Abstract. Background: In the search for anti-viral and antitumour substances from natural resources, antiviral and antitumour activities of licorice root extract and purified ingredients were investigated. Materials and Methods: Viability of cells was determined by 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide method. Antiviral activity was quantified by the selectivity index, defined as the ratio of the 50% cytotoxic concentration (CC$_{50}$) to the 50% effective concentration against human immunodeficiency virus (HIV) or herpes simplex virus (HSV)-infected cells (EC$_{50}$). The tumour specificity was calculated by the ratio of CC$_{50}$ against human normal oral cells to that against human oral squamous cell carcinoma cell lines. Licorice flavonoids and lower molecular polyphenols were subjected to quantitative structure–activity relationship analysis. Results: Alkaline extract of licorice root had higher anti-HIV activity than did water extracts, confirming our previous reports. On the other hand, water extract, especially the flavonoid-rich fraction, had higher anti-HSV activity than did the alkaline extract. The flavonoid-rich fraction was more cytotoxic against human oral squamous cell carcinoma cell lines compared to normal oral cells, suggesting their tumor-specific cytotoxicity. Conclusion: The present study suggests that water and alkaline extracts of licorice root exert different mechanisms of actions against these two viruses. Physicochemical properties, rather than the category of compounds, may be important in determining their anti-HSV activity.

We previously reported that lignin–carbohydrate complex fractions prepared by acid precipitation of the alkaline extracts of various plants potently protected cells from the cytopathic effect of human immunodeficiency virus (HIV) infection (1), and from the damage induced by UV irradiation (2). Crude alkaline extract of the leaves of Sasa senanensis Rehder (SE) (3, 4), green tea leaf, oolong tea leaf, orange flower (5) and licorice root (6) had comparable anti-HIV and anti-UV activity to that of lignin–carbohydrate complex fractions (3, 4). On the other hand, hot-water extracts of various teas and orange flower (5) and Kampo medicines (7) exhibited much lower anti-HIV and anti-UV activity, suggesting the superiority of alkaline extraction over hot-water extraction in obtaining higher amounts of anti-HIV and anti-UV substances. We recently performed quantitative structure–activity relationship analysis of the cytotoxic activity of flavonoids derived from the licorice root against human oral malignant and non-malignant cells (8). Although there are many articles on exist antiviral and antitumor activity of licorice products, most of them have not considered the chemotherapeutic index, which reflects both the cytotoxicity and efficacy. Furthermore, the antitumor activity of alkaline extract of licorice root has not yet been reported to our knowledge.

Based on the above, here we compared three aspects of biological activity (namely anti-HIV, anti-HSV and antitumor activity) of different licorice root extracts and purified components, according to the corresponding selectivity indices (SI). Polymethoxyflavonoids, including tricin, which has a broad antiviral spectrum (9-12), and SE, with potent anti-HIV and anti-HSV activity (13), were used as reference compounds.
Materials and Methods

Materials. The following chemicals and reagents were obtained from the indicated companies: Dulbecco’s modified Eagle’s medium (DMEM) (Gibco BRL, Grand Island, NY, USA); fetal bovine serum (FBS), 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT), RPMI-1640 medium, azidothyminidine (AZT), 2’,3’-dideoxycytidine (ddC), resveratrol, melphalan (Sigma-Aldrich Inc., St. Louis, MO, USA); 3,3’,4’,5,6,7,8-heptamethoxyflavone, nobiletin, tangeretin, sudachitin, methotrexate, dimethyl sulfoxide, dextran sulfate (5 kDa) (Wako Pure Chemical Ind., Ltd., Osaka, Japan); curdlan sulfate (79 kDa; Ajinomoto Co. Inc., Tokyo, Japan); acyclovir, epigallocatechin gallate, curcumin, coumaric acid (Tokyo Chemical Industry Co. Ltd, Tokyo), tricin (Carbosynth ltd., Berkshire, UK). Glycyrrhizin acid and glycyrrhetinic acid were provided by Maruzen Pharmaceuticals (Shibuya, Tokyo, Japan). Culture plastic plates and dishes (96-well) were purchased from Becton Dickinson Labware (Franklin Lakes, NJ, USA). Bottled teas were obtained from a grocery store, and their osmotic pressures were measured by use of a cryoscopic osmometer (OSMOMAT030; Gonotec GmbH, Berlin, Germany).

