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- Review -

Antiviral Activity of Plant-derived Natural Products against Influenza Viruses

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Influenza viruses are zoonotic respiratory pathogens, and influenza infections have caused a substantial burden on public health systems and the livestock industry. Although currently approved seasonal influenza vaccines have shown potent protection efficacy against antigenically well-matched strains, there are considerable unmet needs for the efficient control of viral infections. Enormous efforts have been made to develop broadly protective universal influenza vaccines to tackle the huge levels of genetic diversity and variability of influenza viruses. In addition, antiviral drugs have been considered important interventions for the treatment of viral infections. The viral neuraminidase inhibitor oseltamivir is the most widely used antiviral medication to treat influenza A and influenza B viruses. However, unsatisfactory clinical outcomes resulting from side effects and the emergence of resistant variants have led to greater attention being paid to plants as a natural resource for anti-influenza drugs. In particular, the recent COVID-19 pandemic has underpinned the need for safe and effective antiviral drugs with a broad spectrum of antiviral activity to prevent the rapid spread of viruses among humans. This review outlines the results of the antiviral activities of various natural products isolated from plants against influenza viruses. Special focus is paid to the virucidal effects and the immune-enhancing effects of antiviral natural products, since the products have broad applications as inactivating agents for the preparation of inactivated vaccines and vaccine adjuvants.

Key words : Antiviral drug, influenza virus, natural product, virucidal effect

Introduction

Influenza virus belongs to *Orthomyxoviridae* family and possesses a negative-sense, single-stranded, and segmented RNA genome [76]. Influenza viruses can be divided into four types, A, B, C, and D, according to the antigenicity of NP and M genes. Influenza A viruses show a wide range of hosts including mammals and avian species, showing 18 subtypes of hemagglutinin (HA) and 11 subtypes of neuraminidase (NA) genes, which together define the subtypes of influenza A viruses such as A/H1N1 and A/H3N2. Influenza A viruses present the most notable pathogenicity among humans due to seasonal epidemics and occasional pandemics. In each season, A/H1N1 and A/H3N2 strains co-circulate among humans, causing many hospitalizations and deaths. Influenza A viruses are responsible for intermittent pandemics in last 100 years in history such as the 1918 Spanish flu and the 2009 H1N1 pandemic [58]. Influenza B viruses show very limited antigenic diversity and their pathogenicity is relatively weak in humans compared to influenza A viruses. Influenza B viruses are divided into two distinct lineages, Victoria lineage and Yamagata lineage, but their antigenic distance to each other is much closer than between any two subtypes of influenza A viruses [31]. One or both influenza B virus lineages circulate in each influenza season, but no influenza B virus pandemic has been reported thus far [61]. In addition to influenza epidemics and pandemics, avian influenza viruses such as A/H5N1, A/H7N9, and A/H9N2 have posed serious threats to public health due to their substantial levels of mortality and morbidity in humans. Although it has been suggested that avian influenza viruses rarely transmit to humans, careful monitoring and continuous surveillance on human transmission is needed.

Vaccination has been considered as the most cost-effective

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measure to prevent influenza virus infections. However, influenza viruses constantly undergo antigenic changes by genetic mutation of their RNA genome. In addition, the segmented RNA genomes of influenza viruses can be mixed among different strains, giving rise to a novel strain to which most population has little or no preexisting immunity. Apart from the incomplete protection efficacy of influenza vaccines especially in the immunocompromised and the elderly people, the antigenic variability of influenza viruses make antiviral drugs very imperative. There are three major types of anti-influenza drugs, NA inhibitors, M2 ion channel blockers, and RNA polymerase inhibitors [60, 80](Fig. 1). NA inhibitors inhibit the enzyme activity of the viral NA and thus block the viral egress from the virus-infected cells. M2 ion channel blockers inhibit viral replication in cells by preventing the acidification of the virion. Additionally, nucleoside or nucleotide analogs exert anti-influenza activities by inhibiting the viral RNA polymerase that plays a vital role in the replication and transcription of the viral genome in cells. However, the wide use of chemical antiviral drugs sometimes lead to the occurrence of drug-resistant strains and various side effects have also been reported.

Owing to safety and antiviral activities, natural products from plants have been considered as attractive alternatives to chemical synthetic drugs. Increasing number of studies have suggested that plants can serve as a natural source of safe and effective antiviral drugs against diverse viruses [2, 22]. In general, empirical evidence on antiviral activities of natural products from plants provides insights into in-depth molecular biology studies on precise mechanisms for antiviral activity. This review summarizes the results of the antiviral activity of plant-derived natural compounds against influenza viruses. Furthermore, the virucidal effects and immune enhancing effects of natural products and their potential application into the development of inactivated viral vaccines and vaccine adjuvants are also discussed.

Main Text

Clinically approved antiviral drugs against influenza viruses

Since the first approval of antiviral drug, idoxuridine, for clinical use in 1963, more than 90 antiviral drugs have been approved for the treatment of nine human-infecting viruses (HIV, HBV, HCV, HCMV, HSV, HPV, RSV, VZV, and influenza virus) [15]. Considering that more than 200 human viruses have been recognized thus far, the development of new safe and effective antiviral drugs are urgently needed

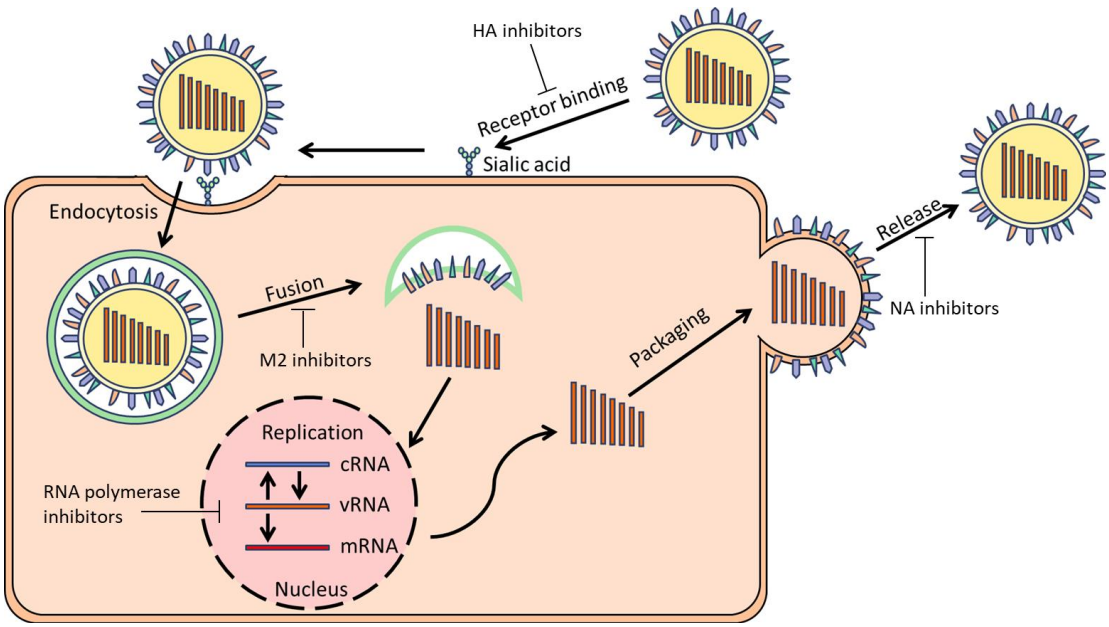


Fig. 1. Influenza virus lifecycle and sites of action of antiviral compounds. HA inhibitors prevent the binding of HA to the receptor and thus block viral attachment and entry to the cell. M2 ion channel blockers inhibit the acidification of virion and impede the release of viral genome into the cytoplasm. Influenza viral RNA polymerase complex replicates viral RNAs and transcribes mRNAs in the nucleus. The viral RNA-dependent RNA polymerase represents the attractive target of antiviral drugs. NA inhibitors inhibit NA which cleaves sialic acid receptors and thus promotes the release of viral particles from infected cells.

