroxycholesterol, Del: Delphinidin

13) S. Martin *et al.* *Br J Pharmacol*, 139, 1095-1102 (2003)

9. Promotion of Hair Growth

With regards to hair growth, activation of dermal papilla cells of hair follicle is relatively important in promoting growth of hair matrix cells (Fig. 33). Proliferation of dermal papilla cells strongly influences the process of hair growth. It is suggested that increasing expression of genetic factors (e.g. FGF-7, VEGF, IGF-1) in the anagen phase of hair growth may promote hair growth.



FGF-7	keratinocytes growth factor, directly influences on dermal papilla cells in hair follicles which promote hair growth. It was reported that expression of FGF-7 was down-regulated in dermal papilla cells of thinning hair.
VEGF	Vascular endothelial growth factor, promote the capillary network surrounding hair follicles providing nutrients to the hair matrix cells.
IGF-1	Insulin-like Growth Factor-1, plays important role in growth. Suppress the transition period of catagen and telogen of hair cycle by inhibiting apoptosis.

Fig.33 Mechanism of hair growth

(1) Dermal Papilla Cells Proliferation effect

In-vitro experiment was conducted to examine the effect of Maqui Berry Extract on the proliferation of dermal papilla cells. As shown in Fig. 34, dermal papilla cells proliferation increase with increasing concentration of Maqui Berry Extract. It is believed that promoting dermal papilla cells proliferation at hair follicles is important in the promotion of hair growth.

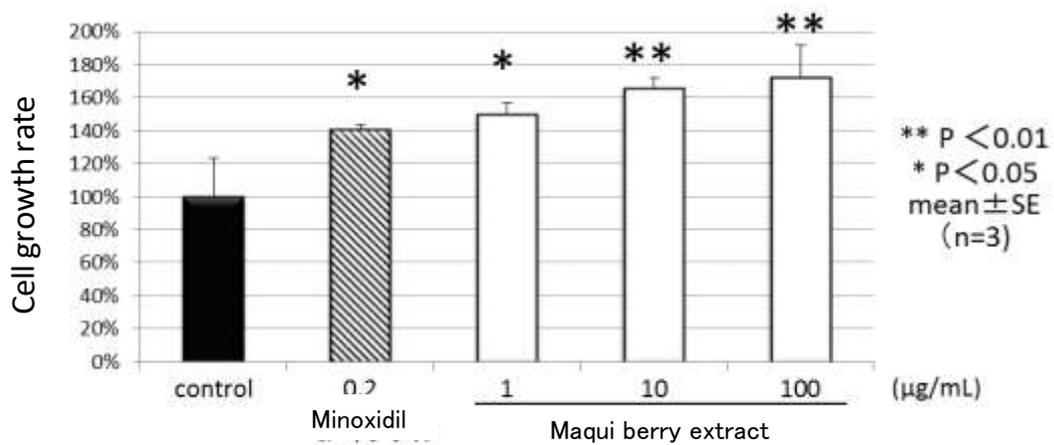


Fig.34 The Effect of Maqui Berry Extract on Dermal Papilla Cells Proliferation (minoxidil – prescribed medication for hair loss is used as positive control)

Besides, the effect of Maqui Berry Extract was compared with other anthocyanin-rich extract, namely black currant extract and bilberry extract. As shown in Fig. 35, Maqui Berry Extract demonstrated a significant effect on dermal papilla cells proliferation upon comparison.

