Asia is the Mine of Natural Antiviral Products for Public Health

R.R. Hafidh[1], A.S. Abdulamir[1], F. Jahanshiri[2], F. Abas[1,3], F. Abu Bakar[1,3,*] and Z. Sekawi[4]

[1]*Institue of Bioscience;* [2]*Department of Microbiology, Faculty of Biotechnology and Biomolecular Sciences;* [3]*Department of Food Science, Faculty of Food Science and Technology and* [4]*Department of Medical Microbiology and Parasitology, Faculty of Medicine and Health Sciences, Universiti Putra Malaysia, 43400 UPM Serdang, Selangor Darul Ehsan, Malaysia*

Abstract: This review highlights a wide range of research on antiviral natural products in Asia in the period from 2000 to 2008. It focuses on the most important findings in this field specifically on the plants' components with potential antiviral activity against wide range of pathogenic DNA and RNA viruses. In addition, the difference between Asians and other populations regarding their habits of using natural products containing antiviral activity instead of allopathic medicine has been reviewed briefly. In conclusion, Asian continent serves as the most important source for providing the candidate natural products for the future antiviral drugs which could save the lives of millions in the world.

Key Words: Antiviral, alternative medicine, polyphenols, flavonoids, tannin, anti-RNA virus, anti-DNA virus and Asia.

INTRODUCTION

There is a long history of medicinal use of plants in Asian countries, some of which have proved useful to humans as pharmaceuticals. Asia seems to be the most promising region for discovering novel biologically-active substances from its flora [1-3]. Long before, mankind discovered the existence of microbes and the idea that certain plants had healing potential. Indeed, these plants contained what we would currently characterize as antimicrobial principles [4]. There is currently a large and ever-expanding global population base that prefers the use of natural products in treating and preventing medical problems [5, 6]. There is currently a worldwide upsurge in the use of herbal preparations and the active ingredients isolated from medicinal plants in health care. The World Health Organization has been promoting traditional medicine as a source of less expensive and comprehensive medical care, especially in developing countries [7]. Natural products have also been successful in drug development. Over 50% of the best-selling pharmaceuticals in use today are derived from natural products [8]. Natural products from plants traditionally have provided the pharmaceutical industry with one of its most important sources of lead compounds and up to 40% of modern drugs are derived from natural sources, using either the natural substance or a synthesized version. Currently, over a 100 new products are in clinical development, particularly as anti-cancer agents and anti-infectives [5, 6, 9]. This has influenced many of pharmaceutical companies to produce new antimicrobial formulations extracted from plants or herbs. The bioactive molecules occur in plants as secondary metabolites and as defense mechanisms against predation, herbivores, fungal attack, microbial invasion and viral infection [10]. Interestingly, most of the plant species have shown strong positive ethnopharmacological correlation with the traditional knowledge [6]. The ethnopharmacology knowledge serve as an innovative and powerful discovery engine for newer, safer and affordable medicines [11].

The aim of this review is to give a comprehensive outlook on the increasing role of medicinal plants in the public health of Asia and an overview of the research done in the continental Asia on the antiviral natural products. This review encompassed introduction, methodology, regional comparison of the medicinal plants uses, Asian traditional diet with antiviral activity, plants components with antiviral activity, the discovered natural antiviral products against DNA and RNA viruses in Asia. Many examples are mentioned discussing the biological potential and antiviral activities of some of the most prominent plants and herbs that were investigated in elegant Asian research. Furthermore, the complete list of research conducted in Asia on antiviral plants, herbs, and natural products' components has been listed in Table **1** which summarizes those studies based on the plant's scientific and the family name as well as the tested virus.

METHODOLOGY

Data were collected using Pubmed, Scopus, and Google Scholar. Endnote program was used to edit reference formatting. Moreover, the central library of University Putra Malaysia was used as an extra source for retrieving books and needed resources.

Regional Comparison of the Uses of Medicinal Plants

India is one of the twelve mega diversity countries in the world. Therefore it has a vital stake in the conservation and the sustainable utilization of its biodiversity resources [12]. Malaysia is also considered as one of the twelve mega diversity countries of the world which together contain at least 60

*Address correspondence to this author at the Department of Food Science, Faculty of Food Science and Technology, Universiti Putra Malaysia, 43400 UPM Serdang, Selangor Darul Ehsan, Malaysia; Tel: 00-60-3 8946 8375; Fax: 00-60-3 8942 3552; E-mail: fatimah_upm_fst@yahoo.com

1876-391X/09

Table 1. Summarize the Efforts Done by Asian Scientists in The Period 2000 to 2008 to Isolate Natural Antiviral Agents From Asian Plants

Scientific Name of the Plant with Antiviral Activity	Family Name	Name of the Virus	References
Aglaia odorata	Meliaceae	Herpes simplex virus type 1	[67]
Agrimonia pilosa	Rosaceae	Herpes simplex virus type 1	[70]
Allium oreoprasum	Alliaceae	Influenza virusi	[65, 66]
Androsace strigilosa	Primulaceae	Influenza viruses	[65, 66]
Ardisia chinensis	Myrsinaceae	Coxsackie B3	[60]
Ardisia squamulosa	Myrsinaceae	Herpes simplex virus type 1 & type 2 and Adenoviruses	[68]
Artemisai princeps var. *orientalis*	Asteraceae	Herpes simplex virus type 1 & type 2 and Adenoviruses	[68]
Asparagus filicinus	Asparagaceae	Influenza viruses	[65, 66]
Astilbe rivularis	Saxifragaceae	Herpes simplex virus	[65, 66]
Astragalus membranaceus or *Radix Astragali*	Fabaceae	Rotavirus, Herpes simplex virus type1 and Cytomegalovirus	[72, 73, 90]
Bergenia ciliate	Saxifragaceae	Herpes simplex virus	[65, 66]
Bergenia ligulata	Saxifragaceae	Influenza virus	[55, 65, 66]
Blumea laciniata	Asteraceae	Respiratory syncytial virus	[70]
Boesenbergia pandurata	Zingiberaceae	Epstein-Barr virus	[33]
Boussingaultia gracilis var. *pseudobaselloides*	Basellaceae	Herpes simplex virus type 1 & type 2 and Adenoviruses	[68]
Bupleurum kaoi	Apiaceae	Cytomegalovirus B1	[109]
Cassiope fastigiata	Ericaceae	Herpes simplex virus	[65, 66]
Centella asiatica	Hydrocotylaceae	Herpes simplex virus type 1 & type 2	[64]
Chaenomeles sinensis	Rosaceae	Influenza viruses	[42]
Citrus hystrix	Rutaceae	Epstein-Barr virus	[33]
Ecklonia cava	Alariaceae	Human immunodeficiency virus type 1	[53]
Elephantopus scaber	Asteraceae	Respiratory syncytial virus	[70]
Elsholtzia rugulosa	Lamiaceae	Influenza A (H3N2) virus	[59]
Elytranthe globosa	Loranthaceae	Herpes simplex virus type 1 and Polioviurs	[69]
Elytranthe maingayi	Loranthaceae	Herpes simplex virus type 1 and Polioviurs	[69]
Elytranthe tubaeflora	Loranthaceae	Herpes simplex virus type 1 and Polioviurs	[69]
Eucommia ulmoides	Eucommiaceae	Human immunodeficiency virus type 1	[54]
Holoptelia integrifolia	Ulmaceae	Herpes simplex virus	[65, 66]
Hypericum japonicum	Hypericaceae	Influenza A (H3N2) virus	[79]
Kaempferia parviflora	Zingiberaceae	Degue virus	[120]
Laggera pterodonta	Asteraceae	Respiratory syncytial virus	[70]
Languas galanga or Alpinia galanga	Zingiberaceae	Epstein-Barr virus	[33]
Maclura cochinchinensis	Moraceae	Herpes simplex virus type 1 & type 2	[64]
Mangifera indica	Anacardiaceae	Herpes simplex virus type 1 & type 2	[64]
Melastoma malabathricum	Melastomataceae	Herpes simplex virus type 1 and Polioviurs	[69]
Moringa oleifera	Moringaceae	Herpes simplex virus type 1	[43, 44, 67]

(Table 1) contd….

