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# Natural Phenolics as Antiviral Agents

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**Abstract:**Viral diseases, such as influenza diseases, are the mainreasons of deathin humans all over the world, despite the fast progressin human medicine. The lack of effective therapiesand/or vaccines for several viral infections, and therapid emergence of new drug-resistant viruses haveled tofind out effectivechemotherapeutic agents to deal these viral diseases. Recentadvances in the understanding of both the cellularand molecular mechanisms of virus replication haveprovided the basis for novel therapeutic strategies.Many natural products such as phenolic compounds have received greatattention for screening and identifying antiviral activity, andsome have been shown to have great medicinal valuein preventing viral diseases. There are severalpotentially useful medicinal plants and herbswaiting to be evaluated and exploited for therapeuticapplications against genetically and functionallydiverse virus families. This review focuses on influenza viruses andantiviral phenolic compounds from medicinal plants(herbs), while paying particular attention to promisingcompounds in preclinical and clinical trials.
**Keywords:** Antiviral agents, influenza, phenolic compounds.

## Introduction

The term of antiviral agents has been known in much broad terms such as substances other than a virus or virus including vaccine or specific antibody which can have either a protective or therapeutic effect to the clear detectable advantage the virus infected host [1]. Viral infections are considered the main cause for morbidity and death worldwide. Infectious viral diseases are major threats to public health and remain major dangers all over the world so far because the viruses spread and mutate very rapidly[2,3]. Lack of specific treatment for viral diseases and restricted biological activity of several drugs have led to using vaccines as preventive agents[4]. The traditional treatment for these illnesses includes various medicaments but resistant pattern of some pathogenic viruses worsen thisstrategy and these drugs also have some severe side effects on patients. So, for these reasons it was necessary to find out new sources to treat these cases [5].

The herbal medicines are considered to be one of the most important approaches in folk medicine. The people use the plants and the natural products directly for their needs to treat much illness. In recent decades, medicinal plants are found in a specific position for being the great sources of drug discovery, irrespective of its categorized group;trees, shrubs or herb. At this time, the use of folk medicines for their therapeutic effects, is not only restricted to the developing countries but also to different communities. According to a report of WHO,up to 80 % of the people living in rural regions use the medicinal plants as main health care system and their practices depended on their knowledge of traditional use of medicinal plants[6].Regarding to a FAO report, at least 25 % medicaments used in modern pharmacopeia, are derived from plant products and many other drugs (synthetic analogues) are being developed on prototype compounds derived from medicinal plants. Drug development programs of pharmaceutical industry play an important role of natural products as more than 50 % of all new clinical drugs are derived from natural products [7].

The wide prescription of herbal drugs is mainly due to their effectiveness, less side effects and relatively low cost. Therapeutic uses of medicinal plants in differentdisease also have an additional important advantage of their easy availability and thus the traditional medical users widely use medicinal plants in their day to day practice [1].

Plant phenolics are defined as a group of natural compounds characterized by having phenol structural units. These compounds include flavonoids, phenolic acids, tannins, etc.[8].Phenolics are plant secondary products and commonly found in different plant and foods as fruits, vegetables, food grains, seeds, flowers, tea, honey and forages. Long time ago, preparations comprise these secondary metabolites as the principal physiologically active components have been used to treat human ailments. A number of traditional plant derived folk medicines rich in phenolics used before to cure disorders of the blood pressure, antiviral infections, stomach problems, antiseptic action,cover of burns, inflammation and inhibition of direct acting mutagens. Phenolics and flavanoids are the major chemical groups with antiviral activity against different diverse virus families such as retroviridae, hepadnaviridae, hespervirides, HIV virus, influenza virus, herpes simplex virus, dengue virus, polio virus, etc. For antiviral activity of phenolic compounds, presence of hydroxyl group and ester groups are very necessary. The phenolics with more hydroxyl groups and 3, 4, 5-three methoxy derivatives show anti-viral and anti-rabies activity while alkyl-esters of gallic acid (gallates), epicathechin responsible for anti-herpetic activity. These compounds represent novel leads, and future studies may cause the development of a pharmacologically approved antiviral agents or group of agents[9,10].

According to both the recent worldwide interest in plant phenolics and the current demand for plants to treat disease, also when taking into account that there is a great lack of knowledge about the antiviral drugs. The present review will focus on antiviral activity of natural phenolics derived from plants.

**1. Viral diseases:**

Viruses are one of the maindangers for both humans and animals. Acute and recurrent viral infections are spreading all over the world and cause wide range of diseases from mild to severe and in some cases they can become life threat in immunocompromised infected human or animal. They pass into the living body and rechange body's metabolism to generate large copies of their genome and proteins[9].

**1.1. Viruses:**

Viruses are obligateintracellular parasites, which contain bundles of gene strands of either DNA or RNA, and may be surrounded by a lipid-containing cover[11].Viruses exploit the host cell environment to propagate new viruses. However, viruses are considered specific because for each virus there is a unique living organism and cell to infect. The virus that causes AIDS never causes hepatitis. The viruses that cause Lassa fever, Ebola fever and acquired immunodeficiency syndrome (AIDS) are examples that researchers call hot agents viruses because these kinds of viruses spread easily, kill sometimes swiftly, and for which there is no cure or vaccine yet.

Viruses use numerous invasion strategies. Each strain of virus has its own unique configuration of surface molecules. These surface molecules work like keys in a lock, enabling viruses to enter into host by precisely fitting the molecules on their surfaces to those on the membranes of the target cells. The success of viruses in evolution has been assured by four general attributes; genetic variation, variety in means of transmission, efficient replication within host cells, and the ability to persist in host. As a consequence viruses have adapted to all forms of life and have occupied numerous ecological niches resulting in widespread diseases in humans, livestock and plants[12].

**1.2. Virus classification:**

Similar to the classification systems used for cellular organisms, virus classification is still subject of ongoing debate and proposals. This is mainly due to the pseudo-living nature of viruses, which are not yet definitively classified as living or non-living. As such, they do not fit neatly into the established biological classification system in place for cellular organisms.

Viruses are mainly classified by phenotypic characteristics, such as morphology, nucleic acid type, mode of replication, host organisms, and the type of disease they cause. Currently there are two main schemes

used for the classification of viruses: theInternational Committee on Taxonomy of Viruses (**ICTV**) system and **Baltimore** classification system, which places viruses into one of seven groups. Accompanying this broad way of classification are specific naming conventions and further classification guidelines set out by the International Committee on Taxonomy of Viruses.

