Anti-herpes simplex virus activities of traditional Chinese medicines, used in Yunnan and Tibetan provinces of China

Michiko JO,[a] Norio NAKAMURA,[a] Masahiko KUROKAWA,[b,c] Katsuko KOMATSU,[a] Kimiyasu SHIRAKI,[b] and Masao HATTORI*[a]

[a]*Institute of Natural Medicine, University of Toyama, 2630 Sugitani, Toyama, 930-0194, Japan.* [b]*Department of Virology, University of Toyama, 2630 Sugitani, Toyama 930-0194, Japan.* [c]*Present address: Department of Biochemistry, School of Pharmaceutical Sciences, Kyushu University of Health and Welfare, 1714-1 Yoshino, Nobeoka, Miyazaki 882-8508, Japan.*
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One hundred and sixty-eight traditional Chinese medicines collected in the Yunnan and Tibetan provinces were screened for their anti-herpes simplex type 1 (HSV-1) activity by using a plaque reduction assay using Vero cells. Of these, 24 extracts exhibited appreciable inhibitory activities against HSV-1. They were further examined for their therapeutic efficacies in mice infected with HSV-1; mice were infected cutaneously with HSV-1 and the extracts were orally administrated three times daily. Among them, nine extracts of *Terminalia chebula* (T42), *Tripterygium hypoglaucum* (Y42M), and *Moghania philippinensis* (Y86M), and a water extract of *Tripterygium hypoglaucum* (Y44H) delayed the development and progression of skin lesions. Methanol extracts of *Cassia fistula* (T59), and *Choerospondias axillaries* (T73), and water extracts of *Begonia evansiana* (Y27H), *Maytenus fookerii* (Y60H) and *Potentilla griffithii* (Y63H) showed therapeutic effects. These extracts may be candidates for the development of anti-HSV-1 compounds.

Key words herpes simplex virus, Chinese medicine, plaque reduction.

Introduction

Herpes simplex virus type 1 (HSV-1) is a major cause of morbidity in humans. It causes a variety of diseases ranging in severity from mild to debilitating and life threatening. After primary infection, HSV-1 establishes latency in sensory and autonomic neurons innervating mucosal membranes.[1] This location can then serve as a site for recurrent infection that may be provoked by a number of stimuli such as sunlight, stress, febrile illnesses, and immunosuppression. However, a significant risk of drug resistance has been observed during therapy, especially for chronic infection and for immunocompromised patients.[2] The treatment against HSV-1 is generally taken with acyclovir (ACV) and other nucleoside derivatives. However, these agents develop resistant mutant HSV-1 strains and thus, there is a need to develop new anti-HSV agents that complement ACV.

As reported from a large number of antiviral screenings on medicinal plants,[3-6] several compounds with anti-HSV activity were isolated from their extracts, including polysaccharides, triterpenes, polyphenols and fatty alcohols.[7-13] In our previous papers, we reported that some Chinese traditional medicines such as *Centella asiatica, Mangifera indica* and *Stephania cepharantha* were active against HSV.[14,15] Especially, morphinane alkaloid, FK-3000, isolated from *S. cepharantha* showed potent activity in the plaque reduction assay with a 50% inhibitory concentrations of plaque formation (IC_{50}) value of 18.0 μg/ml.[15] This prompted us to further search potential anti-HSV candidates from Chinese traditional medicinal plants. In this study, 168 Chinese traditional medicinal plants were collected in the Yunnan and Tibetan provinces. Since these plants are characterized as the plants that grow in harsh conditions, such as low oxygen pressure, intense ultraviolet rays and low temperature, they are expected to be novel natural resources with different characteristics from lowland plants. Some of these extracts have been demonstrated to exhibit inhibitory effects on viral enzymes essential for the proliferation of human immunodeficiency virus (HIV) reverse transcriptase (RT) and hepatitis C virus (HCV) RNA-dependent RNA polymerase.[16]

In the present paper, we report the results of screening of methanol and water extracts prepared from the 168 plants for their inhibitory activity against HSV-1 using a plaque reduction assay. Further, the extracts showing appreciable anti HSV-1 activity *in vitro* were evaluated for their therapeutic efficacies in HSV-1 infected mice.

Materials and Methods

Cell and virus. Vero cells (E6 strain) were grown and maintained in Eagle's minimum essential medium (MEM) supplemented with 5% (v/v) and 2% (v/v), respectively, heat-inactivated calf serum in a humidified 5% CO_2 incubator at 37℃. The wild-type HSV-1 (7401H strain) was propagated in Vero cells using a low multiplicity of infection as previously described.[4]

Medicinal plants. Chinese traditional medicinal plants used in this report were collected in Yunnan and Quig-hai Provinces of China during 2000 for the Japan Society for the Promotion of Science (JSPS) Project of Overseas Survey on Ethnical Medicines. These specimens are deposited in the Herbarium of Materia Medica of Toyama Medical and Pharmaceutical University.[16-18]

*To whom correspondence should be addressed. e-mail : saibo421@ms.toyama-mpu.ac.jp

Preparation of extracts. The crude drugs were pulverized and extracted separately with water and methanol. The solution was evaporated in vacuo to give a residue, which was dissolved in DMSO and used for the following assays.

