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# The History of Licorice Applications in Maruzen Pharmaceuticals Co., Ltd.

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Additional information is available at the end of the chapter

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## Abstract

Licorice is the root and stolon of the genus *Glycyrrhiza* plants. Licorice has a long and storied history of use in both Eastern and Western cultures for over 4000 years. Licorice extracts and its principal component, glycyrrhizin, have been used extensively in foods, tobacco, and cosmetics and in both traditional and herbal medicines. Since its start-up in 1938, our company has been working on extracting and purifying the flavoring, sweetening, cosmetic, and medicinal constituents from licorice. At first, we were manufacturing licorice extracts for soy sauce. Recently, our company has developed new licorice products, such as antioxidative and antimicrobial products for foods from hydrophobic licorice extracts; whitening, antioxidative, and antityrosinase products for cosmetics from hydrophobic licorice extracts; antiaging products for cosmetics from licorice leaves; and some disease-suppression products for agriculture and fishery by water-soluble licorice flavonoids. This chapter presents the history of several kinds of food and cosmetic applications from many extracts and purified constituents from licorice plants in our company.

**Keywords:** *Glycyrrhiza* plants, glycyrrhizin, flavonoids, application, foods, cosmetic

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## 1. Introduction

Licorice (liquorice in British English, Gancao in Chinese, the leguminous plant (Fabaceae)) is a perennial plant grown in the Mediterranean region, the Middle East, Central Asia, Russia, and China. Licorice is the root and stolon of the genus *Glycyrrhiza* plants. Licorice has a long and storied history of use in both Eastern and Western cultures for over 4000 years. Licorice extracts and its principle component, glycyrrhizin, have been used for extensive use in foods, tobacco, and cosmetics and in both traditional and herbal medicines [1–3]. As for the compo-

nents of *Glycyrrhiza* species, about 100 kinds of triterpenoid saponins and sapogenins and about 300 kinds of phenolic compounds have been reported [4]. Since its start-up in 1938, our company has been working on extracting and purifying the flavoring, sweetening, cosmetic and medicinal constituents from licorice. In the early days, the licorice extracts have been used in the food industry, which includes soy sauce and salted foods. Also the licorice extracts and glycyrrhizin have been used in the cosmetic industry. Recently, our company has developed new licorice products, such as antioxidative and antimicrobial products for foods from hydrophobic licorice extracts; whitening, antioxidative and antityrosinase products for cosmetics from hydrophobic licorice extracts; antiaging products for cosmetics from licorice leaves; and some-disease suppression products for agriculture and fishery by water-soluble licorice flavonoids. Our company is a world-leading company to supply a high quality of licorice products and has a historical know-how of licorice-derived product manufacturing (Figure 1). This chapter presents the history of several kinds of food and cosmetic applications from many extracts and purified constituents from licorice plants in our company.

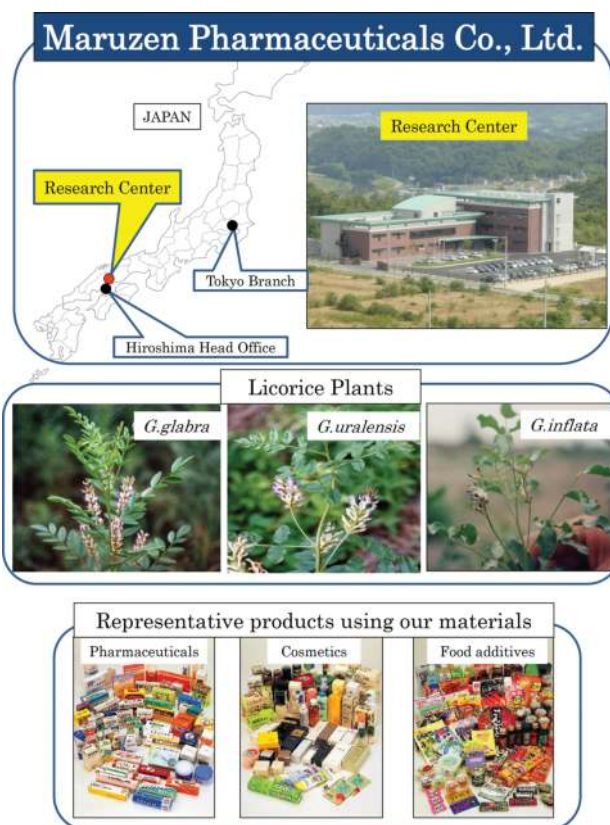


Figure 1. Maruzen Pharmaceuticals profile, licorice plants, and its formulated finished products.

## 2. Glycyrrhizin-producing *Glycyrrhiza* plants

Licorice is applied to the root and stolon of some *Glycyrrhiza* species. Among around 30 species of genus *Glycyrrhiza* [5], the three species of genus *Glycyrrhiza* are commercially used, because they contain glycyrrhizin, which is the main sweetening and bioactive agent. The water-soluble constituents of the three *Glycyrrhiza* species, *G. uralensis*, *G. glabra*, and *G. inflata* are almost same. On the other hand, the hydrophobic extract from the three *Glycyrrhiza* species contains the species-specific flavonoids (Table 1) [6]. The structures of these compounds are shown in Figure 2. Glycyrrhizin is a conjugate of two molecules, glucuronic acid and glycyrrhetic acid, an oleanane-type triterpene. Glycyrrhizin is found in the thickening root and the stolon, but not in the seed, leaf, and stem. Hydrophobic flavonoids are found in the peel of the root and stolon.

<i>Glycyrrhiza</i> plant	Distribution	Water soluble constituents		Hydrophobic flavonoids
		Saponin	Flavonoids	
<i>G. uralensis</i>	China, Mongolia, Kazakhstan	Glycyrrhizin	Liquiritigenin and its glycosides isoliquiritigenin and its glycosides	Glycycomarin licocoumarone
<i>G. glabra</i>	Mediterranean region Middle East Central Asia	Glycyrrhizin	Liquiritigenin and its glycosides isoliquiritigenin and its glycosides	Glabridin glabrene
<i>G. inflata</i>	Xinjiang(China)	Glycyrrhizin	Liquiritigenin and its glycosides isoliquiritigenin and its glycosides	Licochalcone A licochalcone B

Table 1. Distribution and constituents of the three kinds of *Glycyrrhiza* species.