Scientific Name of the Plant with Antiviral Activity	Family Name	Name of the Virus	References
Mussaenda pubescens	Rubiaceae	Respiratory syncytial virus	[70]
Myrica rubra	Myricaceae	Influenza virus	[78]
Nerium indicum	Apocynaceae	Influenza virus and Herpes simplex virus	[65, 66]
Ocimum basilicum	Lamiaceae	Adenoviruses and Enteroviruses	[56]
Piper aduncum	Piperaceae	Herpes simplex virus type 1 and Polioviurs	[69]
Pithecellobium clypearia	Leguminosae	Herpes simplex virus type 1	[70]
Prunella vulgaris	Lamiaceae	Human immunodeficiency virus type 1	[51, 52]
Prunus mume	Rosaceae	Influenza A (H3N2) and (H1N1) viruses	[61]
Punica granatum	Lythraceae	Herpes simplex virus type 1	[70]
Salvia miltiorrhiza	Lamiaceae	Enteroviruses 71	[111]
Schefflera octophylla	Araliaceae	Respiratory syncytial virus	[70]
Scurrula ferruginea	Loranthaceae	Herpes simplex virus type 1 and Polioviurs	[69]
Scutellaria baicalensis	Lamiaceae	Influenza A (H3N2) and B viruses	[40, 41]
Scutellaria indica	Lamiaceae	Respiratory syncytial virus	[70]
Selaginella sinensis	Selaginellaceae	Respiratory syncytial virus	[39]
Serissa japonica	Rubiaceae	Herpes simplex virus type 1 & type 2 and Adenovirus	[68]
Stemona tuberose	Stemonaceae	Degue virus	[120]
Thymus linearis	Lamiaceae	Herpes simplex virus	[65, 66]
Ventilago denticulata	Rhamnaceae	Herpes simplex virus type 1	[67]
Verbascum thapsus	Scrophulariaceae	Influenza virus	[65, 66]

percent of the world's known species [13, 14]. The flora of Malaysia is exceedingly rich and is conservatively estimated to contain about 12,500 species of flowering plants and more than 1,100 species of ferns and fern allies. Many of these species are unique and are found nowhere else in the world [15]. Malaysia has about 1300 medicinal plant products registered by the Ministry of Health and are available in market [16]. Although these medicinal plants or herbs have been used widely as a cure or for health since the olden days, they are now widely used in the pharmaceutical, health and food industry. These herbs are also used in cooking, cosmetics, perfumes and as flavors [17] . On the other hand in Europe, about 1500 species of medicinal and aromatic plants have widely been used in Albania, Bulgaria, Croatia, France, Germany, Hungary, Poland, Spain, Turkey, and United Kingdom. The Maltese islands constitute an apt example where medicinal plants are widely used in every day life as part of folk medicinal remedies [18]. Another study found that a total of only 985 species have been catalogued in several Mediterranean countries, Albania, Algeria, Cyprus, Egypt, Italy, Morocco, and Spain, of which only 406 have medicinal use [19]. By this simple comparison, we can estimate the much larger number of plant species found in Asian countries than in other world countries.

Countries in Africa, Asia and Latin America use traditional medicine (TM) to help meet some of their primary health care needs. In industrialized countries, adaptations of traditional medicine are termed “Complementary“ or “Alternative” medicine (CAM) [20] . The WHO fact sheet 2003 gave a short description on different countries around the world and the practicing of the alternative medicine by their people:

- In China, traditional herbal preparations account for 30%-50% of the total medicinal consumption.
- In Ghana, Mali, Nigeria and Zambia, the first line of treatment for 60% of children with high fever resulting from malaria is the use of herbal medicines at home.
- In Europe, North America and other industrialized regions, over 50% of the population have used complementary or alternative medicine at least once.
- In San Francisco, London and South Africa, 75% of people living with HIV/AIDS use TM/CAM.
- 70% of the population in Canada have used complementary medicine at least once.

- In Germany, 90% of the population has used a natural remedy at some point in their life. Between 1995 and 2000, the number of doctors who had undergone special training in natural remedy medicine had almost doubled to 10,800.
- In the United States, 158 million of the adult population use complementary medicines and according to the USA Commission for Alternative and Complementary medicines, US $17 billion was spent on traditional remedies in 2000.
- In the United Kingdom, annual expenditure on alternative medicine is US$ 230 million.
- The global market for herbal medicines currently stands at over US $ 60 billion annually and is growing steadily [21].

Traditional Asian Diet with Antiviral Activity

Recent physiological, pharmacological and biochemical studies support the wisdom of the traditional dietary practices. Phytochemicals from fruits and vegetables have shown to exert varied beneficial biological actions [22]. For instance, flavonoids, the most common polyphenols found in plants, have been associated with biological effects such as antibacterial, antiviral, anti-inflammatory, antiplatelet, antioxidant, free radical scavenging, and vasodilatory effects [23, 24].

There are a lot of commonly used plants in Asia with proved antiviral activity. Most of them are traditionally used by Asians to treat viral infections and proved by Asian scientists as effective antiviral agents with various modes of action. One of the widely known antivirals is turmeric which is derived from the plant *Curcuma longa* L. (Zingiberaceae). Turmeric is a gold-colored spice commonly used in Indian subcontinent, not only for health care but also for the preservation of food and as a yellow dye for textiles [25]. Since the time of Ayurveda (1900 Bc) numerous therapeutic activities have been assigned to turmeric for a wide variety of diseases and conditions, including those of the skin, pulmonary, and gastrointestinal systems, aches, pains, wounds, sprains, and liver disorders [26]. Extensive research within the last half century has proven that most of these activities, once associated with turmeric, are due to curcumin [27]. Curcumin has been shown to exhibit antioxidant [28], anti-inflammatory [29], antiviral [30], antibacterial [31], and antifungal [32] activities. Considering the recent scientific bandwagon, multitargeted therapy is better than monotargeted therapy for most diseases. Therefore, curcumin can be considered an ideal "Spice for Life" [26]. Other commonly used plants by Asians are *Boesenbergia pandurata* (Roxb.) Schltr. (Zingiberaceae), *Languas galanga or Alpinia galanga* (L.) Willd. (Zingiberaceae) and *Citrus hystrix* DC. (Rutaceae) which are edible plants that are commonly used as flavors or condiments in various Thai food dishes. They are known to exert strong anti-promoting activity in a test of tumor promoter-induced Epstein-Barr virus (EBV) activation [33].

Plants Components with Antiviral Activity

During the past decade, potent agents have become available against viral infections. But the increasing clinical use has been associated with the emergence of drug-resistant strains. Additionally dose-limiting toxic effects have been observed [34, 35]. Plants have had a long evolution of resistance against viral agents which has led to alternative directions in drug development. Therefore, extracts of plants and phytochemicals are getting more important as potential sources for viral inhibitors during the recent decade. Extensive studies have shown that medicinal plants of several parts of the world contain compounds active against viruses that cause human diseases [36-38].

Flavonoids

Amentoflavone and three other flavonoids are active natural components which were isolated from the ethanol extract of *Selaginella sinensis* (Desv.) Spring (Selaginellaceae). Amentoflavone showed potent antiviral activity against respiratory syncytial virus (RSV), with an IC50 of 5.5 μg/ml. The contents of amentoflavone in nine species of *Selaginella* were determined by reversed-phase HPLC in which *S. sinensis* showed a higher content, up to 1.13% [39]. These highly promising results may give a hope to produce natural drug to treat patients, i.e. infants, immune suppressed, etc., who are unable to use ordinary available anti-RSV agents in order to avoid the side effect may develop from such treatment. The major constituents of *Scutellaria baicalensis* Georgi (Lamiaceae), a widely used herb in traditional medical systems of China and Japan, are found to be flavonoids: baicalein, baicalin, and wogonin. These components have been associated with various properties, for example: antioxidant, anti-inflammatory, antithrombotic, antibacterial and antiviral, particularly against influenza A (H3N2) and B viruses [40, 41]. Other active polyphenols with anti-influneza virus activity was isolated by Sawai *et al*. 2008 from 50% ethanol extract of the fruit of *Chaenomeles sinensis* (Thouin) Koehne (Chinese Quince, Rosaceae), which is widely used as a traditional Chinese medicine to treat throat diseases. Partial purification showed that the active components are high molecular weight polyphenols which neutralizes influenza virus by inhibiting hemagglutination activity and by suppressing NS2 protein synthesis [42]. Other potent antiviral flavonoids isolated from *Moringa oleifera* Lam. (Drumstick or moringa leaves, Moringaceae) which is a highly valued plant, distributed in many countries of the tropics and subtropics. It has an impressive range of medicinal uses with high nutritional value. *M. oleifera* is being employed in the antiviral treatment particularly in South Asia. The *Moringa* plant provides a rich and rare combination of zeatin, quercetin, beta-sitosterol, caffeoylquinic acid and kaempferol which may attribute to its antiviral activity particularly on Herpes simplex virus type 1[43, 44].

