Chapter 6

Applicability of Licorice Extracts for Treatment of Oral Diseases, Evaluated by Simplified *In Vitro* Assay Systems with Oral Cells

Hiroshi Sakagami, Takao Kato, Kunihiko Fukuchi, Taisei Kanamoto, Shigemi Terakubo, Hideki Nakashima, Hirokazu Ohno and Masaji Yamamoto

Additional information is available at the end of the chapter

http://dx.doi.org/10.5772/67435

Abstract

Licorice extracts contain various useful substances for oral health. Alkaline extract showed potent anti-HIV activity, whereas flavonoid-rich water extracts showed potent anti-HSV activity, closely correlated with polarizability, ionization potential, a number of ring systems, atomic number and mass. Licorice flavonoids showed higher tumor-specificity against human oral squamous cell carcinoma as compared with human normal oral mesenchymal cells. Glycyrrhiza, at noncytotoxic concentrations, potently inhibited the IL-1β-induced inflammation in cultured human gingival and periodontal ligament fibroblasts. Glycyrrhizin, a major component of Glycyrrhiza, showed the highest UV-protected activity. The results suggest the possible applicability of licorice extracts for several oral diseases and cosmetic products.

Keywords: herb extract, licorice, flavonoids, antiviral activity, tumor-specificity, anti-UV activity, anti-inflammatory activity, oral environment

1. Introduction

The oral cavity (mouth) includes the lips, cheeks, palate (roof of the mouth), floor of the mouth, and the part of the tongue in the mouth (oral tongue). The oral cavity is important for food digestion and speech, the taste buds for sensing different tastes, and the mouth for breathing, drinking, facial expressions, and social interactions.

It is now accepted that the improvement of oral environment leads to the enrichment of quality of life. The number of healthy teeth in aged people well correlates with longevity. In Japan, many of Kampo medicines and component herb extracts have been applied to various oral diseases. However, the selection of Kampo medicines in each case is mostly depended on the experiences of attending physicians, due to the absence of a quantitative evaluation method of their efficacy. This urged us to establish the quick *in vitro* quantification method of antiviral, antitumor, anti-inflammatory, and anti-UV activity, using appropriate cultured cells [1]. These methods were applied to various Kampo medicines and component extracts of plants including licorice to clarify their relative potency.

It was the best to use oral cells for the study of oral diseases. Therefore, we used oral squamous cell carcinoma cell lines and normal oral cells for the study of antitumor activity, and human gingival and periodontal ligament fibroblasts for the study of anti-inflammatory activity. However, we rather used nonoral cells for the study of antiviral and anti-UV activity due to their higher sensitivity (T-cell leukemia and Vero cells for HIV and HSV infection) and also considering the target specificity (skin cells for UV-irradiation).

2. Biological activity of licorice extracts and purified components

2.1. Preparation of flavonoid and chalcone derivatives

For the preparation of licorice flavonoids, the air-dried roots of *Glycyrrhiza inflate* were extracted with MeOH under reflux. The MeOH extracts were dried *in vacuo* and passed through a Diaion HP-20 column, eluting sequentially with H_2O, 50% EtOH and EtOH. The 50% EtOH eluate was chromatographed on a silica gel column ($CHCl_3$:MeOH:H_2O = 10:5:1) to give two fractions. The latter fraction was chromatographed on an octa decyl silyl (ODS) column (MeOH:H_2O, 45:55) to give three fractions. From the first fraction, liquiritin apioside, liquiritigenin 7-apiosylglucoside, liquiritin, and neoliquiritin were obtained with high-performance liquid chromatography (HPLC) (YMC-Pack Pro C18; MeOH:H_2O, 40:60 and CH_3CN:H_2O, 22:78). From the second fraction, isoliquiritin apioside, licurazid, isoliquiritin, and neoisoliquiritin were obtained with HPLC (YMC-Pack Pro C18, MeOH:H_2O, 50:50, and CH_3CN:H_2O, 30:70). From the third fraction, liquiritigenin and isoliquiritigenin were obtained with recycling HPLC (JAI-gel GS-310, MeOH) [2]. The structures of these compounds are shown in **Figure 1**.

For the preparation of water and alkaline extracts, the licorice roots (*Glycyrrhiza glabra* harvested in Afghanistan) were extracted for 20 h with water (not adjusted or adjusted to pH 9 or pH 12) at room temperature. These extracts were filtered through a membrane filter (pore size: 5 μm), neutralized with NaOH or H_2SO_4 and then dried to obtain the water extract (A), alkaline (pH 9.0) extract (B) and alkaline (pH 12.0) extract (C) at the yield of 18.0, 20.3, and 21.7%, respectively (**Figure 2A**). Two alkaline solutions (pH 9.0 and pH 12.0) extracted these ingredients at higher yield than water extraction. Glycyrrhizic acid was the most abundant compound in these extracts, followed by liquiritin apioside and licurazid (**Figure 2b**) [3].

http://dx.doi.org/10.5772/67435

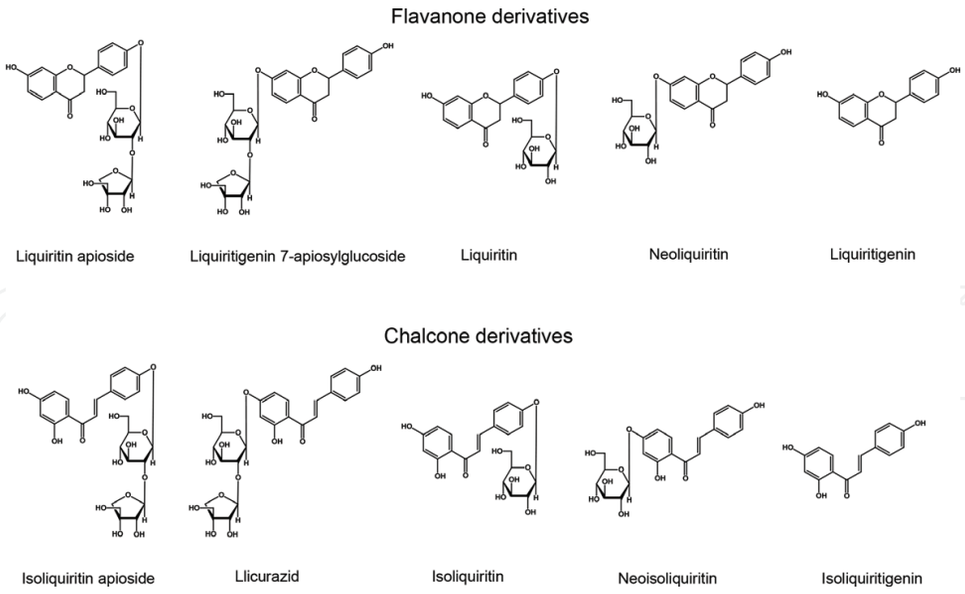
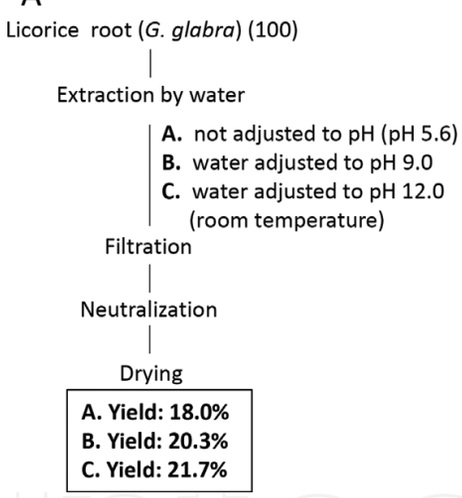


Figure 1. Structures of licorice root flavonoids.