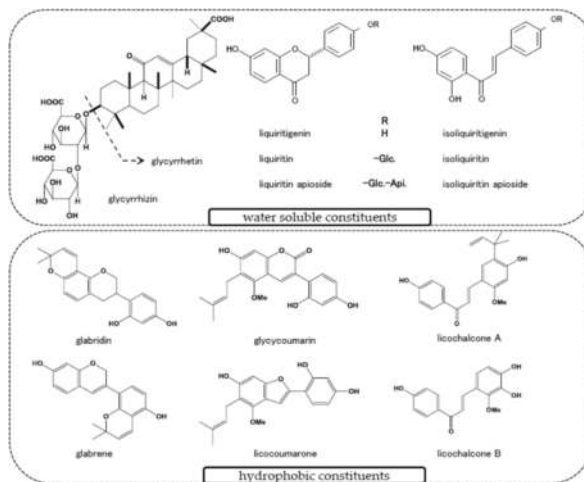


Figure 2. Structures of the constituents of the three kinds of *Glycyrrhiza* species.

### 3. Application of licorice products for foods

Pontefract cakes are made of licorice extract, molasses, sugar, and flour in the Yorkshire town of Pontefract, England, during the seventeenth century. In the nineteenth century, it was used extensively for confectionery [7]. Licorice extracts and glycyrrhizin have the following properties [8]:

1. High-intensity sweetener, glycyrrhizin possesses about 200 times the sweetness potency of sucrose
2. Improving foam stabilization and head-forming characteristics
3. Masking effect of bitter aftertaste
4. Flavor-enhancing effect
5. Nonfermented sweetener
6. Noncaramelization
7. Heat stable
8. Soften the saltiness
9. Depression of freezing point
10. Full-bodied umami and sweetening

Therefore, licorice extracts and glycyrrhizin are used as food additives in a variety of foods such as alcohol beverages, nonalcohol beverages, chewing gum, candy, chocolate, sweet snacks, ice cream, soy sauce, Japanese pickled vegetables, seafood delicacies, steamed fish paste, and sausages in Japan.

Research work by our company in 1994 resulted in a yeast that selectively hydrolyzed the terminal  $\beta$ -glucuronyl linkage of glycyrrhizin to yield glycyrrhetic acid 3-*O*-mono-glucuronide (MGGR), a potent sweetener (relative sweetness to sucrose:  $\times 950$ , **Table 2**) [9, 10]. Moreover, MGGR has a flavor-enhancing property (**Table 3**) [11].

Comparable sucrose concentration (%)	In water		In 5% salt solution	
	Glycyrrhizin	MGGR	Glycyrrhizin	MGGR
2	250	1400	—	—
4	170	950	500	2800
6	170	950	500	2800
8	150	730	440	2400
10	100	500	320	1400

**Table 2.** Relative sweetness intensity in water or 5% salt solution.

	Cocoa	Chocolate	Whipped cream
Blank	2*	2*	16**
MGGR	7*	8*	27**
Same	1*	0*	17**

\*No. of panelist felt delicious.

\*\*Delicious score.

**Table 3.** Flavor-enhancing effect of MGGR against cocoa, chocolate, and whipped cream.

#### 4. Application of licorice products for pharmaceuticals and cosmetics

Glycyrrhizin has also demonstrated antiviral, antimicrobial, anti-inflammatory, antiallergy, antiulcer, antitussive, hepatoprotective, and blood pressure-increasing effects *in vitro* and *in vivo*. In addition, glycyrrhizin is effective in treating hyperlipidemia and inflammation-induced skin hyperpigmentation and is effective in preventing neurodegenerative disorders and dental caries [2].

Therefore, licorice extract, glycyrrhizin, and its derivatives are extensively used in the preparation of cosmetics in Japan. Glycyrrhizin as well as licorice extracts, glycyrrhetin, stearyl glycyrrhethinate, and succinyloxy -glycyrrhethinate (carbenoxolone) are used in drugs, quasi-drugs, and cosmetics. Glycyrrhizin and its salts are used in eye drops, lotions, and tonics as they are soluble in water. On the other hand, glycyrrhetic acid and its derivatives are used in creams, milky lotions, and sun oils as they are soluble in oil.

#### 5. Application of hydrophobic extract of licorice for cosmetics and foods

Recently, the species-specific flavonoids from hydrophobic extracts of *G. uralensis*, *G. glabra*, and *G. inflata* were found. The primary active ingredient isolated from *G. uralensis* was glycycomarin, that isolated from *G. glabra* was glabridin, and that isolated from *G. inflata* was licochalcone A (**Figure 1**). In the course of our studies on the further application of licorice as a cosmetic ingredient, we studied new dermatological availabilities in the hydrophobic extracts of licorice containing licochalcone A isolated from *G. inflata* and glabridin isolated from *G. glabra*.

##### 5.1. Efficacies of hydrophobic extract from *G. inflata* (HPGI)

The primary active ingredient isolated and extracted from *G. inflata* is licochalcone A, an oxygenated retrochalcone that exhibits antimicrobial, antioxidative, anti-inflammatory, antiparasitic activity, and antitumorigenic activity [12, 13].

We indicate several efficacies of hydrophobic extracts from *G. inflata* (HPGI), especially in dermatological uses. The efficacy of HPGI against sebum-induced skin trouble was assessed

in several experiments (**Table 4**) [14]. An androgenic hormone, testosterone, is converted into a pharmacologically active compound, dihydrotestosterone, by an intracellular enzyme, testosterone 5 $\alpha$ -reductase. Dihydrotestosterone combines with the androgen receptor of the sebaceous gland to increase the sebum. Ultimately, the increase of sebum-secretion promotes skin trouble through the oxidative damage and increase of acne fungus.

Tests	Inhibitory effect (IC <sub>50</sub> : ppm)
Testosterone 5 $\alpha$ -reductase activity	18.7
Androgen receptor	5.8
Lipase activity	43.6
Phospholipase A activity	0.38
SOD-like activity	7.0
Antimicrobial activity against <i>P. acnes</i>	15.6 (MIC)

**Table 4.** Inhibitory effects of HPGI on sebum-induced skin trouble experiments.

(1) Inhibitory test of testosterone 5 $\alpha$ -reductase. As shown in **Table 4**, in an assay of the inhibitory effect against testosterone 5 $\alpha$ -reductase activity, HPGI demonstrated the potent inhibitory activity. The inhibitory ability was more effective than positive controls (ethynyl estradiol (IC<sub>50</sub>: 31.5 ppm) and benzyl peroxide (IC<sub>50</sub>: 129 ppm)).

