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NOTE

Inhibitory Effect of Extracts from 33 Medicinal Herbs Against TMV and CMV Infection

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Abstract

Extracts of 33 medicinal herbs belonging to 28 different families used as precious resources in the Northeast Asia were tested for their antiviral activities against two major plant viruses. Twenty one methanol extracts from 19 different families were found to have a antiviral activity against tobacco mosaic virus (TMV) and cucumber mosaic virus (CMV) when tested on indicator plants under greenhouse conditions. Most of these extracts have weak activities at the concentration used. But the methanol extracts of *Phellinus linteus* exhibited potent (98.7±1.3%) antiviral activity against TMV infection and also showed 97.2±2.2% activity against CMV infection. The methanol extracts of the gall of *Rhus javanica* also showed strong inhibitory efficacy over 98.7±1.1% against TMV or CMV infection. Further research is needed to elucidate the active constituents of these medicinal herbs which may be useful in the development of new and effective antiviral agent against plant viruses.

Key words medicinal herb, antiviral activity, tobacco mosaic virus (TMV), cucumber mosaic virus (CMV)

Introduction

To control plant viral diseases, researchers have been investigated many inhibitors derived from various higher plants (Kubo *et al.*, 1990; Hudson, 1990; Stevens, 1992; Ito *et al.*, 1992), a microorganism (Klement *et al.*, 1966; Yeo *et al.*, 1997) and some mushroom (Aoki *et al.*, 1993). Several substances have been reported as plant viral inhibitors, such as milk (Chester, 1934), polysaccharides (Tomaru and Ohkawa, 1975; Sano, 1999). However, effective materials are mostly proteins or polysaccharides, which have little potential for the control of plant viruses under the field condition. Many traditional medicinal herbs have been reported to have potent antiviral activity and some of them have already been used to treat animals and people who suffer from viral infection (Hudson, 1990, Venkateswaran *et al.*, 1987), because they virtually constitute a rich source of bioactive (Fukuchi *et al.*, 1989; Spedding *et al.*, 1989; De Rodriguez *et al.*, 1990). However, little work has been done to control plant viruses by using these medicinal herbs in spite of their excellent pharmacological signification. Extracts of medicinal herbs used as precious resources in the northeast asia regions had never been evaluated for activity against plant viruses. 33 herb species were collected and extracts screened for antiviral activity. Screening were accomplished by evaluating the effects of extracts on tobacco mosaic virus (TMV) and cucumber mosaic virus (CMV) infectivity in local lesion assays. Here we present the first report of the strong inhibition of TMV or CMV infection by extracts of *Phellinus linteus* and Galla Rhois.

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The Guaranteed 33 medicinal herbs were purchased at kyungdong Oriental Drug market, Seoul, Korea and listed in Table 1. The phamacological and industrial importance of medicinal herbs used were described in detail elsewhere (Ahn, 1998 ; Namba, 1986). They were finely powdered using a blender. Each sample (500 g) was extracted twice with methanol at room temperature and filtered (Toyo filter paper No 2, Toyo Roshi) to give an extract. The filtrate was concentrated *in vacuo* at 40℃ using a rotary vacuum evaporator (EYELA Autojack NAJ-160, Japan), and freeze-dried.

To test the antiviral activity the samples, Each of

Table 1. Inhibitory activity of MeOH extracts from medicinal herbs against TMV and CMV