[77]. There are eight antiviral drugs approved for the treatment of influenza virus infection, which are divided into three groups; M2 ion channel inhibitors (amantadine and rimantadine), NA inhibitors (zanamivir, oseltamivir, peramivir, and laninamivir octanoate), and RNA polymerase inhibitors (ribavirin and favipiravir) (Fig. 2). M2 ion channel inhibitor amantadine is the first antiviral drug for the treatment of influenza virus. Although amantadine and its synthetic derivative rimantadine have reached market, frequent occurrence of resistant viruses has made them virtually excluded from clinical options [57]. Zanamivir was developed by computer-aided design to inhibit the viral NA enzyme activity [67]. The clinical approval of NA inhibitor zanamivir was followed by the subsequent development and market launches of other NA inhibitors, which are currently the most widely used anti-influenza drugs. Ribavirin and favipiravir are synthetic nucleoside analogues that show broad spectrum antiviral activity against diverse RNA viruses in addition to influenza virus. Ribavirin and favipiravir in their triphosphate forms inhibit the influenza viral RNA polymerase [20, 35].

Anti-influenza activity of natural products isolated from popular plant foods

Anti-influenza activity of green tea catechins

Green tea is one of most widely consumed beverages and has shown to provide various health benefits such as antioxidant, antimicrobial, and anticancer effects [50]. A number of studies have demonstrated that catechins, polyphenol compounds abundantly found in green tea, show the broad-spectrum of antiviral activity against different viruses including human immunodeficiency virus (HIV), herpes simplex virus (HSC), Ebstein-Barr virus (EBV), hepatitis B virus (HBV), hepatitis C virus (HCV), and influenza virus (Fig. 3) [71]. Among catechins, epigallocatechin-3-gallate (EGCG) has been shown to play a key role in antiviral activity through multifaceted working mechanisms depending on viruses. A study showed that EGCG inhibited the endonuclease activity of influenza RNA polymerase and that the galloyl group of EGCG fit well into the endonuclease domain of influenza RNA polymerase in docking simulation [37]. It was also showed that EGCG inhibited the influenza viral hemagglutinin (HA) and caused physical damage to the viral membrane, thus hindering viral attachment to cellular receptors and also fusion event essential for successful viral infection to cells [34, 65]. *In vivo* testing on a mouse model showed that the oral administration of EGCG reduced morbidity and mortality following influenza virus infection [46]. With well-known safety and bioavailability, EGCG could be developed into safe and effective antiviral agents for prophylactics and therapeutics for influenza virus infection. The virucidal effect and immune-enhancing effect of EGCG are discussed below.

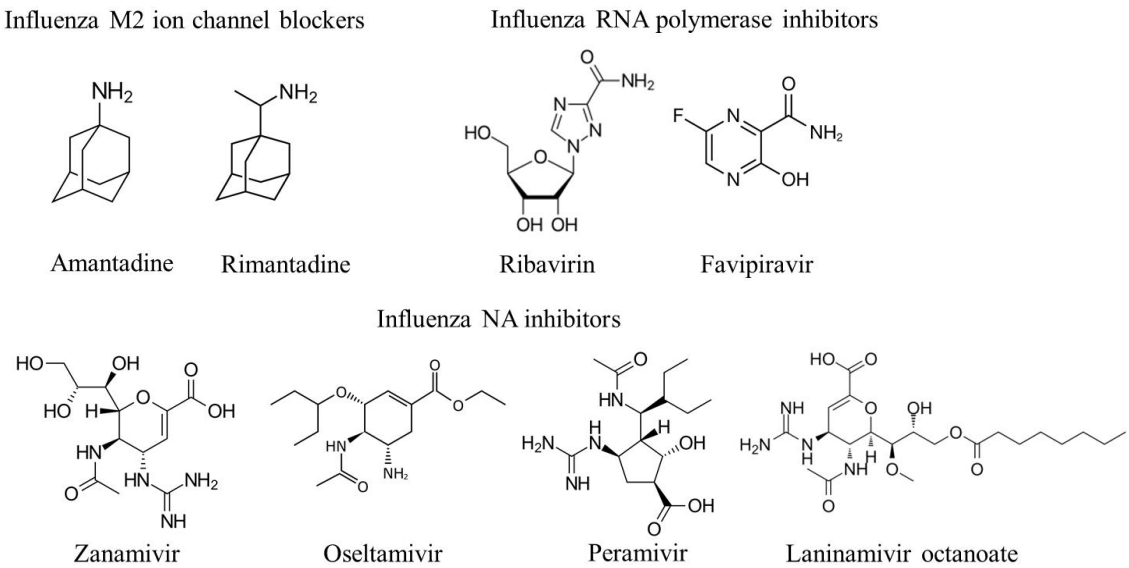


Fig. 2. Chemical structures of anti-influenza drugs approved for clinical use. Influenza M2 ion channel blockers (amantadine and rimantadine) inhibit the transport of hydrogen ions into virion interior and prevent viral uncoating process. Influenza RNA polymerase inhibitors (ribavirin and favipiravir) inhibit the viral RNA polymerase and halt the transcription and replication of the viral RNAs. Influenza NA inhibitors (zanamivir, oseltamivir, peramivir, and laninamivir octanoate) inhibit the viral NA enzyme activity and prevent viral egress.

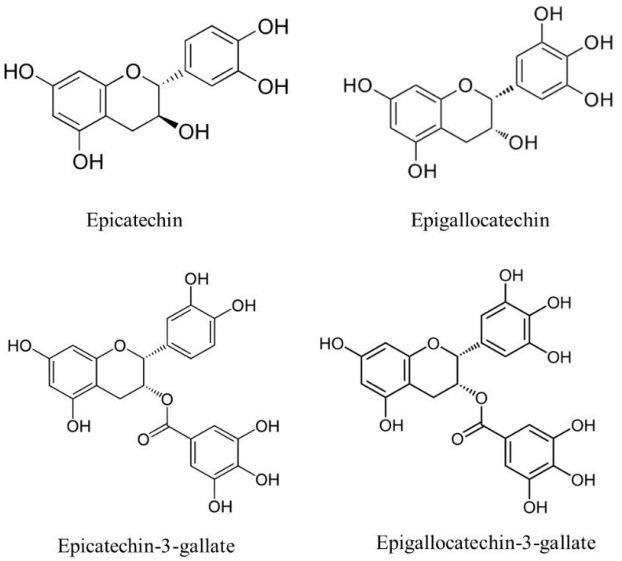


Fig. 3. Chemical structures of green tea catechins. Green tea catechins have been shown to exert anti-influenza virus activity by multiple mechanisms. Epigallocatechin-3-gallate (EGCG) shows the most potent antiviral activity against diverse influenza viruses.