A



B

	Content (%)		
	Water extraction (A)	Alkaline extraction pH 9.0 (B)	Alkaline extraction pH 12.0 (C)
Glycyrrhizic acid	8.84	10.91	9.88
Liquiritin apioside	1.52	2.55	2.82
Liquiritigenin 7-apiosylglucoside	0.65	1.36	0.76
Liquiritin	0.38	0.74	0.42
Neoliquiritin	0.1	0.24	0.18
Liquiritigenin	0.16	0.13	0.09
Isoiquiritin apioside	0.93	0.9	0.34
Licurazid	1.81	1.1	2.47
Isoliquiritin	0.08	0.09	0.06
Neoisoliquiritin	0.06	0.05	0.11
Isoliquiritigenin	0.04	0.03	0.03

Figure 2. (A) Fractional preparation of water and alkaline extracts from licorice root (*G. glabra*) and distribution of major ingredients. (B) Major ingredients in each extract. Cited from Ref. [3].

2.2. Antiviral activity

2.2.1. Anti-HIV activity

Human T-cell leukemia virus I (HTLV-I)-bearing CD4-positive human T-cell line MT-4 was cultured in suspension in an RPMI-1640 medium supplemented with 10% FBS and infected with human immunodeficiency virus (HIV)-1_{IIIB} at a multiplicity of infection of 0.01. HIV- and mock-infected MT-4 cells were incubated for 5 days with different concentrations of extracts and the relative viable cell number was determined by MTT assay. The

concentrations that reduced the viable cell number by 50% (CC_{50}) and that increased the number of viable HIV-infected cells to 50% of control (EC_{50}) were determined from the dose-response curve. The anti-HIV activity was evaluated by the selectivity index (SI) (SI = CC_{50}/EC_{50}). Infection of MT-4 cells with HIV-1 reduced the cell viability to almost zero. Alkaline extract of licorice root (*G. Glabra*) showed the protective effect on HIV infection (SI > 9.2), although its anti-HIV activity was much lower than that of anti-HIV agents [azidothymidine (AZT), 2′,3′-dideoxycytidine (ddC)] (SI = 3029–11,715) and an alkaline extract of the leaves of *Sasa senanensis* Rehder (SE) (SI = 42.7) (left column, **Table 1**). On the other hand, water extract of licorice root, flavonoid-rich fraction, glycyrrhizin acid, glycyrrhetinic acid, flavonoids (iquiritin apioside, liquiritin 7-apiosylglucose, liquiritin, neoliquiritin, liquiritigenin, isoliquiritin apioside, lucurzid, isoliquiritin, neoisoliquiritin, isoliquiritigenin), and polymethoxyflavonoids (tricin, 3,3′,4′,5,6,7,8-heptamethoxyflavone, nobiletin, tangeretin, sudachitin) were all inactive (SI < 1), due to their higher cytotoxicity [4].

	Anti-HIV activity (SI)	**Anti-HSV activity**			**Antitumor activity (TS)**	
		SI		**Maximum cell recovery (%)**	**Epithel. Tumor/ Mesen. normal (Method I)**	**Gingiva. Tumor/ Gingiva. normal (Method II)**
		Method I	**Method II**			
Licorice root extracts						
Water extract (μg/ml)	>1.3	>< 1	>4.6	60	>< 1	>< 1
Purified fraction of water extract (μg/ml)	<1	3.4	6.8	50	>3.5	4.2
Flavonoid-rich fraction of water extract (μg/ml)	<1	4.6	9.2	69	5.5	5.2
Water extract (different lot) (pH 5.6) (μg/ml)	>< 1	<1	2.0	57	>< 1	>< 1
Alkaline (pH 9.0) extract (μg/ml)	>3.0	<1	3.2	62	>< 1	>< 1
Alkaline (pH12.0) extract (μg/ml)	>9.2	<1	<1	49	>< 1	>< 1
Glycyrrhizin acid (μg/ml)	>1.2	<1	<1	ND	>< 1	>< 1
Glycyrrhetinic acid (μg/ml)	<1	<1	<1	ND	1.1	1.1
Licorice flavonoids						
Liquiritin apioside (μg/ml)	>< 1	>5	>500	79	>< 1.0	
Liquiritin 7-apiosylglucose (μg/ml)	>< 1	>< 1	>21	58	1.0	
Liquiritin (μg/ml)	<1	<1	2.8	55	>1.7	
Neoliquiritin (μg/ml)	<1	<1	2.9	59	>< 0.9	
Liquiritigenin (μg/ml)	<1	2.4	>6	77	2.0	

http://dx.doi.org/10.5772/67435

	Anti-HIV activity (SI)	Anti-HSV activity			Antitumor activity (TS)	
		SI		Maximum cell recovery (%)	Epithel. Tumor/ Mesen. normal (Method I)	Gingiva. Tumor/ Gingiva. normal (Method II)
		Method I	Method II			
Isoliquiritin apioside (μg/ml)	><1	23.1	>455	82	><1.1	
Lucurzid (μg/ml)	><1	><1	>667	65	9.0	
Isoliquiritin (μg/ml)	<1	21.4	128.6	97	2.2	
Neoisoliquiritin (μg/ml)	<1	8.0	10.9	87	><0.9	
Isoliquiritigenin (μg/ml)	<1	<1	2.0	54	4.4	
Licochalcone A (μg/ml)					2.0	
Polymethoxyflavonoids						
Tricin (μM)	<1	5.8	7.0	67	><1.0	><1.0
3,3',4',5,6,7,8-Heptamethoxyflavone (μM)	<1	<1	1.1	50	2.0	2.8
Nobiletin (μM)	<1	<1	<1	42	2.0	1.8
Tangeretin (μM)	<1	<1	<1	45	3.0	2.4
Sudachitin (μM)	<1	<1	<1	42	2.9	1.7
Positive control						
AZT (μM)	11,715					
ddC (μM)	3029					
Epigallocatechin gallate (μM)		<1	4.7	62		
Chlorogenic acid (μM)		<1	<1	49		
Coumaric acid (μM)		<1	<1	69		
Curcumin (μM)		1.9	ND	75		
Resveratrol (μM)		<1	2.8	55		
Doxorubicin (μM)					69.9	54.8
5-FU (μM)					>8.9	>28.8
Methotexate (μM)					>170	>45
SE (%)	42.7	8.7	10.2	100	1.6	1.5

Table 1. Anti-HIV, anti-HSV, and antitumor activity of licorice extracts. Cited from Ref. [4].

