A novel procedure for the identification of a fraction with anti-herpes simplex virus type 1 activity *in vivo* from hot-water extract of traditional medicines, *Geum japonicum* THUNB.

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Abstract

We have developed a novel procedure to identify a fraction with antiviral activity *in vivo* from the hot water (HW)-extract of traditional medicines. In this procedure, sera obtained from guinea pigs administered with the HW-extract of *Geum japonicum* THUNB. (GJ) inhibited the growth of herpes simplex virus type 1 (HSV-1) in Vero cells. Further ethylacetate (EtOAc)- and ethanol-extractable fractions prepared from these sera also exhibited anti-HSV-1 activity, but fractions separated based on the other chemical properties such as acid and alkali did not. The EtOAc-extractable fraction directly from GJ-HW-extract also exhibited anti-HSV-1 activity in both growth inhibition assay and a cutaneous HSV-1 infection model in mice. The EtOAc-extraction used in serum fractionation was found to be applicable in the preparation of a fraction with anti-HSV-1 activity *in vivo* from GJ-HW-extract. Our anti-HSV-1 assay and fractionation using guinea pig serum would be useful in the identification of fractions with anti-HSV-1 activity *in vivo* from HW-extracts. Furthermore we have chemically characterized anti-HSV-1 agents in the GJ-EtOAc-extractable fraction and found that they are weakly acidic compounds and that their anti-HSV-1 activities were lost by the treatment with $FeCl_3$ or diazomethane. Thus the physicochemical characterization of anti-HSV-1 agents in serum fractions would be helpful for the efficient preparation and purification of possible anti-HSV-1 agents directly from GJ.

Key words Traditional medicines, Herpes simplex virus, Antiviral activity in serum, Assay of anti-HSV-1 agent, Hot water extracts, *Geum japonicum* THUNB., Fractionation by chemical treatment of serum.

Abbreviations DMSO, dimethylsulfoxide ; EtOAc, ethylacetate ; EtOH, ethanol ; GJ, *Geum japonicum* THUNB. ; HSV-1, herpes simplex virus type 1 ; HW-extract, hot water-extract ; MEM, Eagle's minimum essential medium ; PFU, plaque forming units ; TFA, trifluoroacetic acid.

Introduction

Herpes simplex virus type 1 (HSV-1) causes labial herpes, genital herpes, keratitis, and encephalitis. The herpetic infection is common in humans and a major cause of morbidity especially in immunosuppressed patients with the acquired immunodeficiency syndrome or in transplant recipients.[1-4] Traditional medicines have been

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administered orally in the form of their hot water (HW)-extracts for various diseases in human. Information on their adverse reactions has been historically accumulated in traditional therapy.[5] We have selected 12 HW-extracts as possible candidates for anti-HSV-1 traditional medicines.[6] Thus the HW-extracts would be used safely also for the treatment of human HSV-1 infection as anti-HSV-1 medicines without major adverse reactions as long as they exhibit therapeutic anti-HSV-1 activity.

In the preparation of fractions with biological activity *in vivo* from HW-extracts according to the conventional way, they should be first fractionated by a series of differential extraction procedures using various organic solvents or various pH conditions. Then the biological activity in a series of the separated fractions should be examined *in vitro* and then their effectiveness *in vivo* should be identified. As we reported that 20 of 32 HW-extracts with anti-HSV-1 activity *in vitro* were not effective *in vivo*,[6] it is not clear whether active HW-extracts *in vitro* are absorbed and exhibit their biological activity *in vivo*. Thus the fractions of HW-extracts which are not absorbed *in vivo* would be also difficult to be expected as the fractions with biological activity *in vivo*, even though they exhibit strong biological activity *in vitro*. Therefore we have developed a novel procedure that can first assess the biological activity of fractions absorbed from alimentary tracts and characterize the chemical properties of active agents in their fractions by using serum obtained from guinea pigs administered with HW-extracts.

