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REVIEW

Anti-influenza Agents from Plants and Traditional Chinese Medicine

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Influenza is a serious threat to health in all parts of the world. The control and treatment of influenza depends mainly on chemical or biochemical agents and, to date, some anti-influenza agents have been isolated from plants as a result of chemical and pharmacological studies. These agents include a variety of polyphenols, flavonoids, saponins, glucosides and alkaloids. Traditional medicine focuses on the use of herbs and traditional Chinese medicine has performed well in clinical practice and shows a potential in the therapy of influenza and its symptoms. The present paper reviews some constituents and extracts from plants and traditional Chinese medicine with anti-influenza activity. Copyright © 2006 John Wiley & Sons, Ltd.

Keywords: influenza; traditional Chinese medicine; medicinal plants; review.

INTRODUCTION

Influenza or 'flu' is an infection of the respiratory tract that affects millions of people every year. Influenza is caused by a virus that can only reproduce in living cells which they infect. There are three types of influenza virus: A, B and C. Influenza A can infect humans and other animals, while influenza B and C infect humans only. Influenza A virus is essentially an avian virus that has crossed into mammals. Birds have the greatest number and range of influenza strains, but avian hemagglutinins sometimes appear in pig, human and horse influenza strains.

The center or core of the influenza virus contains RNA which includes all the genes necessary for the virus to survive and reproduce within host cells. Each strand of the influenza virus RNA is covered with a protein coat called nucleoprotein. The entire core of the virus is covered by a matrix protein membrane which, in turn, is covered by a fatty or lipid envelope. Two different types of protein molecules, called the hemagglutinin or H protein and the neuraminidase or N protein, protrude through the fatty envelope like spikes. The H and N proteins help the influenza virus attach to and enter the cell which it infects. The body recognizes these H and N proteins as foreign and attacks the virus by producing antibodies against them. A virus is named by its type (i.e. influenza A, B or C), the place where the virus originated, the laboratory number, and the year it was collected. The influenza A virus is also named by the type of H and N proteins it carries and the animal from which it was isolated, if not a person.

Vaccines are the mainstay of prophylactic treatment for influenza, but do have significant drawbacks as they are needed annually, the vaccines and the circulating virus strains must be closely matched, and there are also well recognized vaccine failures. In immunocompetent young subjects, vaccines are around 80–90% effective in protecting against infection, however, in the elderly, where most of the mortality occurs, vaccines are only around 50% effective (Johnston, 2002).

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MECHANISM AND PRESENT DRUGS FOR THERAPY OF INFLUENZA

Because the influenza virus can only reproduce in the host cell, antiviral drugs need to penetrate cells and selectively inhibit viral replication without cytotoxicity. Some synthetic drugs such as amantadine and rimantadine (influenza A inhibitors) and zanamivir and oseltamivir (neuraminidase inhibitors) have been available for decades, but all have side effects and limitations. Therefore, more attention has been paid to natural, active substances. Herbal remedies play a fundamental role in traditional medicine in China, Korea, Japan, India and the rural areas of Colombia, where they are often the therapeutic of choice to cure influenza and concomitant infections (Gao *et al*., 2002).

Herbal drugs act as anti-influenza agents in two ways: inactivating or restraining the virus directly and inhibiting the virus indirectly by inducing interferon or regulating immune function. Although some compounds and extracts do not exhibit direct antiviral activity *in vitro*, they are used to enhance or regulate immune function in resisting viral infection. First, they promote phagocytosis by the reticuloendothelial system to enhance the immune function of the cell. Second, the compounds induce interferons. Third, they enhance macrophage activation and stimulate the production of IL-1.

The activity of a direct inhibiting virus can usually be tested by *in vitro* tests including viral replication testing, cell survival rate and damage extent testing, tests for membrane fusion, protein anabolic effect testing, RNA anabolic effect testing and assay of sialidase

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activity. Animal experiments, embryo culture studies and preventive and therapeutic efficacy tests are also applied to evaluate either direct or indirect antiviral activities. Many studies on screening anti-influenza active constituents and extracts have been carried out with these tests and methods. Table 1 lists some natural extracts and compounds with anti-influenza activity. This article reviews these works and results as follows.