Ten Kampo medicine and 25 constituent herb extracts showed little or no anti-HIV activity (SI = 1–4) (**Table 2**) [15], possibly due to the fact that most of them were prepared by hot water extraction.

Name	Glycyrrhizin content (mg/g)	LPS contamination (ng/g)	Anti-UV activity (SI)		Anti-HIV activity SI
			HSC-2	HaCaT	
Constituent herb extracts					
Alisma rhizome	<0.1	<2	><1.0	11	><1.0
Asiasarum root	<0.1	2	><1.0	>13	<1.0
Astragalus root	<0.1	17	><1.0	>13	><1.0
Atractylodes lancea rhizome	<0.1	16	<0.8	<0.1	>1.9
Bupleurum root	<0.1	17	<0.7	<0.1	><1.0
Cimicifuga rhizome	<0.1	15	>1.4	9.7	<1.0
Cinnamon bark	<0.1	13	<0.6	7.1	<1.0
Cnidium rhizome	<0.1	18	><1.0	>9.2	>1.5
Coptis rhizome	<0.1	16	1.5	13	<1.0
Gardenia fruit	<0.1	15	>8	>23	>2.7
Ginger	<0.1	14	<0.8	<0.3	><1.0
Ginseng	<0.1	18	><1.0	>7.1	><1.0
Glycyrrhiza	175.4	19	4.3	4.4	<1.0
Japanese Angelica root	<0.1	18	><1.0	>3.9	>1.1
Japanese Gentian	<0.1	16	>1.1	>20	><1.0
Jujube	<0.1	16	><1.0	>3	><1.0
Peony root	<0.1	16	><1.0	>18	<1.0
Phellodendron bark	<0.1	14	<0.1	10	<1.0
Pinellia tuber	<0.1	<2	><1.0	>3.7	><1.0
Platycodon root	<0.1	18	<0.8	<0.15	><1.0
Polyporus sclerotium	<0.1	19	>1.0	>26	>4.4
Poria sclerotium	<0.1	<2	><1.0	4.8	><1.0
Rehmannia root	<0.1	10	><1.0	>7.1	><1.0
Saposhnikovia root	<0.1	13	>1.3	>20	><1.0
Scutellaria root	<0.1	<2	<0.9	38	<1.0
Chinese medicines					
Byakkokaninjinto	7.2	17	><1.0	3.5	<1.0
Hangesyashinto	16.2	9	>4.9	>28	<1.0

Name	Glycyrrhizin content (mg/g)	LPS contamination (ng/g)	Anti-UV activity (SI)		Anti-HIV activity SI
			HSC-2	HaCaT	
Hotyuekkito	0.2	11	><1.0	>5.1	><1.0
Juzentaihoto	7	18	><1.0	>9.6	<1.0
Kikyoto	50.3	18	1.05	4.4	<1.0
Ninjinyoeito	3.5	19	><1.0	23	><1.0
Rikkosan	24.1	>200	>1.2	>6.8	<1.0
Saireito	7	17	>3.4	>19	<1.0
Shosaikoto	9.2	19	>4.3	34	<1.0
Unseiin	<0.1	>200	>4.9	>23	<1.0
Glycyrrhizin		<1	20.6	36.4	>2.0
Vitamin C			44	200	
AZT					17,850

Table 2. Anti-UV and anti-HIV activity of Kampo medicine and constitutional plant extract. Cited from Refs. [15, 16].

2.2.2. Anti-HSV activity

We have recently established the simple *in vitro* assay method of antiherpes simplex virus (HSV) activity. Vero cells, isolated from the kidney of African green monkey (*Cercopithecus aethiops*), [5] were infected with HSV-1 (multiplicity of infection = 0.01). First, HSV-1 and test samples were mixed and stood for 20 min, and the mixture was then added to the adherent Vero cells. After incubation for 4 days, the relative viable cell number was quantified by MTT reagent to yield CC_{50}, EC_{50}, and selectivity index (SI) ($SI = CC_{50}/EC_{50}$). By infection with HSV-1, the cell viability dropped to 34.1 ± 8.9% (18.3–51.1%) (n = 33). Addition of licorice root extracts recovered the cell density to 51–64% of control level. The incomplete recovery of cell viability urged us to adopt the following two methods for measuring EC_{50}. In method 1, EC_{50} was defined as the concentration at which the viability was restored to the midpoint between that of HSV-infected cells and that of mock-infected cells. In method II, EC_{50} was defined as the concentration at which the viability was restored to 50% that of mock-infected cells (see example in **Figure 3**) [4].

It was unexpected that water extract of licorice root [SI = >< 1 (method I); 4.6 (method II)] showed higher anti-HSV activity than alkaline extract (pH 12) (SI < 1 and < 1). Among water extracts, flavonoid-rich fraction showed the highest anti-HSV activity (SI = 4.6; 9.2). Among licorice flavonoids, liquiritin apioside (SI >5; >500), isoliquiritin apioside (SI >23.1; >455), lucurzid (SI >< 1; >667) and isoliquiritin (SI = 21.4; 128.6) showed the highest anti-HSV activity (center column in **Table 1**).

Among five polymethoxyflavonoids, the SI value for tricin (SI = 5.8; 7.0) (**Figure 1**) was comparable with that of SE, while the other four polymethoxyflavonoids (3,3′,4′,5,6,7,8-heptamethoxyflavone, nobiletin, tangeretin, sudachitin) had little or no anti-HSV activity

(SI < 1; < 1–1.1) (**Table 1**). Among lower molecular polyphenols, epigallocatechin gallate, a major compound in green tea, had some anti-HSV activity (SI < 1; 4.7), followed by resveratrol (SI < 1; 2.8).