We have previously screened 142 HW-extracts for their anti-HSV-1 activity *in vitro* and 12 HW-extracts of them were found to exhibit their therapeutic anti-HSV-1 activity *in vivo*.[6] HW-extract of *Geum japonicum* THUNB. (GJ) is one of the 12 HW-extracts with anti-HSV-1 activity *in vitro* and *in vivo*. It is possible that anti-HSV-1 agents in the GJ-HW-extract may act antivirally *in vivo* such as acyclovir.[7] To identify a fraction containing such anti-HSV-1 agents, we have separated fractions with anti-HSV-1 activity *in vivo* from both serum and GJ-HW-extract. We found that the ethylacetate (EtOAc)-extractable fractions of both serum and GJ-HW-extract exhibited anti-HSV-1 activity *in vitro* and *in vivo*. We demonstrated that our procedure using serum is useful to identify and separate fractions with antiviral activity *in vivo* from HW-extracts. Furthermore we reported that major anti-HSV-1 agents of the GJ-EtOAc-extractable fraction are weakly acidic compounds and that their anti-HSV-1 activity are lost by the treatment with $FeCl_3$ and diazomethane.

Materials and Methods

Viruses and cells : HSV-1 (Seibert strain[8] or 7401H strain[9]) was propagated in Vero E6 cells. The infected cultures were frozen and thawed three times, and centrifuged at 3,000 rpm for 15 min. Their supernatants were stored at -80°C until use.[6, 10] Vero cells were grown and maintained in Eagle's minimum essential medium (MEM) supplemented with 5 % and 2 % calf serum, respectively.

Preparation of GJ-HW-extract : Dried GJ (whole plant) was purchased from Tochimoto Tenkaido (Osaka, Japan). HW-extracts were prepared from the dried plant as described previously[6] : the dried materials (5 kg) were boiled under reflux and then the aqueous extracts were filtered and lyophilized. The amount of lyophilized materials was 1027 g. The lyophilized extract was suspended in distilled water at 10 mg/ml, boiled for 10 min, and then centrifuged at 3,000 rpm for 15 min. The supernatant was used for the following assays as GJ-HW-extract.

Administration to guinea pigs : Female Hartley guinea pigs weighing 300-350 g were purchased from Sankyo Labo Service Co., Ltd., Tokyo, Japan. The animals were anesthetized using ether as an anesthetic agent after starvation for 24 h before administration of GJ-HW-extract. As it is difficult to predict which part of alimentary tracts the antiviral agents of HW-extracts are absorbed from, we assessed their possible absorption from the stomach, and small and large intestines of guinea pigs simultaneously. The

abdomen of anesthetized guinea pigs was opened, and each 10 ml (20 mg/ml) of the HW-extract or water was carefully administered simultaneously into the stomach, and small and large intestines (total dose, 600 mg/guinea pig) of a guinea pig by a syringe with a 21-gauge needle. The opened abdomen was closed by clips. Whole blood content was collected from the heart at 2 h after injection, and then their sera were inactivated by heating at 56°C for 30 min.

Fractionation and chemical properties of antiviral agents in serum : Fractions of guinea pig serum were separated based on their chemical properties including stability in acidic and alkaline solution as illustrated in Fig. 1. The serum (3.5 ml) was mixed with 1.5 ml of 25 % NH_4OH (alkaline solution) or 0.175 ml of trifluoroacetic acid (TFA, acidic solution). These mixtures were kept for 1 h at 4°C and centrifuged at 3,000 rpm for 20 min. Then each supernatant was dried by lyophilization. The dried samples were reconstituted in 3.5 ml of MEM to adjust the concentration of agents to those of original serum used. To evaluate chemical properties of the anti-HSV-1 agents, the serum was also extracted with organic solvents which is used for the isolation of compounds from natural products[11-13]: the serum was mixed with 6 volume of ethanol (EtOH) and then kept for 1 h at room temperature, or extracted three times with an equal volume of ethylacetate (EtOAc) or ether. The mixtures were centrifuged at 3,000 rpm for 20 min. The separated fractions were evaporated under reduced pressure and lyophilized. All dried samples prepared were dissolved in 0.1 ml of DMSO and then reconstituted in 3.5 ml of MEM.