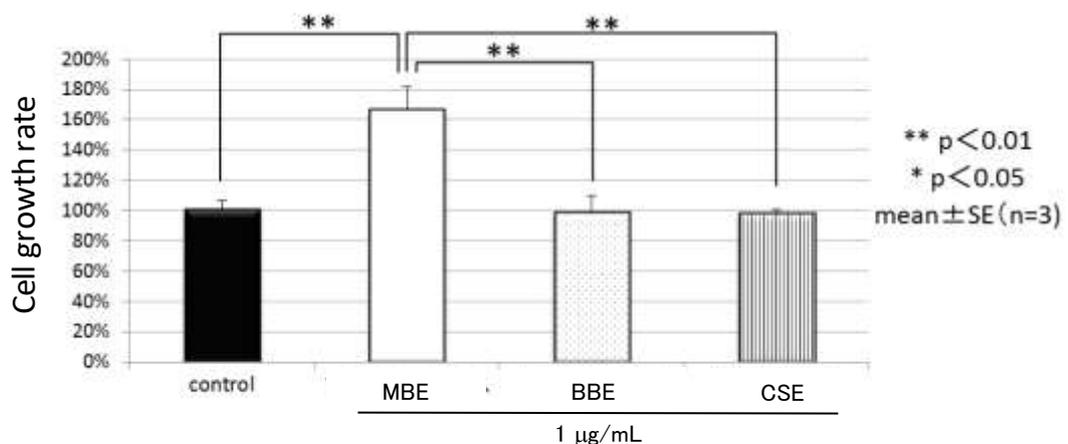


Fig.35 Comparison of anthocyanin-rich extract on dermal papilla cells proliferation

(2) Up-regulation of hair growth genetic factors

Experiment was conducted on the secretion of dermal papilla cells to examine the effect of Maqui Berry Extract on the expression of hair growth genetic factors (FGF-7, VEGF, IGF-1). As shown in Fig. 36, genetic expression of FGF-7, VEGF and IGF-1 significantly up-regulated in samples treated with Maqui Berry Extract. These effects are in fact similar to that of minoxidil, commonly prescribed medication for hair loss.