SE was kindly provided by Daiwa Biological Research Institute Co., Ltd. (Kawasaki, Kanagawa, Japan). One milliliter of SE contained 66.1 mg freeze-dried powder.

Preparation of water and alkaline extracts. Water and alkaline extracts of licorice root (Glycyrrhiza glabra harvested in Afghanistan) were prepared, as described previously (6).

Flavonoid and chalcone derivatives (liquiritigenin 7-apiosylglucoside, liquiritigenin, liquiritiglucoside, liquiritiglucoside, liquiritirin, liquiritigenin, isoliquiritigenin, isoliquiritigenin, licurazid, isoliquiritigenin, neosoliquiritigenin, isoliquiritigenin) were prepared from the MeOH extract of air-dried roots of G. inflata, by Diaion HP-20 and octadecylsilyl (ODS) column, as described previously (8).

Assay for anti-HIV activity. Human T-cell leukemia virus I (HTLV-I)-bearing CD4-positive human T-cell line MT-4, established by Dr. Miyoshi (14) was cultured in RPMI-1640 medium supplemented with 10% FBS and infected with HIV-1 (HIV-1) at a multiplicity of infection of 0.01. HIV- and mock-infected MT-4 cells (5×10⁴ cells/96-microwell) were incubated for 5 days with different concentrations of extracts and the relative viable cell number was determined by MTT assay. The concentration that reduced the number of viable uninfected cells by 50% (CC₅₀) and that which increased the number of viable HIV-infected cells to 50% of that of control (mock-infected, untreated) cells (EC₅₀) were determined from the dose–response curve with mock-infected and HIV-infected cells, respectively. The anti-HIV activity was evaluated by the SI, which was calculated using the following equation: SI=CC₅₀(EC₅₀) (15).

Qsar analysis. The logSI value was calculated from -logCC₅₀value and -logEC₅₀value, as described previously (20). In order to determine the correlation coefficient, we used the data of method II and the following descriptors calculated by Dragon software ver 6.0.16 (Talete srl, Pisani, Milano, Italy): MATS5p (Moran autocorrelation of lag 5 weighted by polarizability; category, 2D autocorrelations) (21); GATS5p (Geary autocorrelation of lag 5 weighted by polarizability; category, 2D autocorrelations) (22); GATS5i (Geary autocorrelation of lag 5 weighted by ionization potential; category, 2D autocorrelations) (21); NRS (number of ring systems; category, Ring descriptors) (23): J_Dz(Z) (Balaban-like index from Barysz matrix weighted by atomic number; category, 2D matrix-based descriptors) (24); and J_Dz(m) (Balaban-like index from Barysz matrix weighted by mass; 2D matrix-based descriptors) (25).

Statistical treatment. Experimental values are expressed as the mean±standard deviation (SD). Statistical analysis was performed by using Mann–Whitney U-test. A value of p<0.05 was considered to be significant. The relation between anti-HSV activity and chemical...
descriptors was investigated using simple regression analyses by JMP Pro version 12.2.0 (SAS Institute Inc., Cary, NC, USA).

Results

Anti-HIV activity. Infection of MT-4 cells with HIV-1 reduced viability to almost zero (13, 14). Among licorice extracts, water extract, its purified fraction and flavonoid-rich fractions had no protective effect on HIV infection (SI<1) (Table I). Alkaline extract exhibited potent anti-HIV activity (SI>9.2), although the anti-HIV activity of alkaline extract was lower than that of SE (SI=42.7) and anti-viral agents (dextran sulfate, curdlan sulfate, AZT, ddC) (SI=3029-11715). The anti-HIV activity increased with increasing pH of the extracting solution ((SI=><1, >3 and >9.2 at pH 5.6, 9 and 12, respectively).