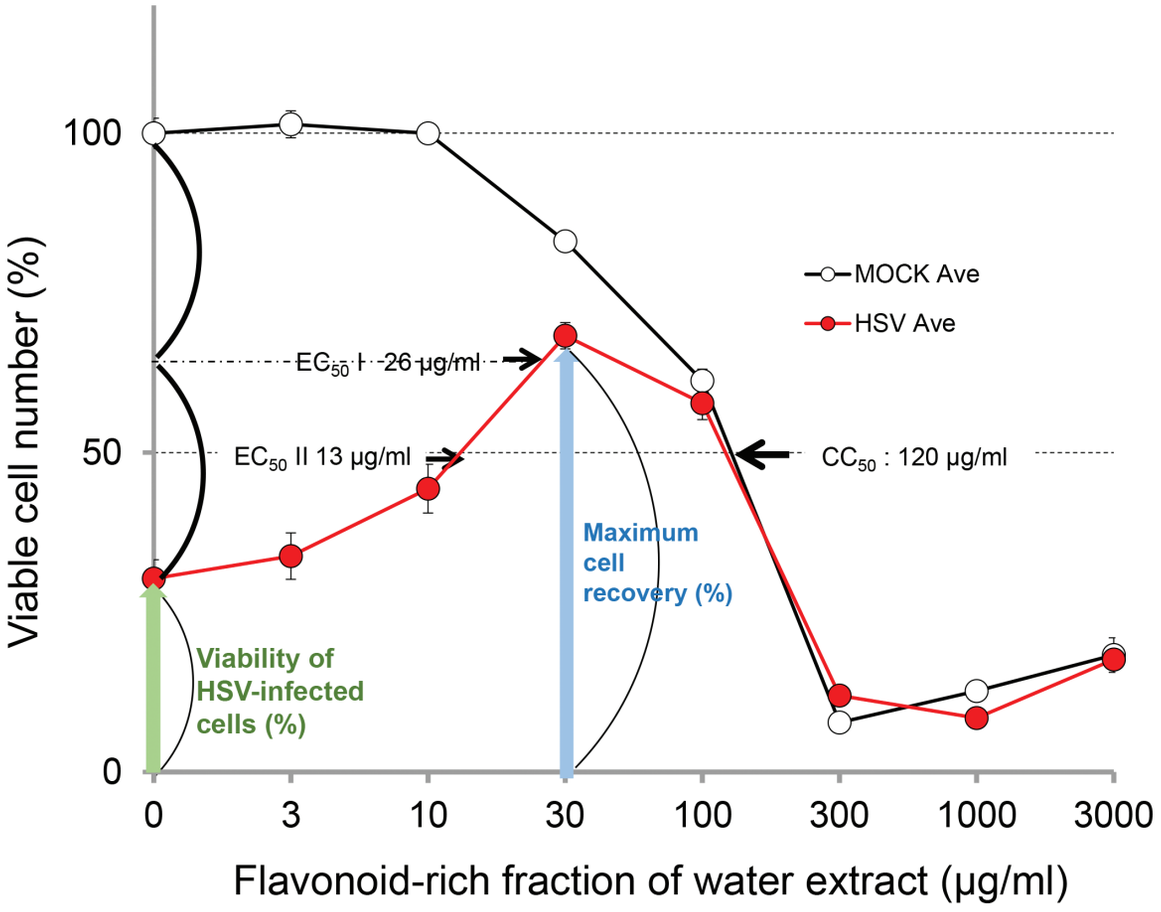


Figure 3. Anti-HSV activity of flavonoid-rich fraction of water extract of liquorice root. Each value represents the mean ± SD of triplicate assays. The 50% effective concentration (EC_{50}), determined by method I or II (see **Table 2**), and 50% cytotoxic concentration (CC_{50}) is indicated by arrows. Cited from Ref. [4]. Cited from Ref. [3].

The quantitative structure-activity relationship (QSAR) analysis [4] demonstrated that anti-HSV activity of licorice flavonoids and lower molecular weight polyphenols correlated well with six chemical descriptors that represent polarizability (MATS5p, GATS5p) [6, 7], ionization potential (GATS5i) [7], a number of ring systems (NRS) [8], and atomic number (J_Dz(Z)) and mass (J_Dz(m) [9] (r^2 = 0.684, 0.627, 0.624, 0.621, 0.619 and 0.618, respectively, $p < 0.0001$) (**Figure 4**). This result suggests that the physicochemical properties, rather than the category of compound, are important for determining anti-HSV activity.

2.3. Antitumor activity

The tumor-selectivity index (TS) was calculated by dividing the mean CC_{50} against normal cells by the mean CC_{50} against tumor cells. We used the following two methods. Method I:

http://dx.doi.org/10.5772/67435

human gingival fibroblast (HGF) + human periodontal fibroblast (HPLF) + human pulp cells (HPC) (normal mesenchymal cells) versus Ca9-22 + HSC-2 + HSC-3 + HSC-4 (human oral squamous cell carcinoma) (epithelial cells). Method II: HGF (gingival normal mesenchymal cells) versus Ca9-22 (gingival tumor epithelial cells) [10] (**Table 1**). We have confirmed that the TS value reflects the antitumor activity, based on the finding that antitumor drugs have extremely higher TS values [11].