Anti-influenza activity of curcumins

Curcumin is a polyphenol compound found in the rhizome of *Curcuma longa* and is commonly used as a spice and coloring agent in foods (Fig. 4). Curcumin has attracted great research interests because of its various health beneficial activities including antioxidant, anti-inflammation, anticancer, and antiviral effects [55]. A number of studies have shown that curcumin exerts antiviral activity against diverse family of viruses including both RNA viruses and DNA viruses and enveloped and non-enveloped viruses [3, 55]. It has been shown that curcumin possesses multiple inhibitory activity against influenza viruses, including inhibition of the viral HA function [7], suppression of NF-kB signaling pathway necessary for influenza virus replication in cells [52], and the inhibition of the viral NA function [38]. These results together suggest that curcumin has potential to serve as effective options for anti-influenza drugs.

Anti-influenza activity of saponins

Naturally occurring saponins are a large group of triterpene and steroid glycosides possessing several biological and pharmacological properties. A study reported the antiviral activities of soyasaponin II isolated from soybean against HSV-1, HCMV, HIV-1, and influenza virus (Fig. 4) [26]. The study showed that the antiviral activity of soyasaponin II was not due to the inhibition of a specific viral target but to virucidal effect. Similar results were obtained from the following study, in which saponins isolated from the seeds of *Camellia sinensis* inactivated influenza A and B viruses in a dose-dependent manner [27]. It was shown that three saponins with 3-*O*-beta-chacotriosyl residue showed potent anti-influenza effect by inhibiting the interaction between the viral HA and receptor sialic acid [64]. Structure-activity relationship stud-

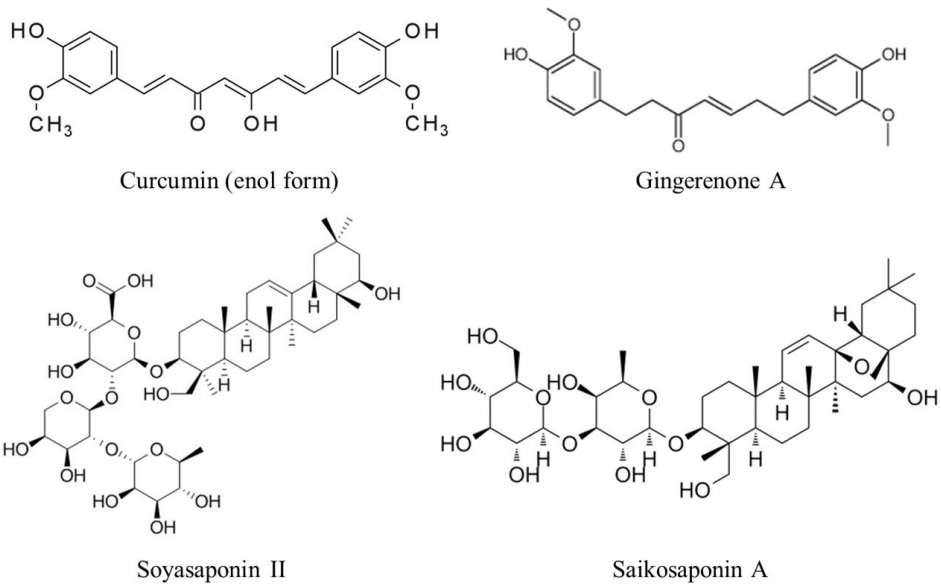


Fig. 4. Chemical structures of curcumin, gingerenone A, soyasaponin II, and saikosaponin A. Curcumin inhibits influenza HA and NA proteins and also suppresses NF-kB signaling pathway. Soyasaponin II displays virucidal effect, and saikosaponin A suppresses NF-kB signaling pathway and pro-inflammatory cytokine production. Gingerenone A inhibits Janus kinase 2 signaling pathway essential for influenza viral replication in cells.

ies demonstrated that chacotriosyl residue and the chlorogenin moiety of saponin was important for the antiviral activity [17, 44]. Another study described the multiple mechanisms of saponin on anti-influenza activity [9]. The study showed that saikosaponin A downregulated NF-kB signaling and pro-inflammatory cytokine production (Fig. 4). In addition, saikosaponin A selectively decreased lung neutrophil and monocyte recruitment during the early stage of innate immune responses upon influenza virus infection. Several saikosaponins isolated from the root of *Bupleurum marginatum var. stenophyllum* also showed potent anti-influenza activity, and the structure-activity relationship was also suggested [21]. In addition, saponins isolated from *Parispolyphylla var. yunnanensis* and *Burkea africana* also showed anti-influenza activity *in vitro* and *in vivo* [47, 56].

Anti-influenza activity of natural products from garlic and ginger

Garlic *(Allium sativum)* is widely consumed as foods and has also been used as traditional remedy against various infectious diseases. Several *in vitro* and *in vivo* studies showed that garlic extracts had antiviral activity against a wide range of viruses including influenza viruses. The water and ethanol extracts of garlic showed antiviral activity against influenza viruses [6], and garlic oil also reduced influenza viral replication in cell culture [12]. Organosulfur compounds (OSCs) of garlic have been identified as key components for antiviral activity against diverse viruses (Fig. 5) [59]. Diallyl trisulfide, a garlic-derived OSC, was shown to have anti-influenza activity *in vitro* and *in vivo* [48]. Both pre-treatment and post treatment of the diallyl trisulfide on A549 cells reduced influenza viral titers, increased antiviral genes such as RIG-I, IRF-3, and interferon-β, and decreased the expression of pro-inflammatory cytokines in cell culture and mice. Given that the broad antiviral activity OSCs of garlic against diverse viruses, it is likely that other OSCs also exhibit anti-influenza activity similar to diallyl trisulfide.

Ginger (*Zingiber officinale*) is a common spice and widely used medical plant and was shown to exert broad antiviral activity. The water extracts of ginger have antiviral activity against human respiratory syncytial virus [5]. Sesquiterpenes isolated from ginger antiviral activity against rhinovirus [16, 36]. There is only one study reporting anti-influenza activity of natural products from ginger, in which gingerenone A, a compound derived from ginger roots, was shown to exert anti-influenza activity by suppressing Janus kinase 2 signaling pathway that is crucial for influenza virus replication (Fig. 4) [69].