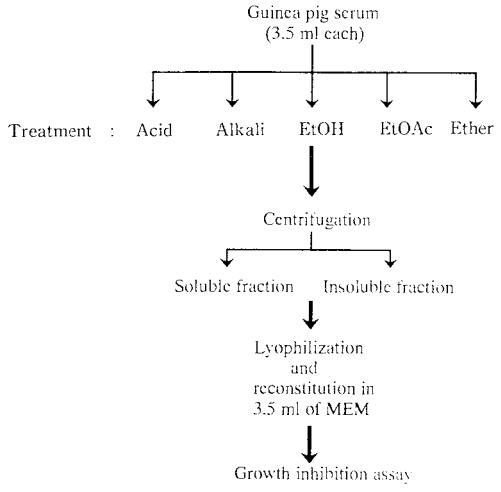

Fig. 1 Anti-HSV-1 assay of serum fractions. Sera obtained from water- and GJ-HW-extract-administered guinea pigs were simultaneously treated with each solvent.

Growth inhibition assay of HSV-1: Anti-HSV-1 activity in guinea pig sera or their fractions were determined by the growth inhibition assay of HSV-1. Monolayers of Vero cells in 25 cm^2 plastic flasks were infected with HSV-1 (Seibert strain) at 0.01 PFU/cell for 1 h. The infected cells were washed three times with MEM and incubated in MEM containing 25 % of guinea pig serum or reconstituted medium from serum fractions. After 24 h incubation at 37°C, the cultures were frozen and thawed three times and centrifuged at 3,000 rpm for 15 min. Virus yields in the culture were determined by the plaque assay of their supernatants on Vero cells.[14]

Anti-HSV-1 assay of EtOAc-extractable fraction directly from GJ-HW-extract : GJ-HW-extract was fractionated as shown in Fig. 2. HW-extract was extracted three times with an equal volume of EtOAc. The EtOAc-extractable fraction was collected and evaporated. The dried EtOAc-extractable fraction was further extracted sequentially with ether and chloroform. The dried ether- and chloroform-extractable fractions and their unextractable fractions were dissolved in dimethylsulfoxide (DMSO) at 10 mg/ml and examined for their anti-HSV-1 activity in the growth inhibition assay.

Therapeutic efficacy in mouse HSV-1 infection model: Female BALB/c mice (6-week-old, Sankyo Labo Service Co., Ltd., Tokyo, Japan) were infected with HSV-1 strain 7401H (1×10^6 PFU/mouse) after scarification in the area of the shaved right midflank with a 27-gauge needle as described previously.[6,9] The EtOAc-extractable fraction of GJ-HW-extract (1 or 5 mg) or water was orally administered in a volume of 0.25 ml/mouse once at eight hours before and three times (approximately 8 hours interval) daily for 8 suc-

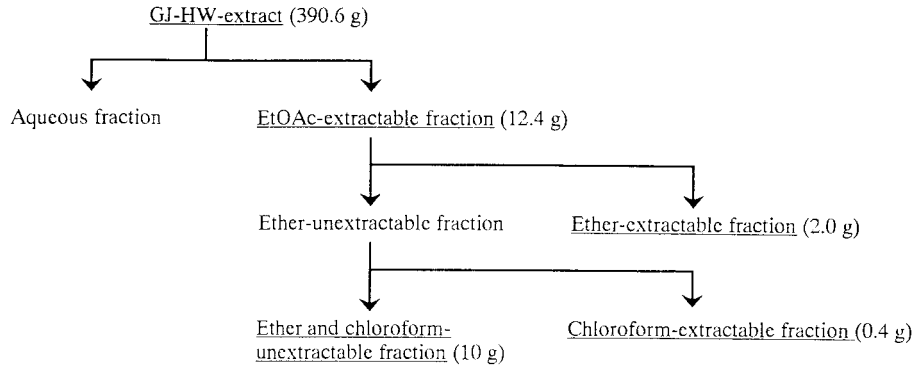

Fig. 2 Fractionation of GJ-HW-extract. Number in parentheses represents each recovered amount. Underlined fractions were examined for anti-HSV-1 activity by growth inhibition assay as shown in Figs 3 and 4.