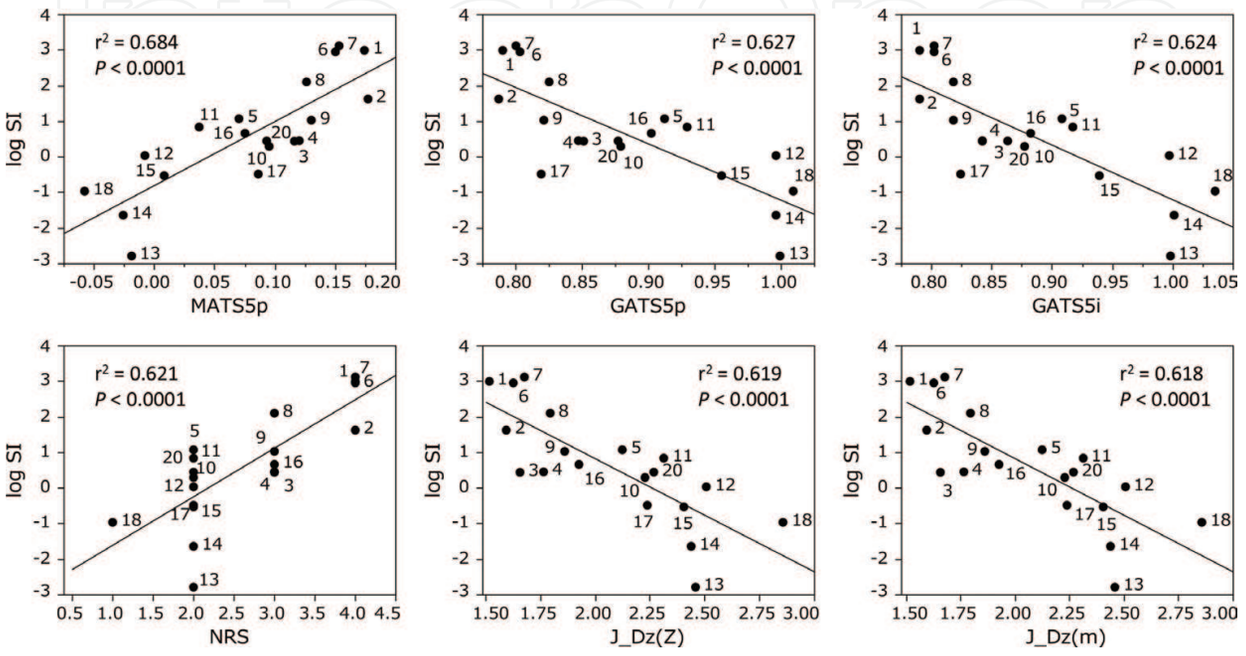


Figure 4. QSAR analysis of anti-HSV activity (defined as SI value determined by method II) of licorice flavonoids, polymethoxyflavonoids, and low molecular weight flavonoids. The log SI value was calculated from -logCC_{50} value and -logEC_{50} value. 1, liquiritin apioside; 2, liquiritin 7-apiosylglucose; 3, liquiritin; 4, neoliquiritin; 5, liquiritingenin; 6, isoliquiritin apioside; 7, lucurzid; 8, isoliquiritin; 9, neoisoliquiritin; 10, isoliquiritigenin; 11, tricin; 12, 3,3′,4′,5,6,7,8-heptamethoxyflavone; 13, nobiletin; 14, tangeretin; 15, sudachitin; 16, epigallocatechin gallate; 17, chlorogenic acid; 18, coumaric acid; 20, resveratrol. The data of curcumin, 19, was not included since the SI value could not be obtained (center column, **Table 1**). Cited from Ref. [4].

Among licorice root extracts, flavonoid-rich fraction of water extract showed the highest TS [TS = 5.5 (method I); 5.2 (method II)], although this value was much lower than that of popular antitumor drugs (doxorubicin, 5-fluorouracil, methotrexate, melphalan: TS = 8.9–170). Among licorice flavonoids, neoisoliquiritin apioside and isoliquiritigenin exhibited the highest antitumor activity (TS = 4.4–9.0), which was well correlated with their solvation energy (r^2 = 0.659) (QSAR analysis) [2]. Five polymethoxyflavonoids (tricin, 3,3′,4′,5,6,7,8-heptamethoxyflavone, nobiletin, tangeretin, and sudachitin) were weakly tumor selective (TS = 1.0–3.0) (right column, **Table 1**) [4].

2.4. Anti-inflammatory activity

We found that interleukin (IL)-1β stimulated the production of prostaglandin (PG)E_2, interleukin (IL)-6, IL-8, and monocyte chemoattractant protein-1 (MCP-1) by human gingival fibroblast (HGF)

to much higher extent than that achieved by LPS prepared from *Escherichia coli* and *Porphyromonas gingivalis* [12]. Glycyrrhiza extract significantly inhibited the IL-1β-stimulated PGE_2 production by HGF cells (EC_{50} = 95 μg/ml, CC_{50} > 4000 μg/ml; SI = CC_{50}/EC_{50} > 22) (**Figure 5A**). Similarly, glycyrrhiza extract significantly inhibited the IL-1β-stimulated PGE_2 production by human periodontal ligament fibroblasts (HPLF) cells (EC_{50} = 32 μg/ml, CC_{50} = 1878 μg/ml; SI = CC_{50}/EC_{50} = 59) (**Figure 5B**) [13]. The endospecy test with LAL reagent revealed that LPS contamination in glycyrrhiza and glycyrrhizin was very low (19 ng/g and <1 ng/g, respectively) (**Table 2**) [14].

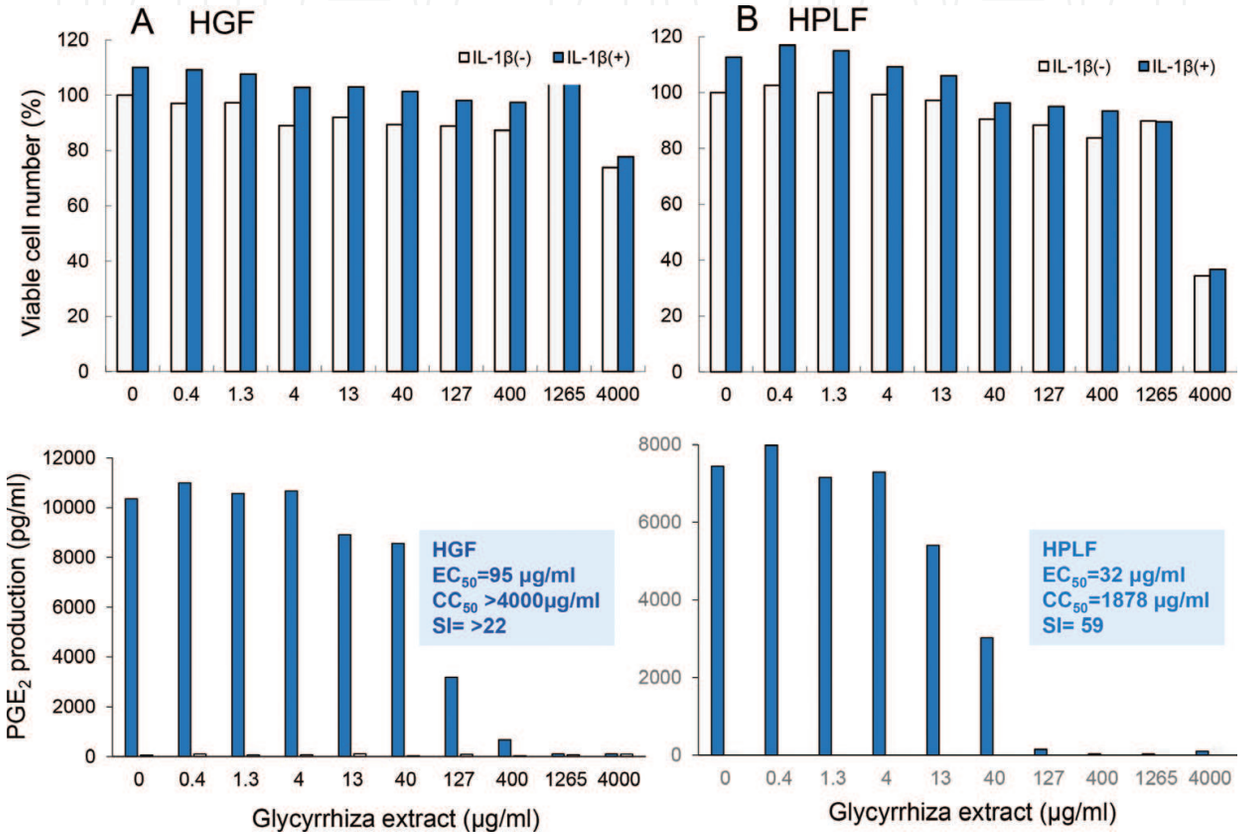


Figure 5. Anti-inflammatory activity of glycyrrhiza extract. HGF (A) and HPLF (B) were incubated for 24 h without (control) or with 5 ng/ml IL1-β in the presence of the indicated concentrations of glycyrrhiza extract, and the relative viable cell number was determined by the MTT method, and the extracellular PGE_2 production was determined by ELISA (Kato, unpublished data).