Glycyrrhizin acid, glycyrrhetinic acid, licorice flavonoids (iquiritin apioside, liquiritin 7-apiosylglucose, liquiritin, neoliquiritin, liquiritigenin, isoliquiritin apioside, lucurzid, isoliquiritin, neoisoliquiritin, isoliquiritigenin), tricin and other popular polymethoxyflavonoids (3,3’,4’,5,6,7,8-heptamethoxyflavone, nobiletin, tangeretin, sudachitin) were all inactive (SI <1), due to higher cytotoxicity (Table I).

Anti-HSV activity. Infection of Vero cells with HSV-1 reduced cell viability to 34.1±8.9% (18.3-51.1%) (n=33) (Table II). This incomplete reduction of viability may be due to the slower spread of HSV infection than the growth of Vero cells.

We first confirmed that the addition of alkaline extract of the leaves of Sasa senanensis Rehder (SE) (used as positive control) to HSV-infected cells had potent anti-HSV activity [SI=6.0±2.7 (3.6-9.3) (method I) and SI=8.0±2.8 (4.5-11.4) (method II)], increasing cell viability to 96.4±5.1% (93-100%) (Table II). This incomplete reduction of viability may be due to the slower spread of HSV infection than the growth of Vero cells.

In contrast, licorice root extracts, licorice flavonoids, polymethoxyflavonoids, and low molecular weight polyphenols achieved significantly (p<0.05) lower recovery of cell viability after HSV infection (viability of 51.0-64.0%) (Table II). The lower recovery urged us to adopt two different methods for measuring the anti-HSV activity (method I and II). The higher anti-HSV activity of water
Table 1. Anti-human immunodeficiency virus (HIV) activity of licorice root extract. Each value was obtained by dose–response experiments of triplicate assays. The selectivity index (SI) was calculated using the following equation: $SI = \frac{CC_{50}}{EC_{50}}$, where $CC_{50}$ was the concentration that reduced the number of viable uninfected cells by 50% and $EC_{50}$ was the concentration that increased the number of viable HIV-infected cells to 50% that of control (mock-infected, untreated) cells. SE: Alkaline extract of the leaves of Sasa senanensis Rehder; AZT: azidothymidine; ddC: 2',3'-dideoxycytidine.

| Licorice root extracts                  | $CC_{50}$ in mock-infected cells | $EC_{50}$ in HIV-infected cells | SI       |
|----------------------------------------|----------------------------------|---------------------------------|----------|
| Water extract (μg/ml)                  | >500                             | 387                             | >1.3     |
| Purified fraction of water extract (μg/ml) | 61.09                           | >500                             | <1       |
| Flavonoid-rich fraction of water extract (μg/ml) | 56.04                           | >500                             | <1       |
| Water extract (different lot) (pH 5.6) (μg/ml) | >500                             | >500                             | <1       |
| Alkaline (pH 9.0) extract (μg/ml)      | >500                             | 165.79                           | >3.0     |
| Alkaine (pH12.0) extract (μg/ml)       | >500                             | 54.1                             | >9.2     |
| Glycyrrhizin acid (μg/ml)              | >500                             | 420.8                            | >1.2     |
| Glycyrrhetic acid (μg/ml)              | >500                             | 500                              | <1       |

| Licorice flavonoids                    |                                  |                                 |          |
|----------------------------------------|----------------------------------|---------------------------------|----------|
| Liquiritin apioside (μg/ml)            | >500                             | >500                             | <1       |
| Liquiritin 7-apiosylglucose (μg/ml)    | >500                             | >500                             | <1       |
| Liquiritin (μg/ml)                     | 28.82                            | >500                             | <1       |
| Neoliquiritin (μg/ml)                  | 11.75                            | >500                             | <1       |
| Liquiritigenin (μg/ml)                 | 10.2                             | >500                             | <1       |
| Isoapisoisoliquiritin apioside (μg/ml) | >500                             | >500                             | <1       |
| Lucurzid (μg/ml)                       | >500                             | >500                             | <1       |
| Isoliquiritin (μg/ml)                  | 9.6                              | >500                             | <1       |
| Neoisoliquiritin (μg/ml)               | 9.39                             | >500                             | <1       |
| Isoliquiritigenin (μg/ml)              | 3.86                             | >500                             | <1       |