Tannins

An increasing portion of patients with human immunodeficiency virus (HIV) infection and/or AIDS cannot use currently FDA-approved anti-HIV drugs, including the reverse transcriptase and protease inhibitors, due to the several metabolic side effects and the rapid rate of virus mutation and subsequent emergence of drug-resistant HIV variants which threaten the longer-term efficacy of HIV treatment [45, 46]. Thus, it is essential to develop new anti-HIV agents with a target different from the HIV reverse transcriptase and protease [47]. Type 1 HIV (HIV-1) envelope fusion-active glycoprotein gp41 participates in the fusion of the virus with the host cell [48-50]. In 2002 researchers from China used conformation-specific monoclonal antibody to screen for inhibitors of the gp41 six-helix bundle formation from aqueous extracts of nine Chinese medicinal herbs with antiviral activity. They found that the extracts of two herbs, *Prunella vulgaris* L. (Lamiaceae) and *Rhizoma cibotte*, have potent inhibitory activity. The important finding was that the viral inhibitory activity of these two herb extracts was significantly caused by Tannins. These results suggested that tannin may be one of the major inhibitors of the HIV-1 gp41 six-helix bundle formation in the herb extracts and that tannin may inhibit HIV-1 entry by disrupting the gp41 six-helix bundle formation [51, 52]. Recently, Artan *et al*. (2008) used tannin from *Ecklonia cava* Kjellman (Paddle Weed) which is an edible marine brown alga with a broad range of bioactivities that belong to the family Alariaceae. Unlike most of other tannins, the bioactive 6,6'-bieckol, the main phloroglucinol derivative of this genus, exhibited no cytotoxicity at concentrations that inhibited HIV-1 replication almost completely. Accordingly, the potentially effective 6,6'-bieckol might be employed as a drug candidate for developing new generation therapeutic agents against HIV [53]. In a similar study, samples from *Eucommia ulmoides* Oliver (Eucommia, Eucommiaceae) have potent inhibitory activity [54]. All these studies proved the fact that using tannin could be a promising new anti-HIV natural drug.

Asian scientists investigated the effect of tannin on influenza virus. Rajbhandari *et al*. (2003) demonstrated that the methanol water extract from rhizomes of *Bergenia ligulata* (Wall) Engl. (Saxifragaceae), a plant used in Nepalese ethnomedicine, inhibited *in vitro* replication of influenza virus in a dose dependent manner. The pretreatment of cells with *B. ligulata* extract was shown to be most effective to prevent cell destruction. The extract inhibited viral RNA synthesis and reduced viral peptide synthesis at 10 μg/ml. They found that virus inhibitory effect is related to the presence of condensed tannins in the extract [55]. Moreover, further studies on the effect of tannin on different virulent viruses are still in need to develop natural and potent antiviral drug from cheap resources.

Apigenin, Linalool, and Urolic Acid

As scientific evidence for the use of the Chinese medicinal herb as folk remedy in treating viral disorders, including ocular, respiratory and hepatic infections, extracts and selected purified components of *Ocimum basilicum* L. (Sweet Basil) belonging to family Lamiaceae were examined. The result showed that they have a broad spectrum of anti-DNA and anti-RNA virus activities. The aqueous extract of *O. basilicum* and its selected purified components, such as apigenin, linalool and ursolic acid, were found to have different degrees of potency against adenoviruses and enteroviruses [56]. The lack of effective drugs for the treatment of adenoviral and enterovirus 71 (EV71) infections [57, 58] motivates exploiting the extracts and the purified components of *O. basilicum* as potential therapeutic agents for the treatment of these diseases [56]. In 2008 Liu *et al*. isolated five active constituents belong to flavonoids family from *Elsholtzia rugulosa* Hemsl. (Lamiaceae), a common Chinese herb, which is widely used in the treatment of cold and fever, namely apigenin, luteolin, apiin, galuteolin and luteolin 3'-glucuronyl acid methyl ester. The influenza virus neuraminidase (NA) activity and *in vitro* antiviral activity assays were established using a cytopathic effect (CPE) reduction method. The findings showed that they all possessed anti-influenza virus properties. Among them, apigenin and luteolin exhibited the highest activities against influenza virus (H3N2) with IC(50) values of 1.43 μg/mL and 2.06 μg/mL, respectively [59].

Polysaccharides

Ardisia chinensis Benth (Myrsinaceae) is a medicinal plant traditionally used in the area of Yao minority in Southern China. The *in vitro* antiviral activities of extracts and fractions from *Ardisia chinensis* were tested by the cytopathic effect (CPE) reduction assay. As a result, both the aqueous extract and the 95% ethanol extract of *Ardisia chinensis* showed *in vitro* antiviral activity against Coxsackie B3 (Cox B3) virus to different extents, and the aqueous extract possessed more potent activity than the ethanol extract. Bioassay-guided fractionation revealed that the antiviral activity of *Ardisia chinensis* was attributed mainly to its high polar fractions, and finally identified to be a polysaccharide. The *Ardisia chinensis* polysaccharide (ACP) fractionated from the aqueous extract exhibited a significant antiviral effect against Cox B3 with a 50% inhibitory concentration IC50 of 3.9 μg/mL and a selective index (SI) over 256. Preliminary characterization indicated that ACP is a neutral polysaccharide in which d-glucose is the major component. The average molecular weights of ACP were determined to be 40037 Da (Mw), 28297 Da (Mn) and 33758 Da (Mp) by gel permeation chromatography [60]. Other new anti-influenza virus polysaccharide component is lectin-like molecule(s) isolated from the fruit-juice concentrate of *Prunus mume* Siebold & Zucc. (Japanese Plum, Rosaceae) which showed strong *in vitro* anti-influenza activity against human influenza A viruses before viral adsorption, but not after viral adsorption, with 50% inhibitory concentration (IC50) values against A/PR/8/34 (H1N1), A/Aichi/2/68 (H3N2) and A/Memphis/1/71 (H3N2) viruses. These compounds may prevent and reduce infection with human influenza A virus, possibly *via* inhibition of viral hemagglutinin attachment to host cell surfaces by its lectin-like activity [61].

Anti-DNA Virus

Herpes Simplex Virus and Adenovirus

One of the potential DNA viruses is Herpes simplex virus type 1 (HSV-1) which causes labial herpes, keratitis, and encephalitis. The herpetic infection is common to humans and a major cause of morbidity especially in immunosuppressed patients [62, 63]. Thai medicinal plants have been investigated on the scientific basis and evaluated as the candidates for anti-HSV agents. A study in Thailand investigated a number of Thai medicinal plants including: *Centella asiatica* (L.) Urb. (Pegaga or gotu kola leaves, Hydrocotylaceae), *Maclura cochinchinensis* (Lour.) Cornor (Cockspur thorn stem, Moraceae), and *Mangifera indica* L. (Mango leaves, Anacardiaceae). These plants are recommended as remedies for herpesvirus infection and have been used in primary health care for centuries. The investigation was conducted to disclose their intracellular activities against herpes simplex viruses (HSV). *C. asiatica*, *M. cochinchinensis*, and *M. indica* contained both anti-HSV-1 and -2 activities, as determined by plaque inhibition assay. The data of this study supports the therapeutic potential of these plant extracts [64]. In another study on Nepalese traditional medicine carried out by Rajbhandari and his colleagues in 2001, the *in vitro* anti HSV activity of selected plants were screened and evaluated. They used the methanolic and methanolic-aqueous extracts derived from medicinal plants in their experiment. *Holoptelia integrifolia* (Roxb.) Planch. (Ulmaceae), *Nerium indicum* L. (Apocynaceae), *Astilbe rivularis* Buch.-Ham. ex D.Don (Saxifragaceae), *Bergenia ciliate* (Haw.) Sternb. (Saxifragaceae), *Cassiope fastigiata* (Wall.) D.Don (Ericaceae) and *Thymus linearis* Benth (Lamiaceae) exhibited considerable antiviral activity against herpes simplex virus. None of these extracts showed cytotoxic effects. *H. integrifolia* extract demonstrated partial protease inhibitory activity [65, 66]. Other plant extracts, which have been found effective against cutaneous HSV-1 infection in mice are extracts of *Aglaia odorata* Lour. (Chinese rice flower leaves, Meliaceae), *Moringa oleifera*, *Ventilago denticulata* Willd. (Leaves, Rhamnaceae). These three plant extracts contain the active compounds that would be effective in the treatment of cutaneous HSV-1 infection. Particularly, they were effective against thymidine kinase-deficient HSV-1 and phosphonoacetate-resistant HSV-1 strains. The treatment with higher doses of these plant extracts than 750 mg/kg per day showed more effective therapeutic anti-HSV-1 efficacy in mice. The antiviral components of many plant extracts are selectively absorbed from alimentary tracts and elicited an HSV-1 inhibitory effect which is not associated with toxicity. Toxicity of these plant extracts were not observed in treated mice. Therefore, these plant extracts may be evaluated to be possible candidates of new plant extracts for anti-herpes simplex virus infection [67]. In Taiwan, scientists tried to search for new antiviral agents from traditional medicine. The hot water (HW) extract was used for *Boussingaultia gracilis* var. *pseudobaselloides* (Hauman) L.H.Bailey in Lawr (Basellaceae), *Serissa japonica* (L. f.) Lam. (Rubiaceae), *Ardisia squamulosa* Presl. (Myrsinaceae) and *Artemisai princeps* var. *orientalis* (Pamp.) Hara (Asteraceae), traditionally used medicinal plants in Taiwan, were evaluated for their *in vitro* anti-herpes simplex viruses (HSV; including HSV-1 and HSV-2) and anti-adenoviruses (ADV; including ADV-3, ADV-8 and ADV-11) activities with a XTT-based colorimetric assay [68]. Results showed that the tested HW extracts exhibited anti-HSV and anti-ADV activities at different magnitudes of potency. *B. gracilis* and *S. japonica* had broad spectrum of antiviral activity while *A. squamulosa* and *A. princeps* were more effective in inhibiting ADV-8 replication than the other four viruses. Cell cytotoxic assay demonstrated that all tested HW extracts had CC50 values higher than their EC50 values. It was concluded that those traditionally used medicinal plants in Taiwan possessed antiviral activity [68]. Other scientists in Indonesia evaluated the anti-HSV-1 activity of the methanolic extracts from some Indonesian medicinal plants. *Melastoma malabathricum* L. (Melastomataceae), the Indonesian Loranthaceae species: *Elytranthe tubaeflora* Ridl., *E. maingayi* Tiegh., *E. globosa* Blume. and *Scurrula ferruginea* (Jack) Dans. The extract of these plants exhibited an attractive anti-DNA antiviral activity [69].