2.5. Anti-UV activity

We measured the ability of test samples to protect the UV-induced injury (referred to as anti-UV activity), using UV-sensitive cell line HSC-2 cell. We found that glycyrrhizin, a major component of Glycyrrhiza, exhibited very high anti-UV activity (SI = 20.6) (**Figure 6A**, **Table 2**). In order to determine whether there is any correlation of anti-UV activity and glycyrrhizin content, we investigated the concentration of glycyrrhizin in the plant extracts and Kampo medicines by HPLC [JASCO PU-980 pump, a JASCO UV-970 UV/VIS detector, Inertsil ODS-3 column, 254 nm; mobile phase: 2.5% acetic acid (40: 60)]. Twenty-five plant extracts, except for Glycyrrhiza (175.4 mg/g), did not contain detectable amounts of glycyrrhizin, whereas 10

http://dx.doi.org/10.5772/67435

Kampo medicines (Byakkokaninjinto, Hangesyashinto, Hotyuekkito, Juzentaihoto, Kikyoto, Ninjinyoeito, Rikkosan, Saireito, Shosaikoto, Unseiin) contained up to 50.3 mg/g of glycyrrhizin, possibly due to the inclusion of Glycyrrhiza (**Table 2**). However, there was no clear-cut relationship between the anti-UV activity and glycyrrhizin content of Kampo medicines and constituent plant extracts [15] (**Table 2**).

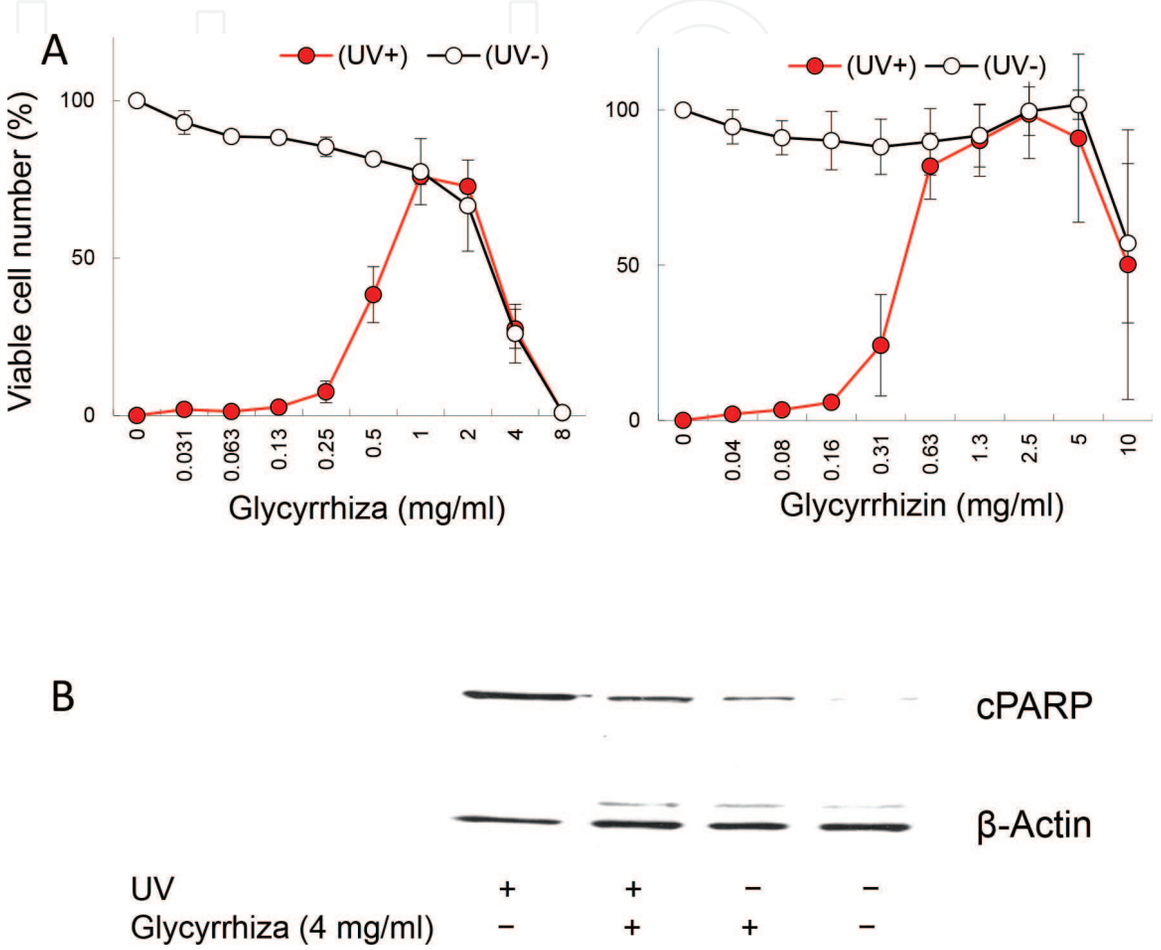


Figure 6. (A) Anti-UV activity of glycyrrhiza (left) and glycyrrhizin (right), (B) protective effect of glycyrrhiza on the UV-induced apoptosis in HSC-2 cells (western blot analysis). Cited from Ref. [15].

Western blot analysis demonstrated that UV irradiation induced the production of cleaved PARP, indicating the activation of caspase-3/-7 in HSC-2 cells, and that glycyrrhiza inhibited the UV-induced caspase activation (**Figure 6B**).

Since it is preferable to use skin-derived cells for the determination of anti-UV activity, we investigated the anti-UV activity of Kampo medicines using human immortal skin keratinocyte cell line HaCaT. We found that the HaCaT cell system gave nearly 1 order higher SI value than the HSC-2 system, maintaining good correlation of anti-UV activity measured between these two cell lines ($r^2 = 0.33$) (**Figure 7A**). There was some correlation between the anti-UV activity (defined as SI value) and absorbance at 253.7 nm in both systems ($r^2 = 0.14$ and 0.24, respectively) (**Figure 7B**), suggesting that some part of anti-UV activity comes from the direct absorption of UV.