| Polymethoxyflavonoids                 |                                  |                                 |          |
|----------------------------------------|----------------------------------|---------------------------------|----------|
| Tricin (μM)                            | 354.71                           | >500                             | <1       |
| 3,3',4',5,6,7,8-Heptamethoxyflavone (μM) | 64.58                           | >500                             | <1       |
| Nobiletin (μM)                         | 51.01                            | >500                             | <1       |
| Tangeretin (μM)                        | 130.77                           | >500                             | <1       |
| Sudachitin (μM)                        | 9.8                              | >500                             | <1       |

| Positive control                      |                                  |                                 |          |
|----------------------------------------|----------------------------------|---------------------------------|----------|
| SE (%)                                 | 1.11                             | 0.026                           | 42.7     |
| Dextran sulfate (μg/ml)                | >1,000                           | 0.215                           | >4,651   |
| Curdian sulfate (μg/ml)                | >1,000                           | 0.163                           | >6,135   |
| AZT (μM)                               | 46.858                           | 0.004                           | 11,715   |
| ddC (μM)                               | 2,198.73                         | 0.726                           | 3,029    |

effect of licorice root [SI=><1 (method I), 4.6 (method II)] than alkaline extract (pH 12) [SI<1(method I), <1 (method II)] was unexpected. Flavonoid-rich fraction of water extract had the highest anti-HSV activity [SI=4.6 (method I), 9.2 (method II)]. Among licorice flavonoids, liquiritin apioside [SI=5 (method I), >500 (method II)], isoliquiritin apioside [SI=23.1 (method I), >455 (method II)], lucurzid [SI<1 (method I), >667 (method II)] and isoliquiritin [SI=21.4 (method I), 128.6 (method II)] had the highest anti-HSV activity.

Among five polymethoxyflavonoids, the SI value for tricin [SI=5.8 (method I), 7.0 (method II)] (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 (method I), <1~1.1 (method II)] (Table II).

Among lower molecular polyphenols, epigallocatechin gallate, a major compound in green tea, had some anti-HSV activity [SI<1 (method I), 4.7 (method II)], followed by resveratrol [SI<1 (method I), 2.8 (method II)] (Table II). This finding prompted us to survey the anti-HSV activity of commercially available bottles of tea extracts. Toasted tea (undiluted), oolong tea (10-fold diluted), green tea (commercial name: “tasty” tea) (undiluted), green tea (commercial name: “healthy” green tea) (10-fold diluted), black tea (commercial name: “afternoon tea”) (10-fold
Table II. Anti-herpes simplex virus (HSV) activity of licorice root extract. The selectivity index (SI) was calculated using the following equation: 

\[ SI = \frac{CC_{50}}{EC_{50}} \]

where \( CC_{50} \) was the concentration that reduced the number of viable uninfected cells by 50% and \( EC_{50} \) was the concentration at which the viability was restored to the midpoint between that of HSV-infected cells and that of mock-infected cells (Method I), or at the point where the viability was restored to 50% that of mock-infected cells (Method II). Each value was obtained by dose–response experiments of triplicate assays.

ND: The SI value could not be determined due to the viability of HSV-infected cells exceeding 50%. SE: Alkaline extract of the leaves of Sasa senanensis Rehder. Maximum cell recovery (%) was calculated at the concentration that induced maximum recovery of viable cells. Maximum cell recovery (%) and viability of HSV-infected cells (%) are explained in the legend of Figure 1.