In Southern Mainland China, screening of aqueous extracts from 21 traditionally used medicinal herbs were done to evaluate their antiviral activities against human herpes simplex virus type 1 (HSV-1) using a cytopathic effect (CPE) reduction assay. Three extracts from *Agrimonia pilosa* Ledeb. (Rosaceae), *Pithecellobium clypearia* (Jack.) Benth. (Leguminosae) and *Punica granatum* L. (Lythraceae), respectively, showed anti-HSV-1 activity, which was possibly contributed by the polyphenolic compounds in the herbal extracts [70]. *Radix Astragali* (root of Astragalus; Huangqi) is a traditional Chinese medicine commonly used as an immunostimulant, hepatoprotective, diuretic, antidiabetic, analgesic, expectorant, and sedative drug. The species of *Radix Astragali* have been defined as *Astragalus membranaceus* (syn. *Astragalus propinquus* Schischkin) and *A. membranaceus* var. *mongholicus* (family: Fabaceae) in Pharmacopoeia of China [71]. The antiviral effect of A. *membranaceus* decoction was investigated by observing the inhibition of HSV-1-induced cytopathic effect in response to treatment with the decoction. The (IC50) was 0.98 g/ml, with the therapeutic index (TI) of 128. The decoction has obvious HSV-1-inhibiting efficacy and low cytotoxicity. This study proved the scientific evidence of the traditional uses of A. *membranaceus* decoction in the treatment of different viral infections in China [72]. Other study on A. *membranaceus* as a complementary antiviral agent to the allopathic treatment found that Cytomegalovirus (CMV-AD169 strain) may be inhibited or killed by ganciclovir and A. *membranaceus in vitro* [73]. Back to the fact that there is still great deficiency in the treatment drugs with specific effect for CMV. This result may enhance the level of treatment in patients undergoing bone marrow transplantation in which CMV remains the most common infectious causes of morbidity and mortality**.**

Anti-RNA Virus

Influenza Virus

Neuraminidase inhibitor resistant influenza virus has recently emerged and circulated in untreated individuals. Influenza virus evolution is causing antiviral susceptibility to change. Vigilance, policy review and development of new anti-influenza drugs are essential [74]. The drugs currently in use are effective against seasonal influenza virus infection, and some cases have been used in the treatment of patients infected with the avian H5N1 influenza virus. However, it is becoming clear that the emergence of drug-resistant viruses will potentially be a major problem in the future efforts to control influenza virus infection [75]. In an attempt to explore the anti-viral activity of some selected Nepalese traditional medicine plants, Rajbhandari *et al*. in 2001 and 2007 used the Methanolic and methanolic-aqueous extracts against influenza virus/MDCK cells. They tried to screen the ethnopharmacological activity of these plants and evaluated their anti-influenza activity in addition to the anti-HSV activity for some of them which they proved. Two species, *Bergenia ligulata* and *Nerium indicum* showed the highest anti-influenza viral activity with 50% inhibitory dose of 10 µg/ml while *Allium oreoprasum* Schrenk (Alliaceae), *Androsace* *strigilosa* Franch. (Primulaceae), *Asparagus filicinus* Buch.-Ham. ex D.Don. (Asparagaceae), *Astilbe rivularis, Bergenia ciliata* and *Verbascum thapsus* L. (Scrophulariaceae) exhibited strong anti-influenza viral activity. None of these extracts showed cytotoxic effects. Additionally for *B. ligulata* a partial protease inhibitory activity was estimated [65, 66]. In another study, the antiviral activity of 101 Korean medicinal plants against influenza virus type A was investigated. The inhibitory activity of the methanol extracts from these plants was tested by means of a modified haemagglutination inhibition (HI) test. Back to the fact that the influenza surface glycoprotein hemagglutinin (HA) is a potential target for antiviral drugs because of its key roles in the initial stages of infection: receptor binding and the fusion of virus and cell membranes [76]. Four of 101 tested extracts exhibited strong anti-influenza virus type A activity at concentration ranges of 0.78-6.25 mg/ml, 0.78-3.13 mg/ml, 0.78-1.56 mg/ml and 0.0975-0.39 mg/ml, respectively. The four methanol extracts were weakly cytotoxic to red blood cells. These extracts inhibited attachment of the virus to the cell and can therefore be used for prophylaxis [77]. In a recent study on influenza virus hemagglutinin antigen type A subtype (H1N1), and subtype (H3N2), and type B, the ethanol extract of Japanese bayberry tree leaves, *Myrica rubra* Siebold & Zucc. (Myricaceae) was added to culture medium of Madino-Darby canine kidney (MDCK) cells inoculated with influenza virus, and the inhibition of influenza virus replication was measured. Interstingly, *Myrica rubra* leaf ethanol extract showed anti-influenza virus activity irrespective of the influenza type and its subtype [78]. Another recent study on anti-influenza virus subtype (H3N2) was done by *in vivo* experiments using the extract of *Hypericum japonicum* Thunb. (Hypericaceae), which is commonly known as Matted St. John's Wort. The experiments revealed that the intranasal treatment of *H. japonicum* extract at a concentration of 10 g/kg x d markedly inhibited the lung consolidation of mice and prolonged the survival time [79].

Human Rotaviruses

Since 1973, human rotaviruses (HRV) have been recognized as the major etiologic agents of diarrhea in infants and young children under 2 years of age throughout the world [80-86]. In South East Asia, morbidity and mortality from rotavirus diarrhea remains very high, particularly among children under 5 years of age, with the peak in children between 6 and 24 months [80]. In Bangladesh it was estimated that one rotavirus death per 111 to 203 children occurs by the age of 5 years [83]. In China, the annual number of episodes of diarrhea per person is 1.90 and 2.44 in children less than 5 years and in the age group from 6 months to 2 years, respectively, with the major pathogen being rotavirus [84-87]. Recently, it was considered that the interaction of persistent diarrhea and malnutrition is a predominant cause of death in the world, thus, control of diarrhea should be taken beyond treatment based on oral rehydration therapy alone [80, 88]. In Asia, clinical studies proved that many antirotavirus agents have therapeutic effects against rotavirus diarrhea. Not only was infant and child rotavirus diarrhea successfully treated with these antiviral agents, but also adult rotavirus diarrhea, rotavirus encephalitis and damage of cardiac muscles [89-95]. The incidence of rotaviral disease is similar in developed and developing countries but the number of deaths is higher in developing countries. Therefore, cheap and effective drugs are necessary as additional approaches to control this dreadful disease. An effective antirotavirus drug may also be beneficial in immunosuppressed patients and malnourished children who are not recommended to receive rotavirus vaccine, or have persistent diarrhea caused by rotaviruses [96, 97]. Antirotavirus drugs might be particularly useful if they were broadly active against all rotaviruses, rather than just against the G1–G4 serotypes of group A rotaviruses targeted by rotavirus vaccines [98]. Compared with the antirotavirus agents used in the USA and Europe recently, the natural compounds used in Asia, such as cacao pigment (*Theobroma cacao* L., Sterculiaceae), extract of green tea (*Thea chinensis* Sims., Theaceae) or the Chinese herb (*Radix Astragali*, Fabaceae) have been identified as ideal candidates for antiviral medicines because of their lack of toxicity or side-effects and because of their effective and cheapness. Some Chinese herbs also have therapeutic effects on bacterial diarrhea *in vitro* and in clinical studies [99, 100]. The clinical studies, whether *in vitro* or *in vivo*, suggested that the natural compounds, as candidates of antirotavirus drugs, are superior to the other chemical synthetic compounds known till now. Therefore, many natural plants and compounds might be ideal candidates for the use as preventive and therapeutic drugs against rotavirus. The development of antirotavirus agents in Asia is improving each year [98].