Plant species (Scientific name)	Family name	Part used	Inhibitory ratio (%)[a]	
			TMV[b]	CMV
Aconitum pseudolaeve var. *erectum* NAKAI	Ranunculaceae	root	27.9±2.8[c]	31.4±3.5
Aloe vera	Liliaceae	leaf	32.6±3.0	28.6±6.5
Angelica dahurica (FISCH.) BENTH *et* HOOKER.	Apioideae	root	0.0	-[d]
Angelica gigas NAKAI	Apioideae	root	0.0	-
Artemisia argyi LEV. *et* VANT.	Carduoideae	leaf	75.2±3.0	65.7±6.3
Carpesium abrotanoides L.	Compositae	seed	0.0	-
Dryopteris crassirhizoma NAKAI	Aspidiaceae	root	45.4±2.3	42.4±3.1
Ephedra sinica Stapf	Ephedraceae	root	0.0	-
Eugenia caryophyllata THUNB.	Myrtaceae	fruit	0.0	-
Euphorbia pekinensis RUPR.	Euphorbiaceae	root	22.4±4.1	31.1±5.4
Euphorbia sieboldiana Morren *et* decainsne	Euphorbiaceae	root	9.5±3.2	-
Ferula assafoetida L.	Euphorbiaceae	root	0.0	-
Foeniculum vulgare GAERTNER.	Umbelliferae	fruit	26.6±4.0	31.6±2.5
Hydnocarpus anthelmintica PIERRE	Flacourticaceae	root	36.7±5.3	26.3±4.3
Kaempferia galanga L.	Zingiberaceae	root	0.0	-
Melia azedarach var. *japonica* Makino	Meliaceae	bark	1.5±0.5	-
Omphalia lapidescens Schroeter	Polyporaceae	fruit body	47.2±5.1	43.7±3.1
Phellenus linteus	Hymenochaetaceae	fruit body	98.7±1.3	97.2±2.2
Phytolacca esculenta V. HOUTTE	Phytolaccaceae	root	0.0	-
Piper nigrum L.	Piperaceae	fruit	23.1±2.4	35.5±6.4
Pogostemon cablin (BLANCO.) BENTH.	Libiatae	root	30.6±3.3	27.6±8.3
Polygonatum sibiricum REDOUTE *ex* REDOUTE	Liliaceae	root	13.9±3.0	18.5±6.0
Pulsatilla cernua (THUNB) Spreng.var. koreana (Nakai) Y. Lee	Ranunculaceae	root	30.6±2.1	40.3±3.7
Quisqualis indica L.	Combretaceae	root	0.0	-
Rhus javanica L. (Galla Rhois)	Anacardiaceae	gall	98.7±1.1	100.0±0
Rumex crispus L.	Polygonaceae	root	0.0	-
Siegesbeckia glabrescens Makino	Carduoideae	leaf	13.9±2.4	18.5±6.0
Sophora flavescens AIT.	Leguminosae	root	0.0	-
Stemonae sessilifolia (MIQ.) FRANCH. *et.* SAV	Stemonaceae	root	0.0	-
Torreya mucifera S. *et.* Z.	Taxaceae	root	32.1±2.5	28.6±2.1
Ulmus macrocarpa HANCE	Ulmaceae	root	30.6±3.6	37.5±7.7
Veratrum maackii var. japonicum	Liliaceae	root	21.7±4.0	26.5±3.8
Veratrum patulum LOES. fil.	Liliaceae	leaf	5.0±2.1	-

[a] Inhibition ratio = (1- No. of local lesions on tretment/No. of lesions on control) x 100.

[b] Antiviral activity of extracts were tested at concentration of 10 mg/ml. Two hours prior to virus inoculation, the dilution of each extract was sprayed on leaves of host plants.

[c] Each value represents the mean±standard deviation of three replicates.

[d] Not tested.

Nicotiana glutinosa and *Chenopodium amaranticolor* was used for local lesion assay of TMV and CMV infection, respectively. For TMV inoculum, tobacco leaves (0.1 g) of *N. tabacum* cv. Samsun, systematically infected with TMV, were grounded in 20 ml of phosphate buffer (0.01 M, pH 7.0). The sap was filtered through two layers of cheesecloth. The filtrate was used as TMV inoculum. The inoculum of CMV was prepared from *N. tabacum* cv. Samsun NN with similar method mentioned above. Antiviral activity in local lesion host was tested by using the half-leaf method. The diluted sample (conc. 10 mg/ml) was applied with cotton swabs on the upper surface of half leaf, while distilled water was applied on the remaining half as control. The virus was inoculated 2 hours after application of the sample by using carborundum (400 mesh) methods. The symptom on the inoculated leaves was observed at 3~5 days after the inoculation, and effects of the treatment were measured by comparing the number of local lesions between the treated and untreated half leaf. The inhibitory effects of test samples was indicated with inhibition ratio (%) calculated by formula = (1-No. of lesions on treatment/No. of lesions on control) × 100.