Anti-HSV assay *in vitro*. Duplicate cultures of Vero cells in 60 mm dishes were infected with 100 plaque forming units (PFU) of HSV-1, for one hour. Subsequently, cells were overlaid with 5 ml of nutrient media (MEM, 2% calf serum and 0.8% (w/v) carboxymethylcellulose) in either the absence or presence of extracts. The cells were then incubated for 3 d at 37℃. The infected cells were fixed with 5% formalin solution and stained with 0.03% methylene blue. Numbers of plaques were counted under a binocular microscope. Additionally, cytotoxicity of extracts was visually determined by comparing their cell monolayers with that of the control, and scored as follows: (++) complete cell detachment; (+) 50% of cell detachment; (-) no detachment of cells.[4] IC_{50} values were determined from a curve relating the plaque number to the concentration of drugs.

Cytotoxicity assay of drugs in cell culture. Cytotoxicity was examined by the growth inhibition of Vero cells as described previously.[4] The cytotoxicity of the extract was determined at concentrations higher than those used in the plaque reduction assay. Vero cells were seeded at a concentration of 2.5 x 10^4 cells/well in 24-well plates and grown at 37℃ for 2 d. The culture medium was replaced by fresh medium containing the extract at various concentrations, and cells were further grown for 2 d. The cells were treated with trypsin and the number of viable cells was determined by a trypan blue exclusion method. The concentrations of the extracts reducing cell viability by 50% (CC_{50}) were determined from a curve of percent cell viability to the concentration of drugs.

Therapeutic efficacy in mouse HSV-1 infection model. Female BALB/c mice (6-week-old) were purchased from Sankyo Laboratory Service Co., Ltd., Tokyo, Japan. The right midflank of each mouse was clipped and depilated with a chemical depilatory, Hair Remover (Shiseido, Co., Ltd., Tokyo, Japan). One or two days later, the naked skin was scratched using a 27-gauge needle and an HSV suspension containing 1 x 10^6 PFU applied to the scratched area.[19,20] Extracts (5 mg) was orally administered in a volume of 0.2 ml/mouse once at 8 h before and three times daily for 7 successive days after HSV-1 infection. The development of skin lesions and mortality were continuously monitored every 8 h daily and scored as follows: 0, no lesion; 2, vesicles in local lesion; 4, erosion and/or ulceration in local lesion; 6, mild zosteriform lesion; 8, moderate zosteriform lesion; 10, severe zosteriform lesion; and death. The infected mice were held at least for a month after infection. The Student's *t*-test was used to evaluate the significance of differences between control and the extract-treated mice in mean survival times and mean times at which skin lesions were initially scored as 2 or 6 after infection.

Results and Discussion

Inhibitory effect of traditional Chinese medicines on HSV-1 *in vitro*. One hundred and sixty-eight traditional Chinese medicines were extracted with water or methanol and the extracts were screened for their anti-HSV-1 activities using a plaque reduction assay at a concentration of 100 μg/ml (Tables 1 and 2). As reported previously, the maximal serum concentration of glucose, a typical chemical with rapid absorption from alimentary tracts in humans, is approximately 300 μg/ml after oral administration at a dose of 10 g in humans with diabetes mellitus.[21] In addition, some low molecular weight components in extracts may be absorbed as rapidly and efficiently as glucose. Thus, a 100 μg/ml dose in the plaque reduction assay was designed to examine possible antiviral activity at the putative concentration of extracts in serum. Further, the concentration of extracts which reduce plaque forming ability to less than 50% would be expected to be effective *in vivo*.[4] We used a 50% reduction in the screening as our definition of anti-HSV-1 activity. It was postulated that a 100 μg/ml concentration which was active in the plaque reduction assay may also achieve an adequate serum concentration in mice to also render an *in vivo* antiviral activity.[4] Twenty-four extracts including eighteen and six methanol- and water-extracts reduced plaque forming ability to less than 50%, although some of them showed visible cytotoxicity (Table 1 and 2).

We also examined the therapeutic indexes (ratio of CC_{50} to IC_{50} values) of 24 to estimate possible candidates as therapeutic anti-HSV-1 extracts *in vivo* (Table 3). Their IC_{50} values ranged from 20.6 to 134.0 μg/ml and their CC_{50} values varied from 35.2 to 384 μg/ml. The IC_{50} values of 21 extracts were lower than their CC_{50} values, indicating that inhibition of plaque formation was not due to cytotoxity. The therapeutic indexes of these extracts were 1.14 to 8.18. However, methanol extracts of *Aquilaria sinensis* (T18), *Choerospondias axillaries* (T73), and a water extract of *Maytenus fookerii* (Y60H) were cytotoxic to Vero cells and had no anti-HSV-1 activity.