Anti-influenza activity of traditional Chinese medicine

Traditional Chinese medicine (TCM) has long history in treating influenza virus infections. Honeysuckle has been used for the treatment of influenza virus infections for thousands of years in China. Recent study has determined that honeysuckle extracts have antiviral activity against influenza viruses *in vitro* and *in vivo* by inhibiting the viral NA enzyme activity [41]. The study showed that acids and flavonoids derived from honeysuckle inhibited the viral replication in cells and reduced the morbidity and mortality in mice after lethal challenge with influenza virus. The results suggest that the natural products of honeysuckle exert anti-influenza activity *in vitro* and *in vivo*. It has been shown that the polysaccharide extracts of *Radix isaditis*, one of representative TCM, has strong anti-influenza activity [45]. The polysaccharides from *R. isaditis* not only inhibited the viral replication but also reduced the expression of pro-inflammatory cytokines through downregulation of Toll-like receptor 3 expression. A later study showed that glucosinolates and their breakdown products isolated from *R. isaditis* exhibited anti-influenza activity,

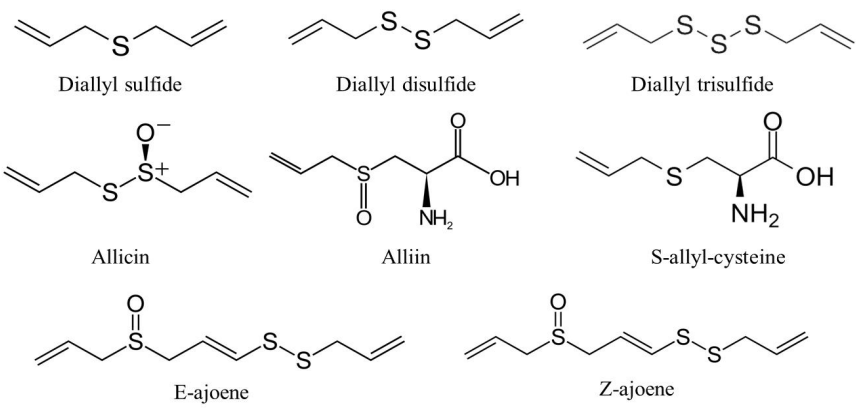


Fig. 5. Chemical structures of organosulfur compounds (OSCs) in garlic. Although the OSCs have been shown to exert broad spectrum of antiviral activity against diverse families of virus, diallyl trisulfide was only reported to have antiviral effect against influenza virus.

although its molecular mechanism was not clearly described [51]. Furthermore, *Terminalia chebula Retz* has been shown to have strong anti-influenza activity [43]. Two related compounds included in *T. chebula*, chebulagic acid and chebulinic acid, were screened as the viral NA inhibitors that were able to reduce the viral replication in cell culture (Fig. 6). Puerarin is a flavonoid compound isolated from *Pueraria lobata* and has many biological functions including antioxidant, anti-inflammation, antitumor, cholesterol lowering, liver protective, and neuroprotective properties (Fig. 6). It was shown that puerarin inhibited the viral NA activity *in vitro* and protected mice against lethal challenge with influenza virus, lowering viral titers and inflammation in the lungs [68]. Overall, anti-influenza activity of TCM seems to depend on the inhibition of the viral NA enzyme activity by polysaccharides compounds.

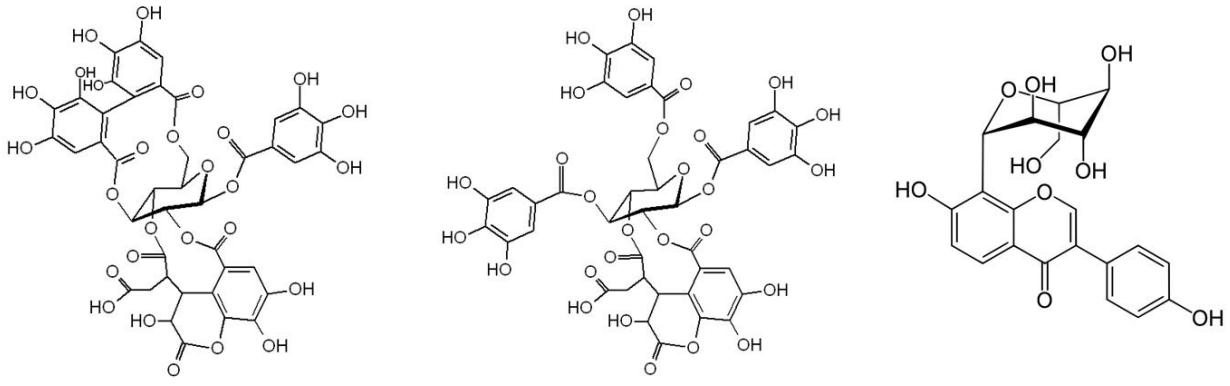
Anti-influenza activity of the natural products from various plants

Natural products with unknown targets

Elaeocarpus sylvestris is distributed in subtropical zones and contains a variety of metabolites including gallate derivatives, coumarins, flavonoids, and sterols [54]. *E. sylvestris* is known to have immunomodulating activity as well as antiviral activity against HCMV and influenza virus [4, 32]. *In vitro* assays revealed that the extracts of *E. sylvestris* and its main ingredients 1,2,3,4,6-penta-*O*-galloyl-β-$_D$-glucose and geraniin strongly inhibited the influenza viral replication (Fig. 7). Also, the molecular docking analysis showed that the two ingredients could interact with 12 influenza viral proteins. The intranasal and the oral administration of the extracts and the ingredients protected mice following lethal influenza virus infection, suggesting that natural compounds contained in *E. sylvestris* could be developed as novel therapeutic agents for the treatment of influenza virus infection. Furanocoumarins isolated from *Angelica dahurica* inhibited the viral replication in cells, without affecting the functions of the viral HA and NA proteins (Fig. 7) [39]. 3-indoleacetonitrile is one of indole derivatives common in cruciferous vegetables such as cabbage, cauliflower, broccoli, and Brussels sprouts. 3-Indoleacetonitrile exerted anti-influenza activity both in cell culture and in a mouse model, although its target or molecular mechanisms has not been defined yet (Fig. 7) [83]. *Poncirus trifoliata* seed extract also displayed anti-influenza acidity in cell culture by inhibiting the viral penetration to cells without affecting the HA-mediated receptor binding, suggesting novel working mechanism [28]. These extracts and compounds were shown to have apparent anti-influenza activity, but their targets or molecular mechanisms have yet to be clarified through further studies.

Natural products possessing inhibitory effects against influenza viral proteins

Influenza HA is most abundant surface glycoprotein that mediates receptor binding rendering viral attachment to cells. In addition, the viral HA induces fusion between the viral and endosomal membrane, thus facilitating the release of viral genome into the cytosol. Embelin, a benzoquinone compound isolated from *Embelia ribes*, demonstrated anti-influenza activity against influenza A and B viruses (Fig. 8) [29]. The embelin exerted effective antiviral activity when added at the early stage of viral infection cycle. In line with this, *in silico* molecular modeling showed that embelin can bind to the receptor binding domain of the viral HA. Polyphenol rich ex-



Chebulagic acid Chebulinic acid Puerarin

Fig. 6. Chemical structures of anti-influenza natural products isolated from plants used in traditional Chinese medicine (TCM). Chebulagic acid, chebulinic acid, and puerarin are anti-influenza components isolated from medical plants used in TCM. The three compounds were shown to have inhibitory effects against influenza NA protein.