(2) Inhibitory effect against androgen receptor.

In this assay, HPGI inhibited the binding of dihydrotestosterone on the receptor at a low concentration and its IC<sub>50</sub> was 5.8 ppm (**Table 4**). From this result, it was indicated that HPGI has a binding ability in the androgen receptor and works an androgen antagonist.

(3) Inhibitory effect against lipase and phospholipase A<sub>2</sub>.

HPGI indicated the inhibition of lipase (IC<sub>50</sub>: 43.6 ppm) and phospholipase A<sub>2</sub> (IC<sub>50</sub>: 0.38 ppm). In this case, the potency of HPGI against phospholipase A<sub>2</sub> was remarkable.

(4) Superoxide dismutase (SOD) like activity.

The suppressant effect of HPGI on active oxygen generation was examined by the reduction of nitro blue tetrazolium (NBT) in a xanthine-xanthine oxidase system. In this assay, HPGI suppressed the generation of active oxygen at low concentration (IC<sub>50</sub>: 7.0 ppm).

(5) Antimicrobial activity against *Propionibacterium acnes*.

In the hair follicle and the sebaceous gland, *P. acnes* produces the chemotactic factor that enhances the migration of neutrophils toward the hair follicle or the sebaceous gland, and causes the development of acne. In addition, *P. acnes* is considered to increase the release of cytokine and T-lymphocyte. These cells and the sebaceous gland also promote damage by acne through allergic reaction. Furthermore, *P. acnes* can multiply in the hair follicle by using the sebum as a nutriment and enhance the production of enzyme, such as lipase, protease, and

hyaluronidase. Among these enzymes, lipase attacks sebum to give free fatty acid and promotes the formation of the primary eruption, comedo. Besides, free radicals generated from fatty acid attack phospholipid in cell membranes result in the formation of chemical mediators, such as prostaglandins and leukotrienes. These mediators derived by the action of phospholipase A<sub>2</sub> enhance the development of acne through inflammatory reaction.

HPGI has antimicrobial activity against *P. acnes* at a low concentration (MIC: 31.3 ppm).

(6) Efficacy assessment in acne patient.

From the above-mentioned results *in vitro* assays, HPGI expected following four actions: (1) inhibitory action of sebum production, (2) antimicrobial action, (3) inhibitory action of allergic and inflammatory response, and (4) suppressant action of oxidative damage.

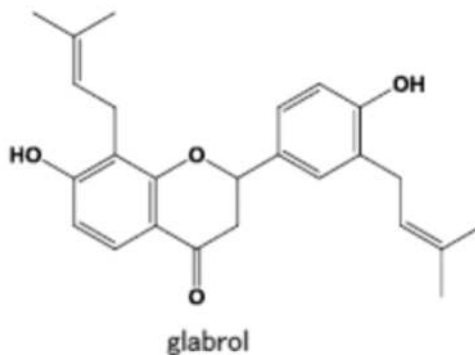
Therefore, we assessed the efficacy of HPGI in acne patients.

Twenty female acne patients received anti-acne gel containing HPGI. All patients applied anti-acne gel onto the whole face twice or three times daily for 2 weeks.

**Table 5** shows the result of efficacy assessment in acne patients. In 17 of the 20 patients tested, the improvement effect was recognized [13].

Efficacy	No. of patients
Marked improvement	6
Improvement	5
Slightly improvement	6
No effect	3
Worse	0

**Table 5.** Result of efficacy assessment of HPGI preparation in acne patients (*n* = 20).



**Figure 3.** Structure of flavonoid in HPGG.

## 5.2. Efficacies of hydrophobic extracts from *G. glabra* (HPGG)

The hydrophobic extract from *G. glabra* (HPGG) contains various flavonoids such as glabridin, glabrene, and glabrol (**Figure 3**). HPGG has been known to have antimicrobial and antioxidant activities [15]. In addition, glabridin has the inhibitory effects on melanogenesis and inflammation [16]. In this section, a depigmenting effect of HPGG *in vitro* and *in vivo* studies was examined [17].

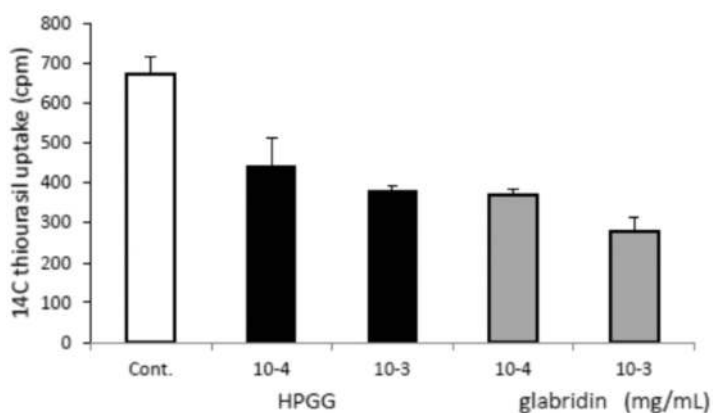
### (1) Inhibitory effect of tyrosinase activity.

We found that HPGG and its constituents had inhibitory effects on tyrosinase activity by absorbance measurement. Their tyrosinase inhibition doses ( $IC_{50}$ : mg/mL) were as follows: glabridin 0.0003, HPGG 0.031, glabrene 0.0046, hydroquinone 0.016, and ascorbic acid 0.21 (**Table 6**) [15]. The latter two compounds are commonly known as depigmenting agents.

Sample	$IC_{50}$ (mg/mL)
HPGG	0.0031
Glabridin	0.0003
Glabrene	0.0046
Glabrol	>0.1
Ascorbic acid	0.21
Kojic acid	0.058
Hydroquinone	0.016

**Table 6.** Inhibition against tyrosinase activity.

### (2) Melanization assay by $^{14}C$ -thiouracil uptake.



**Figure 4.** Melanization assayed by  $^{14}C$ -thiouracil uptake of B-16 melanoma cells.



Melanization was assayed by the incorporation of  $^{14}\text{C}$ -thiouracil into B-16 melanoma cells. Melanization was inhibited by HPGG and glabridin dose-dependently, although glabridin more strongly inhibited it than HPGG (**Figure 4**) [15].

(3) Application in patients with melasma.

