

Screening of A Medicinal Plant Having Antiviral Activity against Influenza A/H5N1

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Objectives

In this study, we aimed to test forty seven oriental medicinal plants with putative antiviral activity against influenza A virus subtype H5N1 in MDCK cell culture.

Materials and Methods

◦ Viruses and cells

H5N1 (A/Vietnam/1194/04 (H5N1)-NIBRG-14) influenza viruses were propagated in the allantoic cavities of 11-day-old embryonated chicken eggs in approved BL-3 containment facility. MDCK cells were cultivated in MEM supplemented with 10% FBS. The procedures for cell culture and virus titration were performed as described elsewhere.

◦ Preparation of plant extracts

The air-dried and finely ground plants (30 g) were extracted with 300 mL of methanol (99.5%) for 24 hr at 25°C on a rotating shaker. After filtration through Whatman No. 2 filter paper, the filtrate was concentrated under reduced pressure and lyophilized, the stored at 4°C (Fig. 1).

◦ Anti-viral activity assay

Confluent monolayers of MDCK cells on the 6-well plates were infected with 100 mL of 100 TCID₅₀/mL of H5N1 viruses. One hour later, plant extracts in MEM media (3 mL) at a non-toxic concentration were added to MDCK cells in well of 6-well plates, and cells were incubated for 48 hr. After this incubation period, virus titers were determined by hemagglutination (HA) assays. The anti-viral activity was defined as a 4 log reduction.

Results

One of the 20 samples tested, the methanol extract of *Forsythia suspensa* Vahl and was shown to inhibit the H5N1 influenza virus (Table 1). Virus titers were 2^7 HA unit in case of sample-free, whereas with methanol extract it were reduced to 2^1 HA unit. This results suggest that *Forsythia suspensa* Vahl as a natural feed additive has a potential to be used in treatment of H5N1 influenza-related disease.

Table 1. Medicinal plants used in the study and anti-viral activity against high pathogenic avian influenza H5N1.

Botanical name	Part used	HA titer reduction
<i>Arctium lappa</i> Linne	seed	-
<i>Kochia scoparia</i> Schrader	seed	-
<i>Forsythia suspensa</i> Vahl	fruit	6 log
<i>Angelica dahurica</i> Bentham et Hooker	root	-
<i>Lonicera japonica</i> Thunberg	aerial	-
<i>Cinnamomum cassia</i> Blume	stem	-
<i>Dictamnus albus</i> Linne	root	-
<i>Notopterygium incisum</i> Ting	root	-
<i>Myrrha</i>	resin	-
<i>Glycyrrhiza glabra</i> Linne	root	-
<i>Prunella vulgaris</i> Linne var. <i>lilacina</i> Nakai	leaf	-
<i>Elsholtzia ciliata</i> Hylander	aerial	-
<i>Sinomenium acutum</i> Rehder et Wilsom	root	-
<i>Coptis japonica</i> Makino	root	-
<i>Kalopanax pictus</i> Nakai	bark	-
<i>Aralia continentalis</i> Kitagawa	root	-
<i>Asparagus cochinchinensis</i> Merrill	root	-
<i>Paeonia suffruticosa</i> Andrews	root	-
<i>Prunus serrulata</i> var. <i>sponitanea</i> Maxim	bark	-
<i>Sophora subprostrata</i> Chun et T. Chen	root	-

- : not detected

Fig. 1. Procedure to prepare extracts from medicinal plants