cessive days after viral inoculation. The EtOAc-extractable fraction (160 or 800 mg) was dissolved in 0.5 ml of DMSO and then suspended in 40 ml of distilled water. Water containing 1.25 % of DMSO was used as the control. The development of skin lesions and death were observed three times daily and the severity of the lesions was assessed as described previously[1,3] : 0, no lesion ; 2, vesicles in local region ; 4, erosion and/or ulceration in local region ; 6, mild zosteriform lesion ; 8, moderate zosteriform lesion ; 10, severe zosteriform lesion ; and death. The Student's t-test was used to evaluate the significance of differences between two groups treated with different drugs in mean survival times and mean times at which skin lesions were initially scored as 2 (vesicles in local region) or 6 (zosteriform lesion) after infection. Statistical differences in the mortality were evaluated using the Kaplan-Meier method and the Wilcoxon test. A p value of less than 0.05 was considered statistically significant.

Characterization of anti-HSV-1 agents : To characterize chemical properties of anti-HSV-1 agents in GJ-EtOAc-extractable fraction, their anti-HSV-1 activity was examined for pH stability. The GJ-EtOAc-extractable fraction (400 mg) was suspended in 20 ml of 20 mM phosphate buffer, pH 8.0. The suspension was centrifuged at 3,000 rpm for 15 min and then the supernatant was filtered (0.45 μm, Kurabo Co., Ltd., Japan). The filtered solution was adjusted to various pH by the addition of 10 M HCl or 10 M NaOH and maintained for 1 h. The solution (2 ml) with various pH was centrifuged at 3,000 rpm for 15 min. The precipitate was dissolved in 2 ml of DMSO. The supernatant (1 ml) was further extracted twice an equal volume of EtOAc. The collected EtOAc-extractable fraction was evaporated and dissolved in 1 ml of DMSO. The aqueous fraction was evaporated to remove the remaining EtOAc and its total volume was adjusted to 1 ml by the addition of distilled water. The anti-HSV-1 activity of all fractions was examined in a plaque reduction assay as described below.

To examine the anti-HSV-1 activity of GJ-EtOAc-extractable fraction based on chemical reactivity, the GJ-EtOAc-extractable fraction was also treated with diazomethane or $FeCl_3$. Diazomethane in ether was prepared immediately before use as described previously.[15] The GJ-EtOAc-extractable fraction (2 mg) in methanol was mixed with 7 ml of the diazomethane solution. The mixture was kept at room temperature for 3 h and then evaporated. For the treatment with $FeCl_3$, the GJ-EtOAc-extractable fraction (0.5 ml) was reacted with 0.25 ml of 1 % $FeCl_3$. The dried materials were dissolved in DMSO for a plaque reduction assay. For the plaque reduc-

tion assay, duplicate cultures of Vero cells in 60 mm plastic dishes were infected with 100 PFU/0.2 ml of HSV-1 (Seibert strain) for 1 h. Then the cells were overlaid with 5 ml of nutrient methylcellulose (0.8%) medium containing various concentrations of samples described above. The HSV-1-infected cultures were incubated for 2-3 days at 37°C. The infected cells were fixed and stained, and the number of plaques was counted.[8]

Results

Anti-HSV-1 activity of serum obtained from guinea pigs administered with GJ-HW-extract

Table I shows the effects of guinea pig sera on the growth of HSV-1 in Vero cells. The sera from GJ-administered guinea pigs reduced the virus yield of HSV-1 significantly as compared with those from water-administered guinea pigs (the controls). The guinea pig serum did not show cytotoxicity at a concentration of 25 % used in the growth inhibition assay (data not shown). The anti-HSV-1 agents of GJ-HW-extract were confirmed to be absorbed from alimentary tracts.