CHEMICAL CONSTITUENTS WITH ANTI-INFLUENZA ACTIVITY ISOLATED FROM MEDICINAL PLANTS

Based on a large body of chemical and pharmacological research, some bioactive compounds have been found in medicinal plants used to treat influenza. A variety of polyphenols, flavonoids, saponins, glucosides and alkaloids isolated from medicinal herbs have been studied extensively and tested for anti-influenza activity, as reflected by their ability to block adherence, penetration, duplication and/or maturation during the course of viral propagation.

Polyphenols

Polyphenol compounds have been shown to inhibit protein or RNA synthesis by the virus. They also restrained viral adsorption. Recent evidence shows that plant polyphenols exhibit antioxidant and radical scavenging properties. Using three separate and complementary methods it was established that a polyphenol-rich extract from the medicinal plant *Geranium sanguineum* L. with strong anti-influenza virus activity, possessed antioxidant and radical scavenging capacities (Sokmen *et al*., 2005).

Table 1. Some anti-influenza virus natural extracts and compounds

Original plant	Part used	Compound or active component	Function
Geranium sanguineum L.	N.a.	A polyphenolic complex	Anti-different influenza virus
Cydonia oblonga Mill.	Fruit	Phenolics	Anti-influenza virus type A
Citrus junos Tanaka (Rutaceae).	Fruit	Flavanone triglycoside	Anti-influenza virus type A
Bupleurum Chinense DC.	Aerial parts	Total flavonoid	Anti-influenza virus type B
	Roots	Extracts	Inhibit proliferation of influenza virus
Polygala tenuifolia Willd.	Roots	Onjisaponins	Mucosal adjuvant
Pinellia ternata (Thunb.) Breit.	Rhizome	Pinellic acid	Mucosal adjuvant
Mangifera indica L.	Leaves	Chinonin	Decrease the infectivity of influenza virus A
Solanum tuberosum L.	Rhizome	Anthocyanin	Inactivated IVA and IVB
Uncaria rhynchophylla	N.a.	Indole alkaloid	Anti-influenza A
Thalictrum simplex L.	N.a.	Thalimonine	Anti-H7N7, H7N1
Cynanchum stauntonii	Roots	Volatile oil	Anti-virus A/NWS/33
Sanicula europaea L.	Leaves	Extract fraction	Anti-influenza A/PR/8/34
Hypericum perforatum L.	Aerial parts	Hypericin	Anti-H5N1, H9N2
Mangifera indica L.	Leaves	Chinonin	Anti-influenza virus type A
Schizonepeta tenuifolia Briq.	Spica	Volatile oil and extracts	Effective on relieving pulmonary infection
Arctium lappa L.	Seed	Arctigenin	Inhibit or deactivate influenza virus type A
Forsythia suspensa (Thunb.) Vahl	Fruit	Total flavonoids Forsythosides	Anti-virus
Mentha haplocalyx Briq.	Leaves	Total flavonoids	
Lonicera japonica Thunb.	Floral buds	Total flavonoids Extract	Anti-virus (H9N2)
Glycyrrhiza uralensis Fisch.	Roots	Lignanoids	Anti-virus
		Extracts	Anti-influenza virus type A
		Extracts	Inhibiting IL-1α
Isatis indigotica Fort	Roots, leaves	Active proteins	Anti-influenza virus type A
Houttuynia cordata Thunb.	Whole plant	Extracts injection	Inhibit cell apoptosis by IVA (H3N2)
Toddalia asiatica Lam.	Roots	Extracts	Anti-H1N1 virus
Acacia catechu (L. f.) Willd.	Stem and leaves	Extracts	Anti-influenza virus type A
Ganoderma applanatum (pers) pat.	Whole plant	Extracts	Anti influenza virus FM1
Notopterygium incisum Ting ex H. T. Chang	Rhizome and stem	Extracts	Anti-A/FM/1/47
Chaenomeles speciosa	N.a.	Extracts	Anti-influenza virus type A
Citrus junos	N.a.	Extracts	
Zingiber officinale	N.a.	Extracts	