We have first synthesized an HPGG that contains 40% of glabridin and using this HPGG, 0.1 or 0.2% HPGG creams were made. An open study has been carried out with application of 0.1 or 0.2% HPGG cream twice a day in patients with melasma, senile pigment freckle, and postinflammatory pigmented lesions for 4 months. The efficacy was evaluated by measuring skin lightness (*L* value) with colorimeter before and after the application. The *L* value has a theoretical value ranging from 0 (pure black) to 100 (pure white).

*L* value was significantly improved after the therapy with 0.1% HPGG cream in patients with melasma only (**Table 7**) [15]. However, with 0.2% HPGG cream, not only patients with melasma, but also with the two lesions showed significantly improvement in *L* values (**Table 8**) [15].

Disease	No. of cases	<i>L</i> value before and after the 4 month therapy
Chloasma	20	Before $58.28 \pm 4.06$ $p < 0.05$ After $59.25 \pm 3.61$
Postinflammatory pigmentation	6	Before $57.56 \pm 3.44$ NS After $58.22 \pm 2.46$
Chloasma + PIP	7	Before $60.26 \pm 2.09$ NS After $60.62 \pm 3.44$
Total	33	Before $58.57 \pm 3.65$ $p < 0.05$ After $59.35 \pm 3.39$

**Table 7.** Changes in *L* values by application with 0.1% GPGG cream.

Disease	No. of case	<i>L</i> value before and after the 4 month therapy
Chloasma	12	Before $55.80 \pm 3.09$ $p < 0.01$ After $57.37 \pm 2.68$
Postinflammatory pigmentation	8	Before $56.34 \pm 3.84$ $p < 0.05$ After $57.94 \pm 3.06$
Senile pigment freckle	15	Before $55.75 \pm 3.48$ $p < 0.05$ After $56.99 \pm 2.63$
Total	35	Before $55.90 \pm 3.34$ $p < 0.01$ After $57.33 \pm 2.69$

**Table 8.** Changes in *L* values by application with 0.2% GPGG cream.

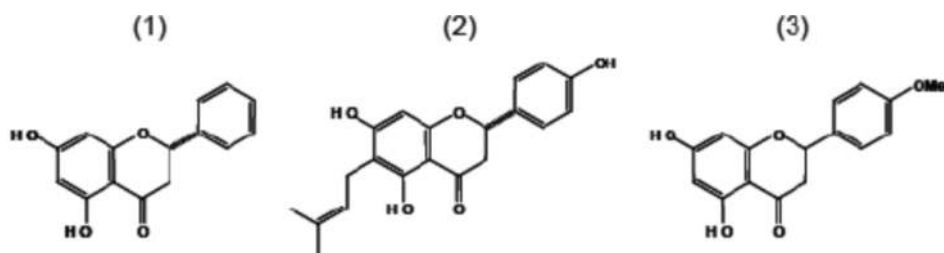
## 6. Application of licorice leaf extract for cosmetics

The aerial parts of licorice are less used in cosmetics. A few phytochemical investigations on the *G. glabra* leaves have reported the presence of several phenolic constituents that are not present in the root [18–20].

We found that licorice extract from *G. glabra* has antiaging effect on human skin [21]. Skin hydration is one of the most important claims in cosmetics as hydrated skin gives an impression of healthy skin. Intracellular lipids in stratum corneum (SC), which are composed mainly of cholesterol, fatty acids, and ceramide, play a crucial role for both water-holding and permeability barrier function in SC. Hyaluronan (HA) is also known to have high water-retaining capacity released to skin hydration, elasticity, and plasticity. It has been reported that HA decreased and was found to be changed in aged skin. Therefore, materials that can modulate ceramide and HA contents in SC could be very effective for aging skin.

### 6.1. Isolation of licorice leaf components

Ten components were isolated from 70% ethanol extract of licorice leaf from *G. glabra*. Three active components out of 10 are shown in **Figure 5** [19].



**Figure 5.** Structure of isolated active components from licorice leaf extract. (1) Pinocembrin, (2) 6-prenyl-naringenin, (3) angophorol.

### 6.2. Effects of licorice leaf extract on mRNA expressions of ceramide-related enzymes

To examine the effects of plant extracts on ceramide synthesis, real-time quantitative RT-PCR analysis was performed on gene expressions of serine palmitoyltransferase long chain base subunit 1 (SPTLC1) and SPTLC2, which were two subunits of serine palmitoyltransferase (SPT) and acid sphingomyelinase (SMPD1). SPT is known to catalyze the rate-limiting step of de novo ceramide synthesis. Acid sphingomyelinase (SMPD1) is also known to convert sphingomyelin into ceramide and plays an important role in ceramide generation for permeability barrier function. Licorice leaf extract and 6-prenyl-naringenin showed the promoting activity on mRNA expressions of SPTLC1, SPTLC2, and SMPD1 in a dose-dependent manner (**Table 9**) [19].

	mRNA expressions (% of control)			
	Licorice leaf extract (µg/mL)		6-prenyl-naringenin (µg/mL)	
	5	20	2.5	5
SPTLC1	113.3 ± 3.5*	132.9 ± 2.7***	113.7 ± 3.7*	148.4 ± 3.7***
SPTLC2	108.8 ± 3.9	127.6 ± 11.6	113.9 ± 14.5	93.8 ± 12.4
SMPD1	117.3 ± 4.1	132.9 ± 8.1*	153.8 ± 9.4**	353.3 ± 5.9***

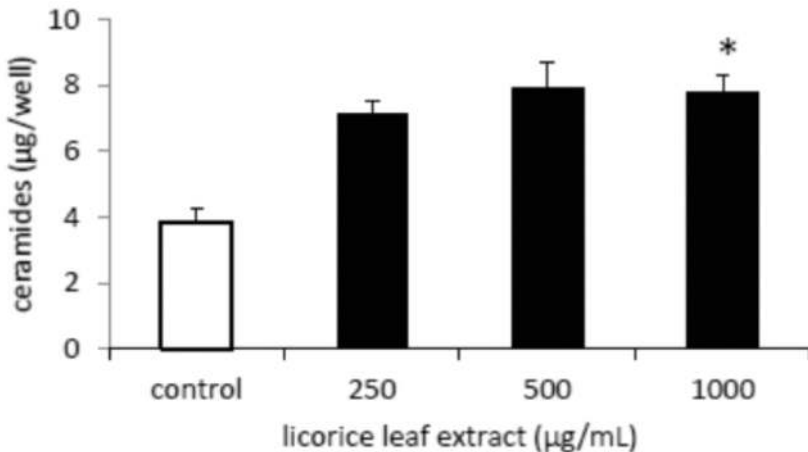
\**p* < 0.05  
 \*\**p* < 0.01  
 \*\*\**p* < 0.001