| Licorice root extracts | EC\(_{50}\) in HSV-infected cells | CC\(_{50}\) in mock-infected cells | Method I | Method II | Method I | Method II | Maximum cell recovery (%) |
|------------------------|----------------------------------|-----------------------------------|----------|----------|----------|----------|----------------------------|
|                        | Viability of HSV-infected cells (%) | CC\(_{50}\) in mock-infected cells |          |          |          |          |                            |
| Water extract (μg/ml)  | 27.0                             | >3,000                           | >3,000   | 650      | <1       | >4.6     | 60                          |
| Purified fraction of water extract (μg/ml) | 27.2                             | 340                               | 100      | 50       | 3.4      | 6.8      | 50                          |
| Flavonoid-rich fraction of water extract (μg/ml) | 30.0                             | 120                               | 26       | 13       | 4.6      | 9.2      | 69                          |
| Water extract (different lot) (pH 5.6) (μg/ml) | 20.0                             | 1,500                             | >3,000   | 740      | <1       | 2.0      | 57                          |
| Alkaline (pH 9.0) extract (μg/ml) | 35.8                             | 1,900                             | >3,000   | 600      | <1       | 3.2      | 62                          |
| Alkaine (pH12.0) extract(μg/ml) | 30.8                             | 1,400                             | >3,000   | >3,000   | <1       | <1      | 49                          |
| Glycyrrhizin acid (μg/ml) | 33.1                             | 300                               | >300     | >300     | <1       | <1      | ND                          |
| Glycyrrhetic acid (μg/ml) | 35.2                             | 52                                | >3,000   | >3,000   | <1       | <1      | ND                          |

Licorice flavonoids

| Liquiritin apioside (μg/ml) | 44.8                             | >3,000                           | 600      | 6        | >5       | >500     | 79                          |
| Liquiritin 7-apiosylglucose (μg/ml) | 34.1                             | >3,000                           | >3,000   | 140      | >1       | >21      | 58                          |
| Liquiritin (μg/ml) | 33.8                             | 14                               | >3,000   | 5        | <1       | 2.8      | 55                          |
| Neoliquiritin (μg/ml) | 41.6                             | 52                               | >3000    | 18       | <1       | 2.9      | 59                          |
| Liquiritigenin (μg/ml) | 45.3                             | 18                               | 7.5      | <3       | >2.4     | >6       | 77                          |
| Isoliquiritin apioside (μg/ml) | 47.9                             | >3,000                           | 130      | 6.6      | 23.1     | >455     | 82                          |
| Licurzid (μg/ml) | 41.5                             | >2,000                           | >3,000   | 3        | <1       | >667     | 65                          |
| Isoliquiritin (μg/ml) | 35.9                             | 18                               | 0.84     | 0.14     | 21.4     | 128.6    | 97                          |
| Neoisoliquiritin (μg/ml) | 20.0                             | 37                               | 4.6      | 3.4      | 8.0      | 10.9     | 87                          |
| Isoliquiritigenin (μg/ml) | 25.2                             | 12                               | >100     | 6        | <1       | 2.0      | 54                          |

Polymethoxyflavonoids

| Tricin (μM) | 18.3                             | 14                               | 2.4      | 2        | 5.8      | 7.0      | 67                          |
| 3,3’,4’,5,6,7,8-Heptamethoxyflavone (μM) | 28.2                             | 110                              | 1,000    | 100      | <1       | 1.1      | 50                          |
| Nobilentin (μM) | 21.6                             | 10                               | >3,000   | >3,000   | <1       | <1       | 42                          |
| Tangeretin (μM) | 32.9                             | 140                              | >3,000   | >3,000   | <1       | <1       | 45                          |
| Sudachitin (μM) | 36.3                             | 18                               | >30      | >30      | <1       | <1       | 42                          |

Polyphenols

| Epigallocatechin gallate (μM) | 29.3                             | 70                               | >300     | 15       | <1       | 4.7      | 62                          |
| Chlorogenic acid (μM) | 33.3                             | 2,000                            | >3,000   | >3,000   | <1       | <1       | 49                          |
| Coumaric acid (μM) | 41.5                             | 660                              | >3,000   | >3,000   | <1       | <1       | 69                          |
| Curcumin (μM) | 51.0                             | 19                               | 10       | ND       | 1.9      | ND       | 75                          |
| Resveratrol (μM) | 47.1                             | 21                               | >3000    | 7.4      | <1       | 2.8      | 55                          |