Respiratory Syncytial Virus

Respiratory syncytial virus (RSV), a member of the *Pneumovirus* genus within the family *Paramyxoviridae*, is the single most important viral respiratory pathogen infecting infants and young children worldwide as well as an important cause of respiratory tract illness in the elderly, transplant patients, and immune suppressed [101]. In Southern Mainland China, Li Y. *et al*. 2004, worked on the aqueous extracts from 21 traditionally used medicinal herbs and they screened them for antiviral activities against human respiratory syncytial virus (RSV) using a cytopathic effect (CPE) reduction assay. Six of the extracts, from *Blumea laciniata* (Roxb.) DC. (Asteraceae), *Elephantopus scaber* L. [excluded] (Asteraceae), *Laggera pterodonta* (Asteraceae), *Mussaenda pubescens* Ait. f. (Rubiaceae), *Schefflera octophylla* (Lour.) Harms (Araliaceae), and *Scutellaria indica* L. (Lamiaceae) exhibited anti-RSV activity with 50% inhibition IC50 concentrations ranging from 12.5 to 32 μg/ml, and selective indices (SI) ranging from 11.2 to 40. In addition to polyphenolic compounds, other constituents present in these extracts may also contribute to their anti-RSV activity [70].

Enteroviruses

Coxsackieviruses (CVs) are important human pathogens, causing a remarkable variety of diseases, from minor common colds to fatal myocarditis, neurological disorders, possibly acute-onset diabetes, certain chronic diseases, including diabetes, dilated cardiomyopathy, inflammatory myopathy, and chronic fatigue syndrome [102-106]. CV group A (CVA) and CV group B (CVB), together with echoviruses and polioviruses, are enteroviruses of the family *Picornaviridae*. CVB1 has been implicated in human diseases of pleurodynia, aseptic meningitis, meningoencephalitis, and myocarditis [107, 108]. *Bupleurum kaoi* (Liu) Chao et

Chuang (Thorough wax root, Apiaceae), which is widely known in Chinese medicine for the treatment of viral and bacterial infection and locally found in Taiwan, was selected to explore its antiviral activity against CVB1 infection. The study concluded that: (i) *B. kaoi* reduces viral infectivity at non-cytotoxic concentrations; (ii) it may interfere with the early stage of the viral infection through viral absorption and penetration; (iii) the inhibition of CVB1 replication after virus infection was through the induction of IFN-α/β expression [109].

Other human enterovirus belonging to family *Picornaviridae* is enterovirus 71 which causes severe outbreaks in Asia with significant morbidity and mortality in children, yet there is no effective treatment [110]. In 2003, Shih *et al.* investigated the anti-enterovirus 71 activity of a protein-bound pigment, allophycocyanin, purified from blue-green algae. Allophycocyanin neutralized the enterovirus 71-induced cytopathic effect in both human rhabdomyosarcoma cells and African green monkey kidney cells. The 50% inhibitory concentration of allophycocyanin for neutralizing the enterovirus 71-induced cytopathic effect was approximately 0.045 +/- 0.012 μM in green monkey kidney cells. The cytotoxic concentrations of allophycocyanin for rhabdomyosarcoma cells and African green monkey kidney cells were 1.653 +/- 0.003 μM and 1.521 +/- 0.012 μM, respectively. A plaque reduction assay showed that the concentrations of allophycocyanin for reducing plaque formation by 50% were approximately 0.056 +/- 0.007 μM and 0.101 +/- 0.032 μM, when allophycocyanin were added at the state of viral adsorption and post-adsorption, respectively. Antiviral activity was more efficient in cultures treated with allophycocyanin before viral infection compared with cultures treated after infection. Allophycocyanin was also able to delay viral RNA synthesis in the infected cells and to abate the apoptotic process in enterovirus 71-infected rhabdomyosarcoma cells with evidence of characteristic DNA fragmentation, decreasing membrane damage and declining cell sub-G1 phase. It is concluded that allophycocyanin possesses antiviral activity and has a potential for development as an anti-enterovirus 71 agent [57].

Further studies on natural anti-enterovius 71 were done in 2007 by Wu *et al.* In this study, the antiviral activities of seven different extracts of *Salvia miltiorrhiza* Bunge (Danshen, Lamiaceae) were determined. The first two extracts, SA1 and SA2, were isolated at room temperature by ethyl acetate and water extraction, respectively, neutralized the enterovirus 71-induced cytopathic effect in Vero, rhabdomyosarcoma, and MRC-5 cells. The 50% inhibitory concentrations for neutralizing the enterovirus 71-induced cytopathic effect were 0.742 +/- 0.042 mg/ml for SA1 and 0.585 +/- 0.018 mg/ml for SA2 in Vero cells. Antiviral activity was more efficient in cultures treated with SA1 or SA2 during viral infection compared to the cultures treated before or after infection, suggesting that these Danshen extracts could interfere with viral entry. SA1 and SA2 were able to inhibit viral RNA synthesis in the infected cells and to reduce the apoptotic process in enterovirus 71-infected Vero cells [111].

In another study the methanolic extracts from some Indonesian medicinal plants were tested against poliovirus which also belongs to enterovirus family. The result showed that besides *Melastoma malabathricum* L. (Melastomataceae), the Indonesian Loranthaceae species: *Elytranthe tubaeflora* Ridl., *E. maingayi* Tiegh., *E. globosa* Blume., *Scurrula ferruginea* (Jack) Dans. and *Piper aduncum* L. (Piperaceae) showed attractive antiviral activity on poliovirus in addition to their anti-HSV-1 activity [69].

Dengue Virus

Dengue fever (DF) is a major infectious disease of tropical countries and the World Health Organization has estimated that approximately 50 million cases of dengue infection occur worldwide every year and about 2.5 billion people – two fifths of the world's population – are now at risk from dengue [112]. Most dengue virus-infected cases are asymptomatic or show minor constitutional symptoms, fever, headache, pain in various body parts, prostration, rash, lymphadenopathy, and leucopenia [113-115]. The severe forms of dengue infections are dengue hemorrhagic fever (DHF) and dengue shock syndrome (DSS) which manifest with bleeding and shock. Children younger than 15 years of age account for 90% of the severe cases in South East Asian countries where the DHF/DSS incidence has increased about 5 fold more rapidly than in the previous 30 years [116, 117]. These severe forms of DV infection have become the leading cause of death among children in Southern Vietnam [118, 119]. In the line of exploring natural products against this deadly disease some Thai herbs including: *Kaempferia parviflora* Wall. Ex. Baker (leaves and stem, Zingiberaceae), *Ellipeiopsis cherrevensis* Fries (Annonaceae) and *Stemona tuberose* Lour. (Stemona leaves and stem, Stemonaceae) were screened for their potential anti dengue virus type-2 (DEN2) properties. The results showed that *E. cherrevensis* did not have any inhibition effects on DEN2 activity in all modes of actions. Although *K. parviflora* and *S. tuberosa* lacked the protection and treatment effects, inhibitory effect on DEN2 activity was shown in virucidal activity. The virucidal value was dose-dependent. These results suggested that DEN2 particles may be inactivated directly by some bioactive compounds in *K. parviflora* and *S. tuberose* [120]. Therefore, more serious studies are crucially needed to find cheap and highly effective natural anti-dengue drug to overcome this critical problem in Asian countries.