**Table 9.** Effects of licorice leaf extract on the mRNA levels of SPTLC, SPTLC2, and SMPD1.

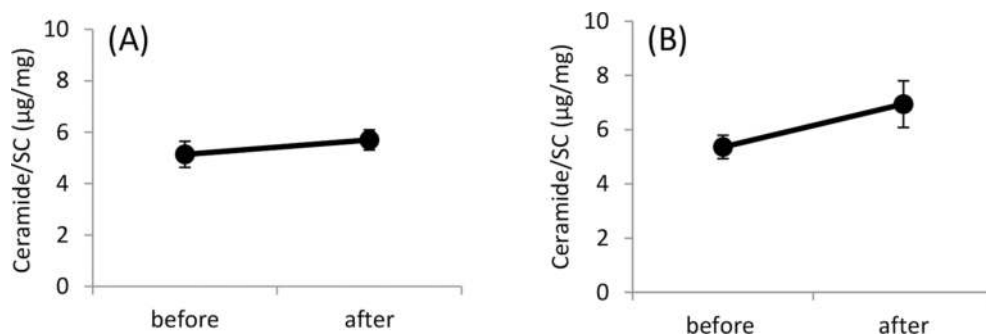
These results indicate that licorice leaf extract may increase de novo biosynthesis of ceramide and hydrolysis of sphingomyelin to ceramide.

### 6.3. Effect of licorice leaf extract on ceramide production in skin-equivalent models and human skin

To examine whether licorice leaf extract has an efficacy on the production of ceramide in skin-equivalent models. The extract dramatically promoted the production of ceramide in skin-equivalent models (**Figure 6**) [19]. For further research to determine the efficacy on the production of ceramide in human skin, 1% licorice leaf extract or placebo lotion was topically applied on healthy volunteers (*n* = 10). The amount of ceramide by the topical application of 1% licorice leaf extract lotion (1.58 µg/mg) was increased as compared to that of placebo lotion (0.56 µg/mg) (**Figure 7**) [19].



**Figure 6.** Effects of licorice leaf extract lotion on ceramide production. \**p* < 0.05 compared with the control.

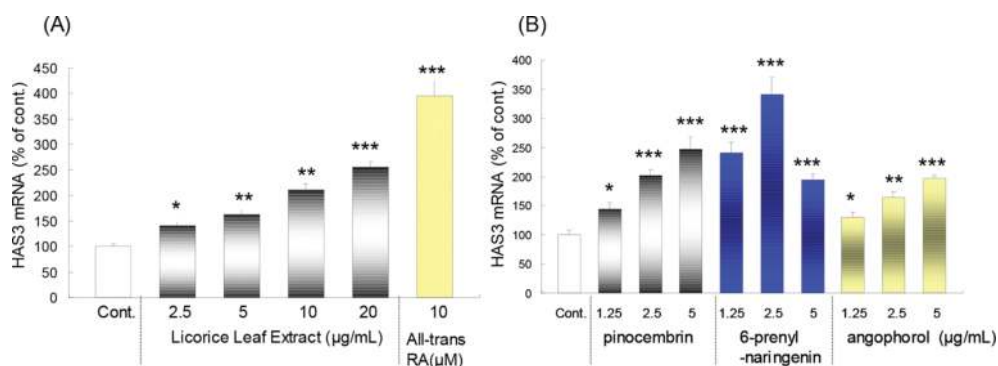


**Figure 7.** Effects of licorice leaf extract lotion on ceramide production. Placebo lotion (A) and 1% licorice leaf extract lotion (B). Values were the mean SEM;  $n = 10$ .

These results suggested that licorice leaf extract has an efficacy on the synthesis of ceramide.

#### 6.4. Effects of licorice leaf extract on mRNA expression of 3-hydroxy-3-methylglutaryl-CoA reductase (HMGCR), a key enzyme on cholesterol biosynthesis

To examine whether licorice leaf extract has a promoting effect on other stratum corneum lipids, real-time quantitative RT-PCR analysis was performed on mRNA expression of HMGCR, the key enzyme for de novo cholesterol synthesis. Treatment with licorice leaf extract showed a significant increase in the expression of HMGCR mRNA by 150% compared with the controls (**Figure 8(A)**). Among three components tested, especially 6-prenyl-naringenin enhanced the expression of HMGCR mRNA in the dose-dependent manner (**Figure 8(B)**) [19]. These results indicate that licorice leaf extract and its components may increase biosynthesis of cholesterol as well as ceramide. Further studies will be needed to determine the promoting activity on cholesterol synthesis.



**Figure 8.** Effects of licorice leaf extract (A) and the components (B) on the mRNA level of HMGCR. \* $p < 0.05$ ; \*\* $p < 0.01$ ; \*\*\* $p < 0.001$ , compared with the controls.

### 6.5. Effects of licorice leaf extract on HA production

Gene expression of HAS3 that relates to hyaluronan biosynthesis was upregulated by the treatment of licorice leaf extract in the dose-dependent manner in normal human epidermal keratinocytes (NHEK) (Figure 9(A)). Three components tested also enhanced the hyaluronan synthase 3 (HAS3) mRNA expression (Figure 9(B)) [19]. All-trans retinoic acid that is known as a stimulator of HA synthesis in NHEK also showed a strong increase in the expression of HAS3 mRNA by 400% compared with the control. To examine whether licorice leaf extract has a promoting activity on HA production, ELISA analysis was performed using cell culture supernatant. These results indicate that licorice leaf extract and its components have a potent stimulation activity for HA production.

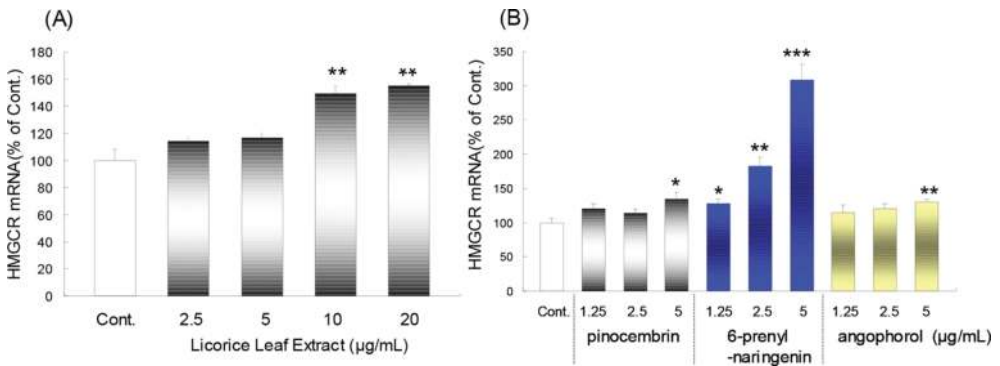


Figure 9. Effects of licorice leaf extract (A) and the components (B) on the mRNA level of HAS3 in NHEK. \* $p < 0.05$ ; \*\* $p < 0.01$ ; \*\*\* $p < 0.001$ , compared with the control.