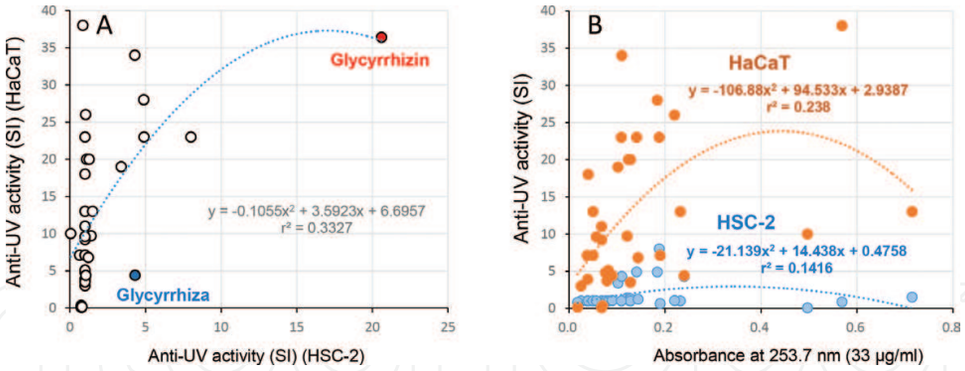


Figure 7. (A) Correlation between anti-UV activities measured in HSC-2 cells and that in HaCaT cells. (B) The correlation of anti-UV activity and absorbance (optical density) measured at 253.7 nm (Kato et al., unpublished data).

Among 10 Kampo medicines, Shosaikoto (SI = 34) showed the highest anti-UV activity, followed by Hangesyashinto (SI > 28), Unseiin (SI > 23), Ninjinyoeito (SI = 23), and Saireito (SI > 19), whereas other four Kampo medicines were much less active (SI < 9.6) (**Table 2**). Among 25 plant extracts, Scutellaria root exhibited the highest anti-UV activity (SI = 38), followed by Polyporus sclerotium (SI > 26), Gardenia fruit (SI > 23), Japanese Gentian (SI > 20), and Saposhnikovia root (SI > 20). Glycyrrhizin also exhibited potent anti-UV activity (SI = 36) (**Table 2**) [16].

3. Conclusions

Alkaline extract of licorice roots showed greater anti-HIV activity than the water extract, while the water extract, especially the flavonoid-rich fraction, showed greater anti-HSV activity than the alkaline extract (**Table 1**), suggesting that water and alkaline extracts might show different site of actions against these two viruses.

It was unexpected that five polymethoxyflavonoids including tricin showed very low level of anti-HSV activity (**Table 1**), since tricin has been reported to show a broad antiviral spectrum [17–20]. It remains to be investigated whether the combination of these compounds with acyclovir [5] or ganciclovir [21] may increase viability of infected cells.

The present study also demonstrated that neoisoliquiritin apioside showed the highest antitumor activity. Isoliquiritin has been reported to inhibit granuloma angiogenesis and tube formation in vascular endothelial cells [22]. Further studies are required to elucidate the mechanism by which this compound induces such high tumor specificity.

We have recently reported that titanium dioxide nanoparticles (TiO_2 NPs) were incorporated into vacuoles of HGF cells and aggravated the gingival inflammation (characterized by the enhanced prostaglandin E_2 production and COX-1 and COX-2 protein expression, and the reduction of intracellular concentrations of amino acid, urea cycle, polyamine, *S*-adenosylmethione and glutathione synthetic pathways) [23]. This finding recommends carful use of dental materials containing TiO_2 NPs for patients with gingivitis or periodontitis. It remains to be investigated whether glycyrrhiza extracts or their fractions can neutralize the aggravation effects of TiO_2 NPs.

http://dx.doi.org/10.5772/67435

More than 80 reports have investigated the anti-inflammatory effect of Kampo medicines (reviewed in Refs. [24] and [25]). However, basic research and clinical studies for the treatment of oral diseases are much less [26–30], and little is known about the relative potency of Kampo medicines. Potent antiviral, anti-inflammatory, antitumor, and anti-UV activities of licorice extracts, demonstrated here, suggest their possible applicability for treating several oral diseases and manufacturing cosmetic products.

QSAR analysis is a useful technique to predict the most active three-dimensional structure. The next step is to synthesize the compound with predicted structure and then investigate the biological activity. This prediction-synthesis-confirmation cycle should be repeated until obtaining the expected biological activity (**Figure 8**). The applicability of such compounds on oral diseases should be tested.

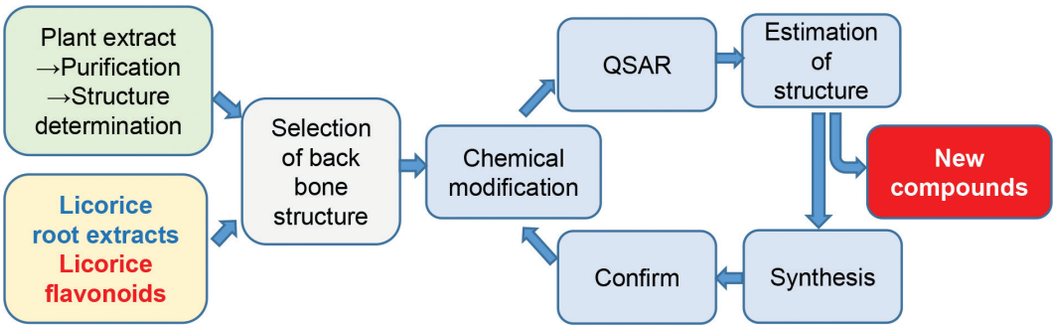


Figure 8. Scheme of manufacturing new anti-HSV agents from licorice extracts.

Author details

Hiroshi Sakagami[1,*], Takao Kato[2], Kunihiko Fukuchi[3], Taisei Kanamoto[4], Shigemi Terakubo[4], Hideki Nakashima[4], Hirokazu Ohno[5] and Masaji Yamamoto[5]

*Address all correspondence to: sakagami@dent.meikai.ac.jp

1 Division of Pharmacology, Meikai University School of Dentistry, Japan

2 Department of Oral and Maxillofacial Surgery, Kanazawa Medical University, Japan

3 Graduate School, Showa University, Tokyo, Japan

4 St. Marianna University School of Medicine, Kanagawa, Japan

5 Research Center, Maruzen Pharmaceuticals Co., Ltd., Japan

References

[1] Sakagami H: Biological activities and possible dental application of three major groups of polyphenols. J Pharmacol Sci. 2014;**126**:92–106.

[2] Ohno H, Araho D, Uesawa Y, Kagaya H, Ishihara M, Sakagami H, Yamamoto M: Evaluation of cytotoxicity and tumor-specificity of licorice flavonoids based on chemical structures. Anticancer Res. 2013;**33**:3061–3068.

[3] Ohno H, Miyoshi S, Araho D, Kanamoato T, Terakubo S, Nakashima H, Tsuda T, Sunaga K, Amano S, Ohkoshi E, Sakagami H, Satoh K, Yamamoto M: Efficient utilization of licorice root by alkaline extraction. In Vivo. 2014;**28**:785–794.

[4] Fukuchi K, Okudaira N, Adachi K, Odai-Ide R, Watanabe S, Ohno H, Yamamoto M, Kanamoto T, Terakubo S, Nakashima H, Uesawa Y, Kagaya H, Sakagami H: Antiviral and antitumor activity of licorice root extracts. In Vivo. 2016;**30**:777–786.