Of the 24 extracts, the methanol extract of *Tripterygium hypoglaucum* or *Tripterygium forrestii* (Y42M) showed the highest therapeutic index, 8.18. Plants of the genus *Tripterygium* (Celastraceae) have been used as a remedy for a treatment of auto-immune diseases and an insecticide in traditional Chinese medicine.[22,23] In the recent study, alkaloids of *T. hypograucum* were reported to induce apoptosis of HL-60 cells through c-myc and NF-κB signaling pathways.[24]

The methanol extracts of *T. chebula* (T42) and a botanical name unknown sample (T61) with anti-HSV-1 activity *in vitro* also exhibited anti HIV RTase acitivity, anti HCV RdRp activity.16) Thus some of 24 extracts that showed anti-HSV-1 activity *in vitro* were suggested to contain biological active components.

Inhibitory effect of traditional Chinese medicines on HSV-1 *in vivo*. Twenty four extracts that reduced plaque forming ability to less than 50% were examined for their therapeutic efficacy in a cutaneous HSV-1 infection model in mice (Table 4). Although the methanol extract of *A. sinensis* (T18) was active *in vitro* (Table 3), it was not enough to prepare the animal test samples. The 23 extracts

Table 1. Antiviral activities of methanol extracts of Tibetan medicinal plants determined by a plaque reduction assay.

Extract NO.	Local name	Botanical name	Part used	MeOH extract	
				% Plaque formation	Cytotoxicity
T1	止瀉木子	*Holarrhena antidysenterica* Wall. ex A. DC.	seed	ND	++
T2	紫鉚子	*Butea monosperma* (Lan.) Kuntze	mature seed	115	-
T3	芒果核	*Mangifera indica* L.	seed	ND	+
T4	蒲桃	*Syzygium cumini* (L.) Skeels	fruit	ND	++
T5	紫草茸	*Laccifer lacca* Kerr.	extraction	109	-
T6	黒種草子	*Nigella glandulifera* Freyn or *N. sativa* L.	mature seed	96.2	-
T7	香旱芹	*Cuminum cyminum* L.	mature fruit	**0**	+
T8	草豆蔻	*Alpinia katsumadai* Hayata	mature seed	ND	++
T9	肉豆蔻	*Myristica fragrans* Houtt	aril	69.2	+
T10	蕃茇	*Piper longum* L.	seed	ND	++
T11	黒云香	*Styrax tonkinensis* (Pierre) Craib ex Hart.	resin	ND	++
T12	乳香	*Boswellia carterii Birdw. or Shorea robusta* Gaertn. f.	resin	ND	++
T13	黄訶子肉	*Terminalia chebula* Retz.	pericarp	ND	+
T14	毛訶子肉	*Terminalia bellirica* (Gaertn.) Roxb.	pericarp	**13.5**	-
T15	余甘子	*Phyllanthus emblica* L.	mature fruit	61.5	+
T16	檀香	*Santalum album* L.	wood	ND	++
T17	紫茉莉根	*Mirabilis himalaica* (Edgew.) Heim.	root	100	+
T18	沉香	*Aquilaria sinensis* (Lour.) Gilg	wood	**37.7**	-
T19	邹木瓜	*Chaenomeles speciosa* (Sweet) Nakai	fruit	74	+
T20	元胡	*Corydalis yanhusuo* W. T. Wang	tuber	78.8	+
T21	紫草	*Onosma hookeri* C. B. Clarke	root	83.8	++
T22	天竺黄	*Bambusa textilis* Mc. clure or *B. arundinacea* Willd	resin	100	-
T23	冰片	*Dryobalanops aromatica* Gaertn. f.	resin	80	-
T24	紅花	*Carthamus tinctorius* L.	flower	ND	++
T25	桂皮	*Cinnamomum cassia* Presl	bark	85.6	+
T26	山沈香	*Syringa pinnatifolia* Hemsl.	wood	94.2	+
T27	蔵菖蒲	*Acorus calamus* L.	rhizome	92.9	-
T28	手掌参	*Gymnadenia conopsea* (L.) R. Br.	tuber	79.8	-
T29	木鼈子	*Momordica cochinchinensis* (Lour.) Spreng.	seed	80.1	-
T30	馬銭子	*Strychnos nux-vomica* L. or *S. pierriana* A. W. Hill	seed	95.5	-
T31	甘草	*Glycyrrhiza uralansis* Fisch. or *G. glabra* L., *G. inflata* Bat.	root	ND	++
T32	寛筋藤	*Tinospora cordifolia* (Willd.) Hook. f. et Thoms. or *T. sinensis* (Lour.) Merr.	tuber	73	-
T33	木香	*Aucklandia lappa* Decne	root	ND	++
T34	黒冰片	*Sus scrofa* L.	faces	86.4	-
T35	杜鵑花	*Rhododendron anthopogonoides* Maxim.	flower, leaf	71.4	+
T36	大黄	*Rheum palmatum* L.	rhizome	55.8	-
T37	蒺藜	*Tribulus terrestris* L.	mature fruit	ND	++
T38	香附子	*Cyperus rotundus* L.	rhizome	52	+