These results indicate that licorice leaf extract may be a useful ingredient for skin hydration and barrier repair because of their ability to synthesize ceramide through the enhancement of mRNA expressions of SPT and SMPD1 and the increase of mRNA of HMGCR related to cholesterol biosynthesis and the increase of HA production through the enhancement of mRNA levels of HAS3 by its active principles.

## 7. Application of flavonoid-rich water-soluble licorice flavonoids (WSLF) for agriculture and fishery

Table 10 shows the flavonoid composition of water-soluble licorice flavonoids (WSLF) tested. WSLF contains about 10% of the total flavonoids and 10% of glycyrrhizin. General water extracts of licorice contain 2% of the total flavonoids and 10% of glycyrrhizin. WSLF tested has five times higher content of total flavonoids [22].

Compounds	Contents (%)
<b>Flavonoids</b>	
Liquiritin	4–8
Isoliquiritin	1–3
Liquiritigenin	0.5–2.0
Isoliquiritigenin	0.5–2.0
<b>Saponin</b>	
Glycyrrhizin	8–13

**Table 10.** Contents of flavonoids and saponin of water-soluble licorice flavonoids (WSLF).

## 7.1. Control of some fungal foliage diseases of vegetables using WSLF

### 7.1.1. *In vitro* test of WSLF

The antifungal activity *in vitro* of WSLF was evaluated by culture tests using the PDA agar medium. The antifungal activity of WSLF among various pathogens causing fungal foliage diseases on vegetables was investigated. WSLF exhibited the antifungal activity against 15 fungi tested (**Table 11**) [23].

Plants	Diseases	Pathogen	% Inhibition of mycelial growth	
			100 µg/mL	1000 µg/mL
Rice	Blast	<i>Magnaporthe grisea</i>	14	61
	Bakanae disease	<i>Gibberella fujikuroi</i>	37	84
	Sheath blight	<i>Thanatephorus cucumeris</i>	16	71
	Seed and seedling rot	<i>Pythium graminicola</i>	16	17
Tomato	Late blight	<i>Phytophthora infestans</i>	18	55
	Gray mold	<i>Botrytis cinerea</i>	18	45
	Corynespora target spot	<i>Corynespora cassicola</i>	34	67
	Leaf mold	<i>Passalora fulva</i>	29	60
Egg plant	Leaf mold	<i>Mycovellosiella natrassii</i>	19	58
Sweet pepper	Frogeye leaf spot	<i>Cercospora capsici</i>	11	42
Cucumber	Corynespora leaf spot	<i>Corynespora cassicola</i>	20	51
	Anthracnose	<i>Colletotrichum orbiculare</i>	11	71
Melon	Gummy stem blight	<i>Didymella bryoniae</i>	50	50
Spinach	Fusarium wilt	<i>Fusarium oxysporum f. spinaciae</i>	38	59
Potato	Late blight	<i>Phytophthora infestans</i>	29	43

**Table 11.** The antifungal activity of WSLF.

WSLE solution of 20 µL of spore solution and 20 µL was mixed on the slide maintained at 25°C for 20 h. The germinated spores were counted under a microscope. WSLE at 0.1 and 1% inhibited the germination of spores in three kinds of fungi (**Table 12**) [23].

Plants	Diseases	Concentration (%)	Inhibition rate (%)	
			No. 1	No. 2
Sweet pepper	Frogeye leaf spot	0.1	48.4	50.1
		1	87.8	94.2
		Water	2.3	5.9
Egg plant	Leaf mold	1	76.2	72.3
		Water	2.9	4.4
Cucumber	Corynespora Leaf spot	1	62.4	
		Water	26.4	

**Table 12.** Effect of WSLE on the germination of spores.

#### 7.1.2. Control of fungal foliage diseases *in vivo*

Control efficacy of WSLE against seven pathogens was evaluated in pot tests. WSLE solutions were sprayed onto young plants. After air-drying the solutions, the plants were artificially inoculated with the spore suspension on the test pathogen, and incubated at 25°C for a given period. Percent of disease control was assessed after the inoculation of 9–12 days by visually measuring the number of diseasing spot.

Control efficacy of WSLE among seven pathogens exhibited 80–100% at 1% (**Table 13**) [24]. In the pot test, WSLE showed excellent control of diseases caused by various pathogens.

Plant disease	No. of disease spot	Inhibition rate (%)
<b>Tomato <i>Corynespora</i> target spot</b>		
WSLE 1%	30	98
Water	1926	
<b>Tomato late blight (no. 1)</b>		
WSLE 1%	0	100
Water	9.3	
<b>Tomato late blight (no. 2)</b>		
WSLE 1%	0	100
Water	22.8	
<b>Cucumber <i>Corynespora</i> leaf spot</b>		
WSLE 1%	506.0	80

Plant disease	No. of disease spot	Inhibition rate (%)
Water	2541.0	
<b>Cucumber anthracnose</b>		
WSLE 1%	4.0	97
Water	134.5	
<b>Cucumber downy mildew</b>		
WSLE 1%	0	100
Water	31.1	
<b>Sweet pepper frog-eye leaf spot</b>		
WSLE 1%	17.5	97
Water	520.0	

**Table 13.** Efficacy of WSLE against 6 pathogens.

## 7.2. Efficacy of WSLE on fish diseases

### 7.2.1. Antibacterial activity of WSLE against fish disease causing bacteria *in vitro*

The antibacterial activity of WSLE was examined by the agar dilution method, which ranged from 32 to 1024  $\mu\text{g}/\text{mL}$  against 33 kinds of bacteria. As shown in **Table 14**, WSLE inhibited the growth of Gram-positive bacteria with MIC values of 128–512  $\mu\text{g}/\text{mL}$ . Whereas of the Gram-negative bacteria 17 kinds of bacteria were sensitive and nine kinds of bacteria were insensitive to the inhibitory effect [25].