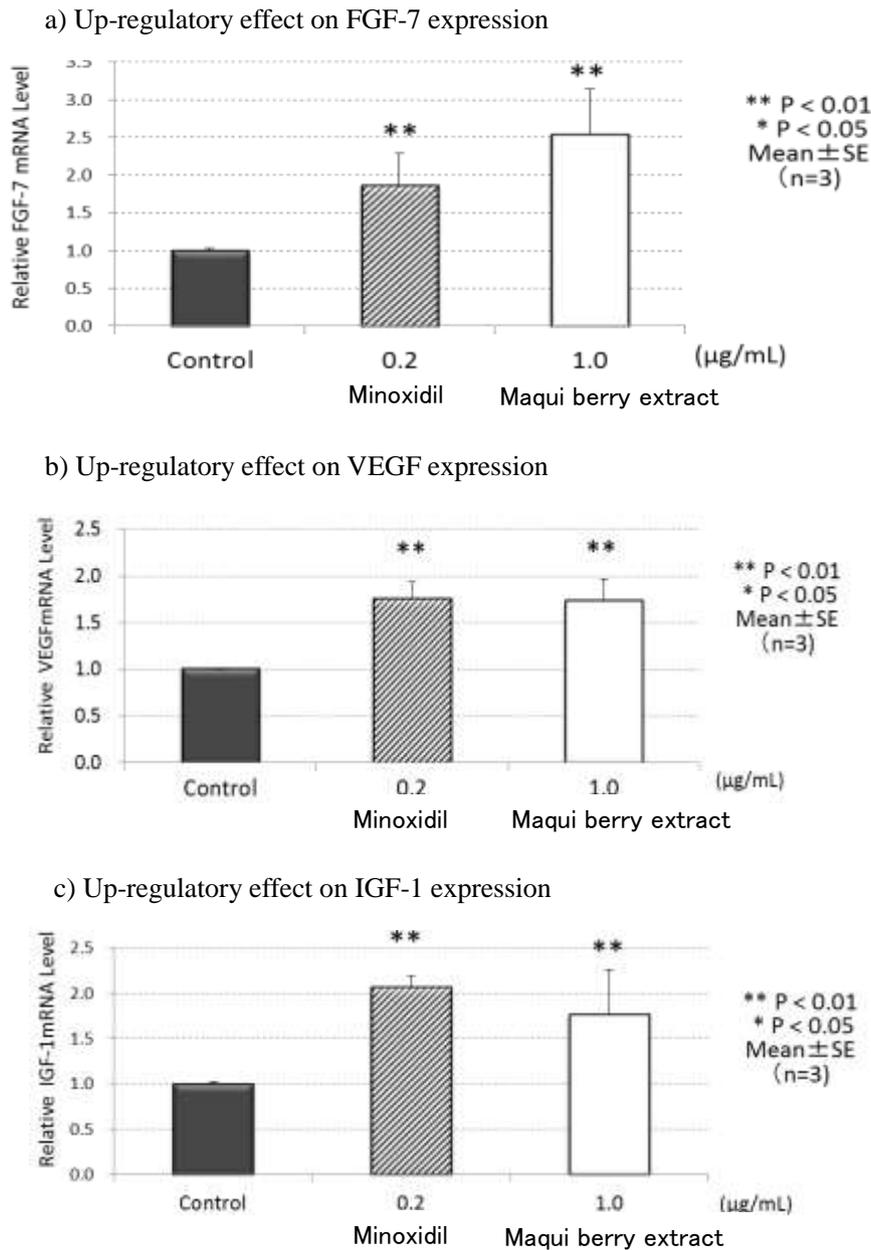


Fig. 36 The effect of Maqui Berry Extract on hair growth genetic factors

Based on above findings, Maqui Berry Extract is expected to promote hair growth and hair thickness.

10. Anti-photo aging of the skin

The effect of Maqui Berry Extract on photo-aging of skin was studied using fibroblasts cells and photo-aging is induced by UVB-irradiation. Results showed that Maqui Berry Extract effectively inhibited UVB-induced cell damage of fibroblasts cells. Meanwhile, MMP-1 is the gene coded for interstitial collagenase, an enzyme that breaks down collagen. Upon UV-irradiation, expression of MMP-1 is up-regulated thus accelerating the degradation of collagen. As shown in Fig. 38, expression of MMP-1 is down-regulated in the presence of Maqui Berry Extract, preventing the degradation of collagen.

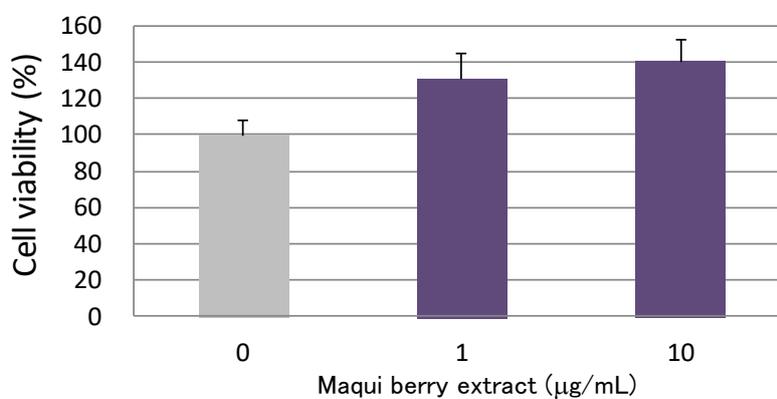


Fig. 37 The Effect of Maqui Berry Extract on UVB-induced cell damage

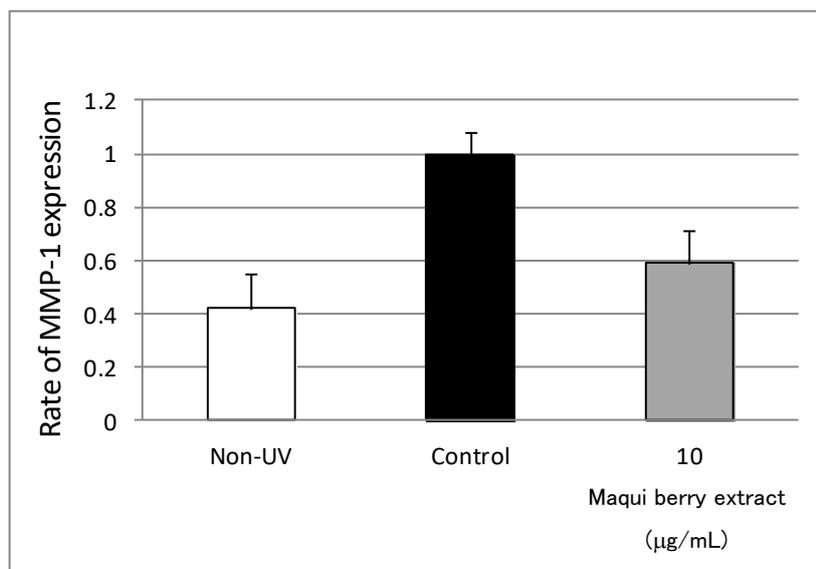


Fig. 38 The Effect of Maqui Berry Extract on the expression of MMP-I upon UVB-irradiation