T39	骨砕補	*Drynaria sinica Diels or D.baronii* (Christ) Diels	rhizome	71.2	-
T40	鬼臼果	*Sinopodophyllum emodii* (Wall. ex Royle) Ying	mature fruit	ND	++
T41	天冬	*Asparagus cochinchinensis* (Lour.) Merr.	tuber	100	+
T42	藏青果	*Terminalia chebula* Retz.	fluit	**0**	+
T43	胡芦巴	*Trigonella foenum-graecum* L.	mature seed	79.9	-
T44	党参	*Codonopsis pilosula* (Franch.) Nannf.	root	72	-
T45	黄芪	*Astragalus mongholicus* Bunge	root	84.4	-
T46	檳榔	*Areca catechu* L.	mature seed	ND	+
T47	黄精	*Polygonatum cirrhifolium* (Wall.) Royle	rhizome	73.4	-
T48	白沙参	*Adenophora lilifolioides* Pax. et Hoffm.	root	68.2	-
T49	角茴香	*Hypecoum leptocarpum* Hook.f.et Thoms. or *H. erectum* L.	whole plant	85.1	+
T50	木通	*Aristolochia moupinensis* Franch. or *A. macrocarpa* C. Y. Wu et S. Y. *Wu, A. griffithii* Thoms. ex Duch.	stem	ND	++
T51	螃蟹	*Potamiscus loshingense* Wu	whole plant	ND	+
T52	木棉花	*Bombax malabaricum* DC. or *B. ceiba* L.	flower	ND	++
T53	藏木香	*Inula helenium* L. or *I. racemosa* Hook. f.	root	ND	++
T54	藏茜草	*Rubia wallichiana* Decne. or *R. tibelica* Hook.f., *R. cordiholia* L.	aril part	67	-
T55	酸勝果	*Embelia laeta* (L.) Mez.	fruit	ND	++
T56	紫檀香	*Pterocarpus indicus* Willd. or *P. santalinus* L. f.	wood	ND	ND
T57	藏野姜	*Hedychium spicatum* Ham.ex Smith	rhizome	ND	++
T58	榼藤子	*Entada phaseoloides* (L.) Merr.	mature seed	77.9	-
T59	腊腸果	*Cassia fistula* L.	fruit	**4**	+
T60	藏商陸	*Phytolacca acinosa* Roxb.	root	**47.4**	+
T61	紅檳榔	*Boranical name unknown*	seed	**4**	+
T62	棱子芹	*Pleurospermum hookeri* (C. B. Clarke) var. *thomsonii* C. B. Clarke	root	90.3	-
T63	文冠木	*Xanthoceras sorbifolia* Bunge	stem	92.2	+
T64	風毛菊	*Ixeris chinensis* (Thunb.) Nakai	whole plant	ND	++
T65	川烏	*Aconitum carmichaeri* Debx.	tuber	98.1	-
T66	藏蔓菁	*Brassiea rapa* Pasg.	underground part	76.6	-
T67	天南星	*Arisaema flavum* (Forsk.) Schott.	tuber	91	-
T68	白花竜胆	*Gentiana algida* Pall.	flower	74	-
T69	白芹艽花	*Gentiana straminea* Maxim.	flower	70.8	-
T70	甘粛雪霊芝	*Arenaria kansuensis* Maxim.	stem, rhizome	71.4	-
T71	懸鈎藤	*Rubus biflorus* Buch.-Ham. ex Smith or *R. kokoricus* Hao.	stem	85.7	-
T72	五綫緑織花	*Meconopsis quintuplinervia* Regel	flower	**26**	+
T73	広酸棗	*Choerospondias axillaris* (Roxb.) Burtt et Hill	mature fruit	**49.4**	+
T74	波棱爪子	*Herpetospermum pedunculosum* (Sex.) Bail.	seed	81.8	-
T75	美麗烏頭	*Aconitum brunneum* Hand.-Mazz.	tuber	79.2	-

Cytotoxicity: (-) no detachment of cells from the surface of dish; (+) 50% of cell detachment; (++) complete cell detachment;
ND, not determined.
Underlines represent extracts that reduced plaque formation to ≥ 50 %

Table 2. Antiviral activities of methanol and water extracts of Yunnan medicinal plants determined by a plaque reduction assay.