N. B. References are given in the text.

A polyphenolic complex (PC), isolated from the Bulgarian medicinal plant *Geranium sanguineum* L., was shown to have selective anti-influenza activity *in vitro*. Although the action was directed against an early stage of infection (within 3 h of infection), the process directly affected was not identified (Julia and Alan, 1998). The selectivity of antiviral action was confirmed by the variation in sensitivity of different influenza viruses to PC and the selection of variants with reduced drug sensitivity.

Polyphenols in fruits also showed antiviral activity. Hamauzu *et al.* (2005) showed that Chinese quince (*Cydonia oblonga* Mill.) had the largest amount of phenolics consisting mainly of high polymeric procyanidins. Quince had considerable amounts of hydroxycinnamic derivatives mainly composed of 3-caffeoylquinic acid and 5-caffeoylquinic acid and polymeric procyanidins. They found apple (variety *Fuji*) had the least amount of phenolics, mainly 5-caffeoylquinic acid and monomeric and oligomeric procyanidins. The antioxidant functions of Chinese quince and quince phenolic extracts were superior to those of chlorogenic acid standard or ascorbic acid evaluated in both the linoleic acid peroxidation system and the DPPH radical scavenging system. However, those extracts were less effective than apple phenolics or (-)-epicatechin in the linoleic acid peroxidation system. On the other hand, Chinese quince phenolics showed the strongest anti-influenza viral activity on the hemagglutination inhibition test.

Flavonoids

Flavonoid compounds mainly inhibit both the activity of neuraminidase and membrane fusion. By bioactivity-guided fractionation, a new flavanone triglycoside, naringenin 7-O-(2′,6′-di-O-alpha-rhamnopyranosyl)-beta-glucopyranoside (**1**), as well as hesperetin 7-O-(2′,6′-di-O-alpha-rhamnopyranosyl)-beta-glucopyranoside (**2**), hesperidin (**3**) and narirutin (**4**) were isolated from the fruits of *Citrus junos* v. *Tanaka*, Rutaceae) (Kim *et al.*, 2001). In addition, (**2**) was reported for the first time from this plant and was found to inhibit the influenza A virus.

The total flavonoids from the aerial part of *Bupleurum chinense* DC. proved to have an inhibitory effect on the influenza B virus (Feng *et al.*, 2002). The results of histopathological observation showed that, compared with the control groups, the pathological process in the lungs of the infected mice was reduced markedly. Although the effect was observed in all the dosage groups of the total flavonoids, the improved effect in the dosage group 0.09 g/kg was the most evident.

The anthocyanin of red-fleshed potato (*Solanum tuberosum* L.) inactivated both influenza viruses A (IVA) and B (IVB) (Hayashi *et al.*, 2003). The IC_{50} of red-fleshed potato anthocyanin was 48 μg/mL (IVA) and 54 μg/mL (IVB). The IC_{50} of pelanin was 107 μg/mL (IVA) and 83 μg/mL (IVB). The antiviral activities of pelanin against influenza viruses were lower than the red-fleshed potato anthocyanin. In previous reports, blackcurrant anthocyanins showed an additive antiviral effect. Therefore, it was believed that the antiviral activity of the potato anthocyanin comes from the additive or synergistic effect of each anthocyanin pigment with other coexisting pigments.

Saponins

Takayuki *et al.* reported that the extract from the root of *Polygala tenuifolia* Willd. contained potent mucosal adjuvant activity. The active substances were purified and identified as onjisaponins A, E, F and G. After 4 weeks, mice inoculated intranasally (i.n.) with onjisaponin (10 μg) and the influenza vaccine (10 μg) showed an increase in serum hemagglutination-inhibiting (HI) antibody titers of 3–14 times greater than the control mice administered vaccine alone (Takayuki *et al.*, 2001). The results of this study suggest that onjisaponins may provide safe and potent adjuvants for intranasal inoculation of influenza HA and diphtheria–pertussis–tetanus vaccines.