Positive control

| SE (%)(v/v) | 38.0                             | 0.25                             | 0.054    | 0.033    | 4.6      | 7.6      | 93                          |
| SE (%)(v/v) | 25.2                             | 0.45                             | 0.052    | 0.044    | 8.7      | 10.2     | 100                         |
| SE (%)(v/v) | 36.1                             | 0.2                              | 0.056    | 0.044    | 3.6      | 4.5      | 100                         |
| SE (%)(v/v) | 50.0                             | 0.3                              | 0.076    | 0.048    | 3.9      | 6.3      | 89                          |
| SE (%)(v/v) | 28.9                             | 0.5                              | 0.054    | 0.044    | 9.3      | 11.4     | 100                         |

Anti-tumor activity. Antitumor activity was determined by the ratio of cytotoxicity against four oral squamous cell lines vs. three normal oral cell types or the ratio of cytotoxicity against

diluted) and freshly made green tea (100-fold diluted) completely inhibited the cytopathic effect of HSV-infection, whereas barley tea was inactive (Figure 2).
Table III. Tumor specificity (TS) of licorice root extracts. Each value was obtained by dose–response experiments of triplicate assays. HGF, Human gingival fibroblast; HPC, human pulp cells; HPLF, human periodontal ligament fibroblast; oral squamous cell carcinoma cell lines: Ca9-22 (derived from gingival tissue), HSC-2, HSC-3 and HSC-4 (derived from tongue); CC50, 50% cytotoxic concentration; 5-FU, 5-fluorouracil.