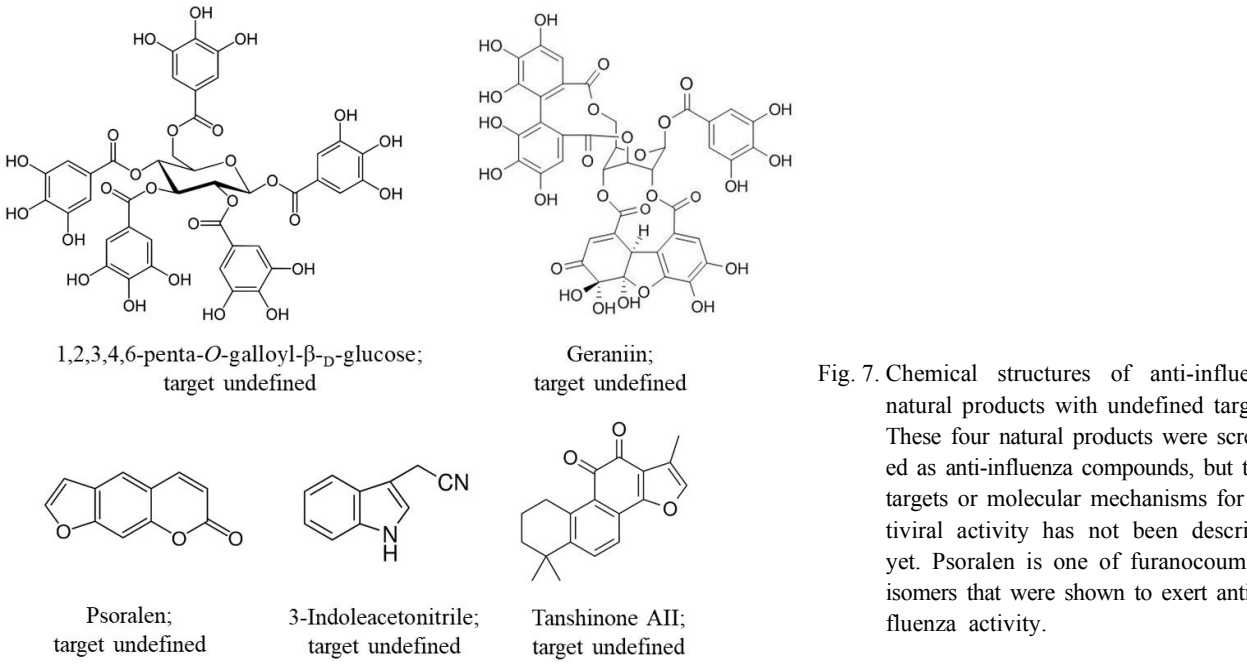


Fig. 7. Chemical structures of anti-influenza natural products with undefined targets. These four natural products were screened as anti-influenza compounds, but their targets or molecular mechanisms for antiviral activity has not been described yet. Psoralen is one of furanocoumarin isomers that were shown to exert anti-influenza activity.

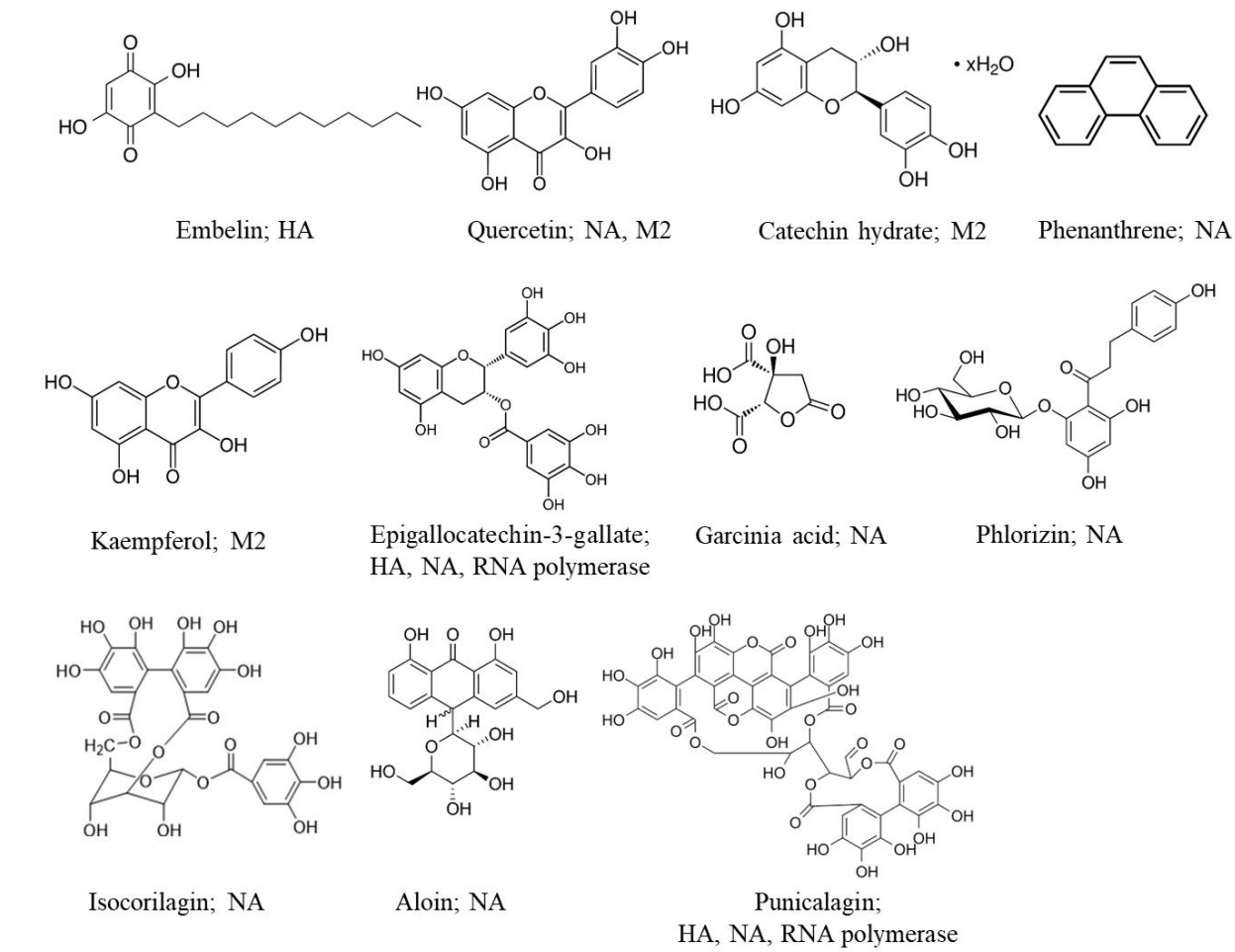


Fig. 8. Chemical structures of anti-influenza natural products with defined viral targets. Natural products and their defined influenza viral target(s) are shown. Quercetin, EGCG, and punicalagin have multiple viral targets.

tracts from *Cistus incanus* also exerted potent anti-influenza activity in cell culture and mice by preventing HA binding to cellular receptors, but responsible ingredients were not clearly defined [18].

M2 ion channel causes the acidification of the interior of influenza virion in the endosome, and thus facilitates the release of viral ribonucleoproteins into the cytosol. *Aloe vera* ethanol extracts showed anti-influenza activity by inhibiting M2 ion channel-induced autophagy, and quercetin, catechin hydrate, and kaempferol were identified as M2 inhibitory components (Fig. 8) [14]. A docking simulation suggested that quercetin, catechin hydrate, and kaempferol could bind to M2 ion channel with higher affinity than known M2 inhibitors.