The International Committee on Taxonomy of Virusesbegan to devise and implement rules for the naming and classification of viruses early in the 1990s, an effort that continues to the present day. The ICTV is the only entity charged by the International Union of Microbiological Societies (IUMS) with the task of developing, refining, and maintaining universal virus taxonomy. The system shares many features with the classification system of cellular organisms, such as taxon structure. Viral classification starts at the level of order and follows as thus, with the taxon suffixes given in italics:

**Order**(-*virales*), **Family**(-*viridae*), **Subfamily**(-*virinae*), **Genus**(-*virus*) and **Species .**

Six orders have been established by the ICTV: the *Caudovirales, Herpesvirales, Mononegavirales, Nidovirales, Picornavirales, and Tymovirales*. These orders span viruses with varying host ranges. *Caudovirales* are tailed dsDNA (group I) bacteriophages, *Herpesvirales* contains large eukaryotic dsDNA viruses, *Mononegavirales* includes non-segmented (-) strand ssRNA (Group V) plant and animal viruses, *Nidovirales* is composed of (+) strand ssRNA (Group IV) viruses with vertebrate hosts, *Picornavirales* contains small (+) strand ssRNA viruses that infect a variety of plant, insect, and animal hosts, and *Tymovirales* contains monopartite ssRNA viruses that infect plants.Other variations occur between the orders, for example, *Nidovirales* are isolated for their differentiation in expressing structural and non-structural proteins separately. However, this system of nomenclature differs from other taxonomic codes on several points. A minor point is that names of orders and families are italicized, as in the International Code of Botanical Nomenclature (**ICBN**).Most notably, species names generally take the form of [Disease] *virus*.The establishment of an order is based on the inference that the virus families contained within a single order have most likely emerged from a common ancestor. The majority of virus families remain unplaced. Baltimore classification[13]is a classification system that places viruses into one of seven groups depending on a combination of their nucleic acid (DNA or RNA), strandedness (single-stranded or double-stranded), sense, and method of replication. Viruses must generate mRNAs from their genomes to produce proteins and replicate themselves, but different mechanisms are used to achieve this in each virus family. Viral genomes may be single-stranded (**ss**) or double-stranded (**ds**), RNA or DNA, and may or may not use reverse transcriptase (RT). Additionally, **ss**RNA viruses may be either sense(**+**) or antisense (**−**). This classification places viruses into seven groups[13]:

♦ I: **dsDNA viruses** (e.g. Adenoviruses, Herpesviruses, Poxviruses).
♦ II: **ssDNA viruses** (+)sense DNA (e.g. Parvoviruses).
♦ III: **dsRNA viruses** (e.g. Reoviruses).
♦ IV: **ssRNA viruses** (+)sense RNA (e.g. Picornaviruses, Togaviruses).
♦ V: **ssRNA viruses** (−)sense RNA (e.g. Orthomyxoviruses, Rhabdoviruses).
♦ VI: **ssRNA-RT viruses** (+)sense RNA with DNA intermediate in life-cycle (e.g. Retroviruses).
♦VII: **dsDNA-RT viruses** (e.g. Hepadnaviruses).

Messenger RNA (mRNA) consists of a sequence of the nucleotide bases Adenine (**A**), Cytosine (**C**), Guanine (**G**), and Thymine (**T**). This sequence of nucleotides is called a message because the cytoplasm of a cell translates mRNA into a protein by interpreting each sequence of 3 nucleotide bases into an aminoacid that forms the protein. The term positive-stranded RNA is used for viruses where the RNA has message sense and can act as a messenger RNA (mRNA). The term negative-stranded RNA is used for viruses where the RNA is the complement of the message sense and the message is constructed by creating a complementary sequence of the nucleotide bases where cytosine (**C**) is complementary to guanine (**G**) and thymine (**T**) is complementary to adenine (**A**) (Fig.1).

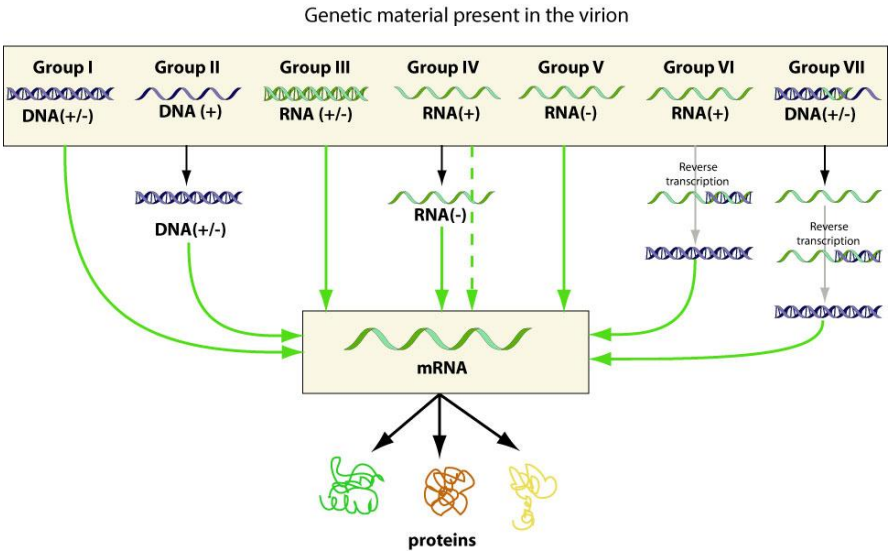



**Fig.(1). Baltimore Classification**

**Influenza virus:**

Influenza, commonly referred to as the flu, is an infectious disease caused by RNA viruses of the family*Orthomyxoviridae* (the influenza viruses), that affects birds and mammals. Seasonal epidemic outbreaks of human influenza viruses occur annually during autumn and winter and cause estimated 250.000 – 500.000 deaths worldwide each year according toWorld Health Organization (WHO). The most common symptoms of the disease are chills, fever, sore throat, muscle pains, severe headache, coughing, weakness/fatigue and general discomfort.In addition to the epidemic form of the disease, pandemic outbreaks occur regularly. Beside the current swine origin influenza virus (SOIV) H1N1V outbreak, three of these pandemics occurred in the last century, in 1918, 1957, and 1968 resulting in millions of deaths within the human population [14,15,16].

Three distinct types of influenza virus; **A**, **B**, and **C**, have been identified. Together these viruses, which are antigenetically distinct from one another, comprise their own viral family, **Orthomyxoviridae**. Most cases of the flu, especially those that occur in epidemics or pandemics, are caused by the influenza A virus, which can affect a variety of human and animal species, but the B virus, which normally is only found in humans, is responsible for many localized outbreaks. The influenza C virus is morphologically and genetically different than the other two viruses and is generally nonsymptomatic, so is of little medical concern. The influenza A, B and C viruses can be distinguished on the basis of antigenic differences between their nucleocapsid (NP) and matrix protein (M).