11. Bioavailability

S.Talavera et al. conducted a study on the absorption anthocyanin in the rat stomach. Absorption of various anthocyanins in the stomach was compared. As illustrated in Fig. 39, in general, delphinidin glycosides were better absorbed as they are potent antioxidants among anthocyanins ¹⁴⁾.

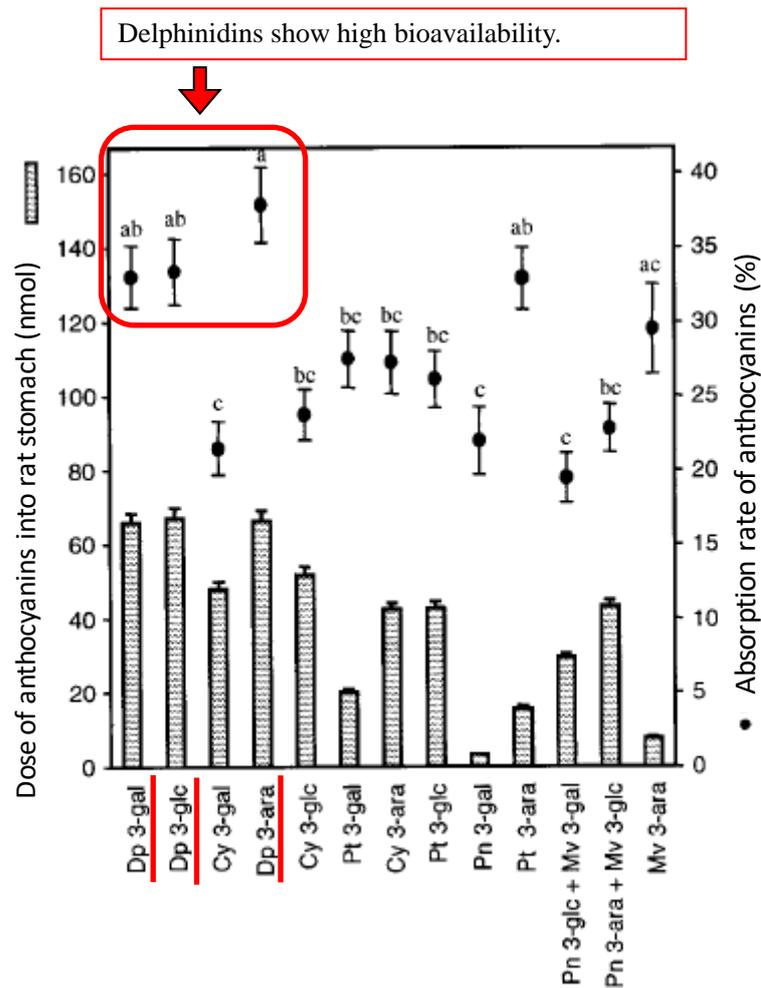


Fig.39 Absorption of anthocyanins ¹⁴⁾

Dp: Delphinidin, Cy: Cyanidin, Pt: Petunidin,
 Pn: Pelargonidin, Mv: Malvidin,
 gal: galactose, glc: glucose, ara: arabinose

14) S. Talavera et al., *J. Nutr.* 133, 4178-4182 (2003)

12. Stability

(1) Heat Stability

Heat stability of Maqui Berry Extract-P35 was evaluated by measuring the anthocyanin content after heating at 80°C and 100°C for hours. As shown in Fig. 40, content of anthocyanins was stable upon heating at 80°C while 15% decrease in the content was observed upon heating at 100°C.