NA is the second abundant surface proteins in the influenza virus virion and has enzymatic activity that cleaves glycosidic linkage to sialic acid, thus promoting viral egress from infected cells. Oseltamivir is the inhibitor of influenza NA and is the most widely used to treat the influenza virus infection. Therefore, identification of novel NA inhibitors from natural compounds paves the way to the development of safe and effective anti-influenza drugs. Garcinia acid is an ingredient contained in *Garcinia atroviridis* (Fig. 8). Garcinia was shown to strongly inhibit viral NA and was suggested to bind to the triad Arg residues of the viral NA, as indicated by molecular docking analysis [49]. *Balanophora involucrata* has been known to possess anti-inflammatory and antibacterial functions. Recently, quercetin and phloridzin isolated from ethyl acetate fraction of *B. involucrate* extracts were newly identified as influenza NA inhibitors against influenza B viruses (Fig. 8) [66]. Flavonoids isolated from *Pinus densiflora* was shown to exert anti-influenza activity with strong NA inhibitory activity [23]. Isocorilagin, a polyphenolic compound isolated from *Canarium album*, displayed antiviral activity against diverse strains of influenza A viruses (Fig. 8) [8]. The study showed that isocorilagin inhibited NA activity and was suggested to bind to the highly conserved residues in the active site of NA. *Geranii Herba* ethanol extracts and its component geraniin showed NA inhibitory activity against both influenza A and B viruses [13]. The ethanol extract of *Caesalpinia decapetala* demonstrated anti-influenza activity against in cell culture and a mouse model, and NA inhibitory activity was suggested as a main antiviral mechanism [82]. Phenanthrene derivatives isolated from *Bletilla striata* displayed inhibitory effect against the viral NA without affecting HA function (Fig. 8) [62]. Aloin is colored compound found in *Aloe vera* and was shown to inhibit influenza viral NA activity and boost host T cell immunity specific to viral HA (Fig. 8) [30].

Several natural products were shown to target multiple influenza viral proteins (Fig. 8). *Epimedium koreanum* is a popular plant in Korea for treating various diseases. A recent study has shown that the water extract of *E. koreanum* has anti-influenza activity [11]. The study showed that the extracts of *E. koreanum* inhibited HA-mediated viral attachment to cells and also inhibited NA enzyme activity, suggesting that the extracts exert anti-influenza activity at multiple infection stages. The extracts reduced influenza viral infectivity by 10~20-folds compared to control, suggesting virucidal effect, although its molecular mechanisms or key ingredients were not determined. Catechins are polyphenolic compounds found in green tea (*Camellia sinensis*) and have been reported to many beneficial effects such as antioxidant, antimicrobial, anti-allergic, anti-inflammatory, and cancer activity [63]. Epigallocatechin-3-gallate (EGCG), one of catechins of green tea, has been shown to broad antiviral activity including influenza virus (Fig. 8). EGCG was shown to exert anti-influenza activity by inhibiting multiple viral proteins including HA, NA, and RNA polymerase. Furthermore, it was shown that EGCG resulted in physical damage to the viral membrane [34]. Of note, EGCG effectively remove the infectivity of the influenza virus by covalent cross-linking to HA [40]. Similar to EGCG, punicalagin, a polyphenol component isolated from *Punica granatum* exhibited anti-influenza activity by inhibiting HA, NA, and viral RNA polymerase (Fig. 8) [24, 42].

Natural products affecting host signaling pathways

Cirsimaritin is a flavonoid compound found in *Artemisia scoparia* (Fig. 9). Cirsimaritin inhibited viral replication of influenza virus in cell culture, but showed no inhibitory effect on the functions of viral HA and NA [78]. Instead, cirsimaritin was found to suppress the activation of JNK/MAPK and p38/MAPK, thereby downregulating the expression levels of pro-inflammatory cytokines. Thus, cirsimaritin inhibited influenza virus replication by affecting host signaling pathways associated with inflammation. Arctiin is a lignan compound found in many plants of the *Asteraceae* family and has been shown to have multiple pharmacological properties (Fig. 9). A study demonstrated that arctiin inhibited viral replication of influenza virus in cells, and this antiviral activity was attributed to suppression of inflammation signaling pathways in the virus infected cells [84]. Eleutheroside B1 is a coumarin compound found in red tea and green tea, and potatos

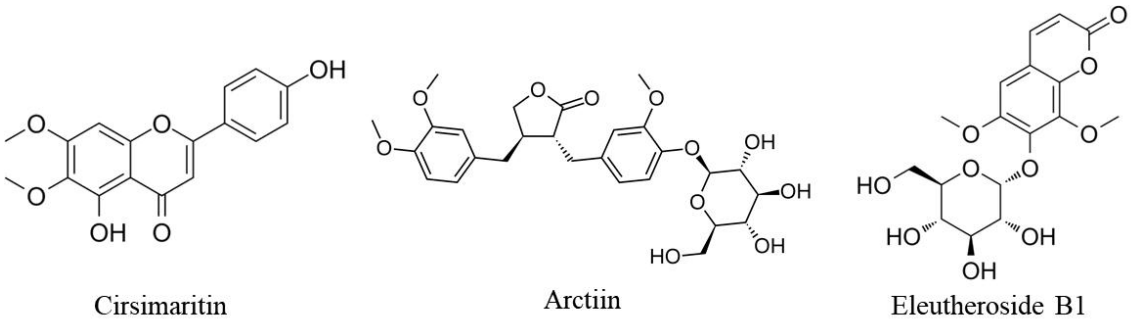


Fig. 9. Chemical structures of anti-influenza natural products affecting host signaling pathways. Cirsimaritin, arctiin, and eleutheroside B1 were shown to have anti-influenza activity by suppressing host signal pathways associated with inflammation responses upon influenza virus infection.

(Fig. 9) [79]. Eleutheroside B1 was shown to affect *N*-glycan biosynthesis, chemokine signaling pathway, interaction between cytokine and receptor, and also target the subunit of host RNA polymerase II to inhibit the production of influenza virus genes. Sesquiterpene fraction isolated from *Laggera pterodonta* acted on the early stage of virus infection but precise working mechanism was not determined [72]. The sesquiterpenes suppressed p38/MAPK pathway and reduced the expression of pro-inflammatory cytokines in cell culture. Flavonoids from *Mosla scabra* alleviated pulmonary inflammation induced by influenza virus infection in mice by affecting pro-inflammatory signaling pathways [81]. Taken together, natural products capable of suppressing inflammation caused by viral infection can potentially inhibit the viral replication.

Virucidal effect of anti-influenza natural products

Virucidal effect means the ability to remove the viral infectivity irreversibly by direct contact between virus particles and compounds. A compound has virucidal effects when the compound permanently impairs the infectivity of a virion outside cells, thus preventing viral attachment or entry into cells. It should be noted that all antiviral compounds do not show virucidal effect because the majority of antiviral compounds reported thus far target viral proteins or cellular proteins via non-covalent or reversible binding modes. The virucidal effects of a certain compound against influenza viruses can be easily measured by plaque inhibition assay since only infectious influenza virus particles can form plaques on cell cultures. There are a couple of reported examples of natural products isolated from plants demonstrating virucidal effect on influenza virus. Tanshinone IIA is the major lipophilic component isolated from *Salvia miltiorrhiza Bunge*, which is currently used to treat myocardial infarction, angina pectoris, stroke, diabetes, sepsis, and other conditions (Fig. 7). It was shown that tanshinone IIA demonstrated virucidal activity against influenza virus [19]. The study showed that incubation of tanshinone IIA with influenza viruses effectively removed the viral infectivity, as indicated by plaque reduction assay. While molecular docking suggested that tanshinone IIA could bind to influenza HA and NA proteins, its precise mechanism on virucidal effect was not determined. Green tea catechins also showed potent virucidal effect against influenza virus [40]. Incubation of green tea extracts with influenza virus eliminated the plaque forming ability of the virus. The study showed that green tea extracts irreversibly inactivated influenza virus, as compared to oseltamivir that is a reversible inhibitor of the viral NA. Mass spectrometry analysis revealed that catechins covalently bound to the Cys residues of the viral HA, which was suggested as an explanation for virucidal effect of catechins. Virucidal effect of natural products can be further applied into the development of inactivated viral vaccines. Currently, highly toxic carcinogens such as formaldehyde and β-propiolactone are used as inactivating agents to inactivate viruses when preparing inactivated vaccines. Natural products possessing virucidal effects such as catechins could serve as novel inactivating agents for the development of viral vaccines with improved safety.