Extract NO.	Local name	Botanical name	Part used	MeOH extract		Water extract	
				% Plaque formation	Cytotoxicity	% Plaque formation	Cytotoxicity
Y1	金糸馬尾黄連	*Thalictrum glandulosissimun* (Fin.et Gagn.) W. T. Wang et S. H. Wang	underground part	**2.6**	+	92	-
Y2	雪山竹そん	*Phallus impudicus* Linn.ex Pers.	fungus	102	-	97.4	-
Y3	紅豆杉皮	*Taxus mairei* (Lemee et Levl.) S. Y. Hu or *Taxus Wallichinana* Zucc.	bark	**0**	+	ND	ND
Y4	紅景天	*Rhodiola sacra* (Prain ex Hamet) S. H. Fu	underground part	92.2	-	ND	ND
Y5	黄連	*Thalictrum glandulosissimum* (Fin.et Gagn.) W. T. Wang et S. H. Wang	underground part	108	+	103	-
Y6	蔵菖蒲	*Acorus calamus* L.	rhizome	**46.8**	+	92.1	-
Y7	雪蓮花	*Sausurea namikawae* Kitam.	whole plant	103	++	92.1	-
Y8	海棠果	*Malus yunnanensis* (Franch.) Schneid. or *M. prunifolia* (Willd.) Borkh.	fruit	ND	++	121	-
Y9	樹花菜	*Botanical name unknown*	whole plant	ND	++	116	-
Y10	香菇	*Lentinus edodes* (Berk.) Sing.	fungus	ND	++	133	-
Y11	石花菜	*Eucheuma gelatinae* (Esp.) J. Ag.	alga	ND	+	124	-
Y12	牛肝菌	*Strobilomyces floccopus* (Vahl. ex Fr.) Karst. or *S. grevillei* (Kl.) Sing. or *S. luteus* (Linn. ex Fr.) Gray	fungus	94.8	-	117	-
Y13	青陽参	*Cynanchum otophyllum* Schneid.	root	84.4	-	98.7	-
Y14	雪山地参	*Lycopus lucidus* Turcz.	rhizome	103	-	108	-
Y15	樹蝴蝶	*Lobaria retigera* Trevis.	fungus	ND	++	122	-
Y16	維西黒木耳	*Auricularia auricula* (L. ex Hook.) Underw.	fungus	51.9	-	94.7	-
Y17	野党参	*Codonopsis subscaposa* Kom.	root	97.4	-	108	-
Y18	雲南三七花	*Panax notoginseng* (Burkill) F. H. Chen	flower	94.8	-	103	-
Y19	鹿心雪茶	*Cladonia fallax* Abbayes	whole plant	103	-	100	-
Y20	紅藤	*Sargentodoxa cuneata* (Oliv.) Rehd. ex Wils.	wood	ND	+	ND	+
Y21	黄橋本	*Ligusticum delavayi* Franch.	root, rhizome	ND	+	109	-
Y22	苞心草	*Rhodobryum giganteum* (Schwaegr.) Par.	whole plant	51.9	+	105	-
Y23	雪連花	*Saussurea laniceps* Hand.-Mazz.	whole plant	ND	++	113	-
Y24	草薢	*Smilax ferox* Wall. ex Kunth	rhizome	75.3	-	93.4	-
Y25	白雪茶	*Thamnolia vermicularis* (Sw.) Ach. ex Schaer.	whole plant	129	-	82.6	-
Y26	雲木香	*Aucklandia lappa* Dence.	root	140	-	89.5	-
Y27	山海棠	*Begonia evansiana* Andr.	wood	ND	++	**30.2**	+
Y28	重楼	*Paris polyphylla* Smith var. *yunnanenis* (Franch.) Hand.-Mazz.	rhizome	92.2	-	ND	+
Y29	馬鞭草	*Verbena officinalis* L.	whole plant	66.2	-	100	-
Y30	蜜桶花	*Brandisia cauliflora* Tsoong et Lu	branch, leaf	100	-	93	-
Y31	青木香	*Akebia trifoliata* (Thunb.) Koidz. var. *australis* (Diels) Rehd.	branch	114	-	80.2	-
Y32	血藤	*Schisandra henryi* Clarke var. *marginalis* A.C.Smith or *S. henryi* Clarke var. *yunnanenis* A.C.Smith	branch	76.6	-	98.8	-
Y33	大発汗	*Millettia bonatiana* Pamp.	branch	87	-	95.3	-
Y34	虎掌草	*Anemone rivularis* Buch.-Ham.	branch	77.9	-	97.7	-
Y35	心不甘	*Tupistra chinensis* Bak.	rhizome	77.9	-	83.7	-
Y36	胃友	*Sarcococca ruscifolia* Stapf	branch, leaf	88.3	-	100	-
Y37	燕尾草	*Passiflora cupiformis* Mast.	stem	84.2	-	ND	+
Y38	五爪金竜	*Tetrastigma hypoglaucum* Planch.	stem	ND	+	ND	+
Y39	青蒿	*Artemisia annua* L.	whole plant	88.4	-	83.7	-
Y40	八角蓮	*Dysosma versipellis* (Hance) M.Cheng	rhizome	ND	++	ND	++
Y41	黒骨頭	*Periploca calophylla* (Wight) Falc.	branch, leaf	ND	++	ND	++
Y42	雷公藤	*Tripterygium forrestii* Loesen. or *T.hypoglaucum* (Levl.) Hutch.	branch	**0**	+	ND	+
Y43	見血飛	*Toddalia asiatica* (L.) Lam.	branch	ND	+	108	-
Y44	紫金皮	*Tripterygium hypoglaucum* (Levl.) Hutch.	bark	ND	++	**33.7**	-
Y45	鉛地楓	*Plumbago zeylanica* L.	branch	57.9	-	ND	+
Y46	麗南参	*Botanical name unknown*	root	91.6	-	72.1	-