The important characteristics that distinguish influenza A, B, and C are:

1. Influenza A viruses naturally infect humans and several other mammalian species, including swine, horses, and variety of avian species. Influenza B virus appears to naturally infect only humans. Influenza C has been isolated mainly from humans but swine in China.
2. The surface glycoproteins of influenza A viruses exhibit much greater amino acid sequence variability than their counterparts in the influenza B viruses. Influenza C virus has only a single multifunctional glycoprotein.
3. There are morphological features that distinguish influenza A and B viruses from influenza C viruses.
4. Through the strategy by which influenza A, B, and C viruses encode their proteins are similar overall, each virus type has distinct mechanisms for encoding proteins specific to the virus type.
5. Influenza A and B viruses each contain eight distinct RNA segments, whereas influenza C viruses contain seven RNA segments.

**1.4. Structure of influenzavirus:**

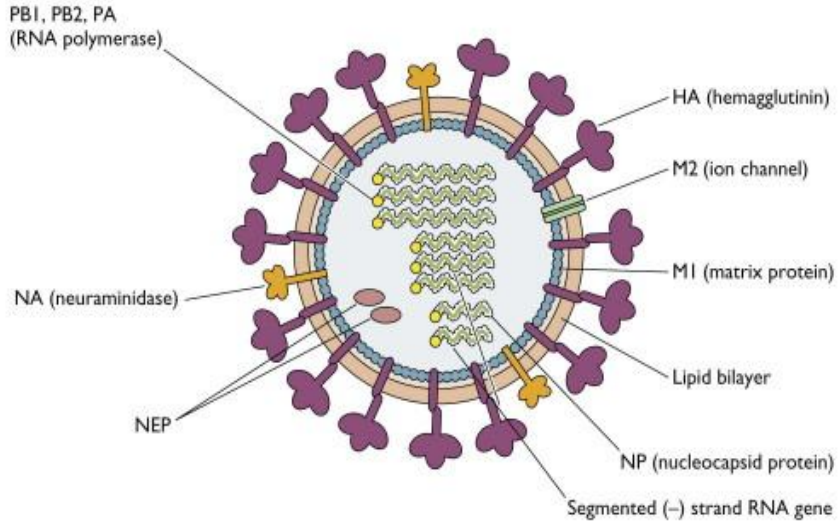



**Fig.(2). The structure of an influenza virus (virion)**

The influenza virion (as the infectious particle as called) is roughly spherical and enveloped. The outer layer is a lipid membrane which is taken from the host cell in which the virus multiplies. In addition to spikes which are inserted into the lipid membrane, these spikes are glycoproteins, because they consist of protein linked to sugars, known as HA (hemagglutinin) and NA (neuraminidase). These are the proteins that determine the subtype of influenza virus such asA/H1N1. The HA and NA are very important in the immune response against the virus. The NA protein is the target of the antiviral drugs Relenza and Tamiflu. Also, M2 protein (ion channel) is embedded in the lipid membrane and it is the target of the antiviral adamantanes; amantadine and rimantadine.

Viral protein which is named M, or matrix protein is found after the lipid membrane. This protein, which forms a shell, gives strength and rigidity to the lipid envelope. Within the interior of the virion, the viral RNAs are found. Eight of them for influenza A viruses. These are the genetic material of the virus; they code for one or two proteins. Each RNA segment, as they are called, consists of RNA joined with several proteins. These RNA segments are the genes of influenza virus. These 8 segments encode for BP1, BP2, PA, HA, NP, NA, M (M1, M2) and NS (NS1, NS2) (Fig. 2).

Mutations in the antigenic structure of the influenza virus have resulted in a number of different influenza subtypes and strains. Specific varieties of the virus are generally named according to the particular antigenic determinants HA and NA. Sixteen types of HA (H1 to H16) and 9 of NA (N1 to N9). The designation **H5N1** refers to a specific combination of hemagglutinin and neuraminidase subtypes.

Two mechanisms, **antigenic drift** and **antigenic shift**, occur in influenza viruses and are responsible for the highly variable nature of the virus. **Antigenic drift** occurs regularly and entails the minor substitution of one or a few amino acids that encode for the HA and NA antigenic sites. This mechanism allows influenza viruses to slightly change, and subsequently the host's neutralizing antibodies are no longer effective. Antigenic drift accounts for the variations each year in the seasonal virus, and this constant variation is why annual vaccinations are recommended for humans. **Antigenic shift** occurs less commonly but creates more concern because it could potentially lead to a novel HA influenza subtype within an animal population, either from genetic reassortment or from virus recombination in a host species (e.g., pig). If infection of the host with 2 distinct influenza viruses occurs, recombination of those two organisms may take place. This is possible with influenza viruses because their genomes are segmented, therefore each gene is a separate piece of genetic material. The new influenza virus may have certain characteristics of both original viruses; consequently, the pathogenicity of the new virus would be difficult to predict and, if easily transmitted, could lead to a pandemic. The ability of two distinct influenza viruses to hybridize is particularly concerning when this process involves virulent subtypes such as the H5N1. The recombination of virulent subtype influenza virus could lead to an infectious organism that is adapted to the humanhost Med., **7**: 501–510[17,18].

### 1.5. Influenza (A) virus:

This genus has one species (influenza A virus). Wild aquatic birds are the natural hosts for a large variety of influenza A. Occasionally, viruses are transmitted to other species and may then cause destruction outbreaks in domestic poultry or give rise to human influenza pandemics. The type A viruses are the most harmful human pathogens among the three influenza types and cause the most severe disease. Type A viruses are divided into subtypes based on differences of two surface proteins called hemagglutinin (**H**) and neuraminidase (**N**). There are 16 different hemagglutinin subtypes and 9 neuraminidase subtypes. The designation **H5N1** refers to a specific combination of hemagglutinin and neuraminidase subtypes. Hemagglutinin and neuraminidase have complex protein structures Med., **7**: 501–510[19]..They are antigens that stimulate an immune response, especially the production of antibodies.

- **H5N1** Subtype - bird flu virus.
- **H3N2** Subtype - Hong Kong flu pandemic of 1968.
- **H5N2** Subtype - highly pathogenic in chickens.
- **H3N8** Subtype - frequently found in horses.
- **H2N2** Subtype - Asian flu pandemic of 1957.
- **H7N7** Subtype - 2003 poultry epidemic.
- **H1N1** Subtype - Spanish flu pandemic of 1918-1919.

### 1.6. Transmission of Influenza virus:

Seasonal influenza spreads easily and can sweep through schools, nursing homes or businesses and towns. People who contact influenza are most infective between the second and third days after infection and infectivity lasts for around ten days Med., **7**: 501–510[20]. Influenza can be spread in three main ways: by direct transmission when an infected person sneezes mucus into the eyes, nose or mouth of another person; through people inhaling the aerosols produced by infected people coughing, sneezing and spitting; and through hand-to-mouth transmission from either contaminated surfaces or direct personal contact, such as a hand-shake [21, 22]and they may all contribute to the spread of the virus [23]. In the airborne route, the droplets that are small enough for people to inhale are 0.5 to 5 μm in diameter and inhaling just one droplet might be enough to cause an infection. Although a single sneeze releases up to 40,000 droplets, most of these droplets are quite large and will quickly settle out of the air[22,24].