[5] Sakagami H, Fukuchi K, Kanamoto T, Terakubo S, Nakashima H, Natori T, Suguro-Kitajima M, Oizumi H, Yasui T, Oizumi T: Synergism of alkaline extract of the leaves of *Sasa senanensis* Rehder and antiviral agents. In Vivo. 2016;**30**:421–426.

[6] Moran PAP: Notes on continuous stochastic phenomena. Biometrika. 1950;**37**:17–23.

[7] Geary RC: The contiguity ratio and statistical mapping. Incorp Statist. 1954;**5**:115–145.

[8] Feher M, Schmidt JM: Property distributions: differences between drugs, natural products, and molecules from combinatorial chemistry. J Chem Inf Comput Sci. 2003;**43**:218–227.

[9] Ivanciuc O: QSAR comparative study of Wiener descriptors for weighted molecular graphs. J Chem Inf Comput Sci. 2000;**40**:1412–1422.

[10] Horikoshi M, Kimura Y, Nagura H, Ono T, Ito H: A new human cell line derived from human carcinoma of the gingiva. I. Its establishment and morphological studies. (in Japanese). Japan J Oral Maxillofac Surg. 1974;**20**:100–106.

[11] Suzuki R, Matsuo S, Sakagami H, Okada Y, Shirataki Y: Search of new cytotoxic crude materials against human oral squamous cell carcinoma using NMR metabolomics Anticancer Res. 2014;**34**:4117–4120.

[12] Ono M, Kantoh K, Ueki J, Shimada A, Wakabayashi H, Matsuta T, Sakagami H, Kumada H, Hamada N, Kitajima M, Oizumi H, Oizumi T: Quest for anti-inflammatory substances using IL-1β-stimulated gingival fibroblasts. In Vivo. 2011;**25**:763–768.

[13] Kato T, Segami N, Sakagami H: Anti-inflammatory action of Kampo medicines and component herb extracts in relation to LPS contamination. (In Japanese). 2015 132th Kanto Regional Meeting, the Japanese Pharmacological Society, Chiba, July 2015.

[14] Kato T, Segami N, Sakagami H: Anti-inflammatory activity of hangeshashinto in IL-1β-stimulated gingival and periodontal ligament fibroblasts. In Vivo. 2016;**30**:257–264.

[15] Kato T, Horie N, Matsuta T, Umemura N, Shimoyama T, Kaneko T, Kanamoto T, Terakubo S, Nakashima H, Kusama K, Sakagami H: Anti-UV/HIV activity of Kampo medicines and constituent plant extracts. In Vivo. 2012;**26**:1007–1013.

[16] Kato T, Hino S, Horie N, Shimoyama T, Kaneko T, Kusama K, Sakagami H: Anti-UV activity of Kampo medicines and constituent plant extracts: re-evaluation with skin keratinocyte system. In Vivo. 2014;**28**:571–578.

[17] Wang HK, Xia Y, Yang ZY, Natschke SL, Lee KH: Recent advances in the discovery and development of flavonoids and their analogues as antitumor and anti-HIV agents. Review Adv Exp Med Biol. 1998;**439**:191–225.

[18] Matsuta T, Sakagami H, Satoh K, Kanamoto T, Terakubo S, Nakashima H, Kitajima M, Oizumi H, Oizumi T. Biological activity of luteolin glycosides and tricin from *Sasa senanensis* Rehder. In Vivo. 2011;**25**:757–762.

[19] Murayama T, Li Y, Takahashi T, Yamada R, Matsubara K, Tuchida Y, Li Z, Sadanari H. Anti-cytomegalovirus effects of tricin are dependent on CXCL11. Microbes Infect. 2012;**14**:1086–1092.

[20] Yazawa K, Kurokawa M, Obuchi M, Li Y, Yamada R, Sadanari H, Matsubara K, Watanabe K, Koketsu M, Tuchida Y, Murayama T. Anti-influenza virus activity of tricin, 4′,5,7-trihydroxy-3′,5′-dimethoxyflavone. Antivir Chem Chemother. 2011;**22**:1–11.

[21] Yamada R, Suda H, Sadanari H, Matsubara K, Tuchida Y, Murayama T: Synergistic effects by combination of ganciclovir and tricin on human cytomegalovirus replication in vitro. Antiviral Res. 2016;**125**:79–83.

[22] Kobayashi S, Miyamoto T, Kimura I, Kimura M: Inhibitory effect of isoliquiritin, a compound in licorice root, on angiogenesis in vivo and tube formation in vitro. Biol Pharm Bull. 1995;**18**: 1382–1386.

[23] Garcia-Contreras R, Sugimoto M, Umemura N, Kaneko M, Hatakeyama Y, Soga T, Tomita M, Scougall-Vilchis RJ, Contreras-Bulnes R, Nakajima H, Sakagami H: Alteration of metabolomic profiles by titanium dioxide nanoparticles in human gingivitis model. Biomaterials. 2015;**57**:33–40.

[24] Xia Q, Zhang H, Sun X, Zhao H, Wu L, Zhu D, Yang G, Shao Y, Zhang X, Mao X, Zhang L and She G: A comprehensive review of the structure elucidation and biological activity of triterpenoids from Ganoderma spp. Molecules. 2014;**30**:17478–17535.

[25] Shen Y, Li CG, Zhou SF, Pang EC, Story DF, Xue CC: Chemistry and bioactivity of Flos Magnoliae, a Chinese herb for rhinitis and sinusitis. Review. Curr Med Chem. 2008;**15**:1616–1627.

[26] Hijikata Y, Makiura N, Kano T, Higasa K, Shimizu M, Kawata K, Mine T: Kampo medicine, based on traditional medicine theory, in treating uncured glossodynia: efficacy in five clinical cases. Am J Chin Med. 2008;**36**:853–847.

[27] Liao J, Zhao L, Yoshioka M, Hinode D, Grenier D: Effects of Japanese traditional herbal medicines (Kampo) on growth and virulence properties of Porphyromonas gingivalis and viability of oral epithelial cells. Pharm Biol. 2013;**51**:1538–1544.

[28] Ara T, Honjo K, Fujinami Y, Hattori T, Imamura Y, Wang PL: Preventive effects of a Kampo medicine, orento on inflammatory responses in lipopolysaccharide treated human gingival fibroblasts. Biol Pharm Bull. 2010;**33**:611–616.

[29] Ohshima S, Hatori Y, Honma S, Terasawa K, Saitoh Y, Kobayashi D: Analysis of body constitution of 52 patients with Stevens-Johnson Syndrome (SJS) using Kampo medical questionnaires: prediction of SJS based on body constitution using decision tree. Yakugaku Zasshi. 2011;**131**:745–756.

[30] Sharma R, Tobin P, Clarke SJ. Sharma R, Tobin P, Clarke SJ: Management of chemotherapy-induced nausea, vomiting, oral mucositis, and diarrhoea. Lancet Oncol. 2005;**6**: 93–102.