Recently the results were verified again by Nagai *et al.* Two inoculations of mice with onjisaponin F and influenza HA vaccine at 3 week intervals, significantly increased serum HI antibody and nasal anti-influenza virus IgA and IgG antibody titers compared with inoculating vaccine alone. Intranasal vaccination with onjisaponin F inhibited the proliferation of mouse adapted influenza virus A/PR/8/34 in bronchoalveolar lavages of infected mice (Nagai *et al.*, 2005).

Alkaloids

Alkaloids have shown inhibitive activity on viruses such as HIV, HSV and HBV (Li *et al.*, 2002; Gao *et al.*, 2002) and some alkaloids also showed anti-influenza activity. An indole alkaloid (**1**) from *Uncaria rhynchophylla* was found to exhibit a potent inhibitory effect against influenza A virus *in vitro*, and the essential structural features for activity were elucidated by studying the structure-activity relationship using natural and synthetic derivatives of the indole alkaloids (Hiromitsu *et al.*, 1997).

The pavine alkaloid (-)-thalimonine (Thl), isolated from the Mongolian plant *Thalictrum simplex*, inhibited markedly the replication of influenza virus A/Germany/27, str. Weybridge (H7N7) and A/Germany/34, str. Rostock (H7N1) in cell cultures of chicken embryo fibroblasts. In a number of assays at a non-toxic concentration range 0.1–6.4 μM the alkaloid inhibited viral reproduction in a selective and specific way (selectivity index = 640, 106.6, respectively). Expression of viral glycoproteins hemagglutinin (HA), neuraminidase (NA) and nucleoprotein (NP) on the surface of infected cells, virus-induced cytopathic effects, infectious virus yields, HA production and virus-specific protein synthesis were all reduced. The inhibition was dose-related and depended on virus inoculum. Time action experiments indicated that viral reproduction was markedly inhibited when Thl was added at 4–5 h after infection. No inactivating effect on extracellular virus was found (Serkedjieva *et al.*, 2003a, 2003b).

Other compounds

(*E*, *E*)-2, 4-Decadienal, 3-efhyl-4-methypentanol, 5-pentyl-3H-furan-2-one, (*E*, *Z*)-2, 4-decadienal and 2(3H)-furanone, dihydro-5-pentyl were found to be the major components of the volatile oil from *Cynanchum stauntonii* which exhibited activity against the influenza

virus *in vitro* (IC_{50} = 64 μg/mL). In *in vivo* experiments, it prevented influenza virus-induced deaths in a dose-dependent manner (Yang *et al.*, 2005).

A traditional Japanese herbal (Kampo) formulation, Sho-seiryu-to (Xiao-Qing-Long-Tang in Chinese) showed oral adjuvant activity for nasally administered influenza vaccine. The active substance was isolated from one of the component herbs, the tuber of *Pinellia ternata* (Thunb.)Breit., and was identified as 9S,12S,13S-trihydroxy-10E-octadecenoic acid (pinellic acid) by spectroscopic and synthetic methods (Nagai *et al.*, 2005). Oral administration of pinellic acid to mice given primary and secondary intranasal inoculation of influenza A vaccine enhanced antiviral IgA antibody titers in nasal and bronchoalveolar washes. They also found that onjisaponins and pinellic acid showed negligible hemolytic activity at an effective dose for adjuvant activity.

Hypericin, a phytochemical extracted from common St John's wort herb (*Hypericum perforatum* L.) and related species, has been found recently to kill the H5N1 and H9N2 of avian virus *in vitro*. The survey report of Liang Jianping showed that hypericin at a concentration of 0.744 mg/mL exhibited marked antiviral activity (Li, 2005). Arctigenin, a lignanoid compound in *Fructus Arctii* (*Arctium lappa* L.), inhibited or deactivated IVA *in vitro* (Pang *et al.*, 2004).