Extracts of 33 medicinal herbs belonging to 28 different families used as precious resources in the Northeast Asia were tested for their antiviral activity against two major plant viruses. Twenty one methanol extracts from 19 different families were found to have a antiviral activity against TMV and CMV (Table I). Most of these extracts have weak activities at the concentration used. But the methanol extracts of *Phellinus linteus* exhibited potent antiviral activity (98.7±1.3%) against TMV infection and also showed 97.2±2.2% activity against CMV infection. The methanol extracts of the gall of *Rhus javanica* also showed strong inhibitory efficacy over 98.7±1.1% against TMV or CMV infection. The antiviral effect of extracts of these two species were similar or slightly higher than the effects of some natural substances derived from micro-organisms and higher plants previously reported (Takagi and Shimada, 1977; Kalo and Taniguchi, 1987; Kim *at al.* 2006). Therefore, These have the possibility of the development as new inhibitors of plant virus infection. The methanol extracts of *Artemisia argyi* exhibited moderately inhibitory efficacy (75.2±3.0%) against TMV and showed 65.7±6.3% activity against CMV infection. The extracts of *Aconitum pseudolaeve, Aloe vera, Dryopteris crassirhizoma, Euphorbia pekinensis, Euphorbia sieboldiana, Foeniculum vulgare, Hydnocarpus anthelmintica, Melia azedarach, Omphalia lapidescens, Piper nigrum, Pogostemon cablin, Polygonatum sibiricum, Pulsatilla cernua, Siegesbeckia glabrescens, Torreya mucifera, Ulmus macrocarpa, Veratrum maackii* and *Veratrum patulum* exhibited weak activities against TMV or CMV infections. Other plant extracts, *Angelica dahurica, Angelica gigas, Carpesium abrotanoides Ephedra sinica, Eugenia caryophyllata, Ferula assafoetida, Kaempferia galanga, Phytolacca esculenta, Quisqualis indica, Rumex crispus, Sophora flavescens* and *Stemonae sessilifolia* failed to show antiviral property. In the bio-assay studies with methanol extracts from 33 samples, the antiviral responses varied with medicinal herbs species used. Extracts from *Phellinus linteus* have been used for centuries in traditional korean medicine. It is shaped like a hoof, has a bitter taste, and in the wild grows on mulberry trees. The stem's color ranges from dark brown to black. A paper published by Harvard Medical School, reported that *Phellinus linteus* is a possible anticancer agent. *In vitro* research published by the British Journal of Cancer, demonstrated one possible anticancer mechanism behind *Phellinus linteus*. It was found that the mushroom inhibited the growth of breast cancer cells by way of inhibiting the enzyme AKT as well as inhibiting angio-genesis (Zhu *et al*, 2008). Galla Rhois is a nest of parasitic bug, *Mellaphis chinensis*, in *Rhus javanica*. Galla Rhois has been used for the therapy of diarrhea, peptic ulcer, hemauria, etc., that showed various anti inflammatory activity, and other biological properties (Kim *et al*, 2005).

The present study is the first to investigate pharmaco-gnosy resources for compounds with activity against plant viruses in korea and the first report that extracts of *Phellinus linteus* and Galla Rhois are sources of potent inhibitor against plant virus infection.

It is possible that the elucidation of active constituents in these extracts may provide useful lead to the develop-ment of new and effective antiviral agent.

Literature Cited

Aoki, M., Tan, M., Fukushima, A., Hieda, T., Kubo, S., Takabayashi, M., Ono, K. and Mikami, Y. (1993) Antiviral substances with systemic effects produced by basidiomycetes such as *Fomes fomentarius*. Biosci. Biotech. Biochem. 57:278~282.

Chester, K. S. (1934) Specific quantitative neutralization of the viruses of *tobacco mosaic tobacco ring spot, and cucumber mosaic* immune sera. Phytopathology 24:1180~1202.

De Rodriguez, D. J., J. Chula, C. Simons, M. Armoros, A. M. Veriohe and L. Girre (1990) Search for *in vitro* antiviral activity of a new isoflavone glycoside from Vlex europeus. Planta Med. 50:59~62.

Fukuchi, K., H. Sakagarmi, T. Okuda, T. Hatano, S. Tanuma and K. Kitajima (1989) Inhibition of herpes simplex virus infection by tannis and related compounds. Antiviral Res. 11:285~297.

Hudson, J. B. (1990) Antiviral compounds from plants. Boca Raton, Florida: CRC Press. pp. 200.

Ito, Y., I. Seki, S. Tanifuji and A. Hiramatsu (1992) Inhibition of protein synthesis by antiviral protein from *Yucca recurvifolia* leaves. Biosci. Biotech. Biochem. 57:518~519.