Serum fractions were treated with acid, alkali, or organic solvents, and the treated fractions were examined for their anti-HSV-1 activity in a growth inhibition assay (Table II). The EtOH- and EtOAc-extractable fractions of the serum significantly reduced the virus yields of HSV-1. However the acid- and alkali-treated fractions, and ether-extractable fraction did not exhibit significant anti-HSV-1 activity as compared with their control serum fractions. Therefore the anti-HSV-1 agents of GJ-HW-extract in serum were found to be extracted with EtOH or EtOAc.

Table I Effects of guinea pig sera on the growth of HSV-1 in Vero cells.

Exp. no.	Mean virus yield±S.D. ($\times 10^{-7}$ PFU/ml)	
	Control (water)	GJ-HW-extract
1	6.15±1.94	2.48±0.21 (40.3%)*
2	9.89±0.16	4.28±1.92 (43.3%)*
3	13.7±0.14	3.08±4.26 (22.5%)*

Sera obtained from water- and GJ-HW-extract-administered guinea pigs were assayed for their anti-HSV-1 activity in Vero cells. Virus yield is expressed as a mean±standard deviation (S.D.). Figures in parentheses are the percent of each control virus yield.
*Significant difference from the control, $p<0.05$ by Student's t-test.

Table II Effects of various fractions obtained from guinea pig sera on the growth of HSV-1 in Vero cells.

Fractions	Mean virus yield±S.D. ($\times 10^{-7}$ PFU/ml)	
	Control (water)	GJ-HW-extract
Exp.1		
Acid soluble	7.37±1.41	5.59±0.17(75.8%)
Alkali soluble	3.69±1.02	2.50±0.57(67.8%)
EtOH soluble	2.35±0.33	1.24±0.12(52.8%)*
insoluble	1.33±0.76	2.93±0.05(220.3%)
Ether extract	3.17±1.18	3.31±0.41(104.4%)
insoluble	14.6±3.75	14.3±3.25(97.9%)
Exp.2		
EtOAc extract	5.24±0.10	2.65±0.68(50.6%)*
insoluble	3.52±0.83	3.24±0.73(92.0%)

Each fraction was prepared from serum of guinea pigs administered with water or GJ-HW-extract. Sera obtained from three guinea pigs were assayed for anti-HSV-1 activity (yield reduction) independently. Figures in parentheses are the percent of each control virus yield.
*Significant difference from the control, $p<0.01$ by Student's t-test.

However the acid- and alkali-treated fractions, and ether-extractable fraction did not exhibit significant anti-HSV-1 activity as compared with their control serum fractions. Therefore the anti-HSV-1 agents of GJ-HW-extract in serum were found to be extracted with EtOH or EtOAc.

Anti-HSV-1 activity of EtOAc-extractable fraction directly from GJ-HW-extract

As the EtOAc-extractable fraction of guinea pig serum showed anti-HSV-1 activity (Table II), the anti-HSV-1 activity of EtOAc-extractable fraction directly from GJ-HW-extract was confirmed in the growth inhibition assay (Fig.3). The GJ-EtOAc-extractable fraction was found to inhibit the growth of HSV-1. The 90 % inhibitory dose of the EtOAc-extractable fraction was about one-fourth of that of the HW-extract. Even when the GJ-EtOAc-extractable fraction was further extracted sequentially with ether and chloroform,