Chinonin, the major component of mango leaf extract (*Mangifera indica* L.), showed an obvious comprehensive inhibitive effect against human influenza virus A as well as inhibition of its proliferation after adsorption, and the median effective concentration (EC_{50}) for the two effects were 0.70 and 0.76 mg/mL, respectively. It was found that chinonin was weakly effective in preventing viral adsorption and was ineffective at killing the virus. Over time, the inhibitive effect of chinonin against the influenza virus A weakened at low concentration, and remained unchanged at high concentration (not less than 1.4 mg/mL). Also, chinonin had a remarkable capacity to decrease the infectivity of the influenza virus A (Li and Zhen, 2005).

SOME EXTRACTS FROM PLANTS WITH ANTI-INFLUENZA POTENTIAL

Compared with those isolated constituents mentioned, other work has been carried out using crude extracts. Although the compounds contributing to the antiviral effects are still unclear, this does not detract from the effectiveness of these extracts on anti-influenza.

Boiled water extracts of *Chaenomeles speciosa*, *Citrus junos* and *Zingiber officinale* exhibited marked antiviral activity (Park and Lee, 2005). Boiled water extracts of the two Korean medicinal plants, *Citrus junos* and *Zingiber officinale*, have strong anti-influenza virus type A activity and no cytotoxic effects, and they may inhibit attachment of the virus to the cell and may be used for prophylaxis.

An experiment was performed to detect the antiviral activities of crude fruit extracts of wild *Ribes nigrum* L. (Kurokarin extract) against influenza virus types A and B (Knox *et al.*, 2003). Kurokarin extract was prepared as follows: fruits of *Ribes nigrum* L. were heated at 50 °C in a heating tank, and then ground under anaerobic conditions. The extracts were centrifuged, and the supernatant fluid was filtered and sterilized, and then added into the culture medium containing the cells. The concentration of extract required to inhibit the plaque formation of both IVA and IVB by 50% (IC_{50}) was 3.2 μg/mL. Both IVA and IVB were directly inactivated up to 99% by 10 μg/mL of the extract at pH 2.8, and 95% to 98% by this dose at pH 7.2. The growth of IVA in cells treated with 10 and 100 μg/mL of the extract for 6 h after infection was completely suppressed. Virus titers in culture fluids of the cells treated with 100 μg/mL of Kurokarin extract for 1 h at 8–9 h after infection were suppressed completely, indicating that the extract inhibited the virus release from the infected cells.

The influence of *Sanicula europaea* L. extracts on influenza virus growth has been investigated in MDCK cells (Kadir *et al.*, 1996). Fractions I, II and III separated from *Sanicula europaea* L. extract with sephadex column chromatography were found to be non-toxic against MDCK cells. The growth of influenza A/PR/8/34 was completely inhibited by these fractions, while that of influenza B/Lee/40 was not affected. Fractions II and III were shown not to have a direct virucidal activity on influenza A/PR/8/34. Influenza A/Vic/1/75 produced microscopic plaques in the presence of the extract. *In vitro* RNA synthesis with viral RNA-dependent RNA polymerase was also inhibited by a water soluble extract of *Sanicula europaea* L. These observations suggest that the *Sanicula europaea* L. extract contains an anti-influenza virus agent.

TRADITIONAL CHINESE MEDICINE FOR INFLUENZA

Traditional Chinese medicinal concept of influenza

In the traditional Chinese medicinal (TCM) system, many symptoms of influenza belong to 'exterior syndrome', (a TCM term for conditions with fever, headache, general soreness, anhidrosis or unsmooth perspiration and floating pulse, etc., caused by exogenous pathogenic factors). It is believed that heat-clearing and detoxification are connected with eliminating the virus, while the support of healthy energy is concerned with enhancing immunity (Li and Liu, 2004). According to this frame of reference, influenza is treated by drugs for relieving 'exterior syndrome' and heat-clearing drugs are used as antibiotics (see Table 2).

It should be mentioned that Chinese traditional medicine employs especially complex formulae which contain multiple ingredients to treat various symptoms. These formulae are effective both as antiviral agents and in immunomodulation and are believed to ameliorate or prevent adverse side-effects linked to the toxicity of individual drugs.