Y47	鶏矢藤	*Paederia scandens* (Lour.) Merr.	stem	94.7	-	95.3	-
Y48	蜜桶花	*Brandisia cauliflora* Tsoong et Lu	branch, leaf	72.6	-	82.6	-
Y49	木通	*Clematis armandii* Franch.	heart wood	94.7	-	90.7	-
Y50	小麻葯	*Piper hancei* Maxim.	whole plant	65.3	-	68.6	-
Y51	土大黄	*Rumex nepalensis* Spreng.	rhizome, root	57.9	-	96.5	-
Y52	大紅袍	*Kadsura longipedunculata* Finet et Gagn.	branch	ND	-	**26.7**	-
Y53	土茯苓(野蓧根)	*Polygonum cymosum* Trev.	root	68.4	++	ND	+
Y54	紫金標	*Ceratostigma willmottianum* Staph	branch	ND	-	79.1	+
Y55	滇白前(瓦草)	*Silene asclepiadea* Franch. (= *Melandrium viscidulum var. szechuanense* (Williams) Hand.-Mazz.)	root	ND	++	ND	-
Y56	血地胆(醤頭)	*Dioscorea cirrhosa* Lour.	root	ND	+	ND	+
Y57	藜芦(披麻草)	*Veratrum taliense* Loesen. f. or *V.mengtzeanum* Loesen. f.	root	**40**	-	97.7	+
Y58	雲南美登木	*Maytenus fookerii* Loes.	leaf	62.1	-	69.8	-
Y59	雲南夢芙木	*Rauvolfia yunnanensis* Tsiang	root	93.7	-	84.9	-
Y60	雲南美登木	*Maytenus fookerii* Loes.	branch	ND	-	**48.8**	-
Y61	化石丹茶	*Botanical name unknown*	branch, leaf	74.7	-	**47.7**	+
Y62	白花蛇舌草	*Hedyotis diffusa* Willd. or *H.tenelliflora* Bl. or *H.corymbosa* (L.) Lam.	root	105	-	86	+
Y63	翻白葉	*Potentilla griffithii* Hook. f. var. *velutina* Card.	whole plant	ND	+	**23.3**	-
Y64	黄柏(木蝴蝶茎,枝)	*Phellodendron chinense* Schneid.	branch, stem	85.3	-	104	-
Y65	大百解	*Iris tectorum* Maxim.	branch	86.3	-	124	-
Y66	岩白菜	*Bergenia purpurascens* (Hook.f. et Thoms.) Engl.	root	ND	+	93.2	+
Y67	穿山竜	*Dioscorea nipponica* Makino	rhizome	ND	+	59	+
Y68	野生天麻	*Gastrodia elata* Bl.	rhizome	77.9	-	89.8	-
Y69	珠子参	*Panax japonicus* C. A. Meyer var. *major* (Burk.) C. Y. Wu et K. M. Feng	rhizome, root	92.6	-	124	-
Y70	竹黄(竹菌)	*Engleromyces goetzii* P. Henn	fungus	82.1	+	104	-
Y71	珠子参十黄精	*Panax japonicus* C. A. Meyer var. *major* (Burk.) C. Y. Wu et K. M. Feng	rhizome, root	77.9	-	106	-
Y72	槲寄生	*Viscium coloratum* (Kom.) Nakai	whole plant	75.8	-	116	-
Y73	雲黄連	*Coptis teetoides* C. Y. Cheng	rhizome	84.2	-	107	-
Y74	山半夏	*Typhonium trilobatum* (L.) Schott	root	100	-	93.2	-
Y75	蔵菖蒲	*Acorus calamus* L.	rhizome	**5.3**	+	103	-
Y76	山慈菇	*Tinospora yunnanensis* S. Y. Hu	root	122	-	113	-
Y77	黄芩	*Scutellaria amoena* C. H. Wright	root	69.7	-	83	+
Y78	馬蹄香	*Valeriana jatamansi* Jones	root	89.5	-	113	-
Y79	o to ha ba	*Botanical name unknown*	whole plant	118	-	107	-
Y80	水田七	*Tacca plantaginea* (Hance) Drench.	root	90.8	-	81.8	-
Y81	山豆根	*Menispermum dauricum* DC.	root	101	-	103	-
Y82	香白	*Heracleum scabridum* Franch.	root	94.7	-	115	-
Y83	白狗腸	*Campsis grandiflora* (Thunb.) Loisel.	stem	76.3	-	108	-
Y84	葛根	*Pueraria phaseoloides* (Roxb.) Benth.	root	90.8	-	100	-
Y85	半枝蓮	*Scutellaria barbata* D. Don	whole plant	88.2	-	113	-
Y86	千斤拔	*Moghania philippinensis* (Merr. et Rolfe) Li	root	**0**	+	109	-
Y87	紅葱	*Eleutherine americana* Merr. et Heyne	bulb	**14.5**	-	107	-
Y88	天山雪蓮花	*Saussurea medusa* Maxim.	whole plant	98.7	-	140	-
Y89	新疆雪蓮花	*Saussurea involucrata* Kar. et Kir.	whole plant	ND	+	97.4	-
Y90	雲南丹参	*Salvia yunnanensis* C. H. Wright	underground part	ND	++	89.8	-
Y91	大黄	*Rheum tanguticum* Maxim. ex Balf.	rhizome	**15.8**	-	93.2	-
Y92	蔵紅花	*Crocus sativas* L.	flower	121	-	ND	ND
Y93	蔵茵陳	*Swertia mussotii* Franch.	whole plant	ND	ND	115	-