### 1.7. Replication of Influenza virus:

As shown in Fig.3. viruses can replicate only in living cells [25]. Influenza infection and replication is a multi-step process:

1. The virus has to bind to the cell andenter, then deliver its genome to a site where it can produce new copies of viral proteins and RNA, assemble these components into new viral particles, and, last, exit the host cell [26]. Influenza viruses bind through hemagglutinin onto sialic acid sugars on the surfaces of epithelial cells, typically in the nose, throat, and lungs of mammals, and intestines of birds.
2. After the hemagglutinin is cleaved by a protease, the cell imports the virus by endocytosis[27]. Once inside the cell, the acidic conditions in the endosome cause that a part of the hemagglutinin protein fuses the viral envelope with the vacuole's membrane, then the M2 ion channel allows proteins to move through the viral envelope and acidify the core of the virus, which causes the core to dissemble and release the viral RNA and core proteins [26]. The viral RNA (vRNA) molecules, accessory proteins and RNA-dependent RNA polymerase are then released into the cytoplasm[28].
3. These core proteins and vRNA form a complex that is transported into the cell nucleus, where the RNA-dependent RNA polymerase begins transcribing complementary positive-sense vRNA[29].
4. The vRNAeither is exported into the cytoplasm and translated or remains in the nucleus.
5. Newly synthesized viral proteins are either secreted through the Golgi apparatus onto the cell surface (in the case of neuraminidase and hemagglutinin, step 5b) or transported back into the nucleus to bind vRNA and form new viral genome particles.
6. Other viral proteins have multiple actions in the host cell, including degrading cellular mRNA and using the released nucleotides for vRNA synthesis and also inhibiting translation of host-cell mRNAs [30]. Negative-sense vRNAs that form the genomes of future viruses, RNA-dependent RNA polymerase, and other viral

proteins are assembled into a virion. Hemagglutinin and neuraminidase molecules cluster into a bulge in the cell membrane. The vRNA and viral core proteins leave the nucleus and enter this membrane protrusion.

7. The mature virus buds off from the cell in a sphere of host phospholipid membraneacquiring hemagglutinin and neuraminidase with this membrane coat[31].

After the release of new influenza viruses, the host cell dies. Because of the absence of RNA proofreading enzymes, the RNA-dependent RNA polymerase that copies the viral genome makes an error roughly every 10 thousand nucleotides, which is the approximate length of the influenza vRNA. Hence, the majority of newly manufactured influenza viruses are mutants; this causes antigenic drift[19].

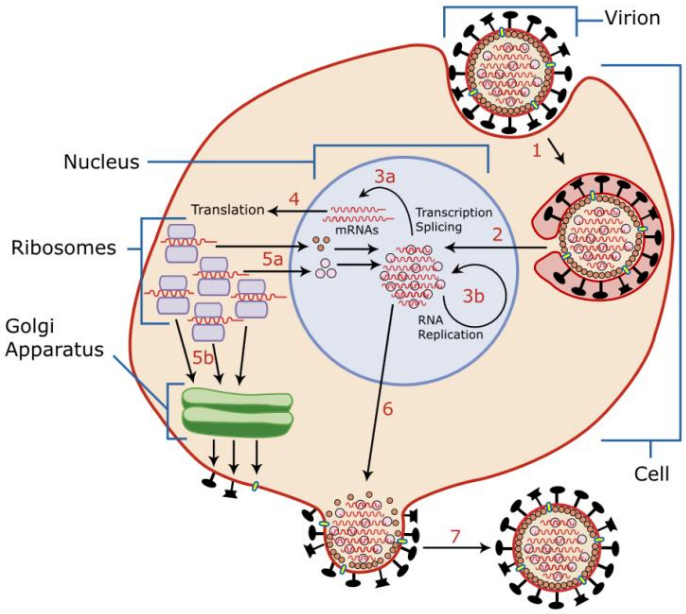



**Fig.(3). Replication of Influenza A virus**

### 1.8. Antiviral drugs for influenza:

The number of anti-influenza virus agents has increased in the past few years, and many chemical and biological anti-influenza agents have been investigated. The infected people with flu have to get plenty of rest, drink a lot of liquids, avoid using alcohol and tobacco and, if necessary, take medications such as acetaminophen (paracetamol) to relieve the fever and muscle aches associated with the flu. Children and teenagers with flu symptoms (particularly fever) should avoid taking aspirin during an influenza infection (especially influenza type B), to avoid Reye's syndrome, a rare but potentially fatal disease of the liver. Since influenza is caused by a virus, antibiotics have no effect on the infection, unless prescribed for secondary infections such as bacterial pneumonia. Antiviral medication can be effective, but some strains of influenza can show resistance to the standard antiviral drugs[32,33].

### 1.9. Mode of action of anti-influenzadrugs:

Drugs for influenza can induce their effect through different mechanisms as follows:

### I. Inhibitors of haemagglutinin:

Haemagglutinin is a glycoprotein located on the surface of influenza virion. During the initial step of infection. Haemagglutinin molecules bind specifically to host cell sialic acid receptors and enable entry of the virus into cell cytoplasm by fusion of viral and cell membranes [34]. The binding interaction of haemagglutinin with cellular receptors can be efficiently inhibited by macromolecules composed of multiple sialic acid residues conjugated to glycan, glycopeptide or polyacrylamide backbones [35].Haemagglutinin-mediated membrane fusion has been successfully blocked by a large variety of small organic compounds as quinones[36].

The sialidase fusion protein DAS181 is an entirely broad spectrum haemagglutinin inhibitor that enzymatically removes sialic acid receptors from respiratory epithelium cells, preventing virus attachment [37].

**II- M2 ion channel blockers:**

M2 ion channel is a transmembrane viral protons that mediates the selective transport of proteins into the interior of the influenza virion. Conductance of protons acidifiers the internal space of the viral particle and facilitate the haemagglutinin-mediated membrane fusion which in turn results in the uncoating of the influenza nucleocapsid and import of the viral genome into the nucleus[30].Adamantanes are potent M2 channel blockers, which are known as the first synthetic anti-influenza drugs. Two adamantane derivatives (amantadine and rimantadine) have been licensed for influenza control and are commercially available under the trademarks Symmetrel® and Flumadine®, respectively [33].