Some herbs used to treat 'exterior syndrome'

Herbs that relieve exterior syndrome include Ephedra herb (*Ephedra sinica Stapf*), Ramulus cinnamomi (*Cinnamomum cassia Presl*), thinleaf milkwort root

Table 2. Medicines for influenza in traditional Chinese medicinal therapy

Function		Representative herbs botanical name
Relieving exterior syndrome	Acrid-warm herbs relieving superficies	*Ephedra sinica Stapf, Cinnamomum cassia Presl, Schizonepeta tenuifolia* Briq. and *Saposhnikovia divaricata* (Turcz.) Schischk.
	Acrid-cold herbs relieving superficies	*Bupleurum chinense* DC., *Pueraria lobata* (Willd.) *Ohwi, Arctium lappa L.*
Heat-clearing drugs		*Forsythia suspensa* (Thunb.) Vahl, *Isatis indigotica* Fort, *Lonicera japonica* Thunb., *Houttuynia cordata* Thunb.

N. B. References are given in the text.

(*Schizonepeta tenuifolia* Briq.), *divaricate saposhnikovia* root (*Saposhnikovia divaricata* (Turcz.) Schischk.), bupleurum root (*Bupleurum chinense* DC.), pueraria (*Pueraria lobata* (Willd.) Ohwi) and *Fructus Arctii* (*Arctium lappa* L.). Volatile oil and spica extracts of *Schizonepeta tenuifolia* Briq. were proved to be effective in relieving pulmonary infection and exhibited a protective effect on IVA infected mice (Ni *et al*., 2004). An antiinflammatory agent composed of extracts of bupleurum root (*Bupleurum chinense* DC.), *Flos Lonicerae* (*Lonicera japonica* Thunb.) giant knotweed rhizome (*Polygonum cuspidatum* Sieb.et Zucc.) etc., completely inhibited the proliferation of influenza virus in MDCK cells at a concentration of 160 μg/mL (Liu *et al.*, 2003). The medicine given orally at a dosage of 0.1–1.2 g/kg for 5 days significantly decreased the mortality rate and prolonged the titer ($p < 0.01$). This effect is similar to that of virazole at the same dosage level.

Based on the guide of Chinese traditional medicinal theory, many classical Chinese medicinal formulae of 'diaphoresis relieving superficies' show good curative effect on influenza nowadays. Ma-Xing-Gan-Shi-Tang is mainly composed of extracts of Ephedra herb (*Ephedra sinica Stapf*), armeniacae semen (*Prunus armeniaca*), licorice root (*Glycyrrhiza uralensis* Fisch.) and *Gypsum Fibrosum*. The formula enhanced the IFN-γ secretion in influenza A virus infected mice. The percentage of the T cell subset was also regulated (Lu *et al*., 2005). Bupleuri and Ramuli Cinnamomi decoction increased the survival rate of FMI virus infected mice (Ding and Jin, 2004). Puerariae decoction which is composed of pueraria (*Pueraria lobata* (Willd.) Ohwi), Ephedra herb (*Ephedra sinica Stapf*), *Ramulus Cinnamomi* (*Cinnamomum cassia Presl*), Paeonia (*Paeonia 1actiflora* Pall.), licorice root (*Glycyrrhiza uralensis* Fisch.), *Rhizoma Zingiberis Recens* (*Zingiber officinale* Rosc.) and *Fructus Jujuba*e (*Ziziphus jujuba* Mill.) has been used to cure influenza for thousands of years in China. Puerariae decoction was found to maintain the body weight and relieve the fever of IV infected mice by way of inhibiting the production of IL-1α. Although this decoction did not depress viral multiplication directly in lung cells, it reinforced the activity of interferon in order to inhibit IL-1α. Because IL-1α is only produced in a great quantity at the primary period of viral infection, Puerariae decoction should be taken earlier than other drugs (Wang, 2000; Wang, 1998).