CONCLUSIONS

Asia seems the arsenal for the future antiviral agents extracted from natural plants products. Nevertheless, with all the scientists' efforts to find effective and inexpensive antiviral agents from natural products which lack the common side effects of commercially available antiviral agents, this field still far away from its goal. The research on some life threatening viruses like Dengue virus didn't cover the increased need to find highly effective anti-dengue virus till now, in spite of the frequent outbreaks of such viruses in Asia. On the other hand, natural antiviral research on other viruses like HSV, influenza, RSV was more predominant in this area. Surprisingly, most of the scientifically-proved successful researches done on antiviral activities of natural products in Asia have not been traced actively by the international pharmacological companies nor these researches were optimized and converted to clinical trials. Taken together, this field, first, needs more attention from the international health organizations to convert the proven useful extracts into approved antiviral drugs. Second, there is a grave need to pursue long-term planning of research on a wide range of potential antiviral plants present in Asia or in other parts of the world that still need exploration and discovery.

REFERENCES

[1] Hoareau L, DaSilva EJ. Medicinal plants: a re-emerging health aid. Electron J Biotechnol 1999; 2: 56-70.

[2] Perry LM, Metzger J. Medicinal Plants of East and Southeast Asia. Massachusetts, Cambridge, USA: MIT Press 1980.

[3] Murakami A, Ohigashi H, Koshimizu K. Chemoprevention, insights into biological mechanisms and promising food factors. Food Rev Int 1999; 15: 335-95.

[4] Rios JL, Recio MC. Medicinal plants and antimicrobial activity. J Ethnopharmacol 2005; 100: 80-4.

[5] Jassim SAA, Naji MA. Novel antiviral agents: a medicinal plant perspective. J Appl Microbiol 2003; 95: 412-27.

[6] Gautam R, Saklani A, Jachak SM. Indian medicinal plants as a source of antimycobacterial agents. J Ethnopharmacol 2007; 110: 200-34.

[7] Use of Traditional Medicine in the Region: Regional Office for the Western Pacific. [homepage on the internet]. WHO [Accessed Dec 2008]. Available from: http://www.wpro.who.int/sites/trm/data/sample.htm

[8] Schuster BG. A new integrated program for natural product development and the value of an ethnomedical approach. J Altern Complement Med 2001; 7 (Suppl 1): S61-72.

[9] Harvey AL. Natural products in drug discovery. Drug Discov Today 2008; 13: 894-901.

[10] Cox PA. Ethnopharmacology and the search for new drugs. Ciba Found Symp 1990; 154: 40-7, discussion 7-55.

[11] Patwardhan B. Ethnopharmacology and drug discovery. J Ethnopharmacol 2005; 100: 50-2.

[12] Arora S, Kaur K, Kaur S. Indian medicinal plants as a reservoir of protective phytochemicals. Teratog Carcinog Mutagen 2003; (Suppl 1): 295-300.

[13] Wiart C, Akaho E, Hannah M, *et al*. The rainforest of Southeast Asia, particularly Malaysia, is endowed with several medicinal plants species. Am J Chin Med 2005; 33: 683-5.

[14] Noweg T, Abdullah A. Forest plants as vegetables for communities bordering the Crocker Range National Park. ASEAN Review of Biodiversity and Environmental Conservation (ARBEC) (January-March), 2003; 1-18.

[15] Chang YS, Rasadah MA. Inventory, Documentation and Status of Medicinal Plants Research in Malaysia. In: Batugal PA, Kanniah J, Lee SY, Oliver JT, Eds. Medicinal Plants Research in Asia the Framework and Project Workplans. Malaysia: International Plant Genetic Resources Institute 2004; pp. 120-6.

[16] Kulip J. An ethnobotanical survey of medicinal and other useful plants of Murats in Sabah, Malaysia. Telopea 2003; 10: 81-98.

[17] Malaysia's National Policy on Biological Diversity [homepage on the internet]. ARBEC [Accessed Dec 2008]. Available from: http://www.arbec.com.my

[18] Lanfranco G. Popular use of medicinal plants in the Maltese islands. Insula 1992; 1: 34-5.

[19] Gonzalez-Tejero MR, Casares-Porcel M, Sanchez-Rojas CP, *et al*. Medicinal plants in the Mediterranean area: synthesis of the results of the project Rubia. J Ethnopharmacol 2008; 116: 341-57.

[20] Weeks LC, Strudsholm T. A scoping review of research on complementary and alternative medicine (CAM) and the mass media: looking back, moving forward. BMC Complement Altern Med 2008; 8: 43.

[21] Traditional Medicine: Fact Sheet. [homepage on the internet]. WHO. [Accessed Dec 2008]. Available from: http://www.who.int/mediacentre/factsheets/fs134/en/

[22] Krishnaswamy K, Raghuramulu N. Bioactive phytochemicals with emphasis on dietary practices. Indian J Med Res 1998; 108: 167-81.

[23] Harborne JB. Advances in flavonoid research since 1992. Phytochemistry 2000; 55: 481-504.

[24] Simonetti P, Gardana C, Pietta P. Plasma levels of caffeic acid and antioxidant status after red wine intake. J Agric Food Chem 2001; 49: 5964-8.

[25] Krishnaswamy K. Traditional Indian spices and their health significance. Asia Pac J Clin Nutr 2008; 17 (Suppl 1): 265-8.

[26] Aggarwal BB, Sundaram C, Malani N, Ichikawa H. Curcumin: the Indian solid gold. Adv Exp Med Biol 2007; 595: 1-75.

[27] Aggarwal BB, Harikumar KB. Potential therapeutic effects of curcumin, the anti-inflammatory agent, against neurodegenerative, cardiovascular, pulmonary, metabolic, autoimmune and neoplastic diseases. Int J Biochem Cell Biol 2008; 41: 40-59.

[28] Ak T, Gulcin I. Antioxidant and radical scavenging properties of curcumin. Chem Biol Interact 2008; 174: 27-37.

[29] Menon VP, Sudheer AR. Antioxidant and anti-inflammatory properties of curcumin. Adv Exp Med Biol 2007; 595: 105-25.

[30] Rai D, Yadav D, Balzarini J, De Clercq E, Singh RK. Design and development of curcumin bioconjugates as antiviral agents. Nucleic Acids Symp Ser (Oxf) 2008; (52): 599-600.

[31] Rai D, Singh JK, Roy N, Panda D. Curcumin inhibits FtsZ assembly: an attractive mechanism for its antibacterial activity. Biochem J 2008; 410: 147-55.

[32] Mishra S, Narain U, Mishra R, Misra K. Design, development and synthesis of mixed bioconjugates of piperic acid-glycine, curcumin-glycine/alanine and curcumin-glycine-piperic acid and their antibacterial and antifungal properties. Bioorg Med Chem 2005; 13: 1477-86.

[33] Tiwawech D, Hirose M, Futakuchi M, *et al*. Enhancing effects of Thai edible plants on 2-amino-3, 8-dimethylimidazo(4,5-f)quinoxaline-hepatocarcinogenesis in a rat medium-term bioassay. Cancer Lett 2000; 158: 195-201.

[34] Tisdale M. Monitoring of viral susceptibility: new challenges with the development of influenza NA inhibitors. Rev Med Virol 2000; 10: 45-55.

[35] Reusser P. Herpesvirus resistance to antiviral drugs: a review of the mechanism, clinical importance and therapeutic options. J Hosp Infect 1996; 33: 235-48.

[36] Sindambiwe JB, Calomme M, Cos P, *et al*. Screening of seven selected Rwandan medicinale plants for antimicrobial and antiviral activities. J Ethnopharmacol 1999; 65: 71-7.

[37] Semple SJ, Reynolds GD, O'Leary GD, Flower RLP. Screening of Australian medicinal plants for antiviral activity. J Ethnopharmacol 1998; 60: 163-72.

[38] Kott V, Barbini L, Cruanes M, *et al*. Antiviral activity in Argentine medicinal plants. J Ethnopharmacol 1999; 64: 79-84.

[39] Ma SC, But PP, Ooi VE, *et al*. Antiviral amentoflavone from *Selaginella sinensis*. Biol Pharm Bull 2001; 24: 311-2.

[40] Broncel M. Antiatherosclerotic properties of flavones from the roots of *Scutellaria baicalensis Georg*. Wiad Lek 2007; 60: 294-7.

[41] Nagai T, Suzuki Y, Tomimori T, Yamada H. Antiviral activity of plant flavonoid, 5,7,4'-trihydroxy-8-methoxyflavone, from the roots of *Scutellaria baicalensis* against influenza A (H3N2) and B viruses. Biol Pharm Bull 1995; 18: 295-9.