Bacteria	MIC ( $\mu\text{g}/\text{mL}$ )	Bacteria	MIC ( $\mu\text{g}/\text{mL}$ )
<b>Gram-positive bacteria</b>			
<i>Carnobacterium piscicola</i>	256	<i>Staphylococcus epidermidis</i>	512
<i>Nocardia asteroides</i>	256	<i>S. aureus</i>	256
<i>Bacillus cereus</i>	256	<i>Lactococcus garvieae</i>	128
<i>B. brevis</i>	256		
<b>Gram-negative bacteria</b>			
<i>Aeromonas hydrophila</i>	>1024	<i>Vibrio. damsela</i>	1024
<i>A. salmonicida</i>	1024	<i>V. fisheri</i>	128
<i>Flavobacterium columnare</i>	64	<i>V. fluvialis</i>	>1024
<i>E. psychrophilum</i>	64	<i>V. carchariae</i>	>1024
<i>Flexibacter maritimus</i>	256	<i>V. harveyi</i>	1024
<i>Pseudomonas chloraruphis</i>	>1024	<i>V. ichthyenteri</i>	512
<i>Photobacterium damsela</i>	1024	<i>V. ordalli</i>	256



Bacteria	MIC (µg/mL)	Bacteria	MIC (µg/mL)
<i>Enterococcus cloacae</i>	>1024	<i>V. parahaemolyticus</i>	1024
<i>Escherichia coli</i>	>1024	<i>V. penaeicida</i>	256
<i>Klebsiella planticola</i>	>1024	<i>V. proteolitycus</i>	>1024
<i>Salmonella typhimurium</i>	1024	<i>V. splendidus</i>	512
<i>Vibrio alginolyticus</i>	>1024	<i>V. tubiashii</i>	256
<i>V. anguillarum</i>	1024	<i>V. vulnificus</i>	256

**Table 14.** Antibacterial activity of WSLE.

The MICs of constituents, liquiritigenin, and isoliquiritigenin are shown in **Table 15**. Isoliquiritigen demonstrated significant antibacterial activity against all bacteria tested. In contrast, liquiritigenin exhibited no antibacterial activity against six kinds of bacteria tested [23].

Bacteria	MIC (µg/mL)	
	Liquiritigenin	Isoliquiritigenin
<b>Gram-positive bacteria</b>		
<i>Nocardia asteroides</i>	128	<32
<i>Staphylococcus epidermidis</i>	>128	64
<i>S. aureus</i>	>128	128
<b>Gram-negative bacteria</b>		
<i>Aeromonas salmonicida</i>	>128	>128
<i>Photobacterium damsela</i>	>128	128
<i>Vibrio anguillarum</i>	>128	128
<i>V. harveyi</i>	>128	>128

**Table 15.** Antibacterial activities of constituents in WSLE.

### 7.2.2. Effects of WSLE on nonspecific immune responses and disease resistance against *Edwardsiella tarda* infection in Japanese flounder, *Paralichthys olivaceus*

#### 7.2.2.1. Effects of WSLE on nonspecific immune responses in Japanese flounder, *P. olivaceus*

Healthy Japanese flounder, each weighting about 56 g, was divided into three groups used in 0, 5, and 50 mg/kgBW/day of WSLE. Each diet was fed to three groups once a day for 2 weeks. After 1 and 2 weeks of feeding, five fishes from each group were randomly collected. Blood was drawn from the caudal vein and used for hemolytic and lysozyme activities. Hemolytic activity of WSLE-treated fish was significantly higher ( $P < 0.05$ ) than that of the control fish after 1 and 2 weeks (**Figure 10**) [24]. On the other hand, lysozyme activity showed little change (**Figure 11**) [24].

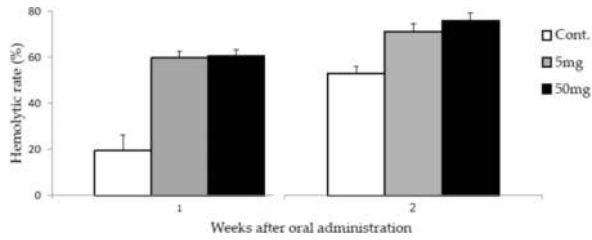


Figure 10. Hemolytic activity of Japanese flounder serum.

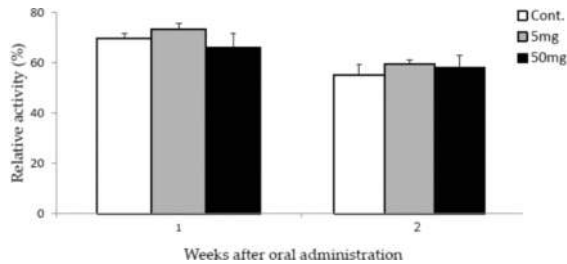


Figure 11. Lysozyme activity of Japanese flounder serum.

Leukocytes were collected from the head kidney and the intestinal tract and used for superoxide anion release and phagocytic activities. The production of the superoxide anion was quantified by the reduction of nitro blue tetrazolium (NBT). WSLE showed significant higher activity than the control group after 1 and 2 weeks (Figure 12) [25]. The activity increased according to time in most groups. The production of the superoxide anion is a method for destroying intracellular bacteria. Phagocytic activities of head-kidney and intestinal tract leukocytes were determined under a microscope by the zymosan-NBT method. Supplementation of WSLE significantly ( $p < 0.05$ ) enhanced the phagocytic activity after 1 and 2 weeks (Figure 13) [26].

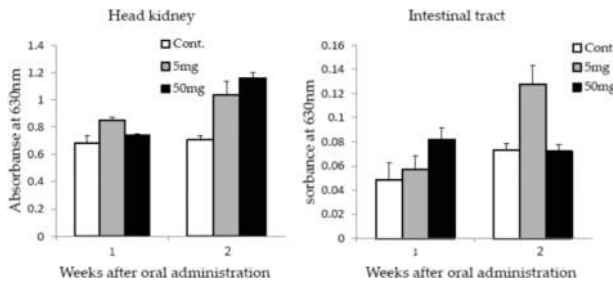
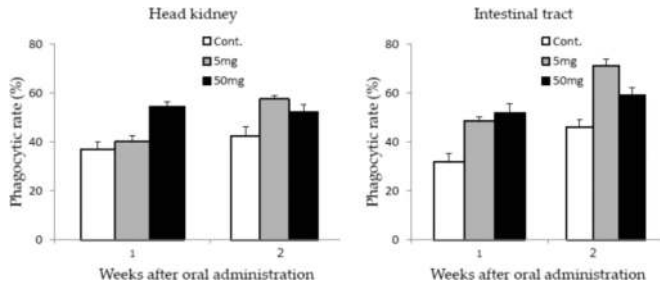


Figure 12. NBT reduction activity of Japanese flounder leukocytes.