**III- Inhibitors of viral RNA polymerase:**

Transcription and replication of the influenza virus genome is carried out by the influenza RNA polymerase holoenzyme, which is characterized by two catalytic activities. Polymerase activity is needed for the elongation of generated RNA chains, whereas endonuclease activity is essential for cleavage of the 5`-capped primer sequence of the host mRNA. The cap is the terminal 7-methylguanosinbonded through a triphosphate group to the host mRNA. This (cap snatching) process is needed for the initiation of viral RNA transcription [39].Influenza RNA polymerase is an extremely suitable target for the development of new broad-specific antivirals because of its highly conserved structure among influenza strains. It is thought that the influenza polymerase plays a crucial role in virus adaptation to humantohuman transmission and, consequently, in the formation of pandemic influenza variants [40]. Two basic classes of RNA polymerase inhibitors have been described based on different mechanisms of action.

The first group is represented by nucleoside analogues for the blocking of viralRNA chain elongation [41].A typicalmember of this group is favipiravir, which is an inhibitor of influenza A,B and C strains, including variants resistant toamantadine or oseltamivir.The second class of antiviral molecules targeting the influenza polymerase is represented by compounds which block the endonuclease and cap-binding domains of the polymerase holoenzyme. These antivirals include cap analogues [39]**,** short capped oligonucleotides [42], and small organic compounds, such as 4-substitued 2,4-phenylbutanoic acid[43]and flutimide isolated from the fungus *Delitschiaconfertaspora*[44].

**IV- Inhibitors of neuraminidase:**

Neuraminidase, also referred to as sialidase, is an antigenic glycoprotein anchored in the surface envelope of the influenza virions, which hydrolytically cleaves the terminal sialic acid from the host cell receptors. Thus, it plays a crucial role in the release of viral progeny from the membranes of infected cells, prevents self-aggregation of virions and facilitates the movement of the infectious viral particles in the mucus of the respiratory epithelia [45,46].Influenza neuraminidase has been established as a key drug target for the prophylaxis and treatment of influenza infections, predominantly for the following reasons:

Firstly, the structure of the influenza neuraminidase active site is highly conserved between influenza A and B strains, making neuraminidase an attractive target for the development of broad-spectrum inhibitors [47].Secondly, resistance to neuraminidase inhibitors develops less commonly than to other anti-influenza drugs. Nevertheless, the intensive application of neuraminidase inhibitors for influenza treatment results in a permanently increasing number of drug-resistant strains [48].Thirdly, in contrast to adamantanes, neuraminidase inhibitors are mostly well tolerated in patients under therapy [49].Finally, neuraminidase protein is a freely accessible target for antiviral molecules with an extracellular mode of action. At present, several licensed anti-influenza medications are available on the market, most notably the inhalant zanamivir with the trademark Releza®, and the orally administered oseltamivir (Tamiflu®) having excellent bioavailability and relatively long half-life *in vivo* [50].

**V- Host cell factor targeting:**

Many human host cell molecules play an important role in influenza virus propagation and, therefore, represent promising targets for the design of new generation inhibitors of the virus-cell interaction. Muller et al.

described in his review 35 cellular factors essential for influenza virus infection for which 57 inhibitors with apparent anti-influenza activity are available[51]. The most intensively studied are the compounds which effectively inhibit intracellular signalling cascades with a resulting negative influence onthe establishment of viral infection [52]Studies have also focused on inhibitors of vacuolar proton-ATPase which render the viral M2 ion channels inactive[53], inhibitors of cellular proteases which block the proteolytic activation of haemagglutinin[54], and blockers of the cellular ubiquitin-proteasome system [55]. Although the development of host factor inhibitors is a promising research strategy tolimit the emergence of drug-resistant mutants, their possible toxic side-effects in vivo need to be carefully studied.

### 1.10. Combination therapy of influenza infections:

Recent *in vitro* and *in vivo* studies have demonstrated that the simultaneous application of two or more anti-influenza drugs with different modes of action, e.g. oseltamivir and amantadine, results in increased virus inhibition and enhanced therapeutic efficiency [56]. Similar findings were made with the combination of influenza virus inhibitors and immunomodulatory agents, especially corticosteroids[57,58]. These observations are in accordance with the hypothesis that the applied drugs exert additive or synergistic anti-influenza effects in the infected cells. Such a combination regimen enables a reduction in the concentration of the individual inhibitory drugs, resulting in decreased drug toxicity and a reduced risk of antiviral resistance emergence in seasonal and pandemic influenza viruses[59]. The principals of the combination therapy can in the future become a crucial strategy not only in the treatment of influenza infections, but also in the therapy of other serious viral, bacterial and parasitic diseases.

## 2. Antiviral activity of natural phenolic compounds:

### 2.1. Chemistry of phenolic compounds:

Phenolic or polyphenol componentsare one of the most numerous and widely distributed groups of constituents in the plant kingdom, with more than eight thousands phenolic structures known so far[60].Polyphenols are natural products of the secondary metabolism of plants. They are emerged biogenetically from two main biosynthetic pathways; the shikimate pathway and the acetate pathway [61].Natural polyphenols can range from simple structure, such as phenolic acids, to highly polymerized metabolites, as tannins. They are found primarily in conjugated form with one or more sugar moiety linked to hydroxyl groups, addition to direct linkages of the sugar unit to an aromatic carbon atom also exist. The sugar moieties can present as monosaccharides, disaccharides, or oligosaccharides. Glucose is the most common sugar moiety is linked in addition to, galactose, rhamnose, xylose and arabinose, as well as glucouronic and galactouronic acids and many other different sugars. Gifferentgroups, such as amines, carboxylic acids and lipids can also bind with other phenolcompounds[62].Polyphenols can be divided into at least tengroupsregarding to their chemical structure.

### 2.2. Classification of phenolic compounds:

**Simple phenolics:**

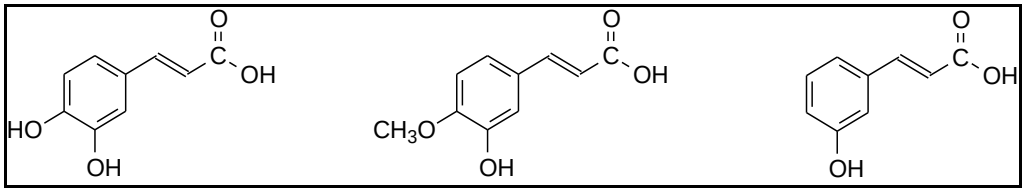
Lowmolecular weight phenolic components are simple phenolic derivatives (**$C_6$**), as phenol itself, cresol, thymol, resorcinol, etc. This group is widespread among different plant species. Phenolics with a **$C_6$-$C_1$** structure, as phenolic acids (e.g., gallic, vanillic, ρ-hydroxybenzoic acids and aldehydes (e.g., vanillin, ρ-hydroxybenzaldehyde) also are fairly common in higher plants. All of these compounds also can be found in a free form or glycosides (Fig. 4).