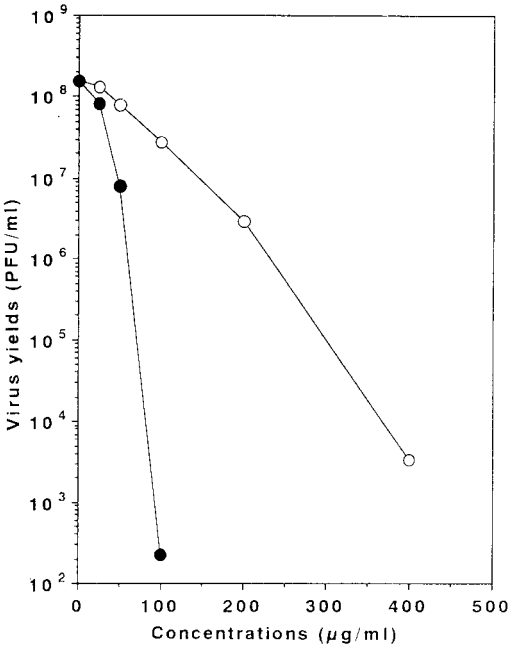

Fig. 3. Effects of GJ-HW-extract and its EtOAc-extractable fraction on the growth of HSV-1 in Vero cells. Open and closed circles represent virus yields in the presence of GJ-HW-extract and its EtOAc-extractable fraction, respectively.

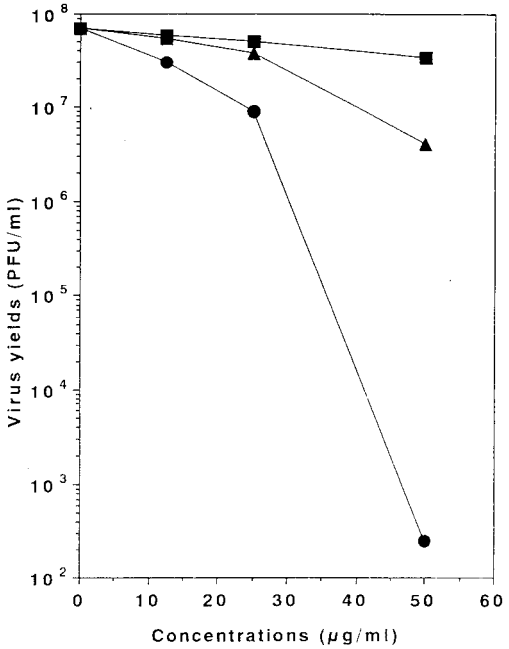

Fig. 4 Effects of ether- and chloroform-extractable fractions, and remaining fraction from EtOAc-extractable fraction on the growth of HSV-1 in Vero cells. Closed triangles, squares, and circles represent virus yields in the presence of ether- and chloroform-extractable fractions, and the remaining fraction, respectively.

major anti-HSV-1 activity was not extracted into ether- and chloroform-extractable fraction (Fig. 4). Thus the specific anti-HSV-1 activity in EtOAc-extractable fraction was increased by removal of fractions extracted with ether and chloroform.

Therapeutic efficacy of GJ-EtOAc-extractable fraction on mouse HSV-1 infection model

Table III shows the therapeutic anti-HSV-1 efficacy of GJ-EtOAc-extractable fraction in a

Table III Effects of EtOAc-extractable fraction from GJ-HW-extract on cutaneous HSV-1 infection in mice.

Exp. NO.	Treatment	Mean time (days±S.D.)			Mortality
		Score 2*	Score 6*	Survival time	
1	Control (water)	3.33±0.50	5.56±0.73	7.00±0.76	8/9
	EtOAc-extractable fraction				
	1 mg/mouse	4.67±0.71**	6.00±0.71	7.86±1.22	9/10
	5 mg/mouse	4.30±0.48**	5.80±0.42	8.75±1.28**	9/10
2	Control	3.09±0.30	5.27±0.47	6.36±0.51	11/11
	EtOAc-extractable fraction				
	1 mg/mouse	3.27±0.47	5.64±0.51	7.09±0.83***	11/11

*Mean time at which score 2 or 6 was first observed after infection.
**Significant difference from the control, $p<0.01$ by Student's t-test.
***Significant difference from the control, $p<0.05$ by Student's t-test.

cutaneous HSV-1 infection model in mice. The GJ-EtOAc-extractable fraction at 1 and 5 mg/mouse significantly prolonged mean survival times and/or delayed the development and progression of skin lesions. The therapeutic efficacy of GJ-EtOAc-extractable fraction coincided with the fact that the anti-HSV-1 activity in serum was in its EtOAc-extractable fraction (Table II).