Heat-clearing drugs

In TCM, although few herbal drugs were found to have a stronger antibiotic capacity than conventional drugs, many herbs were shown to have notable antiviral activity *in vitro* and *in vivo*. Many of them belong to the classification 'heat-clearing drugs'. These herbs are represented by *Forsythia suspensa* (*Forsythia suspensa* (Thunb.) Vahl), isatis root and *Folium Isatidis* (*Isatis indigotica* Fort), *Flos Lonicerae* (*Lonicera japonica* Thunb.) and heartleaf houttuynia herb (*Houttuynia cordata* Thunb.).

The antiviral action of isatis root and *Folium Isatidis* (*Isatis indigotica* Fort) from different idioplasms was studied by Liu Sheng *et al*. (2000). According to hemagglutination titer, it was shown that isatis root and *Folium Isatidis* were effective against influenza A virus in three aspects: direct, therapeutic and preventive action (Hu and Shen, 2001). Moreover, the antiviral activity of crude drugs from different idioplasms varies obviously. The active proteins in isatis root also exhibited antiviral activity of IVA *in vitro*.

Like isatis root and *Folium Isatidis*, heartleaf houttuynia herb (*Houttuynia cordata* Thunb.) was also considered an effective antiviral herb due to its broad-spectrum antiviral activity. Heartleaf houttuynia herb injection was found to inhibit MDCK cell apoptosis induced by IVA (H3N2) (Guo *et al*., 2003). A study of synergistic antiviral activities was carried out using amantadine, ribavirin and heartleaf houttuynia herb. The inhibitory effect of cytopathic effect caused by influenza virus A3 in MDCK cells and the therapeutic effect on BALB/c mice pneumonia caused by influenza virus FM1 were examined and analysed when the three drugs were used alone or associatively. The results showed that the *in vitro* in-jections of amantadine, ribavirin and heartleaf houttuynia herb were 256, 512, 1024 times, respectively, more effective cooperatively than alone (Yan *et al*., 2002).

There are also some traditional Chinese medicinal formulae for 'heat clearing'. Lonicerae and Forsythiae powder is an old TCM anti-flu formulae. Among the nine ingredients, forsythia (*Forsythia suspensa* (Thunb.)

Vahl), *Herba Menthae* (*Mentha haplocalyx* Briq.) fineleaf schizonepeta herb (*Schizonepeta tenuifolia* Briq.) and *Flos Lonicerae* (*Lonicera japonica* Thunb.) contain the most antiviral chemical constituents of total flavonoids at a percentage of 8.23%, 7.39%, 6.82% and 4.48%, respectively (Shi *et al.*, 2001). Besides flavonoids, lignanoid compounds from *Fructus Arctii* (*Arctium lappa* L.) and licorice root (*Glycyrrhiza uralensis* Fisch.) were also contributors to antiviral effects (Shi *et al.*, 2003). Other components such as forsythosides were found to be the main active constituents in *Forsythia suspensa* (Zhao *et al.*, 2005a).

An aerosol containing *Flos Chrysanthemi indici* (*Chrysanthemum indicum* L.), *Flos Lonicerae* (*Lonicera japonica* Thunb.), heartleaf houttuynia herb (*Houttuynia cordata* Thunb.) and others was found to suppress the proliferation of influenza virus in rat lung and have good curative effect in treating upper respiratory tract infection (Ma *et al.*, 2000). A synergistic effect of *Flos Lonicerae* with different herbs on antivirus has been found recently (Wang *et al.*, 2005b). In a test for antiviral effects (H9N2) on chick embryo fibroblasts, extracts of *Flos Lonicerae* could kill virus at 7.81 mg/mL directly. When combined with *Astragalus mongholicus*, the inhibitory action was enhanced significantly.

Other herbal drugs of TCM

Asiatic toddalia root (*Toddalia asiatica* Lam.) showed potent antiviral activities against H1N1 virus, with a 50% effective concentration value of 4.7 mg/L in the MTS assay and 0.9 mg/L in the quantitative PCR assay, respectively (Li *et al.*, 2005). Although the optimal antiviral activity of *Toddalia asiatica* was observed with co-treatment of influenza virus infection, it remained effective even when administered 24 h before and after the initiation of infection.