**ρ-hydroxybenzoic acid Gallic acid (trihydroxybenzoic acid)**

**Fig.(4). Examples of simple phenols**

**Phenylpropanoids:**

Phenylpropanoid derivatives (**$C_6$-$C_3$**) also can be considered as an important group of low molecular weight phenolics. The most knownphenylpropanoidare the hydroxycinnamic acid (ρ-coumaric, caffeic, freulic, sinapic) and its derivatives. Phenylpropanoids are usually covalently linked to cell wall polysaccharides (predominantly ester-linked to arabinose units of hemicellulose) or to the so-called core lignin [63] (Fig. 5).

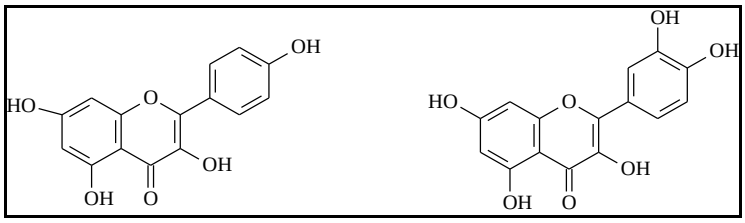


**Caffeic acid** **Ferulic acid** **Coumaric acid**

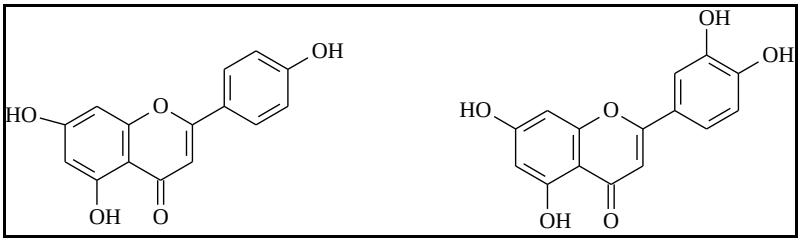
**Fig.(5). Examples of phenylpropanoids**

**Flavonoids:**

Flavonoids represent the most known and widespread group of plant phenolics. The structure of this group is diphenylpropanes (**$C_6$-$C_3$-$C_6$**) and includes two aromatic rings linked through three carbon atoms that usually form an oxygenated heterocycle group. the flavonoids include flavones (e.g. apigenin, luteolin), flavonols (e.g. quercetin, kaempferol, myricitin), and their glycosides. Flavonols occur as ***O***-glycosides with the latter characterized for having a carbon-carbon linkage between the anomeric carbon of a sugar molecule and the C-6 and C-8 carbon of the flavone [64] (Fig. 6).



**Kaempferol (flavonol)** **Quercetin (flavonol)**

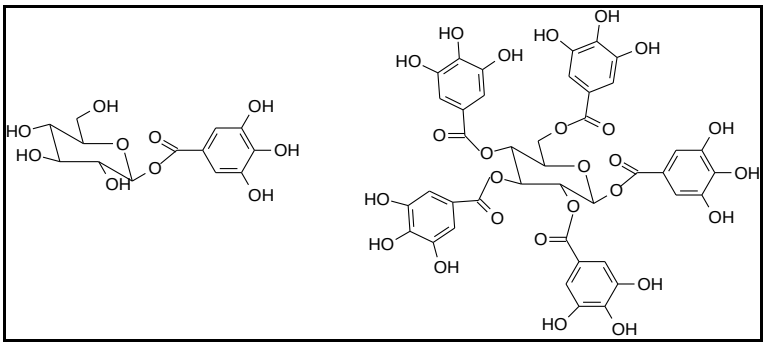


**Apigenin (flavone)** **Luteolin (flavone)**

**Fig.(6). Examples of flavonoids**

**Tannins:**

The word tannin has well established usage in the scientific literature which relates specifically to the application of plant extracts in industry of leather. Tannins are water soluble phenolic compounds having molecular weight ranged between 500 and 3000 Da giving the usual phenolic reactions and having special properties as the ability to precipitate alkaloids, gelatin and other proteins. Tannins are a large group of polyphenolic constituents widely distributed in plant species. Increasing interest in the biological and pharmacological role of these metabolites in the therapeutic effects of traditional medicines [65,66,67]has led to a rapid growth of knowledge in this area. Plant tannins can be divided into two major groups hydrolysable and condensed tannins (Fig. 7):



**1-*O*- galloyl-β-D-glucopyranose 1,2,3,4,6-penta-*O*- galloyl-β-D-glucopyranose**

**Fig.(7). Examples of gallotannins**

**(a)- Hydrolysable tannins**: hydrolysable tannins can be divided into two groups:

**I)-Gallotannins**: the gallotannins are simple polygalloyl esters of glucose (sugar).

**II)-Ellagitannins**: the oxidation coupling of galloyl groups converts to ellagitannins. The simple ellagitannins are esters of hexahydroxydiphenic acid (HHP). HHDP Spontaneouslylactonizes to ellagic acid in aqueous solution (Fig. 8).

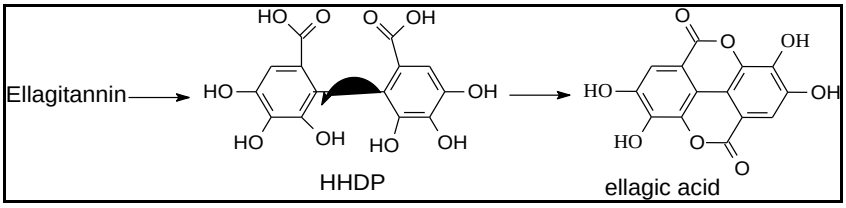
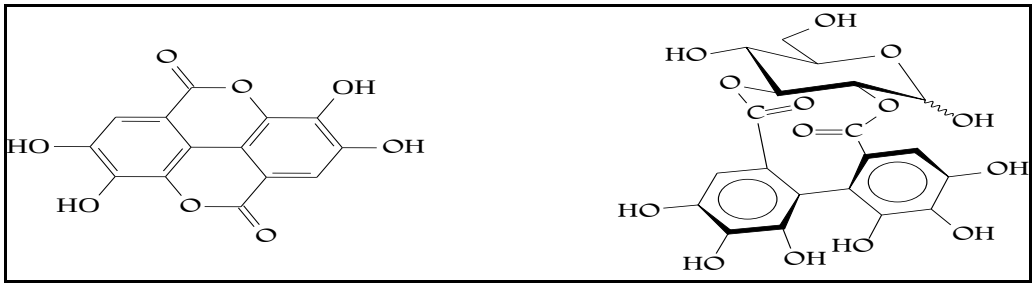