Chemical characterization of anti-HSV-1 agents

The chemical properties of anti-HSV-1 agents in GJ-EtOAc-extractable fraction were characterized. When the GJ-EtOAc-extractable fraction was treated at pH 1 and 12, its anti-HSV-1 activity was reduced but stable at a range of pH 3-11 (Table IV). The major anti-HSV-1 activity was extracted into the EtOAc fractions in acidic conditions but into the aqueous fractions in alkaline conditions. Thus these data suggested that the anti-HSV-1 agents were weakly acidic compounds. When the GJ-EtOAc-extractable fraction was treated with $FeCl_3$, the color of treated solution turned blue and its anti-HSV-1 activity was reduced as compared with that of untreated fraction (Table V). The anti-HSV-1 activity of GJ-EtOAc-extractable fraction was also lost by the treatment with diazomethane (Table V). Therefore the major anti-HSV-1 agents in GJ-EtOAc-extractable fraction were suggested to be weakly acidic compounds with $FeCl_3$- and diazomethane-reactive functional groups such as phenolic and carboxyl groups.

Table IV Anti-HSV-1 activity of GJ-EtOAc-extractable fraction at various pH.

pH	Plaque formation (%)			
	Supernatant	Precipitate	EtOAc	Aq.
1	54.5	78.9		
3	0.0	92.6	0.0	71.1
4	0.0	75.6	0.0	65.7
5	0.0	74.8	0.0	68.6
6	0.0	75.6	0.0	54.1
7	0.0	78.1	73.1	28.5
8	0.0	92.6	71.1	30.6
9	0.0	71.1	66.5	0.0
10	0.0	76.4	74.4	0.0
11	0.0	98.8	81.0	0.0
12	58.7	82.6		

Anti-HSV-1 activity of GJ-EtOAc-extractable fraction was examined for pH stability by plaque reduction assay at 150 μg/ml. Figures represent the percentage of control culture. EtOAc and Aq. represent EtOAc phase and aqueous phase, respectively, in EtOAc extraction of the supernatant.

Table V Effects of diazomethane and $FeCl_3$ on anti-HSV-1 activity of GJ-EtOAc-extractable fraction.

Exp. no.	Fractions	Plaque formation (%)
		100 μg/ml
1	GJ-EtOAc-extractable fraction	0.0
	Diazomethane-treated fraction	57.8
2	GJ-EtOAc-extractable fraction	0.0
	$FeCl_3$-treated fraction	60.5

The range of plaque numbers in untreated controls was 90-110. Figures represent the percentage of control culture.

Discussion

It is essential that the anti-HSV-1 agents in HW-extracts should be absorbed from alimentary tracts to exhibit their anti-HSV-1 activity *in vivo*. Thus in this study we examined the anti-HSV-1 activity in serum and fractionated the serum based on the chemical properties of possible anti-HSV-1 agents. In this fractionation, we used acid (TFA), alkali (NH_4OH), and three organic solvents (EtOH, EtOAc, and ether). TFA and NH_4OH are volatile reagents and could be easily removed from serum fractions by evaporation and lyophilization. We did not need to neutralize them. Thus their use in acid- and alkali-treatments would be useful to adjust the pH and salt concentrations of culture medium in growth inhibition assay. EtOH, EtOAc, and ether are a set of representative organic solvents with weak, middle, and strong hydrophobicity, respectively, among many organic solvents to be used for chemical extraction. We can easily estimate the hydrophobicity of unknown compounds by the

comparison of their solubility into each of the three solvents. Therefore the use of a set of these solvents including TFA and NH_4OH would be convenient to identify the chemical properties of anti-HSV-1 agents in serum.