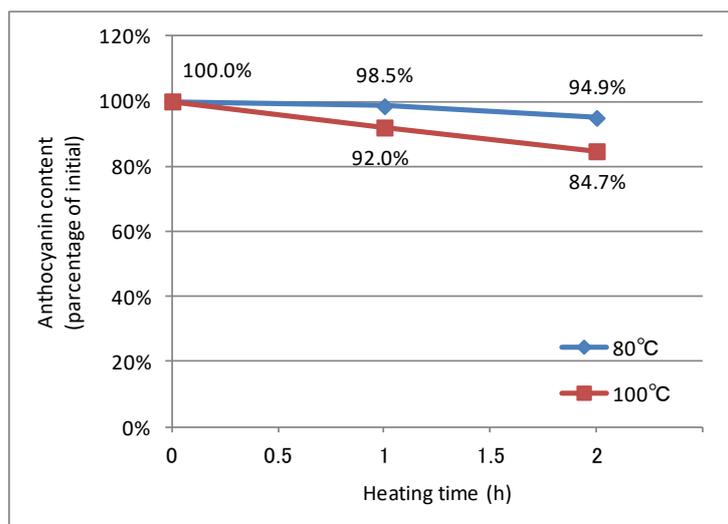


Fig.40 Heat stability of Maqui Berry Extract-P35

(2) Heat stability of aqueous solution of Maqui Berry Extract

The heat stability of aqueous solution of Maqui Berry Extract-P35 in 0.2% citric acid was analyzed. The anthocyanins content of the solution was measured after heating at 40°C, 60°C and 80°C for 60 mins. Content of anthocyanins reduced to 95% and 80% upon heating at 60°C and 80°C respectively (Fig. 41).

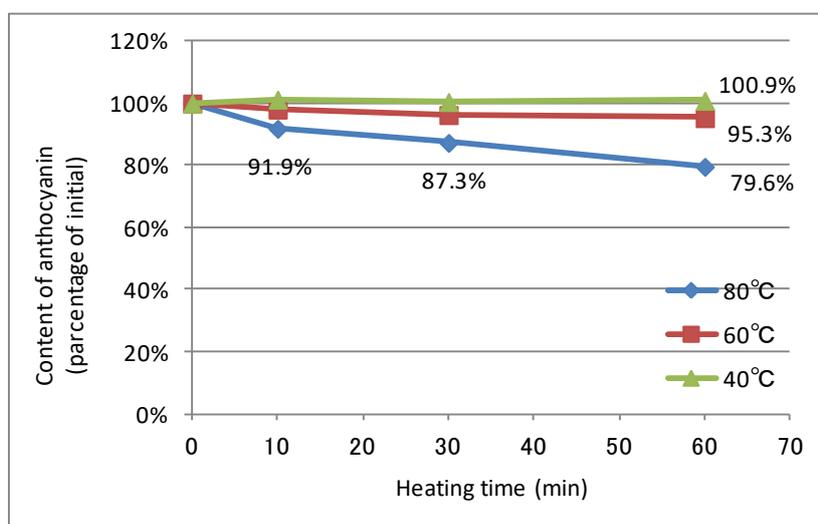


Fig. 41 Heat Stability of Aqueous Solution of Maqui Berry Extract

(3) pH stability

The pH stability of aqueous solution of Maqui Berry Extract was analyzed. Fig. 42 showed the colour changes of the aqueous solution of Maqui Berry Extract at different pH value. Aqueous solution of Maqui Berry Extract changes colour from red to purple, green and yellowish green when the pH changes from acidic to alkaline.



Fig. 42 pH Stability and colour changes of Aqueous Solution of Maqui Berry Extract

13. Nutritional Value

Table 2: Nutritional Value of Maqui Berry Extract

Analyzed Item	100g of edible part	Analysis Method
	Maqui Berry Extract-P35	
Energy	356 kcal	Modified Atwater method
Protein	1.3g	Combustion method
Fatty Acid	0.3g	Acid degradation
Sugar	78.1g	Calculation: 100 - (water + protein + fat + ash)
Sodium	64.7mg	Atomic absorption spectrophotometry
Sodium chloride equiv.	0.2g	Sodium equiv. value
Water	2.0g	Heat drying at atmospheric pressure
Ash	0.7g	Direct incineration
Fiber	17.6g	Prosky method

Standard Conversion factor for Energy expression: Protein 4, fat 9, sugar 4.