**Fig.(8). Ellagitannin**



**Ellagic acid 2,3-hexahydroxydiphenoyl-(α/β)-D-glucopyranose PunicalinPunicalagin**

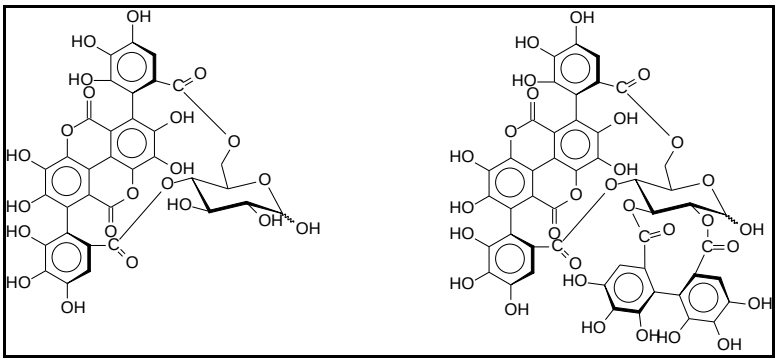
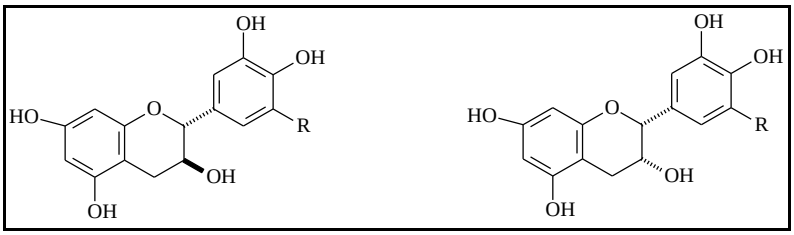


**Fig.(9). Examples of Ellagtannins**

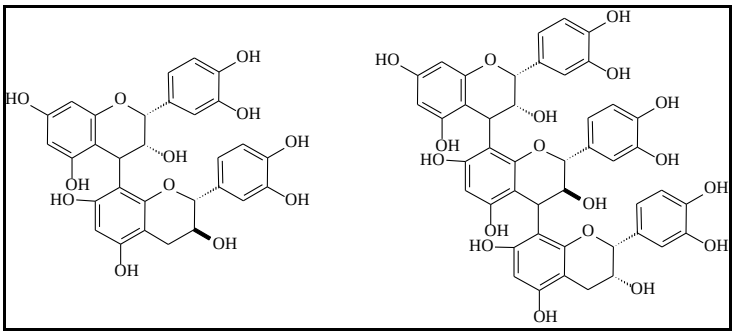
**b)- Condensed tannins**:

Condensed tannins are known as polyflavonoids (flavan-3-ol, or proanthocyanidins). The most widely studies condensed tannins are based on the flavan-3-ol, (–) epicatechin and (+) catechin. Addition of a third phenolic group on the B ring yields eipgallocatechin and gallocatechin (Fig. 10).



**R = H, Catechin** **R= H, Epicatechin**

**R = OH, Gallocatechin** **R = H, Epigallocatechin**



**A proanthocyanidin (Dimmer)** **Aproanthocyanidin (Trimer)**

**Fig.(10). Examples of condensed tannins**

**2.3. Anti-influenza virus activity of natural phenolics:**

Polyphenol compounds have showeddifferent antiviral mechanisms, including inhibition of neuraminidase activity, viral protein, RNA synthesis or membrane fusion.Theseeffects are probably directed against an early stage of viral infection. A lot of these compounds also inhibit viral adsorption to the host cell. As mentioned before, polyphenols of*Reynoutriaellipticа*revealeda high inhibitory neuraminidase activity [68], as determined by a simple neuraminidase assay system developed by Myers [69]. Polyphenols from the leaves of *Folium lsatidis*decreased the pulmonary index and significantly reduction the mortality rate together with prolonging life time in a mouse model[70].Also, polyphenols from *Chaenomelessinensis*fruits gave anti-influenza virus activity by inhibiting hemagglutination activity and by suppressing NS2 protein synthesis [71].Flavonoids are a large class of polyphenolic compounds and are also found in different plant parts, fruits, vegetables and plant-derived beverages. Flavonoid compounds can inhibit both neuraminidase activity and membrane fusion. As early as 1990; 5,7,4`-trihydroxy-8-methoxyflavone, extracted from*Scutellariabaicalensis* root, was showed an influenza virus sialidaseinhibition activity[72].Later, isoscutellarein (5,7,8,4`-tetrahydroxyflavone) isolated from the leaves of *Scutellaria. baicalensis* was also gave potent influenza virus sialidase inhibitory effect when administered orally to mice [73].A new flavanone hesperetin, 7-O-(2``,6``-di-*O*-α-rhamnopyranosyl)-β-glucopyranoside extracted from *Citrus junos* revealed inhibition effect to influenza A virus [74].Recently, flavonoids have received attention due to their various antiviral activities against the influenza virus [75].In particular, quercetin 3-rhamnoside, which is isolated from *Houttuyniacordata*, showed inhibitory effects on influenza A virus replication [76].The juice of the *Agrimoniapilosa*root, has been used to treat colds and coughs [77].The extract of this plant; includingmuch flavonoids such as rutin, hyperoside, catechin, quercetinand quercitrin[78]; was showed inhibition activity against human influenza viruses including H1N1 and H3N2 in embryonated chicken eggs and Madin–Darby canine kidney (MDCK) cells [79].The ethanolicextract of *Cleistocalyxoperculatus* leaves was found to exhibit potential neuraminidase inhibitory effect. Bioassay of this extract directed fractionation led to the isolation of six flavanones [5]. *Geranium sanguineum* L polyphenols have