[42] Sawai R, Kuroda K, Shibata T, Gomyou R, Osawa K, Shimizu K. Anti-influenza virus activity of *Chaenomeles sinensis*. J Ethnopharmacol 2008; 118: 108-12.

[43] Anwar F, Latif S, Ashraf M, Gilani AH. Moringa oleifera: a food plant with multiple medicinal uses. Phytother Res 2007; 21: 17-25.

[44] Miean KH, Mohamed S. Flavonoid (myricetin, quercetin, kaempferol, luteolin, and apigenin) content of edible tropical plants. J Agric Food Chem 2001; 49: 3106-12.

[45] van der Valk M, Reiss P. Metabolic side effects of antiretroviral therapy. Ned Tijdschr Geneeskd 2008; 152: 1260-4.

[46] Gallant JE. Initial therapy of HIV infection. J Clin Virol 2002; 25: 317-33.

[47] Chen TK, Aldrovandi GM. Review of HIV antiretroviral drug resistance. Pediatr Infect Dis J 2008; 27: 749-52.

[48] He Y, Cheng J, Li J, *et al*. Identification of a critical motif for the human immunodeficiency virus type 1 (HIV-1) gp41 core structure: implications for designing novel anti-HIV fusion inhibitors. J Virol 2008; 82: 6349-58.

[49] Noah E, Biron Z, Naider F, Arshava B, Anglister J. The membrane proximal external region of the HIV-1 envelope glycoprotein gp41 contributes to the stabilization of the six-helix bundle formed with a matching N' peptide. Biochemistry 2008; 47: 6782-92.

[50] Liu S, Wu S, Jiang S. HIV entry inhibitors targeting gp41: from polypeptides to small-molecule compounds. Curr Pharm Des 2007; 13: 143-62.

[51] Liu S, Jiang S, Wu Z, *et al*. Identification of inhibitors of the HIV-1 gp41 six-helix bundle formation from extracts of Chinese medicinal herbs *Prunella vulgaris* and *Rhizoma cibotte*. Life Sci 2002; 71: 1779-91.

[52] Lu L, Liu SW, Jiang SB, Wu SG. Tannin inhibits HIV-1 entry by targeting gp41. Acta Pharmacol Sin 2004; 25: 213-8.

[53] Artan M, Li Y, Karadeniz F, Lee SH, Kim MM, Kim SK. Anti-HIV-1 activity of phloroglucinol derivative, 6,6'-bieckol, from Ecklonia cava. Bioorg Med Chem 2008; 16: 7921-6.

[54] Lv L, Sun YR, Xu W, Liu SW, Rao JJ, Wu SG. Isolation and characterization of the anti-HIV active component from *Eucommia ulmoides*. Zhong Yao Cai 2008; 31: 847-50.

[55] Rajbhandari M, Wegner U, Schopke T, Lindequist U, Mentel R. Inhibitory effect of Bergenia ligulata on influenza virus A. Pharmazie 2003; 58: 268-71.

[56] Chiang LC, Ng LT, Cheng PW, Chiang W. Antiviral activities of extracts and selected pure constituents of *Ocimum basilicum*. Clin Exp Pharmacol Physiol 2005; 32: 811-6.

[57] Shih SR, Tsai KN, Li YS, Chueh CC, Chan EC. Inhibition of enterovirus 71-induced apoptosis by allophycocyanin isolated from a blue-green alga *Spirulina platensis*. J Med Virol 2003; 70: 119-25.

[58] Naesens L, Lenaerts L, Andrei G, *et al*. Antiadenovirus activities of several classes of nucleoside and nucleotide analogues. Antimicrob Agents Chemother 2005; 49: 1010-6.

[59] Liu AL, Liu B, Qin HL, Lee SM, Wang YT, Du GH. Anti-influenza virus activities of flavonoids from the medicinal plant *Elsholtzia rugulosa*. Planta Med 2008; 74: 847-51.

[60] Su M, Li Y, Leung KT, *et al*. Antiviral activity and constituent of Ardisia chinensis benth against coxsackie B3 virus. Phytother Res 2006; 20: 634-9.

[61] Yingsakmongkon S, Miyamoto D, Sriwilaijaroen N, *et al*. *In vitro* inhibition of human influenza A virus infection by fruit-juice concentrate of Japanese plum (Prunus mume SIEB. et ZUCC). Biol Pharm Bull 2008; 31: 511-5.

[62] Leung DT, Sacks SL. Current recommendations for the treatment of genital herpes. Drugs 2000; 60: 1329-52.

[63] Galasso GJ, Whitley RJ, Merigan TC. Antiviral Agents and Human Viral Diseases. Philadelphia: Lippincott-Raven 1997.

[64] Yoosook C, Bunyapraphatsara N, Boonyakiat Y, Kantasuk C. Anti-herpes simplex virus activities of crude water extracts of Thai medicinal plants. Phytomedicine 2000; 6: 411-9.

[65] Rajbhandari M, Wegner U, Julich M, Schopke T, Mentel R. Screening of Nepalese medicinal plants for antiviral activity. J Ethnopharmacol 2001; 74: 251-5.

[66] Rajbhandari M, Mentel R, Jha PK, *et al*. Antiviral activity of some plants used in Nepalese traditional medicine. Evid Based Complement Alternat Med 2007; [Epub ahead of print].

[67] Lipipun V, Kurokawa M, Suttisri R, *et al*. Efficacy of Thai medicinal plant extracts against herpes simplex virus type 1 infection *in vitro* and *in vivo*. Antiviral Res 2003; 60: 175-80.

[68] Chiang LC, Cheng HY, Liu MC, Chiang W, Lin CC. *In vitro* anti-herpes simplex viruses and anti-adenoviruses activity of twelve traditionally used medicinal plants in Taiwan. Biol Pharm Bull 2003; 26: 1600-4.

[69] Lohezic-Le Devehat F, Bakhtiar A, Bezivin C, Amoros M, Boustie J. Antiviral and cytotoxic activities of some Indonesian plants. Fitoterapia 2002; 73: 400-5.

[70] Li Y, Ooi LS, Wang H, But PP, Ooi VE. Antiviral activities of medicinal herbs traditionally used in southern mainland China. Phytother Res 2004; 18: 718-22.

[71] Dong TT, Ma XQ, Clarke C, *et al*. Phylogeny of Astragalus in China: molecular evidence from the DNA sequences of 5S rRNA spacer, ITS, and 18S rRNA. J Agric Food Chem 2003; 51: 6709-14.

[72] Sun Y, Yang J. Experimental study of the effect of *Astragalus membranaceus* against herpes simplex virus type 1. Di Yi Jun Yi Da Xue Xue Bao 2004; 24: 57-8.

[73] Liu WJ, Liu B, Guo QL, *et al*. Influence of *Ganciclovir* and *Astragalus membranaceus* on proliferation of hematopoietic progenitor cells of cord blood after cytomegalovirus infection *in vitro*. Zhonghua Er Ke Za Zhi 2004; 42: 490-4.

[74] Lackenby A, Thompson CI, Democratis J. The potential impact of neuraminidase inhibitor resistant influenza. Curr Opin Infect Dis 2008; 21: 626-38.

[75] Sugrue RJ, Tan BH, Yeo DS, Sutejo R. Antiviral drugs for the control of pandemic influenza virus. Ann Acad Med Singapore 2008; 37: 518-24.

[76] Russell RJ, Kerry PS, Stevens DJ, *et al*. Structure of influenza hemagglutinin in complex with an inhibitor of membrane fusion. Proc Natl Acad Sci USA 2008; 105: 17736-41.

[77] Park KJ. Evaluation of *in vitro* antiviral activity in methanol extracts against influenza virus type A from Korean medicinal plants. Phytother Res 2003; 17: 1059-63.

[78] Mochida K. Anti-influenza virus activity of myrica rubra leaf ethanol extract evaluated using Madino-Darby Canine Kidney (MDCK) cells. Biosci Biotechnol Biochem 2008; 72: 3018-20.

[79] Liu N, Hu XL, Meng YR, Zhu YT, Huang BS, Lin PZ. Effect of anti-influenza virus H3N2 of Hypericum japonicum *in vivo*. Zhong Yao Cai 2008; 31: 1022-4.

[80] Sunoto RF. Diarrhoeal problems in Southeast Asia. Southeast Asian J Trop Med Public Health 1982; 13: 306-18.

[81] Bishop RF, Davidson GP, Holmes IH, Ruck BJ. Virus particles in epithelial cells of duodenal mucosa from children with viral gastroenteritis. Lancet 1973; 2: 1281-3.