14. Safety Profile

(1) Residual Agricultural Chemicals

The raw material, Maqui Berry is wild harvested, pesticides are not used. According to the Food Sanitation Act. on 260 items:

Maqui Berry : Not detected

Maqui Berry Extract-P35 : Not detected

Test trustee : Institute for Environment and Health Food Co., Ltd.

Date : May 9, 2012

Report Number : 12042319 -1 (Maqui Berry)

12042319-2 (Maqui Berry Extract-P35)

(2) Acute Toxicity

Acute Toxicity test was conducted according to the Guidelines for Single-Dose Toxicity Tests for Pharmaceutical Products where Maqui Berry Extract 2000mg/kg was orally given to starved mice (male & female ICR, 5 weeks old, weight 20-25) for 14 days. No abnormalities and fatal event observed at 2000mg/kg. No abnormalities of organs observed under macroscopic examination upon autopsy.

LD₅₀ > 2,000mg/kg

15. Recommended dosage

Product Description	Claim	Recommended dosage
Maqui Berry Extract-P35	Eye health Antioxidant Anti-inflammatory Anti-diabetes Prevention of atherosclerosis Hair growth Anti-photo aging	30-60mg

16. Application

	Applications	Claims	Examples
Food	Nutritional Supplement Beauty Food	Eye health Antioxidant Anti-inflammatory Anti-diabetes Prevention of atherosclerosis Hair growth	Beverages Hard & soft capsules, tablets Candies, chewing gums, chocolates, wafers, jellies Ham, sausage, etc.
Cosmetics	Skin care Cosmetics	Anti-photo aging	Lotions, toner, serum, rinse, treatment care, pack, body gel etc.

17. Packing

Product	Packing	Weight
Maqui Berry Extract-P35 (water soluble powder, food grade)	Interior packing: Aluminum bag Exterior packaging: Cardboard	1kg 5kg
Maqui Berry Extract-J (liquid, food grade)	Interior packing: Cubic polyethylene container Exterior packing: Cardboard	1kg 5kg 20kg
Maqui Berry Extract-PC35 (water soluble powder, Cosmetics grade)	Interior packing: Aluminum bag Exterior packaging: Cardboard	1kg 5kg
Maqui Berry Extract-LC (liquid, cosmetics grade)	Interior packing: Cubic polyethylene container Exterior packing: Cardboard	1kg 5kg 20kg

18. Storage

Store in a cool, dry and dark place. Avoid heat and places with high humidity.

It is recommended to finish using the product once open as it is highly hygroscopic. Otherwise, dessicant bag is recommended to be inserted for storage purpose.

19. Expression

<Food>

Maqui Berry Extract-P35

Expression: Maqui Berry Extract

Maqui Berry Extract-J

Expression: Maqui Berry Juice

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

<Cosmetics>

Maqui Berry Extract-PC35

INCI: Aristotelia Chilensis Fruit Extract

Maqui Berry Extract-LC

INCI: Water, Butylene glycol, Aristotelia Chilensis Fruit Extract

PRODUCT STANDARD

PRODUCT NAME

MAQUI BERRY EXTRACT-P35

FOOD

This product is water-soluble powder extracted from maqui berry, the fruit of *Aristotelia chilensis* with water.

It guarantees minimum of 35.0% total anthocyanins and 20.0% total delphinidins.