reported to have strong antiviral activity against influenza virus. Their selective anti-influenza activity was studied*in vitro* and *in vivo*, as well as in chickens and mice [80].While, the polyphenols rich extract of*Cistus incanus*gave a potent antiviral activity in A549 and MDCK cell cultures infected with human influenza and avian strains of different subtypes [81].One of the major polyphenols in the pomegranate juice, Punicalagin, isolated from*Punicagranatum*, showed avirucidalactivity against influenza H3N2 and H1H1 *in vitro*[82].Recently, many studies have also shown antioxidant abilities, such as radical scavenging capabilities, from extracts of *G. sanguineum* in addition to antiviral activity [83].Concerning the active constituents in *Ephedraeherba* extract responsible for antiviral activity on the growth of influenza A/PR/8 in MDCK cells, tannin is strongly suggested to be one of the candidate constituents, based on the complete or partial reversal of the inhibitory activity[84].It has been reported that *Ephedraeherba*extract contains many kinds of condensed tannins and this condensed tannin group is composed of flavan units, mostly consisting of (+)-catechin, (–)-epicatechin (EC), or their analogs. As well as, it is well known that these tannins are found in green tea. Tea-condensed tannins, such as (–)-epigallocatechin gallate (EGCG) and The aflavindigallate, have been mentioned to decrease the growth of influenza A and B viruses [85].Green tea is produced from the leaves of the evergreen plant *Camellia sinensis*, and the major active components of this plant are polyphenolic compounds, as catechins. Catechins include EGCG, (–)-epigallocatechin, (–)-epicatechingallate, and (–)-EC, and EGCG represent approximately 50% of the total concentration of catechins in green tea. From these components, EGCG was considered to be a potent inhibitor of influenza viruses, including H3N2 andH1N1in an MDCK cell culture, and the 3-galloyl group of catechinspossess an important role for observing antiviral activity [86].Grape extracts demonstrated almost complete protection for MDCK cells against influenza at 100 mg/ml without any cytotoxic effects on cells[87].*Limoniumdensiflorum* extract is including polyphenol which demonstrated a potent activity against influenza H1N1 [88].The methanol extract of maca (*Lepidiummeyenii*) which is rich in flavonoid content has antiviral activity against H1N1 influenza similar to those of amantadine antiviral drug[89].*Brassica juncea* water extract was investigated for antiviral activity against influenza virusA/H1N1. At higher nontoxic concentrations, this extract showed maximum antiviral activity against influenza virus A/H1N1[90].

## Conclusion:

A lot of studies in anti-influenza virus natural products have increased rapidly in the past several years as seen by publishing many articles in this issue. Many plant extracts and compounds exhibit activity either directly by antiviral or by stimulating the immune system, or on both two parameters. However, many questions need to reply. One of these problems with current antiviral drug research is that only isolated phytochemicals or specific fractions of the plants are used, rather than plant parts or whole plants. Refined fractions or isolated pure compound of medicinal plants may show certain antiviral activity *in vitro*, but are often in the same time more toxic and less clinically effective than mixture of medicinal plants. In fact, entire plants act very differently in the human body than do the isolated compounds. Traditional Chinese medicine preferably use sent replants, because they are thought to contain synergistic effects that interact with the virus in different methods and neutralize the undesirable effects of any toxic components the plant might include.

It is clearly that, in the practice of traditional Chinese medicine, herbal medicines were prescribed based on the diagnosed symptoms of individual patients without a clear identification of inducing agent, and are therefore not realized to be specific to a specific virus or disease. The constituents of herbal medicines also vary case by case and may even change for each individual patient through the course of the treatment depending on each treatment result. Therefore, it is so difficult to describe a particular herbal composition from previous publications suitable for treating a specific virus. We can think that, the interaction between the components found in medicinal plants and the diseased case of the host might be far more complex than merely the result of a direct antiviral activity exerted by a single chemical compound. So, the pharmacological researches of combinations of medicinal plants have to be encouraged. Traditional combinations of herbal medicines had the potential to become the therapeutics of choice in the future due to the synergistic effect done by the numerous ingredients that inhibit the virus at different stages, improve the impaired immune system.

**Future approaches in antiviral potentials of medicinal plants:**

The importance of herbal medicine can be demonstrated from the fact that depending on the World Health Organization (WHO) estimates, which revealed that up to 80% of the World's population use the medicinal plant for their healthcare needs. The accessibility of an extensive range of potentially active herbs and constituents, to potentiate as anti-influenza agents, may have a leading role in the continued work against the new influenza strain. Programs went for consideration of medicinal plants into the healthcare need of the people should be supported in nations where plants and their products are practiced for medical needs. The nations having notably use of folk medicine have to sponsor specific particular programs dedicated toward new medication discovery from phytochemicals. Improved separation techniques offer possibilities to screen medicinal plant's anti-infectious/antiviral nature. Beforehand, many problems thought to be obstacle in the herbal medicine antiviral drug discovery program are no longer an important. For instance, screening of the antiviral potentials of plant extracts always represent a risk of incidental infection to the workers. Vector-based assay techniques have been very helpful in overcoming such screening obstacles, i.e. recombinant viral vectors mimicking the infection and expressing firefly luciferase marker gene have been broadly used to screen a variety of antivirals[91].A perfect utilization of plants is the production of vaccines and protein-based therapeutics. Several scientific reports recommend that plant could offer a valuable source for the production of pharmaceutical grade peptides/proteins[92]. Since the expression of first subunit vaccine for HBV surface antigen in 1992, many other different vaccine antigens have been successfully expressed inplants and their safety has been assessed in animal and humans[92,93].While, considering this part of plants for treating human viral illnesses one must be careful, as majority of viral vaccines are constituted of lessened or inactivated viral particles. Because of these confinements endeavors have been directed toward expressing coat proteins of different viruses which are presumed to assemble as virus-like-particles (VLP) in plants and are antigenic in nature. Several other issues like fitting handling of protein to be expressed in plants are critical aspects to be considered. There are other issues to be resolved before the translational usage of medicinal plants in the developed world. One such issue is the separation of active constituents associated with the medicinal characteristics of a specific plant. In certain parts of the world crude plant extracts are utilized in healthcare and their efficacy is well-documented with no side effects. Although it will be hard to get these plant extracts approved through international regulatory agencies such as the Food and Drug Administration (FDA) of the United States of America or other comparable European partners, however, for countries with limited resources, government supported investigations will serve as a portal for merging of modern drug discovery with conventional Chinese/Eastern medicine. Moreover, considering the problems faced by the developed world such as drug resistance and failure in finding an effective vaccine for dangerous infectious agent such as HIV, a causative agent for deadly AIDS, phytomedicinal products may give a hope. However, herbal (medicinal plants) preparations are widely used in several parts of the world, individually or in combination, information about the interactions of medicinal plants on living system is rare. It is only experience of the indigenous people using a particular plant/phytochemical product for treating an ailment. Clinical findings such as co-administration of medicinal plants kava-kava and St. John's Wort leading to hepatotoxicity [94]ought to be made accessible to the healthcare providers practicing traditional medicine. For the most part, herbal remedies are seen as harmless, however, many reports recommend hepatotoxicity associated with herbal medication [95].Publication of scientific reports suitable for the cytotoxicities of medicinal plants utilization must to be empowered and incorporated into a universal database system[96,97]. Also, bigger randomized, twofold-blind, placebo-controlled multicenter clinical trials should be conducted before joining of a specific herbal remedy in treating individuals.

There is currently a huge and ever-expanding worldwide population base that prefers the utilization of natural products in treating and preventing medical risks. This has affected numerous pharmaceutical firms to produce new drugs derived from plants or herbs. Currently, plant and herb resources are unlimited, as far as the search for helpful phytochemicals are concerned.

In light of current dangers, the improvement of novel anti-influenza drugs remains a high priority.

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