|                    | Human oral squamous cell carcinoma | Human normal oral cells | TS   |
|--------------------|------------------------------------|-------------------------|------|
|                    | Ca9-22 (A) | HSC-2 | HSC-3 | HSC-4 | Mean (B) | HGF (C) | HPLF (D) | HPC Mean (D/B) | (C/A) |
| Licorice root extracts |          |       |       |       |          |         |          |                  |      |
| Water extract (μg/ml) | >500  | >500  | >500  | >500  | >500  | >500  | >500  | >500  | >1         | >1   |
| Purified fraction of water extract (μg/ml) | 103  | 200  | 69  | 153  | 131  | 438  | >500  | >431  | >456      | 3.5   | 4.2   |
| Flavonoid-rich fraction of water extract (μg/ml) | 51  | 75  | 35  | 71  | 58  | 263  | 347  | 351  | 320      | 5.5   | 5.2   |
| Water extract (different lot) (pH 5.6) (μg/ml) | >500  | >500  | >500  | >500  | >500  | >500  | >500  | >500  | ><1      | <1   |
| Alkaline (pH 9.0) extract (μg/ml) | >500  | >500  | >500  | >500  | >500  | >500  | >500  | >500  | ><1      | <1   |
| Alkaline (pH12.0) extract (μg/ml) | >500  | >500  | >500  | >500  | >500  | >500  | >500  | >500  | ><1      | <1   |
| Glycyrhrizin acid (μg/ml) | >500  | >500  | >500  | >500  | >500  | >500  | >500  | >500  | ><1      | <1   |
| Glycyrhetic acid (μg/ml) | 77  | 61  | 50  | 68  | 64  | 86  | 65  | 63  | 71       | 1.1   | 1.1   |
| Positive control |          |       |       |       |       |         |          |       |          |      |
| Doxorurubicin (μM) | 0.28 | 0.09 | 0.13 | <0.078 | <0.15 | 3.47 | 9.57 | 0.45 | >30.9 | 12.2  |
| 5-FU (μM) | 35 | 208 | 10 | 198 | 113 | >1,000 | >1,000 | >1,000 | >1,000 | >8.9   | >28.8   |
| Methotexate (μM) | 8.97 | 0.19 | <0.13 | <0.13 | 2.35 | >400 | >400 | >400 | >170 | >45     |
| Polymethoxyflavonoids |          |       |       |       |       |         |          |       |          |      |
| 3,3′,4′,5,6,7,8-Heptamethoxyflavone (μM) | 110 | 176 | 81 | 238 | 151 | 305 | 317 | 295 | 306 | 2.0   | 2.8    |
| Nobiletin (μM) | 91 | 189 | 50 | 182 | 128 | 165 | 306 | 302 | 258 | 2.0   | 1.8    |
| Tangeretin (μM) | 131 | 100 | 117 | 91 | 110 | 310 | 365 | 313 | 329 | 3.0   | 2.4    |
| Sudachitin (μM) | 40 | 158 | 56 | 117 | 93 | 67 | 500 | 252 | 273 | 2.9   | 1.7    |
| Tricin (μM) | >500  | >500  | >500  | >500  | >500  | >500  | >500  | >500  | ><1      | <1   |
| Doxorurubicin (μM) | 0.133 | 0.060 | 0.090 | 0.058 | 0.085 | 7.3 | 1.3 | 9.3 | 6.0 | 69.9   | 54.8   |
| 5-FU (μM) | >1,000 | 128 | 79 | >325 | >1,000 | 234 | >745 | >2.3 | >10.9  |      |
| Mephalan (μM) | 92   | 142  | 20 | 28  | 157 | 146 | 154 | 152 | 5.4 | 2.7    |      |
| Oil-soluble fraction of licorice |          |       |       |       |       |         |          |       |          |      |
| (G. glabra) (μg/ml) | 27 | 29 | 28 | 28 | 74 | 75 | 59 | 69.3 | 2.5    |
| (G. uraleansis) (μg/ml) | 11 | 18 | 22 | 17 | 112 | 85 | 42 | 79.7 | 4.7    |
| (G. inflata) (μg/ml) | 32 | 35 | 24 | 30 | 71 | 82 | 61 | 71.3 | 2.4    |
| Licorice flavonoids |          |       |       |       |       |         |          |       |          |      |
| Liquiritin apioside (μg/ml) | >200 | >200 | >200 | >200 | >200 | >200 | >200 | >200 | ><1.0  |      |
| Liquiritin (μg/ml) | 156 | 69 | 128 | 118 | >200 | >200 | >200 | >200 | >1.7    |
| Neoliquiritin (μg/ml) | >200 | >200 | >200 | >200 | >200 | >200 | >158 | >186 | ><0.9  |      |
| Liquiritigenin (μg/ml) | 38 | 49 | 86 | 58 | 145 | 138 | 70 | 118 | 2.0    |
| Isoliquiritin apioside (μg/ml) | >200 | 129 | >200 | >176 | >200 | >200 | >200 | >200 | ><1.1  |      |
| Isoliquiritin (μg/ml) | 43 | 163 | 73 | 93 | >200 | >200 | >200 | >200 | >2.2    |
| Neoisoliquiritin (μg/ml) | 116 | >200 | 200 | >172 | >200 | >200 | >88 | >163 | ><0.9  |      |
| Neoisoliquiritin apioside (μg/ml) | >200 | >200 | >200 | >200 | >200 | >200 | >200 | >200 | >1.0    |
| Neoisoliquiritin apioside (μg/ml) | >200 | >200 | >200 | >200 | >200 | >200 | >200 | >200 | >9.0    |
| Isoliquiritigenin (μg/ml) | 4 | 9.5 | 9.9 | 7.8 | 42 | 37 | 25 | 34.7 | 4.4    |
| Licochalcone A (μg/ml) | 4.1 | 6.3 | 9.5 | 6.6 | 12 | 18 | 10 | 13.3 | 2.0    |
| Glabridin (μg/ml) | 8.5 | 8.9 | 8.8 | 8.7 | 22 | 21 | 11 | 18.0 | 2.1    |
| Glycyrrhizin acid (μg/ml) | >200 | >200 | >200 | >200 | >200 | >200 | >200 | >200 | ><1.0  |      |
| Glycyrhetic acid (μg/ml) | 5.4 | 35 | 36 | 31 | 59 | 68 | 30 | 52.3 | 1.7    |

| SE (%) | 4.4 | 4.9 | 4.9 | 3.8 | 4.5 | 6.5 | 7.5 | 8.2 | 7.4 | 1.6 | 1.5 |
Ca9-22 vs. HGF cells (both derived from gingival tissue) (Table III). Among licorice root extracts, flavonoid-rich fraction of water extract had the highest TS [TS=5.5 vs. normal oral cells, 5.2 vs. HGF cells], although this was much lower than that of popular antitumor drugs (doxorubicin, 5-fluorouracil, methotrexate, melphalan: TS=2.3-170). Oil-soluble fractions of licorice had moderate antitumor activity (TS=2.4-4.7). Among licorice flavonoids, neoisoliquiritin apioside and isoliquiritigenin