<u>Appearance</u>	Deep purple powder with light unique smell.	
<u>Total anthocyanins</u>	Min. 35.0%	(HPLC)
<u>Total delphinidins</u>	Min. 20.0%	(HPLC)
<u>Loss on Drying</u>	Max. 10.0%	(Analysis for Hygienic Chemists, 1g, 105 °C, 2 hr)
<u>Purity Test</u>		
<u>(1) Heavy Metals (as Pb)</u>	Max. 20 ppm	(Sodium Sulfide Colorimetric Method)
<u>(2) Arsenic (as As₂O₃)</u>	Max. 2 ppm	(Standard Methods of Analysis in Food Safety Regulation, The Third Method, Apparatus B)
<u>Standard Plate Counts</u>	Max. 3×10 ³ cfu/g	(Analysis for Hygienic Chemists)
<u>Moulds and Yeasts</u>	Max. 1×10 ² cfu/g	(Analysis for Hygienic Chemists)
<u>Coliforms</u>	Negative	(Analysis for Hygienic Chemists)
<u>Composition</u>	<u>Ingredient</u>	<u>Content</u>
	Maqui berry extract	100%

PRODUCT STANDARD

PRODUCT NAME

MAQUI BERRY EXTRACT-J

FOOD

This product is concentrated juice from maqui berry, the fruit of *Aristotelia chilensis*.

Appearance

Red purple liquid with light unique smell.

Purity Test

(1) Heavy Metals (as Pb)

Max. 20 ppm (Sodium Sulfide Colorimetric Method)

(2) Arsenic (as As₂O₃)

Max. 2 ppm (Standard Methods of Analysis in Food Safety Regulation, The Third Method, Apparatus B)

Standard Plate Counts

Max. 3×10³ cfu/g (Analysis for Hygienic Chemists)

Moulds and Yeasts

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

Coliforms

Negative (Analysis for Hygienic Chemists)

Composition

<u>Ingredient</u>	<u>Content</u>
Maqui berry concentrated juice	100%

PRODUCT STANDARD

PRODUCT NAME

MAQUI BERRY EXTRACT-PC35

COSMETIC

This product is water-soluble powder extracted from maqui berry, the fruit of *Aristotelia chilensis* with water.

It guarantees minimum of 35.0% total anthocyanins and 20.0% total delphinidins.

<u>Appearance</u>	Deep purple powder with light unique smell.	
<u>Total anthocyanins</u>	Min. 35.0%	(HPLC)
<u>Total delphinidins</u>	Min. 20.0%	(HPLC)
<u>Loss of drying</u>	Max. 10.0%	(Analysis for Hygienic Chemists, 1g, 105°C, 2h)
<u>Purity Test</u>		
(1)Heavy Metals (as Pb)	Max. 20 ppm	(The Second Method of The Japanese Standards of Quasi-Drug Ingredients)
(2)Arsenic (as As₂O₃)	Max. 2 ppm	(The Third Method of The Japanese Standards of Quasi-Drug Ingredients)
<u>Standard Plate Counts</u>	Max. 1×10 ² cfu/g	(Analysis for Hygienic Chemists)
<u>Moulds and Yeasts</u>	Max. 1×10 ² cfu/g	(Analysis for Hygienic Chemists)
<u>Coliforms</u>	Negative	(Analysis for Hygienic Chemists)
<u>Composition</u>	<u>Ingredient</u>	<u>Content</u>
	Aristotelia chilensis fruit extract	100%

PRODUCT STANDARD

PRODUCT NAME

MAQUI BERRY EXTRACT-LC

COSMETIC

This product is water-soluble liquid prepared from the extract of maqui berry, the fruit of *Aristotelia chilensis* by aqueous 1,3-butylene glycol.

Appearance

Red purple liquid with light unique smell.

Certification test

(1) Anthocyanin

Dilute 0.1 ml this product in 5 ml methanol and add 0.2 ml hydrochloric acid. After heated the mixture to 80°C, the color of the solution changes to be red.

Purity Test

(1) Heavy Metals (as Pb)

Max. 10 ppm (The Second Method of The Japanese Standards of Quasi-Drug Ingredients)

(2) Arsenic (as As₂O₃)

Max. 1 ppm (The Third Method of The Japanese Standards of Quasi-Drug Ingredients)

Standard Plate Counts

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

Moulds and Yeasts

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

Coliforms

Negative (Analysis for Hygienic Chemists)

Composition

<u>Ingredient</u>	<u>Content</u>
Water	70%
Butylene glycol	29%
<u>Aristotelia chilensis fruit extract</u>	1%
Total	100%

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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