Catechu (*Acacia catechu* (L. f.) Willd.) extract showed significant inhibition on the influenza A virus on chick embryo culture and the hemagglutination inhibition titer was more than eight times higher than the control group (Zhao *et al.*, 2005b). Cytotoxicity of catechu extract was low and the catechu extract significantly inhibited influenza A virus from infecting MDCK cells. The catechu extract had distinct inhibitory functions on the influenza A virus directly, and the hemagglutination inhibition titer was over 16 times higher than the control group.

Mice infected with influenza virus FM1 strain were used to test the antiviral effect of the extract of *Ganoderma applanatum* (Pers) Pat.collected in Yunnan province. The results showed that the extract of *Ganoderma applanatum* collected from the bark of the *Quercus variabilis* tree exerted a significant protective effect on the infected mice (Zhu *et al.*, 1998).

Notopterygium incisum (*Notopterygium incisum* Ting ex H. T. Chang) extended the lifetime of the mice infected with A/FM/1/47 and provided a satisfying protective effect against death ($p < 0.05$). The influenza virus was inactivated directly and the titer was also reduced (Guo *et al.*, 2005).

The mechanism of Yiqi Qingwen Jiedu Heji (composed of *Radix Astragali* (*Astragalus membranaceus* (Fisch.) Bge. var. *mongholicus* (Bge.) Hsiao), *Ephedra herb*, *folium perillae* (*Perilla frutescens* (L.) Britt.), *Radix Scutellariae* (*Scullellaria baicalensis Georgi*), etc.) in resisting influenza immune damage was elucidated by studying its effect on cytokine expression. After the mice were infected with IV FM1, their level of protein expression was higher than that of the control group. Expression of TNF-α, IL-6, IFN-γ reached its peak in 3 days. However, protein expression in the Yiqi Qingwen Jiedu Heji treated group was decreased and the decrease became marked on the third day, compared with the control group. In contrast, the expression of IL-10 in the treated group was increased markedly. These results indicated that Yiqi Qingwen Jiedu Heji can dampen the expression of pro-inflammatory cytokines TNF-α, IL-6 and IFN-γ while increasing the expression of IL-10, thus alleviating inflammatory injury. So the clinical application of such medicine can shorten the course of disease (Wang *et al.*, 2005a).

CONCLUSION

Research in anti-influenza virus natural products has increased dramatically in the past several years as seen by the numerous publications. Many constituents and extracts from plants show activity either directly by antiviral or by affecting the immune system, or on both parameters. However, many questions remain. One problem with current antiviral drug research is that only certain fractions or isolated phytochemicals of the plants are tested, rather than whole plants or plant parts. Refined fractions or isolates of medicinal plants may exhibit certain antiviral activity *in vitro*, but are often more toxic and less clinically effective than combinations of herbal medicines (Gao *et al.*, 2002). In fact, whole plants work very differently in the human body than do the isolates, and traditional Chinese medicine preferably employs whole herbs, because they are thought to contain synergistic elements that interact with the virus in different ways and neutralize the negative effects of any toxic constituents the plant might contain.

It is noted that in the practice of traditional Chinese medicine, herbal medicines were prescribed based on the diagnosed symptoms of individual patients without a clear identification of causative agent, and are therefore not known to be specific to a particular virus or disease. The compositions of herbal medicines also vary case by case and may even change for each individual patient during the course of the treatment according to each treatment result. It is therefore very difficult to describe a particular herbal composition from previous documents suitable for treating a specific virus. We believe that the interaction between the compounds from medicinal plants and the diseased state of the host might be far more complex than merely the result of a direct antiviral activity exerted by a single chemical entity. Therefore, the pharmacological study of combinations of herbal medicines should be encouraged. Traditional formulae containing a combination of herbal medicines hold the potential to become the therapeutics of choice in the future due to the synergistic effect achieved by the multiple ingredients that inhibit the virus at different stages, strengthen the impaired immune system and improve the overall symptoms.

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