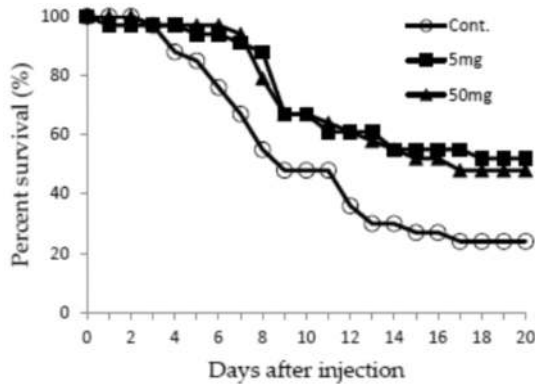


**Figure 13.** Phagocytic activity of Japanese flounder leukocytes.

7.2.2.2. *Effects of WSLE on disease resistance against Edwardsiella tarda infection in Japanese flounder, P. olivaceus*

Healthy Japanese flounder, each weighing about 53 g, was divided into three groups of 33 fishes fed with 0, 5, and 10 mg/kgBW/day of WSLE, respectively. These three groups were fed with each supplementation diet once a day for 10 days. On the 10th day of feeding, these groups were injected intraperitoneally with  $8.0 \times 10^2$  CFU of *E. tarda*.

The cumulative survival rate of the experimental fish following *E. tarda* intraperitoneal challenge is shown in **Figure 14** [27, 28]. The cumulative survival rate was high 48 and 44% when infected fish were fed with 5 and 10 mg/kgBW/day diet and low as 20% in 0 mg/kgBW/day diet fed group, respectively.



**Figure 14.** Effect of WSLE administration on the survival of Japanese flounder experimentally infected with *E. tarda*.

Oral administration of WSLE caused enhancement in humoral (hemolytic and lysozyme) and cellular (phagocytic and superoxide anion release) activities. After 10 days of dietary treatment with WSLE, the fish were challenged by intraperitoneal injection with *E. tarda*, WSLE-treated fish demonstrated increased survival rate.

## 8. Conclusion

Licorice has been used for pharmaceuticals, cosmetics, and food products as water-soluble licorice extract that contains glycyrrhizin, the primary constituent having sweet-taste and various biological activities. Recently, many studies have focused on the licorice ingredients except glycyrrhizin, about 300 phenolic compounds were found from licorice. We investigated licochalcone A extracted from *G. inflata* and glabridin from *G. glabra* in particular. The primary active ingredient isolated and extracted from *G. inflata* is licochalcone A, an oxygenated retrochalcone, which has been associated with various biological properties such as an antioxidant, antimicrobial, as well as anti-inflammatory. As a result, licochalcone A showed several activities such as inhibitory effects of testosterone 5 $\alpha$ -reductase, lipase, and phospholipase A<sub>2</sub>, as well as androgen receptor antagonist, antimicrobial and SOD-like activities, which relate to skin care, especially the suppression of acne formation and development. On the basis of this evidence, a trial of licochalcone A with acne patients was carried out and the efficacy was demonstrated clinically. The primary active ingredient of *G. glabra* is glabridin, a prenylated isoflavonoid, which is one of the most studied licorice flavonoids, a comprehensive literature survey linked to its bioactivities. Glabridin has inhibitory effects on tyrosinase activity, a key enzyme in the production of melanins and melanization using cultured B16 melanoma cells. An open study of glabridin with melasma patients was conducted. The efficacy was evaluated by measuring skin lightness before and after therapy. Glabridin significantly improved after the therapy not only in melasma but also in lesions. The aerial parts of licorice have received scant interest. The few phytochemical investigations on the *G. glabra* leaves have shown the presence of some flavonoids that are not in the roots. We found licorice leaf extract and its component, 6-prenyl-naringenin upregulated both SPTLC1 and SMPD1 mRNA expression related to ceramide synthesis as well as HMGCR mRNA expression related to the cholesterol synthesis. In addition, licorice leaf extract stimulated ceramide production in skin-equivalent models and human skin and promoted HA synthesis by a mechanism that involves upregulation of HAS3 mRNA expression. These results suggested that the licorice leaf extract may be a useful ingredient for skin hydration and barrier repair. There are few reports on licorice extract and glycyrrhizin used in agriculture and fishery. We examined flavonoid-rich water soluble licorice extract (WSLE) in agriculture and fishery uses. In agriculture, WSLE suppressed hyphal elongation of 12 kinds of plant pathogenic fungi and zoospore release from the conidia. In the pot test, WSLE suppressed the number of lesions in six kinds of plant diseases. As a result, we suggested WSLE has the control effects on some fungal diseases of vegetables such as cucumber, tomato, and sweet pepper. In fishery, WSLE and isoliquiritigenin inhibited the fish disease caused by bacteria, especially Gram-positive bacteria. Effects of WSLE on nonspecific immune response of Japanese flounder were investigated. Oral administration of 5 or 50 mg/kgBW/day of WSLE for 2 weeks showed some significant enhance in humoral (hemolytic and lysozyme activities) and cellular (super oxide anion release and phagocytic activities) activities. After 10 days dietary treatment with WSLE, the fishes were challenged by intraperitoneal injection with *E. tarda*, WSLE-treated fish demonstrated increased survival rate.

## 9. Future direction

As for licorice resources, licorice plants are widely found growing wild in regions along the Silk Road. However, according to the recent overharvesting of wild licorice, its habitats are severely disturbed and many of them are degraded or undergoing desertification, especially in China. Therefore, licorice cultivation has been undertaken in China. However, in the present glycyrrhizin and flavonoid content of the cultivated licorice is lower than wild one. In the aim of securing a stable source of licorice, we have to study to obtain the cultivated licorice with same quality of wild licorice.

Over the past half-century, we have been engaged in the development of licorice extracts and its components, and have been offering a number of useful and unique materials to our customers in medicinal, cosmetic, functionary food, and food industries. Elucidation of the constituents and biological activities of both underground and aerial parts in licorice plants have led to the development of many valuable licorice products for various industries. We are further expanding the potential of licorice.

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