Immunomodulating effects of anti-influenza natural products

There are a large body of studies demonstrating immunomodulating effects of catechin EGCG *in vitro* and *in vivo*. Literatures have demonstrated considerably varied and even contrasting results regarding immunomodulating effects of EGCG according to experimental models and settings [70]. While EGCG stimulated multiple TLR signaling pathways in human and murine cells, which is essential for the sub-

sequent activation of innate immune responses and inflammation [75], EGCG inhibited IFN-γ production in human PBMC cells [73] and suppressed the maturation and proliferation of lymphocytes in human and mice models [1]. General observation is that EGCG suppresses IFN-γ production and pro-inflammatory responses, suggesting that EGCG has little impact on boosting cellular immunity. On the other hand, EGCG displayed potent activating effects on humoral immune responses through stimulation of B cell proliferation and antibody production. In a mouse model, catechin treatment led to increased number of antibody-secreting cells in spleen and enhanced antibody responses to foreign antigens [25, 33]. The immune-enhancing properties of EGCG led to further studies on the potentials of EGCG as a vaccine adjuvant. Recombinant influenza HA vaccine mixed with EGCG, vitamin A, and vitamin E induced significantly higher levels of antibodies and specific T cell responses than other adjuvants in a mouse model [53]. The potent adjuvanticity of EGCG was further evaluated in recombinant HA vaccine and inactivated influenza vaccine in a mouse model [10]. In a mouse model, EGCG mixed with HA full-length or globular domain significantly increased neutralizing antibodies and provided complete protection against lethal infection with wild type influenza virus. Of note, EGCG induced robust antibody effector functions that could potentially improve the efficacy and the breadth of protection. These results together show that EGCG has potent immune-enhancing activity. Therefore, EGCG has dual effects of antiviral activity and immune-stimulating activity, which provides promising prospect for developing safe and effective antiviral prophylactic and therapeutic agents using EGCG.

Saponins have been known to possess numerous biological properties such as membrane-permeabilizing, hypocholeterolemic, anticarcinogenic, and immunostimulant. In particular, potent immune stimulating activity of saponins led to studies to a develop saponin-based vaccine adjuvant QS-21, which is a mixture of two isomeric bidesmosidic saponins isolated from the tree bark of *Quillaja saponaria Molina* (Fig. 10). The molecular mechanism underlying the immune stimulating activity of QS-21 remains largely uncharacterized in spite of wide use in various experimental vaccines and clinically approved vaccines. Given previous results that QS-21 directly activated antigen-presenting cell such as dendritic cells and promoted a pro-inflammatory signaling pathways [74], it can be suggested that saponins can stimulate immune responses independent of the presence of antigens.

Antiviral activity can be obtained by different mechanisms as follows; 1) direct inhibition of virus by targeting viral proteins or viral membrane, 2) the suppression of host proteins essential for successful viral replication, 3) the reduction of inflammation induced by viral infection, and 4) the enhancement of innate immune responses upon viral infection. As described above, many of natural compounds isolated from plants have been found to exert multifaceted antiviral mechanisms. It is likely that antiviral compounds possessing both antiviral activity and immune-modulating effects present better options for effective antiviral agents. In addition, various health beneficial effects of antiviral compounds such as antioxidant and anticancer could provide a basis for the development of next-generation antiviral drugs with improved safety

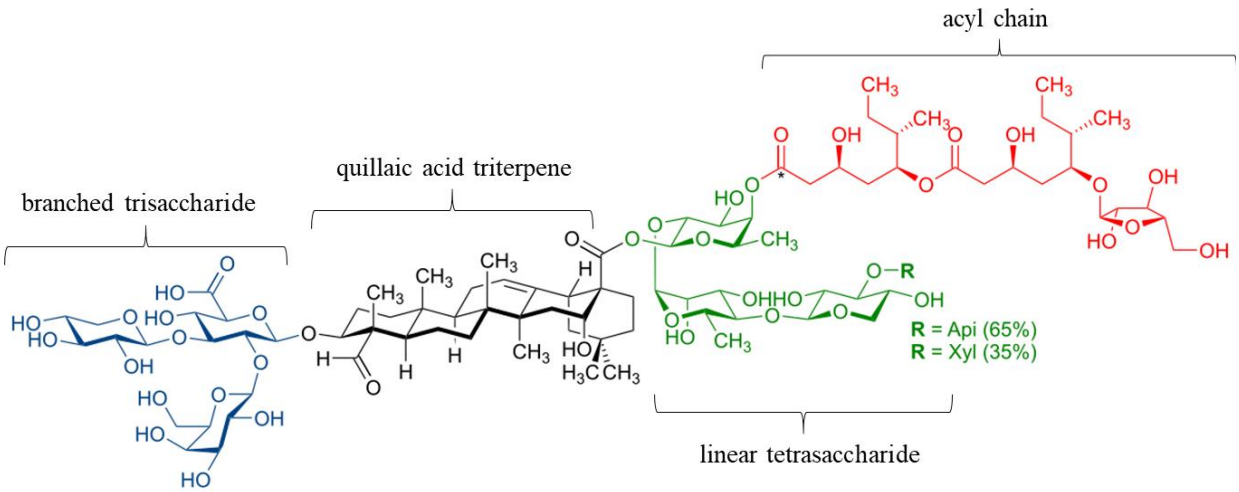


Fig. 10. Chemical structures of QS-21, a saponin-based vaccine adjuvant. QS-21 consists of four chemical groups, a branched trisaccharide, quillaic acid triterpene, a linear tetrasacchride, and an acyl chain. QS-21 is a 65:35 mixture of the apiose and xylose isomers.

and efficacy.

Conclusions

As seen in the recent COVID-19 pandemic, the absence of appropriate antiviral drugs renders most population highly vulnerable to viral infections. Although there are many preexisting antiviral candidates developed for the treatment of viral infections, their efficacy was found to be unsatisfactory for the treatment of the newly emerged COVID-19 virus, suggesting that those candidates including RNA polymerase inhibitors have little antiviral activity against the virus. Plant-derived natural products possessing anti-influenza virus activity discussed in this review are summarized in Table 1. In