Kalo, F and T. Taniguchi (1987) Properties of a virus inhibitor from spinach leaves and mode of action. Ann. Phytopath. Soc. Japan 53:159~167.

Kim, M. W., S. M. Ju, K .J. Kim, Y. G. Yun, D. M. Han, W. S. Kim and B. H. Jeon (2005) Study on the Antileukemic Effect of Galla Rhois. The Kor. Ass. of Oriental Medical Physiol. 19:234~234.

Kim, Y. S., Hwang, E. I., Kim, K. S., Ryu, M. H. and W. H. Yeo (2004) Inhibitory effects of *Acinetobacter* sp. KTB3 on infection of Tobacco mosaic virus in tobacco plants. Plant Pathol. J. 20:293~296.

Klement, Z., J. Kiraly and I. Pozsar (1966) Suppression of virus multiplication and local lesion production in tobacco following inoculation with a saprophytic bacterium. Acta Phytopathol. Acad. Sci. Hung. 1:11~18.

Kubo, S., T. Ikeda, S. Imaizumi, Y. Takanami and Y. Mikami (1990) A potent plant virus inhibitor found in *Mirabilis jalapa* L. Ann. Phytopathol. Soc. Jpn. 56:481~487.

Namba, T. (1986) Colored Illustrations of Wakan-Yaku (The Crude Drugs in Japan, China and the Neighboring Countries), Hoikusha Pub., Osaka.

Sano, Y. (1999) Antiviral activity of alginate against infection by *Tobacco mosaic virus*. Carbohydr Polym 38:183~186.

Spedding, G., A. Ratty and E. J. Middleton (1989) Inhibition of reverse transcriptases by flavonoids. Antiviral Res. 12:99~110.

Stevens, W. A. and T. Reynolds (1992) Plant virus inhibitors from members of the Polygonaceae. Biomedical Letters 47: 269~273.

Takagi, Y. and K. Shimada (1977) Inhibitory effect of aqueous extracts from the saw dust-rice bran media grown mycelia of *Hymenomycetes* on tobacco mosaic virus infection. Ann. Phytopath. Soc. Jpn 43:211~214.

Tomaru, K. and Y. Ohkawa (1975) The inhibitory activity of alginic acid against *Tobacco mosaic virus* infection. Ann. Phytopath. Soc. Japan 41:155~161 (in japanese with English summary).

Yeo, W. H., Y. H. Kim, E. K. Park and S. S. Kim (1997) Physicochemical characteristics and antiviral activity of ASA, an antibiotic produced by *actinomycetes* B25. Korean J. Plant Pathol. 13:63~68.

Venkateswaran, P. S., I. Millman and B. S. Blumberg (1987) Effects of an extract from *Phyllanthus niruri* on hepatitis B and Woodchuck hepatitis viruses: *in vitro* and *in vivo* studies. Proceedings of the National Academy of Sciences of the USA 84:274~278.

Zhu, T., S. H. Kim and C. Y. Chen (2008) "A medicinal mushroom: *Phellinus linteus*". Current Medicinal Chemistry 15:1330~1335.

33종 생약 추출물의 담배모자이크바이러스(TMV)와 오이모자이크바이러스(CMV)에 대한 감염 억제효과

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요 약 동북아시아 지역에서 전통적으로 진귀하게 사용되어온 생약자원 28과 33종의 추출물을 이용하여 주요 식물바이러스에 대한 감염억제활성을 조사하였다. 온실 생물검정 시험결과, 19과 21종의 메탄올 추출물이 담배모자이크바이러스(TMV)와 오이모자이크바이러스(CMV)에 대하여 항바이러스 활성을 보였다. 대부분의 공시한 시료들은 약한 항바이러스 활성은 보였으나, 목질진흙버섯의 메탄올 추출물은 TMV에 대하여 98.7±1.3%의 강한 항바이러스 활성을 보였다. 이는 CMV에 대하여도 97.2±2.2%의 높은 활성을 보였다. 또한, 오배자의 메탄올 추출물은 TMV 및 CMV에 대하여 98.7±1.1%이상의 강한 억제효과를 보였다. 금후의 연구에서는 효과적이고 새로운 형태의 식물바이러스 방제제 개발에 있어서 매우 유용할 수 있는 이들 선발된 생약자원 유래의 항바이러스 활성물질의 구명이 필요하다.

색인어 생약자원, 항식물바이러스활성, 담배모자이크바이러스, 오이모자이크바이러스