We found that the EtOAc-extractable fraction from serum exhibited anti-HSV-1 activity (Table II). The anti-HSV-1 activity in this fraction was consistent with the fact that the EtOAc-extractable fraction from GJ-HW-extract had anti-HSV-1 activity *in vitro* and *in vivo* (Figs. 3 and 4, and Table III). The anti-HSV-1 activities in both serum and GJ-EtOAc-extractable fraction were similarly inactivated in strong acidic and alkaline conditions and not extracted by ether (Tables II and IV, and Fig. 4). The chemical properties of anti-HSV-1 agents were similar in both serum and GJ-EtOAc-extractable fraction. Thus we confirmed the advantage of using serum for the identification of a fraction with anti-HSV-1 activity *in vivo* from GJ-HW-extract. Together with these results, the anti-HSV-1 agents in GJ-EtOAc-extractable fraction were suggested to be weakly acidic compounds which have $FeCl_3$- and diazomethane-reactive functional groups (Table V).

Conventionally HW-extracts are first fractionated by EtOH or methanol, acetone, isopropanol or butanol, EtOAc, chloroform, ether, toluene, hexane etc. in the order of their hydrophilicity or acidic, neutral, or alkaline solution. Then the fractions containing anti-HSV-1 agents are determined by testing all the separated fractions *in vitro*. However since biological activity *in vitro* does not always correlate to that *in vivo*, many fractions with anti-HSV-1 activity *in vitro* must be further examined for their activity *in vivo*. In contrast to the conventional way, our procedure uses serum with the anti-HSV-1 activity *in vivo*. The fractionation of serum was found to be useful to separate a fraction with anti-HSV-1 activity *in vivo* directly from GJ-HW-extract. Such an approach may be available to separate an *in vivo*-active fraction from other HW-extracts which exhibited anti-HSV-1 activity *in vitro* and *in vivo*.[6]

In this study we found that major anti-HSV-1 agents in GJ-HW-extract are weakly acidic compounds with $FeCl_3$- and diazomethane-reactive functional groups (Table V). This result was not inconsistent with the characterization of chemical properties of serum fractions. As our final purpose is to find the anti-HSV-1 agents which are active *in vivo*, such physicochemical properties would be helpful to prepare and purify efficiently possible anti-HSV-1 agents directly from GJ.

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和文抄録

単純ヘルペスウイルス (HSV-1) 感染症に治療効果のある伝統医薬熱水エキスから，生体内で有効な抗 HSV-1 活性を示す分画を得るための同定法を開発した。この方法では，大根草熱水エキスを投与したモルモットから得た血清が，HSV-1 の Vero 細胞での増殖を抑制することが確認され，この血清の酢酸エチル，エタノール抽出分画にも抗 HSV-1 活性が認められた。しかし，酸，アルカリ分画やエーテル分画には抗 HSV-1 活性は認められなかった。また，大根草熱水エキスから直接抽出した酢酸エチル分画は抗 HSV-1 活性を示した。この分画はマウス HSV-1 感染系においても治療効果を示し，その抗 HSV-1 活性成分の酸，アルカリに対する安定性，有機容媒に対する溶解性は血清の酢酸エチル分画と同一であることが確認された。したがって，モルモット血清を用いてその血清分画を行なうことにより，熱水エキスの生体内での抗ウイルス活性分画の同定が行なえることがわかった。さらに，大根草熱水エキスの酢酸エチル分画を化学的に処理することにより，その抗 HSV-1 活性物質がフェノール性化合物などの弱酸性物質であることを明らかにした。今後，血清分画の抗 HSV-1 活性試験から得られる情報が，大根草から直接その抗 HSV-1 活性物質を効率的に分離するために役に立つと思われる。

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