Cytotoxicity: (-) no detachment of cells from the surface of dish; (+) 50% of cell detachment; (++) complete cell detachment;
ND, not determined.
Underlines represent extracts that reduced plaque formation to ≥ 50 %

Table 3. *In vitro* anti-HSV-1 activity of Tibetan and Yunnan medicinal plants.

Extract NO.	Local name	IC_{50} (μg/ml)	CC_{50} (μg/ml)	Therapeutic Index
T7	香旱芹	55.1	117.9	2.14
T14	毛訶子肉	73.8	205.4	2.78
T42	藏青果	64.8	133.8	2.06
T59	腊腸果	71.0	311.6	4.39
T60	藏商陸	66.7	113.9	1.71
T61	紅檳榔	61.4	286.5	4.66
T18	沉香	49.4	35.2	0.71
T72	五綫緑繊花	55.8	227.6	4.08
T73	広酸棗	134.3	113.5	0.85
Y1M	金糸馬尾黄連	48.6	265.6	5.46
Y3M	紅豆杉皮	56.8	384.3	6.77
Y6M	蔵菖蒲	73.4	>400	>5.45
Y42M	雷公藤	20.6	168.2	8.18
Y57M	藜芦(披麻草)	97.0	164.8	1.70
Y75M	蔵菖蒲	55.4	170.1	3.07
Y86M	千斤拔	57.7	203.3	3.52
Y87M	紅葱	67.1	76.2	1.14
Y91M	大黄	72.8	179.1	2.46
Y27H	山海棠	73.2	150.0	2.05
Y44H	紫金皮	54.4	251.3	4.62
Y52H	大紅袍	67.1	332.1	4.95
Y60H	雲南美登木	85.70	59.87	0.70
Y61H	化石丹茶	119.8	143.2	1.19
Y63H	翻白葉	60.4	119.1	1.97

M, methanol extract
H, water extract

involving T73 and Y60H that had no anti-HSV activity *in vitro* (Table3) were examined *in vivo*. In this study, methanol extracts of *T. chebula* (T42), *T. hypograucum* (Y42M), and *Moghania philippinensis* (Y86M), and a water extract of *T. hypoglaucum* (Y44H) delayed the development and progression of skin lesions. Methanol extracts of *Cassia fistula* (T59), and *Choerospondias axillaries* (T73), and water extracts of *Begonia evansiana* (Y27H), *Maytenus fookerii* (Y60H), and *Potentilla griffithii* (Y63H) significantly prolonged mean survival times. Nine extracts showed therapeutic anti-HSV-1 activity in the murine infection model.

For a methanol extract of *Veratrum taliense* (Y57M), most of the mice were dead before skin lesions could be scored, because of the possible toxicity of the extract being administered. This plant was historically known to have potent toxicity. However, Y57M did not show strong cytotoxicity *in vitro* (Table 3). Further T73 and Y60H were toxic *in vitro* (Table 3) but active *in vivo* (Table 4). Thus, the some components of extracts were selectively absorbed from alimentary tracts and metabolized and exerted anti-HSV-1 activity which was not associated with toxicity.[4)]

Fourteen of the 23 extracts examined *in vivo* exhibited anti-HSV-1 activities in the plaque reduction assay but did not show any therapeutic efficacy against HSV-1 infection in mice (Table 4). These results suggest that active constituents of these crude drugs may not be absorbed from alimentary tracts. Even if the anti-HSV-1 constituents are absorbable,

Table 4. Effect of Tibetan and Yunnan medicinal plants on cutaneous HSV-1 infection in BALB/c mice.