[82] Haffejee IE. The epidemiology of rotavirus infections: a global perspective. J Pediatr Gastroenterol Nutr 1995; 20: 275-86.

[83] Unicomb LE, Kilgore PE, Faruque SG, *et al*. Anticipating rotavirus vaccines: hospital-based surveillance for rotavirus diarrhea and estimates of disease burden in Bangladesh. Pediatr Infect Dis J 1997; 16: 947-51.

[84] Ke SR, Li ZY, Meng QH. Some features of diarrhoeal diseases in Heilongjiang Province, China. Chin J Epidemiol 1987; 8: 144-7.

[85] Luo GR, Liu ZC, Ding JZ. Etiological analysis of children with infectious diarrhea. Chin J Pediatr 1998; 36: 252.

[86] Cai LM, Zhang C, Jiang WP, Mao WS, Chen H. Clinical analysis of rotavirus enteritis. Chin J Infect Dis 1998; 16: 49-50.

[87] Yu WL. Household survey of diarrhoeal disease control in P. R. China. Chin J Epidemiol 1989; 10: 257-60.

[88] Bern C, Martines J, De Zoysa I, Glass RI. The magnitude of the global problem of diarrheal disease: a ten year update. Bull WHO 1992; 70: 705-14.

[89] Tong QH, Liu ZM. Effects of treatment of rotavirus encephalitis as complication of rotavirus diarrhea with cimetidine and cerebrolysin in 21 cases. J You Jiang Med Coll Nationalities 1999; 1: 64-5.

[90] Lin HB, Xu ZF. Therapeutic effectiveness of Radix Astragali injection on damage of cardiac muscles in children with rotavirus diarrhea. Strait Pharmaceutic J 1998; 10: 65.

[91] Tang F, Liu M, Li XM. Clinical and experimental study on anti-human rotavirus effects of Xiaoer Zhixie capsule. Tianjing Trad Chin Med 1991; 6: 22-3.

[92] He ST, He FZ, Wu SR. Clinical and experimental study on treatment of rotaviral enteritis with Qiwei Baizhu powder. Chin J Integrated Trad West Med 1996; 16: 132-5.

[93] Zhang WD, Zhou QS, Yang ZL. Clinical and experimental study on Wenchangning oral liquor in treating infantile autumn diarrhea. Chin J Integr Trad West Med 1996; 16: 454-8

[94] Chen SD, Xie XL, Pan QF. Studies on treatment of rotavirus diarrhea with *Valeriana jatamansii jone*. Chin J Pediatr 1985; 23: 129-31.

[95] Yang JD, Zheng SY. Clinical study on treatment of 209 cases of adult diarrhea with Wubai decoction. J Zhangjiakou Med Coll 1997; 14: 103-4

[96] Dodet B, Heseltine E, Mary C, Saliou P. Rotaviruses in human and veterinary medicine. Sante 1997; 7: 195-9.

[97] Troussard X, Bauduer F, Gallet E, *et al*. Virus recovery from stools of patients undergoing bone marrow transplantation. Bone Marrow Transplant 1993; 12: 573-6.

[98] Gu Y, Gu Q, Kodama H, Mueller WE, Ushijmai H. Development of antirotavirus agents in Asia. Pediatr Int 2000; 42: 440-7.

[99] Du F, Liang QF, Lin KS, Liang FP, Wen JH, Huang MT. Effects of KeXieDing Liqour on rotavirus and enteropathic *Escherichia coli*. Chin J Zoonoses 1994; 10: 50-51.

[100] Li YL, Sun GQ, Yang YY. Clinical and experimental studies on treatment of infantile diarrhea with Xiaoer Suxieting liquor in 419 cases. Chin J Integrated Trad West Med 1991; 11: 79-82.

[101] Moore EC, Barber J, Tripp RA. Respiratory syncytial virus (RSV) attachment and nonstructural proteins modify the type I interferon response associated with Suppressor of Cytokine Signaling (SOCS) proteins and IFN-Stimulated Gene-15 (ISG15). Virol J 2008; 5: 116.

[102] Foulis AK, Farquharson MA, Cameron SO, McGill M, Schonke H, Kandolf R. A search for the presence of the enteroviral capsid protein VP1 in pancreases of patients with type 1 (insulindependent) diabetes and pancreases and hearts of infants who died of coxsackieviral myocarditis. Diabetologia 1990; 33: 290-8.

[103] Yousef GE, Isenberg DA, Mowbray JF. Detection of enterovirus specific RNA sequences in muscle biopsy specimens from patients with adult onset myositis. Ann Rheum Dis 1990; 49: 310-5.

[104] Kandolf R, Klingel K, Zell R, *et al*. Molecular pathogenesis of enterovirus- induced myocarditis: virus persistence and chronic inflammation. Intervirology 1993; 35: 140-51.

[105] Muir P, Nicholson F, Illavia SJ, *et al*. Serological and molecular evidence of enterovirus infection in patients with end-stage dilated cardiomyopathy. Heart 1996; 76: 243-9.

[106] Galbraith DN, Nairn C, Clements GB. Evidence for enteroviral persistence in humans. J Gen Virol 1997; 78: 307-12.

[107] Ramsingh AI, Chapman N, Tracy S. Coxsackieviruses and diabetes. Bioessays 1997; 19: 793-800.

[108] Toniolo A, Onodera T, Jordan G, Yoon JW, Notkins AL. Virus-induced diabetes mellitus. Glucose abnormalities produced in mice by the six members of the Coxsackie B virus group. Diabetes 1982; 31: 496-9.

[109] Cheng PW, Chiang LC, Yen MH, Lin CC. *Bupleurum kaoi* inhibits Coxsackie B virus type 1 infection of CCFS-1 cells by induction of type I interferons expression. Food Chem Toxicol 2007; 45: 24-31.

[110] Kuo CJ, Shie JJ, Fang JM, *et al*. Design, synthesis, and evaluation of 3C protease inhibitors as anti-enterovirus 71 agents. Bioorg Med Chem 2008; 16: 7388-98.

[111] Wu BW, Pan TL, Leu YL, *et al*. Antiviral effects of *Salvia miltiorrhiza* (Danshen) against enterovirus 71. Am J Chin Med 2007; 35: 153-68.

[112] Dengue and Dengue Haemorrhagic Fever: Fact Sheet. [homepage on the internet]. WHO [Accessed Dec 2008]. Available from: http://www.who.int/mediacentre/factsheets/fs117/en/

[113] Saleem K, Shaikh I. Skin lesions in hospitalized cases of dengue Fever. J Coll Physicians Surg Pak 2008; 18: 608-11.

[114] Premaratna R, Pathmeswaran A, Amarasekara ND, Motha MB, Perera KV, de Silva HJ. A clinical guide for early detection of dengue fever and timing of investigations to detect patients likely to develop complications. Trans R Soc Trop Med Hyg 2009; 103(2): 127-31.

[115] Potts JA, Rothman AL. Clinical and laboratory features that distinguish dengue from other febrile illnesses in endemic populations. Trop Med Int Health 2008; 13(11): 1328-40.

[116] Kittigul L, Pitakarnjanakul P, Sujirarat D, Siripanichgon K. The differences of clinical manifestations and laboratory findings in children and adults with dengue virus infection. J Clin Virol 2007; 39: 76-81.

[117] Wichmann O, Hongsiriwon S, Bowonwatanuwong C, Chotivanich K, Sukthana Y, Pukrittayakamee S. Risk factors and clinical features associated with severe dengue infection in adults and children during the 2001 epidemic in Chonburi, Thailand. Trop Med Int Health 2004; 9: 1022-9.

[118] Lan NT, Kikuchi M, Huong VT, *et al*. Protective and enhancing HLA alleles, HLA-DRB1*0901 and HLA-A*24, for severe forms of dengue virus infection, dengue hemorrhagic fever and dengue shock syndrome. PLoS Negl Trop Dis 2008; 2: e304.

[119] Tung YC, Lin KH, Chiang HC, *et al*. Molecular epidemiology of dengue virus serotype 2 in the taiwan 2002 outbreak with envelope gene and nonstructural protein 1 gene analysis. Kaohsiung J Med Sci 2008; 24: 398-407.

[120] Phurimsak C, Leardkamolkarn V. Screening for antiviral effect of Thai herbs; *Kaempferia parviflora*, *Ellipeiopsis cherrevensis* and *Stemona tuberosa* against Dengue virus type-2. 31st Congress on Science and Technology of Thailand. Suranaree University of Technology; 2005.

Received: February 10, 2009 Revised: March 15, 2009 Accepted: April 4, 2009