Figure 2. Anti-herpes simplex virus (HSV) activity of different tea extracts. The osmolarity of tea materials were adjusted to 280 mOsm by adding solid NaCl, filtered through Millipore filter to be sterilized prior to the use for experiments. The 50% effective concentration (EC50) was the concentration at which the viability was restored to the midpoint between that of HSV-infected cells and that of mock-infected cells (method I) (A), or as the concentration at which the viability was restored to 50% that of mock-infected cells (method II) (B). Each value represents the mean±S.D. of triplicate assays. PBS, Phosphate-buffered saline; U, undiluted; ×10, 10-fold dilution, ×100, 100-fold dilution. SE ×20, 60 and 200 contained 3, 1, or 0.3 mg/ml dried materials, respectively.
exhibited the highest antitumor activity (TS=4.4-9.0). Five polymethoxyflavonoids (3,3',4',5,6,7,8-heptamethoxyflavone, nobiletin, tangeretin and sudachitin) were weakly tumor selective (TS=2.0-3.0) (Table III).

Discussion

The present study demonstrated that the alkaline extract of licorice roots had greater anti-HIV activity than the water extract (Table I), confirming our previous finding (5, 6), while the water extract, especially the flavonoid-rich fraction, had greater anti-HSV activity than the alkaline extract (Table II). This unexpected finding suggests the different mechanism of actions of water and alkaline extracts against these two viruses.

We next investigated the anti-HSV activity of licorice components. Licorice root flavonoids, especially liquiritin apioside, isoliquiritin apioside, lucurzid, isoliquiritin, showed higher anti-HSV activity than other polymethoxyflavonoids and low molecular weight polyphenols (Table II). We performed the preliminary QSAR analysis of anti-HIV activity using the mixtures of these 19 compounds. Among 1,705 chemical descriptors, six that represent polarizability (MATS5p, GATS5p) (21, 22), ionization potential (GATS5i) (22), number of ring systems (NRS) (24), atomic number (J_Dz(Z)) and mass (J_Dz(m)) (25) correlated well with anti-HSV activity ($r^2=0.684$, 0.627, 0.624, 0.621, 0.619 and 0.618, respectively, $p < 0.0001$) (Figure 3). This result suggests that the physicochemical properties, rather than the category of compound, are important factors determining anti-HSV activity.

We also found that tricin, another four polymethoxyflavonoids and low molecular weight polyphenols incompletely overcame the cytopathic effects of HSV infection (Table II). It remains to be investigated whether combination of these compounds with acyclovir (15) or ganciclovir (25) may increase viability of infected cells.

The present study also demonstrated that neoisoliquiritin apioside had the highest anti-tumor activity. Isoliquiritin has been reported to inhibit granuloma angiogenesis and tube formation in vascular endothelial cells (26). Further studies are required to elucidate the mechanism by which this compound induces such high TS.

In conclusion, the present study suggests that alkaline extraction of licorice root may be applicable for the industrial mass-production of an anti-HIV agent, while the flavonoid-rich fraction of water extract might be used for the treatment of HSV. Further clinical study with licorice product is underway in our laboratory.

Figure 3. Determination of correlation between chemical descriptors and anti-herpes simplex virus (HSV) activity of licorice flavonoids, polymethoxyflavonoids and low molecular weight flavonoids (defined as selectivity index (SI) by method II in Table II). 1. Liquiritin apioside; 2. liquiritin 7-apiosylglucose; 3. liquiritin; 4. neoliquiritin; 5. liquiritigenin; 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 (Table II).
Conflicts of Interest
This study was funded in part by Maruzen Pharmaceuticals (Hiroshi Sakagami), which caused no prejudice against the impartiality of the research reported.

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