Table 1. Plant-derived natural products with anti-influenza activity

Class	Natural products	Viral targets	Effects on host signaling pathways
Natural products from popular plant foods	Epigallocatechin-3-gallate	HA, RNA polymerase, viral membrane, virucidal effect	Suppression of pro-inflammatory signaling pathway, immune-enhancing effect
	Curcumin	HA, NA	Suppression of NF-kB signaling pathway
	Soyasaponin II	Virucidal effect	Unknown
	Saikosaponin A	Unknown	Suppression of pro-inflammatory signaling pathway
	Diallyl trisulfide	Unknown	Increase of antiviral genes, suppression of pro-inflammatory signaling pathway
	Gingerenone A	Unknown	Suppression of Janus kinase 2 signaling pathway
Traditional Chinese medicine	Honeysuckle extracts	NA	Unknown
	Radix isaditis extracts	Unknown	Suppression of pro-inflammatory signaling pathway
	Chebulagic acid	NA	Unknown
	Chebulinic acid	NA	Unknown
	Puerarin	NA	Unknown
Natural products with unknown target	1,2,3,4,6-penta-*O*-galloyl-β-D-glucose	Unknown	Unknown
	Geraniin	Unknown	Unknown
	Psoralen	Unknown	Unknown
	3-Indoleacetonitrile	Unknown	Unknown
	Tanshinone IIA	Virucidal effect	Unknown
Natural products with viral targets	Embelin	HA	Unknown
	Aloe vera ethanol extracts	M2	Unknown
	Quercetin	NA, M2	Unknown
	Catechin hydrate	M2	Unknown
	Kaempferol	M2	Unknown
	Garcinia acid	NA	Unknown
	Phloridzin	NA	Unknown
	Isocorilagin	NA	Unknown
	Phenanthrenes	NA	Unknown
	Aloin	NA	Unknown
	Epimedium koreanum extracts	HA, NA	Unknown
	Punicalagin	HA, NA, RNA polymerase	Unknown
Natural products affecting host signaling pathways	Cirsimaritin	Unknown	Suppression of pro-inflammatory signaling pathway
	Arctiin	Unknown	Suppression of pro-inflammatory signaling pathway
	Eleutheroside B1	Unknown	Inhibition of RNA polymerase II
	Sesquiterpenes	Unknown	Suppression of p38/MAPK pathway and pro-inflammatory signaling pathway

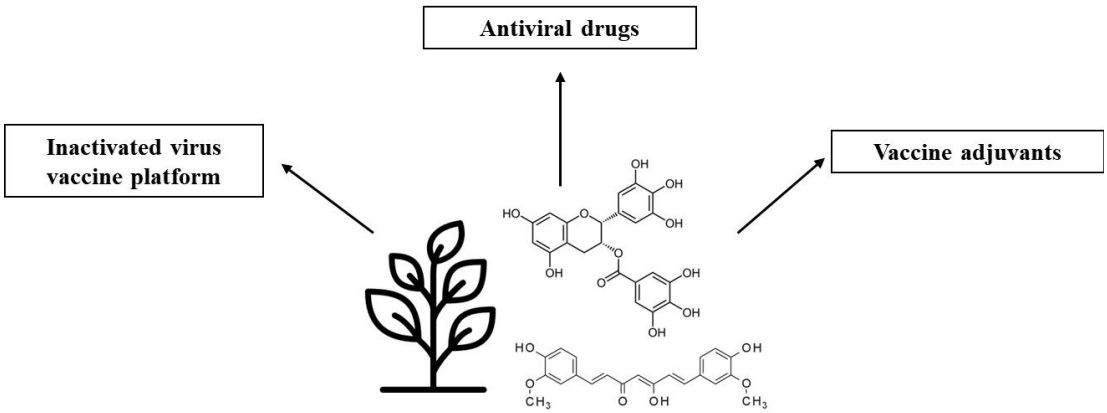


Fig. 11. Possible application of plant-derived antiviral products. Many of plant-derived natural products have antiviral activity and immune-enhancing effect, in addition to well-known health beneficial properties. Therefore, antiviral natural products can be developed into novel antiviral drugs or vaccine adjuvants. Furthermore, natural products with virucidal effect can serve as alternatives to current toxic inactivating agents such as formaldehyde or β-propiolactone for preparing inactivated virus vaccines.

contrast to synthetic chemical drugs, natural products isolated from plants demonstrate a broad spectrum of antiviral activity against different families of viruses. For instance, catechin EGCG has been shown to possess antiviral activity against diverse virus families through multiple independent mechanisms, targeting both viral proteins and host signaling pathways [71]. In addition, curcumins and saponins have also been reported to exert antiviral activity against broad range of viruses [26, 55]. The broad antiviral activity of natural compounds is welcome considering that the effectiveness of synthetic antiviral drugs is limited to only closely related virus strains. Importantly, when a pandemic with a new virus occurs suddenly, broadly reactive antiviral compounds can offer a safety net for public health until a specific antiviral drug is designed and available for clinical use. Frequent uptake of antiviral plants containing antiviral compounds as foods or beverages can provide protection against viral infections.

It remains a great challenge to develop an effective, nontoxic, and well-tolerated antiviral drugs. Plants have long been used as medical purposes or foods and thus natural products from plants would possess better biosafety and bioavailability than synthetic chemical agents. Along with accelerated population aging, use of antiviral drugs based on natural products will enhance patient compliance and drug administration rates, which is important for effective control of infectious diseases at population level, especially in pandemic situation. Although a large body of natural compound have been shown to possess antiviral activity *in vitro*, low efficacy or *in vivo* toxicity in animal and human models remain major causes of clinical failure, providing substantial difficulty in developing clinically relevant antiviral drugs. It is apparent that plants serve as the vast resource of natural compounds with safe and effective antiviral activity. However, low yields, difficulties in extraction and purification steps, and highly complicated structure of the natural products present substantial hurdle for downstream commercialization process. It also remains a great challenge that modifications of structure of the antiviral products are necessary to achieve desired levels of antiviral potency and bioavailability.

In conclusion, natural products from plants represent a rich source of safe and effective antiviral drugs. The broad-spectrum antiviral activity, virucidal effect, in vivo safety, and other health beneficial effects together make them highly attractive candidates for the development of novel antiviral drugs against influenza viruses as well as other diverse families of viruses, vaccine adjuvants, and inactivating agents for inactivated viral vaccines (Fig. 11).

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The Conflict of Interest Statement

The authors declare that they have no conflicts of interest with the contents of this article.

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초록 : 식물 유래 천연물의 인플루엔자에 대한 항바이러스 활성

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인수공통 호흡기바이러스인 인플루엔자바이러스 감염으로 인해 공중보건과 가축산업에 심각한 피해가 지속적으로 발생하고 있다. 인플루엔자 백신 접종을 통해 항원형이 일치하는 바이러스 감염에 대해 우수한 방어면역을 제공하고 있으나, 효과적인 바이러스 감염 제어에는 여전히 큰 공백이 존재하고 있다. 다양한 항원형을 갖는 바이러스에 동시방어가 가능한 범용인플루엔자백신 개발과 함께 바이러스 치료효과를 제공하는 항바이러스제의 개발도 중요한 접근법으로 고려되고 있다. 현재 널리 사용되고 있는 인플루엔자 항바이러스제의 불완전한 치료효과와 내성바이러스의 출현 등의 문제들로 인해 식물 유래 천연물의 항바이러스 활성에 대한 관심이 증가하고 있다. 특히, 현재 진행 중인 코로나-19 팬데믹은 범용적인 항바이러스 활성을 갖는 안전하고 효과적인 항바이러스제 개발의 필요성을 뚜렷이 보여준다. 본 리뷰는 현재까지 보고된 천연물의 항인플루엔자바이러스 활성을 요약하였다. 또한, 항바이러스 활성을 갖는 천연물의 바이러스 사멸활성과 면역증강활성을 이용하는 신규 백신개발과 면역증강제 개발 가능성에 대해서도 분석하였다.