Exp.	Extract NO.	Sample	Mean time (day ± S.D.)			Mortality[c]
			Score 2[a]	Score 6[a]	Survival[b]	
1		control	3.00±0.00	4.75±0.45	6.41±0.79	10/10
	T14	毛訶子肉	3.00±0.00	5.00±0.00	6.17±0.82	10/10
	T60	藏商陸	2.83±0.41	4.83±0.41	6.00±0.63	10/10
	Y3M	紅豆杉皮	3.00±0.00	4.67±0.52	6.00±0.63	10/10
	Y6M	蔵菖蒲	2.83±0.41	4.60±0.55	6.00±0.00	10/10
	Y75M	蔵菖蒲	3.00±0.00	4.67±0.58	6.00±0.00	10/10
	Y91M	大黄	2.80±0.45	5.00±0.00	5.80±0.45	10/10
	Y44H	紫金皮[e]	3.00±0.00	4.25±1.50[d]	5.80±0.45	10/10
	Y52H	大紅袍	3.00±0.00	5.00±0.00	6.25±0.50	10/10
2		control	3.00±0.00	4.17±0.39	6.67±0.65	10/10
	T7	香旱芹	3.14±0.38	4.14±0.38	6.86±0.69	10/10
	T42	藏青果[e]	3.14±0.38	4.71±0.49[f]	6.86±0.69	10/10
	T61	紅檳榔	3.00±0.00	4.43±0.53	6.86±1.21	10/10
	T72	五綫緑繊花	3.00±0.00	4.00±0.00	6.43±0.53	10/10
	Y42M	雷光藤[e]	3.29±0.49	4.86±0.38[d]	7.28±1.38	10/10
	Y57M	藜芦（披麻草）	ND[g]	ND	ND	10/10
	Y86M	千斤拔[e]	3.00±0.00	4.71±0.49[f]	6.57±0.53	10/10
	Y61H	化石丹茶	3.00±0.00	4.29±0.49	6.57±0.53	10/10
3		control	3.00±0.00	4.36±0.50	5.45±0.82	10/10
	T59	腊腸果[e]	3.00±0.00	4.57±0.53	6.42±0.53[f]	10/10
	T73	広酸棗[e]	3.00±0.00	4.71±0.49	6.57±0.53[d]	10/10
	Y1M	金糸馬尾黄連	3.00±0.00	4.57±0.53	5.71±0.49	10/10
	Y87M	紅葱	3.00±0.00	4.29±0.49	6.00±0.58	10/10
	Y27H	山海棠[e]	3.00±0.00	4.86±0.38	6.43±0.53[f]	10/10
	Y60H	雲南美登木[e]	3.00±0.00	4.29±0.49	6.43±0.53[f]	10/10
	Y63H	翻白葉[e]	3.00±0.00	4.29±0.49	6.29±0.49[f]	10/10

[a] Mean time at which score 2 or 6 was first observed after infection. The infected mice which were not scored were excluded from the calculation of mean time. [b] Surviving mice were not included for calculation of mean survival times. [c] Number of dead mice/number of mice tested. [d] Mean times are significantly prolonged ($p<0.01$ *vs.* control by Student's *t*-test). [e] The extracts exhibiting significant therapeutic efficacy in this HSV-1 infection model. [f] Mean times are significantly prolonged ($p<0.05$ *vs.* control by Student's *t*-test). [g] ND, not detected.

they might not be kept at appropriate concentrations necessary for anti-HSV-1 in the blood stream or organs due to pharmacokinetic efficiency including the rate of absorption, metabolism and excretion. This results in that an *in vitro* assay would not always correlate to their therapeutic anti-HSV-1 efficacy *in vivo*.[4] Since most of them showed high tannin contents (data not shown), tannins may contribute to their inhibitory action *in vitro*; the monomeric hydrolysable tannins, oligomeric ellagitannins and condensed tannins, having galloyl groups or hexahyroxydiphnoyl groups, have been reported to have the potent anti-HSV activity.[25]

Since most of traditional Chinese medicines as well as folk medicines have been used for treatment of various diseases and the maintenance of health conditions, it may be expected that the components of these traditional medicines may be candidates for development of anti-viral agents against HSV.

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Japanese abstract

雲南省及びチベット自治区で採集した168種の中国少数民族薬物の水及びメタノールエキスの抗 HSV-1 活性をプラーク減少法 を用いて検討した。24種のエキスに抗 HSV-1 活性が認められ，さらに HSV-1 を経皮感染させたマウスを用い，これらのエキスを約 1 週間経口投与した。その結果，*Terminalia chebula* (T42)，*Tripterygium hypoglaucum* (Y42M)，*Moghania philippinensis* (Y86M) のメタノールエキス，及び，*Tripterygium hypoglaucum* (Y44H) の水エキスに有意な皮膚病変進展の遅延効果が認められた。また，*Cassia fistula* (T59)，*Choerospondias axillaries* (T73) のメタノールエキス，及び，*Begonia evansiana* (Y27H)，*Maytenus fookerii* (Y60H)，*Potentilla griffithii* (Y63H) の水エキスに生存期間の延長が認められた。これらのエキスには，抗 HSV-1 活性を示す化合物が含まれており，新規抗 HSV-1 化合物の探索に有用であると思われる。

*〒930-0194 富山市杉谷 2630
富山大学・和漢医薬学総合研究所 薬物代謝工学 服部征雄